

CRANFIELD UNIVERSITY

**SCHOOL OF INDUSTRIAL
AND MANUFACTURING SCIENCE**

MOHD FAIRULNIZAL MD NOH

ACADEMIC YEARS 2001 – 2004



Electrochemical sensors development for toxic heavy metals

Supervisor: Dr Ibtisam E. Tothill

PhD Thesis

Mac 2005

This thesis is submitted for the degree of Doctor of Philosophy.

ProQuest Number: 10832216

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



ProQuest 10832216

Published by ProQuest LLC (2019). Copyright of the Dissertation is held by Cranfield University.

All rights reserved.

This work is protected against unauthorized copying under Title 17, United States Code
Microform Edition © ProQuest LLC.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

ABSTRACT

Heavy metals are toxic environmental pollutants and the development and application of decentralised monitoring equipment for their real time monitoring is gaining rapid popularity. Research within this thesis presents the work undertaken to develop single-use sensors for on-site detection of toxic heavy metals in various environmental matrices.

The fabricated three-electrode configuration system (graphite carbon/gold-working electrode, carbon-counter electrode and silver/silver chloride - reference electrode) was coupled with square-wave anodic stripping voltammetry (SWASV), constant current stripping chronopotentiometry (CCSCP) or linear sweep voltammetry (LSV) in order to provide a mean of a relatively inexpensive on-site detector for trace levels of lead (II), copper (II), cadmium (II) and arsenic (III). Detections and determinations of these metals were carried out on bare mercury film screen-printed carbon electrode (SPCE), mercury film SPCEs modified with cysteine and bare screen-printed gold electrode (SPGE) using the optimised procedures developed during this investigation. Modifications of mercury film SPCEs with cysteine provided means of increasing the sensitivity of stripping response obtained at the mercury film SPCE. Detection limits of 0.4, 0.7 and 3 $\mu\text{g l}^{-1}$ were estimated for lead (II), cadmium (II) and copper (II) measurements, respectively for 120 s deposition times. For the bare SPGE, which provides mercury-free sensor, detection limits of 2 and 5.8 $\mu\text{g l}^{-1}$ were obtained for lead (II) detection using SWASV at 240 s and 120 s deposition times. Cadmium (II) was found to interfere with lead (II) measurement with SPGE. Arsenic (III) was also detected with SPGE and detection limit of 0.7 $\mu\text{g l}^{-1}$ was obtained. Other metals such as lead (II), copper (II) and mercury (II) were tested for interference study with As (III) measurements. With the optimised working conditions, the results obtained indicate that the screen-printed electrochemical sensors are sensitive and reproducible for the CCSCP, SWASV and LSV determination of lead (II), copper (II), cadmium (II) and arsenic (III) in the microgram per litre – milligram per litre range. The reproducibility of the measurements, which also contributed to the interest in developing the electrochemical sensing devices for metal ions, was below 15 % for the mercury film SPCE, cysteine modified SPCE, and SPGE.

Surface analysis was conducted using Scanning electron microscopy (SEM) in order to study the electrode surface characterisation. The application of the various electrodes to real samples was demonstrated and proved successful for both wastewater and soil extracted samples including *in situ* measurements at a contaminated site in Spain and Germany.

ACKNOWLEDGEMENTS

I would like to express my gratitude to the following people/organisation that helped make this thesis possible:

- Dr. I.E. Tothill for the supervision and scientific support.
- Dr. Rashid O. Kadara for his continued scientific support.
- Prof. M. Valiente (UAB, Barcelona, Spain) and Técnicas de Protección Ambiental S.A. (TPA, Madrid, Spain) for providing the samples and analytical data used as part of the studies.
- My colleagues (Charlie), at SIMS, Greg, as well as others at IBST, Silsoe.
- The Ministry of Science, Technology and Innovation (MOSTI) and Public Service Department, Malaysia for PhD scholarship under human resource development program.
- Arwah abah (semoga Allah merahmati rohmu), emak (kasih sayang dan doa mu penawar duka), Ida (isteri tercinta yang cukup sabar), Balqis and Adlina (pengubat jiwa), keluarga dan kawan-kawan yang sentiasa memberi sokongan dan semangat untuk berjaya.
- Akhir sekali, kepada Allah yang memberi rahmat, iman, kesabaran, pertolongan dan petunjuk untuk menjalani hidup yang sementara ini.

CONTENTS

ABSTRACT	i
ACKNOWLEDGEMENTS	iii
CONTENTS	iv
LIST OF FIGURES	x
LIST OF PLATES	xx
LIST OF TABLES	xx
NOTATION	xxi

CHAPTER 1

1.0	GENERAL INTRODUCTION	2
1.1	OUTLINE	2
1.2	BACKGROUND	2
1.2.1	Cadmium	4
1.2.2	Copper	4
1.2.3	Lead	5
1.2.4	Mercury	6
1.2.5	Arsenic	7
1.3	LEGISLATION CONCERNING HEAVY METALS	7
1.4	ANALYTICAL METHODS FOR HEAVY METAL ANALYSIS	9
1.4.1	Atomic Spectrometry	9
1.4.1.1	Flame AAS	10
1.4.1.2	Graphite furnace or Flameless AAS (GF-AAS)	11
1.4.1.3	Hydride generation AAS	11
1.4.1.4	Inductively coupled plasma optical emission spectrometry (ICP-OES)	12
1.4.1.5	Inductively coupled plasma mass spectrometry (ICP-MS)	12
1.4.2	Visible Spectrophotometry	13
1.4.3	X-ray Fluorescence Spectrometry (XRF)	13
1.4.4	Instrumental Neutron Activation Analysis (INAA)	14
1.4.5	Immunoassay	14
1.4.6	Biosensors	16

1.4.6.1	Cell based biosensors	16
1.4.6.2	Enzyme-based biosensors for the determination of metal ions	17
1.4.7	Electrochemical techniques	19
1.4.7.1	Potentiometry-Ion Selective electrode	20
1.4.7.2	Stripping analysis	21
1.5	ELECTROCHEMICAL METHODS	23
1.5.1	Potentiometry	23
1.5.2	Voltammetry	24
1.5.3	Processes at Electrode - Solution Interface	25
1.5.3.1	Non-Faradaic processes	25
1.5.3.2	Faradaic processes	25
1.5.4	The Rate of Electrode Reaction	26
1.5.4.1	Mass Transport	27
1.5.5	Linear Sweep and Cyclic Voltammetry	27
1.5.5.1	Linear Sweep Voltammetry	27
1.5.5.2	Cyclic Voltammetry	30
1.5.6	Pulsed Voltammetry	33
1.5.7	Chronopotentiometry	33
1.5.8	Chronoamperometry	35
1.5.9	Stripping Analysis	36
1.6	SURFACE ANALYTICAL TECHNIQUES	40
1.6.1	Electron Microscopy	40
1.6.1.1	Scanning electron microscopy	41
1.7	PROJECT AIMS AND OBJECTIVES	41

CHAPTER 2

2.0	FABRICATION AND CHARACTERISATION OF SCREEN-PRINTED ELECTRODES	45
2.1	OUTLINE	45
2.2	INTRODUCTION	45
2.3	EXPERIMENTAL	47
2.3.1	Printing Materials/ Reagents	47

2.3.2 Equipments	48
2.3.3 Safety	49
2.3.4 Fabrication of electrodes	49
2.3.5 Electrochemical measurements	52
2.3.5.1 Voltammetric studies	52
2.3.5.2 Chronopotentiometric measurement	53
2.3.6 Data analysis	53
2.4 RESULTS	52
2.4.1 Voltammetric characterisation of the working electrode	53
2.5 CONCLUSION	62

CHAPTER 3

3.0 HEAVY METAL IONS DETECTION ON MERCURY FILM SCREEN-PRINTED CARBON ELECTRODES	64
3.1 OUTLINE	64
3.2 INTRODUCTION	64
3.2.1 Detection with mercury film screen-printed carbon sensors	66
3.2.2 Detection with unmodified bare screen-printed carbon sensors	68
3.2.3 Detection with other metallic-film screen-printed electrodes	69
3.3 EXPERIMENTAL	73
3.3.1 Apparatus and reagents	73
3.3.2 Risk assessments and codes of practice	73
3.3.3 Soil/sediment sample treatment	73
3.3.4 Water sample treatment	74
3.3.5 Procedure	74
3.3.6 Real sample analysis	75
3.3.7 Data Analysis	75
3.4 RESULTS	76
3.4.1 Mercury film screen-printed carbon electrode – Optimisation of experimental variables for stripping voltammetric detection of cadmium, lead and copper	76
3.4.1.1 Influence of mercury (II)	76

3.4.1.2 Effect of deposition time	78
3.4.1.3 Effect of deposition potential	80
3.4.1.4 Effect of frequency	80
3.4.1.5 Calibration curve for cadmium (II), lead (II) and copper (II)	83
(a) Cadmium (II)	83
(b) Lead (II)	84
(c) Copper (II)	86
3.4.1.6 Sample evaluations	87
3.5 CONCLUSION	90

CHAPTER 4

4.0 HEAVY METAL IONS DETECTION ON CYSTEINE MODIFIED MERCURY FILM SCREEN-PRINTED CARBON ELECTRODES	93
4.1 OUTLINE	93
4.2 INTRODUCTION	93
4.3 EXPERIMENTAL	96
4.3.1 Chemicals	96
4.3.2 Surface characterisation of screen-printed carbon electrode with SEM	97
4.3.3 Measurement procedure	97
4.4 RESULTS	99
4.4.1 Selection of optimum measurement conditions for CCSCP analysis of cadmium (II) and lead (II)	99
4.4.2 Cysteine concentration and reaction mechanism	100
4.4.3 Mercury concentration	103
4.4.4 Deposition potential	105
4.4.5 Deposition time	106
4.4.6 Stripping current	108
4.4.7 Calibration plot, detection limit and precision	109
4.4.8 Effect of lead and copper on cadmium stripping peak	111
4.4.9 Surface characterisation of screen-printed carbon electrode by SEM	112

4.4.10 Application to water and soil-samples extracts	116
4.5 OPTIMISATION OF EXPERIMENTAL VARIABLES FOR STRIPPING CHRONOPOTENTIOMETRIC MEASUREMENT OF COPPER	122
4.5.1 Effect of mercury (II) concentration	122
4.5.2 Effect of cysteine concentration	124
4.5.3 Effect of constant current	125
4.5.4 Effect of deposition time	126
4.5.5 Effect of deposition potential	126
4.5.6 Calibration graph	127
4.5.7 Real samples analysis	129
4.6 CONCLUSION	131

CHAPTER 5

5.0 DETECTION OF LEAD (II) AND CADMIUM (II) USING SCREEN-PRINTED GOLD ELECTRODES	134
5.1 OUTLINE	134
5.2 INTRODUCTION	134
5.3 DETERMINATION OF LEAD (II)	138
5.3.1 Experimental	138
5.3.1.1 Chemicals	138
5.3.1.2 Electrode fabrication	139
5.3.1.3 Procedure	139
5.3.1.4 Soil/Sediment sample treatment	140
5.3.1.5 Surface characterisation of screen-printed gold electrode with SEM	140
5.3.2 RESULTS	140
5.3.2.1 Surface characterisation of screen-printed gold electrode by SEM	140
5.3.2.2 Linear Sweep Voltammetry of lead (II) on screen-printed gold electrode	142
5.3.2.3 Optimisation of measurement parameters	143

5.3.2.4	Interference study	150
5.3.2.5	Application to waste-water and soil extract samples	153
5.4	DETERMINATION OF CADMIUM (II)	159
5.4.1	Results of cadmium (II) analysis	159
5.5	CONCLUSION	163

CHAPTER 6

6.0	DETECTION OF ARSENIC (III) USING SCREEN-PRINTED GOLD ELECTRODES	165
6.1	OUTLINE	165
6.2	INTRODUCTION	165
6.3	EXPERIMENTAL	168
6.3.1	Chemicals	168
6.3.2	Electrode Fabrication	168
6.3.3	Procedure	168
6.4	RESULTS	168
6.5	CONCLUSION	179

CHAPTER 7

7.0	DISCUSSION	181
7.1	INTRODUCTION	182
7.2	FABRICATION, ELECTROCHEMICAL AND SURFACE CHARACTERISATION OF SCREEN-PRINTED ELECTRODE	182
7.2.1	Voltammetric characterisation of the working electrode	183
7.2.2	Surface characterisation of the working electrode	185
7.3	HEAVY METAL IONS DETECTION ON SCREEN-PRINTED ELECTRODES	186
7.3.1	Mercury film screen-printed carbon electrode-stripping voltammetric detection cadmium (II), lead (II) and copper (II)	187
7.4	DETECTION WITH CYSTEINE MODIFIED MERCURY FILM	189

SCREEN-PRINTED CARBON ELECTRODE	
7.5	METAL IONS ANALYSIS DETECTION USING SCREEN-PRINTED GOLD ELECTRODE 192
7.5.1	Lead (II) and Cadmium (II) analysis 192
7.5.2	Arsenic (III) analysis 196

CHAPTER 8

8.0	CONCLUSIONS & FUTURE WORK 200
8.1	OUTLINE 201
8.2	CONCLUSION 201
8.3	FUTURE WORK 204
REFERENCES 206	
PUBLICATIONS 227	

LIST OF FIGURES

Figure	Page
1.1	Time-potential excitation signal in a linear sweep voltammetry experiment. 27
1.2	Typical linear sweep voltammogram showing the resulting current-potential curve 28
1.3	Potential-time excitation signal in cyclic voltammetric experiment 31
1.4	Cyclic votammogram recorded for a reversible redox couple during a single potential scale 31
1.5	Current excitation (a) and potential response (b) for chronopotentiometry 34
1.6	Anodic stripping voltammetry: the potential-time waveform along with the resulting voltammogram 38
1.7	Flowchart outlining the different stages of the work in this thesis 43
2.1	Schematic representation of the fabrication steps for the screen-printed electrode system prepared for this work 50

2.2	Screen-printed gold electrode	50
2.3	Schematic representation of the screen-printing process	51
2.4	Linear sweep voltammograms of (a) 0.1 M HCl + ammonium acetate electrolyte solution on SPCE-Ercon and GCE (b) 0.025 M Tris-HCl/0.1M KCl for SPCE-Acheson and GCE (c) 0.05 M HCl on gold disc electrode and SPGE. Scan range: -1.5 V to 1.5 V, scan rate: 50 mV s ⁻¹ .	55
2.5	Cyclic voltammograms of 0.5 mM potassium ferrocyanide in 0.1 M KCl. SPCE (Acheson and Ercon)(vs. screen-printed Ag/AgCl reference electrode) and GCE (vs. SCE, 3 M KCl). Scan range: -0.6 V to 0.8 V, scan rate: 20 mV/s.	56
2.6	Cyclic voltammograms of 0.5 mM potassium ferrocyanide in 0.1 M KCl. SPGE (vs. screen-printed Ag/AgCl reference electrode) and GDE (vs. SCE, 3 M KCl). Scan range: -0.6 V to 0.8 V, scan rate: 20 mV/s.	56
2.7	Cyclic voltammograms showing the variation in scan rates for 0.5 mM potassium ferrocyanide in 0.1 M KCl on SPCE-Acheson (a) 10, (b) 25, (c) 50, (d) 75 mV s ⁻¹ . Scan range -0.6 V to 0.8 V (vs. screen-printed Ag/AgCl reference electrode)	57
2.8	Cyclic voltammograms showing the variation in scan rates for 0.5 mM potassium ferrocyanide in 0.1 M KCl on SPGE (a) 10, (b) 25, (c) 50, (d) 75 mV s ⁻¹ . Scan range -0.6 V to 0.8 V (vs. screen-printed Ag/AgCl reference electrode)	57
2.9	Plot of peak-peak separation (ΔE_p) versus log v for determining the transfer coefficient (α) of 5 mM ferrocyanide in 0.1 M KCl on (a) SPCE-Ercon and (b) SPGE. CV scan range = -0.6 V to 0.8 V (vs. screen-printed Ag/AgCl reference electrode).	59
2.10	Plot of anodic peak currents versus square root of scan rates for the determination of the electrochemical active area of SPGE. CV scan range = -0.6 V to 0.8 V (vs. screen-printed Ag/AgCl reference electrode).	61

3.1	Effect of mercury concentration on the stripping voltammetric response of $50 \mu\text{g l}^{-1}$ cadmium (II) in 0.1 M HCl/0.5 M ammonium acetate solution. Experimental conditions: deposition potential = -1.1 V, final potential = -0.05 V, deposition time = 120 s, SW frequency = 25 Hz, step potential = 5 mV, SW amplitude = 0.025 V.	77
3.2	Effect of mercury concentration on the stripping voltammetric response of $50 \mu\text{g l}^{-1}$ lead (II) in 0.1 M HCl/0.5 M ammonium acetate solution. Experimental conditions: deposition potential = -1.1 V, final potential = -0.05 V, deposition time = 120 s, SW frequency = 25 Hz, step potential = 5 mV, SW amplitude = 0.025 V.	77
3.3	Effect of mercury concentration on the stripping voltammetric response of $50 \mu\text{g l}^{-1}$ copper (II) in 0.1 M HCl/0.5 M ammonium acetate solution. Experimental conditions: deposition potential = -1.1 V, final potential = -0.05 V, deposition time = 120 s, SW frequency = 25 Hz, step potential = 5 mV, SW amplitude = 0.025 V.	78
3.4	Effect of deposition time on the stripping voltammetric response of $50 \mu\text{g l}^{-1}$ (a) cadmium (II), (b) lead (II) and (c) copper (II). Deposition potential = -1.1 V, SW frequency = 25 Hz, step potential = 5 mV, amplitude = 25 mV, $\text{Hg}^{2+} = 80 \text{ mg l}^{-1}$.	79
3.5	Effect of deposition potential on the stripping voltammetric response of $50 \mu\text{g l}^{-1}$ (a) cadmium (II), (b) lead (II) and (c) copper (II). Deposition time = 120 s, SW frequency = 25 Hz, step potential = 5 mV, amplitude = 25 mV, $\text{Hg}^{2+} = 80 \text{ mg l}^{-1}$.	81
3.6	Effect of frequency on the stripping voltammetric response of $50 \mu\text{g l}^{-1}$ (a) cadmium (II), (b) lead (II) and (c) copper (II). Deposition time = 120 s, SW frequency = 25 Hz, step potential = 5 mV, amplitude = 25 mV, $\text{Hg}^{2+} = 80 \text{ mg l}^{-1}$.	82
3.7	Stripping voltammograms for increasing concentration of cadmium (II), lead (II) and copper (II) in 0.1 M HCl + 0.5 M ammonium acetate along with the blank response. $\text{Hg}^{2+} = 80 \text{ mg l}^{-1}$, deposition time = 120s, deposition potential = -1 V, final potential = 0.05 V, frequency = 20 Hz, SW amplitude = 25 mV, step potential = 5 mV.	83

3.8	Calibration curve for cadmium (II) in 0.1 M HCl + 0.5 M ammonium acetate a) and (b) linear ranges for cadmium (II) at mercury coated SPCE. $\text{Hg}^{2+} = 80 \text{ mg l}^{-1}$, deposition time = 120s, deposition potential = -1 V, final potential = 0.05 V, frequency = 20 Hz, SW amplitude = 25 mV, step potential = 5 mV.	84
3.9	Calibration curve for lead (II) 0.1 M HCl + 0.5 M ammonium acetate a) and (b) linear ranges for cadmium (II) at mercury coated SPCE. $\text{Hg}^{2+} = 80 \text{ mg l}^{-1}$, deposition time = 120s, deposition potential = -1 V, final potential = 0.05 V, frequency = 20 Hz, SW amplitude = 25 mV, step potential = 5 mV.	85
3.10	Calibration curve for copper (II) 0.1 M HCl + 0.5 M ammonium acetate a) and (b) linear ranges for copper (II) at mercury coated SPCE. $\text{Hg}^{2+} = 80 \text{ mg l}^{-1}$, deposition time = 120s, deposition potential = -1 V, final potential = 0.05 V, frequency = 20 Hz, SW amplitude = 25 mV, step potential = 5 mV.	86
3.11	(a) Stripping voltammograms and (b), (c), (d) standard addition plot for the determination of cadmium (II), lead (II) and copper (II) on mercury coated SPCE in one of the extracted soil samples. Depending on the real concentrations, standard solutions of cadmium (II), lead (II) and copper (II) were added in 40, 80 and 120 and 160 $\mu\text{g l}^{-1}$ increments to the sample. $\text{Hg}^{2+} = 80 \text{ mg l}^{-1}$, deposition time = 120s, deposition potential = -1 V, final potential = 0.05 V, frequency = 20 Hz, SW amplitude = 25 mV, step potential = 5 mV.	88
3.12	Correlation plot between SWASV-MFSPCE and ICP-MS for the determination of lead in extracted soil samples.	90
4.1	Effect of cysteine concentration on the constant current stripping chronopotentiometric of cadmium (II) and lead (II). Experimental condition: 50 $\mu\text{g l}^{-1}$ cadmium (II) and lead (II), 10 mg l^{-1} Hg (II), 0.025 M Tris-HCl/0.1M KCl. Deposition potential = -1 V, deposition time = 2 min, constant current = 1 μA .	100

4.2	Stripping chronopotentiograms of cadmium (II) with and without addition of 10 mM cysteine. Experimental condition: 50 $\mu\text{g l}^{-1}$ cadmium (II), 10 mg l^{-1} Hg, 0.025 M Tris-HCl/0.1M KCl. Deposition potential = -1 V, deposition time = 2 min, constant current = 1 μA . Hg-Cd-SPE : Cadmium (II) with mercury on screen-printed electrode. Hg-Cys-Cd-SPE : Cadmium (II) with mercury and addition of cysteine on screen-printed electrode.	101
4.3	Effect of mercury (II) concentration on stripping peak area of 50 $\mu\text{g l}^{-1}$ cadmium (II) and lead (II). Experimental condition: 10 mg l^{-1} Hg, 0.025 M Tris-HCl/0.1M KCl, pH 7.4 & 6.4, respectively. Deposition potential = -1 V, deposition time = 2 min, constant current = 1 μA .	104
4.4	Effect of deposition potential on stripping response of 50 $\mu\text{g l}^{-1}$ cadmium (II) and lead (II) in 0.025 M Tris-HCl/0.1 M KCl. Other of experimental conditions are as Figure 4.2.	105
4.5	Effect of deposition time on the constant current stripping chronopotentiometric of 25 and 50 $\mu\text{g l}^{-1}$ cadmium (II) using 40 mM cysteine. Other experimental conditions as for Figure 4.2.	107
4.6	Effect of deposition time on the constant current stripping chronopotentiometric of 50 $\mu\text{g l}^{-1}$ lead (II) in 0.025 M Tris-HCl/0.1M KCl for fresh samples and 2 days samples. Other experimental conditions as in Figure 4.2.	107
4.7	Effect of stripping current on the chronopotentiometric stripping response of 50 $\mu\text{g l}^{-1}$ cadmium (II) and lead (II) in 0.025 M Tris-HCl/0.1 M KCl.	108
4.8	Calibration plot (a) from 0 to 800 $\mu\text{g l}^{-1}$ and (b) from 0 to 50 $\mu\text{g l}^{-1}$ for increasing concentration of cadmium (II) with 40 mM cysteine (Hg-Cys-Cd) and without using cysteine (Hg-Cd), respectively. Other experimental condition as for 4.2.	110
4.9	Calibration plot for lead (II) from 0.7 to 1000 $\mu\text{g l}^{-1}$ in 0.025M Tris-HCl/0.1M KCl, pH 6.4 with 20 mM cysteine.	111

4.10	Stripping chronopotentiograms of 50 $\mu\text{g l}^{-1}$ Cd (II) (blue line), 50 $\mu\text{g l}^{-1}$ Cd + 50 $\mu\text{g l}^{-1}$ Pb + 50 $\mu\text{g l}^{-1}$ Cu (pink line) and 50 $\mu\text{g l}^{-1}$ Cd + 150 $\mu\text{g l}^{-1}$ Pb + 25 $\mu\text{g l}^{-1}$ Cu (red line). Experimental condition: 10 mg l^{-1} Hg (II), 40 mM Cys in 0.025 M Tris-HCl/0.1M KCl. Deposition potential = -1V, deposition time = 2 min, constant current = 1 μA .	112
4.11	SEM micrographs showing the surface features of various complexes formed on the surface of SPCE at 8000 magnification a) SPCE without amalgam b) 10 mg l^{-1} Hg + 100 $\mu\text{g l}^{-1}$ Cd c) 10 mg l^{-1} Hg + 40 mM Cys and d) 10 mg l^{-1} Hg + 40 mM Cys + 100 $\mu\text{g l}^{-1}$ Cd.	114
4.12	Stripping chronopotentiograms and standard addition plot (inset) for the determination of cadmium (II) in real soil sample using 0.025M Tris-HCl/0.1M KCl, pH 7.4 as buffer with 40 mM cysteine. Other experimental conditions are as for Figure 4.2.	117
4.13	Calibration graph (left) and stripping chronopotentiograms of lead (II) standards from 0 – 400 $\mu\text{g l}^{-1}$ in 0.025 M Tris-HCl/0.1M KCl, pH 6.4. The standards were prepared using Palmsens at Senspol Technical Meeting, Seville, 2002.	119
4.14	Stripping chronopotentiograms and standard addition plot (inset) for the determination of lead (II) in real soil sample using 0.025M Tris-HCl/0.1M KCl, pH 6.4 as buffer with 20 mM cysteine. Other experimental conditions are as for Figure 4.2.	119
4.15	Stripping chronopotentiograms for (a) 50 $\mu\text{g l}^{-1}$ copper (II) in solutions containing HCl at different concentration using MFSPCE. Hg^{2+} = 10 mg l^{-1} , deposition time = 120 s, Cys = 20 mM, deposition potential = -0.9 V, constant current = 1 μA .	123
4.16	Effect of mercury concentration on stripping chronopotentiometric stripping of 50 $\mu\text{g l}^{-1}$ Cu in 2 M HCl.	123
4.19	Effect of cysteine concentration on the stripping chronopotentiometric stripping of 50 $\mu\text{g l}^{-1}$ Cu in 2 M HCl (a) and stripping voltammograms (b).	124

4.18	Effect of constant current on stripping chronopotentiometric of $50 \mu\text{g l}^{-1}$ Cu in 2 M HCl (a) and stripping voltammograms (b).	125
4.19	Effect of constant current on stripping chronopotentiometric of $50 \mu\text{g l}^{-1}$ Cu in 2 M HCl.	126
4.20	Effect of deposition potential on stripping chronopotentiometric of $50 \mu\text{g l}^{-1}$ Cu in 2M HCl.	127
4.21	Calibration graph of Cu (II) in 2 M HCl (a) from $10 - 400 \mu\text{g l}^{-1}$ (b) from $0 - 50 \mu\text{g l}^{-1}$ using Autolab and Palmsens, respectively. The analysis was done using different set of standards prepared at different time and (c) Stripping chronopotentiograms for increasing concentrations of copper (II) in 2 M HCl from $0 - 400 \mu\text{g l}^{-1}$ using Autolab.	128
4.22	Correlation between results obtained by CCSCP and ICP-MS for copper (II) analysis in 2 M HCl.	130
4.23	Results (No. 24) shows correlation between Cu^{2+} level using cysteine modified electrodes and GF-AAS. There is another result (No. 22) from other agency. Results were produced by BfG, Germany.	131
5.1	Typical scanning electron micrographs (1000X magnification) showing the surface features of a screen-printed gold electrode and analysis results.	141
5.2	Cyclic voltammetry behaviour of lead (10 mg l^{-1}) at SPGE in 0.05 M HCl. (a) no lead (b) 0 s, (c) 15 s, (d) 30 s, (e) 45 s and (f) 90 s. The electrodes were cleaned electrochemically with 0.5 M H_2SO_4 /0.01M KCl prior to CV analysis. Deposition was in unstirred solution at -0.7 V deposition potential. Scan rate= 100 mV s^{-1} , equilibrium time = 120s, step potential = 2.44 mV.	142
5.3	Optimisation of deposition time parameters for square-wave analysis of 100 and $50 \mu\text{g l}^{-1}$ lead using Autolab and Palmsens, respectively. Experiments carried out with in 0.05M HCl. Deposition potential = -0.7 V, deposition time = 120 s; frequency = 8 Hz; amplitude = 0.025 V and step amplitude = 0.05 V.	144

5.4	Optimisation of deposition potential parameters for square-wave analysis of 100 and 50 $\mu\text{g l}^{-1}$ lead using Autolab and Palmsens, respectively. Experiments carried out with in 0.05M HCl. Other conditions as in Figure 5.3.	145
5.5	Optimisation of frequency parameters for square-wave analysis of 100 and 50 $\mu\text{g l}^{-1}$ lead using Autolab and Palmsens, respectively. Experiments carried out in 0.05 M HCl. Other conditions as in Figure 5.3.	146
5.6	Optimisation of chloride parameters for square-wave analysis of 100 and 50 $\mu\text{g l}^{-1}$ lead using Autolab and Palmsens, respectively. Experiments carried out with in 0.05M HCl. Other conditions as in Figure 5.3.	147
5.7	Repetitive square-wave stripping voltammograms for 100 $\mu\text{g l}^{-1}$ of lead in 0.1 M HCl. Deposition potential = -0.7 V, deposition time = 120s, frequency = 8 Hz, dep amplitude = 0.02505 V. Cleanup process = 0.8V (for 90s).	148
5.8	Square-wave stripping responses for distinct concentrations of lead from 10 to 50 $\mu\text{g l}^{-1}$ range. Deposition time: 240s. Other conditions as in Figure 5.3.	149
5.9	Square-wave stripping responses for distinct concentrations of lead from 25 to 300 $\mu\text{g l}^{-1}$ range. Deposition time: 120s. Other conditions as in Figure 5.3.	149
5.10	Effect of the addition of Cd (II) on the stripping response of lead (II) in 0.05 M HCl (a). Stripping voltammograms showing the effects of cadmium (II) on the stripping response of lead (II) in 0.05 M HCl.) (b)	151
5.11	Interference study on Pb (II) with other metals; chromium, mercury, arsenic(III), ferum, zinc, copper and calsium.	152
5.12	Typical square-wave voltammograms and for the determination of lead (II) in waste-water sample on SPGE. Standard addition solutions of lead (II) were added in 50, 100, 150 and 200 $\mu\text{g l}^{-1}$ increments to the sample. Other conditions as in Figure 5.4. The	155

error bars represent standard deviation of 3 independent measurements.

- 5.13 Standard addition plot for the determination of lead (II) in waste- 155
water sample on SPGE. Standard addition solutions of lead (II) were
added in 50, 100, 150 and 200 $\mu\text{g l}^{-1}$ increments to the sample. Other
conditions as in Figure 5.4. The error bars represent standard
deviation of 3 independent measurements.
- 5.14 Correlation between SWV and ICPMS for the determination of lead 157
(II) in wastewater and soil samples.
- 5.15 Results on correlation between two methods tested for lead (II) 158
analysis (GF-AAS vs Screen-printed gold electrode (No.24) at
SENSPOL Technical Meeting on Problems related to Diffuse
Pollution Sources: Characterization of Sediment, Dredged Material
and Groundwater, Koblenz, Germany, 28 – 31 October 2003. No. 22
results produced by other group which based on mercury coated
screen-printed electrode.
- 5.16 Stripping voltammograms for 0, 50 and 100 $\mu\text{g l}^{-1}$ Cd (II) in 0.05 M 160
HCl. Deposition potential = -0.7 V, deposition time = 120s,
Frequency = 8 Hz, step potential: 0.05 V; amplitude: 0.025 V.
- 5.17 Graph on the effect of various concentration of HCl on stripping peak 161
of 100 $\mu\text{g l}^{-1}$ Cd (II). Deposition potential = -0.7 V, deposition time =
120s, Frequency = 8 Hz, step potential: 0.05 V; amplitude: 0.025 V.
- 5.18 Repetitive square-wave stripping voltammograms for 50 $\mu\text{g l}^{-1}$ of Cd 162
(II) in 0.05 M HCl. Deposition potential = -0.7 V, deposition
time=120s, frequency = 8 Hz, dep amplitude = 0.02505 V. Cleanup
process= 0.8V (for 90 s).
- 5.19 Calibration Graph for Cd (II) in 0.05 M HCl. Others condition is 162
same in Figure 5.16.
- 6.1 Chronopotentiometry Stripping Analysis of 0 and 50 $\mu\text{g l}^{-1}$ As (III) in 169
1 M HCl. Deposition potential = -0.1 V, deposition potential = 120 s
and stripping current= 1 μA .

6.2	Square-Wave Stripping Voltammetry analysis of 0 and 50 $\mu\text{g l}^{-1}$ As (III) in 1M HCl. Deposition potential = -0.3V, deposition time = 60 s, frequency = 25Hz, step potential = 0.0495 and amplitude = 0.02505V.	170
6.3	Linear sweep voltammetry of 0 $\mu\text{g l}^{-1}$ and 50 $\mu\text{g l}^{-1}$ As (III) in 1 M HCl. Begin potential = -0.3 V, end potential = 0.8 V, step potential = 0.00244, scan rate = 200 mV, equilibrium time = 60 s.	170
6.4	Stripping voltammograms on the effect of chloride ion on linear sweep voltammetry of 50 $\mu\text{g l}^{-1}$ As(III) in . Begin potential = -0.3 V, end potential = 0.8 V, step potential = 0.00244, scan rate = 200 mV, equilibrium time = 60 s.	172
6.5	Effect of deposition time on LSV of 50 $\mu\text{g l}^{-1}$ As (III) in 1 M HCl. Begin potential = - 0.3 V, end potential=0.8 V, step potential = 0.00244, scan rate = 200 mV.	173
6.6	The effect of deposition potential for 50 $\mu\text{g l}^{-1}$ As(III) in 1 M HCl. Other parameters are same as in Figure 6.4.	174
6.7	Effect of scan rate on 50 $\mu\text{g l}^{-1}$ As (III) in 1 M HCl. Other parameters are same as in Figure 6.4.	174
6.8	Effect of other metals on the stripping voltammetry of As (III) in 1 M HCl.	175
6.9	Voltammograms of 100 $\mu\text{g l}^{-1}$ As (III) (a), and addition of 100 $\mu\text{g l}^{-1}$ Pb (II) (b), 200 $\mu\text{g l}^{-1}$ Pb (II) (c) and 1000 $\mu\text{g l}^{-1}$ Pb (II) (d) in 1 M HCl.	176
6.10	Voltammograms of 100 $\mu\text{g l}^{-1}$ As (III), and addition of 100 $\mu\text{g l}^{-1}$ Cu (II), 200 $\mu\text{g l}^{-1}$ Cu (II), 1000 $\mu\text{g l}^{-1}$ Cu (II) in 1 M HCl.	177
6.11	Voltammograms of 100 $\mu\text{g l}^{-1}$ As (III), and addition 200 $\mu\text{g l}^{-1}$ Hg (II), 1000 $\mu\text{g l}^{-1}$ Hg (II) in 1 M HCl.	177
6.12	Votammograms for As (III) from 0, 10, 20, 30, 40, 50, 70,100 and 200 $\mu\text{g l}^{-1}$ As (III) in 1 M HCl. Begin potential = -0.3 V, end potential = 0.8 V, step potential = 0.00244, scan rate = 100 mV, equilibrium time = 60 s.	178

6.13	Calibration graph for As (III) from 0 to 200 $\mu\text{g l}^{-1}$ As (III) in 1 M HCl. Begin potential = -0.3 V, end potential = 0.8 V, step potential = 0.00244, scan rate = 100 mV, equilibrium time = 60 s.	179
------	--	-----

LIST OF PLATES

Plate		Page
2.1	An Autolab (Electrochemical Analyser) (a) and Palmsens (b)	48
2.2	A DEK 248 semi-automatic precision screen-printer	51
4.1	PalmSens with screen-printed carbon electrode	99

LIST OF TABLES

Table		Page
1.1	Guidelines and standards for drinking water quality	8
1.2	Maximum admissible concentration values for heavy metals in soil for United Kingdom, European Union and Japan	9
1.3	Enzyme-based biosensors developed for heavy metals determinations.	19
1.4	Examples of Environmental Applications of Stripping Analysis	22
1.5	The ratio of Fe^{3+} to Fe^{2+} changes.	29
1.6	A tentative classification of stripping electroanalytical schemes	36
1.7	Different techniques of stripping analysis.	37
2.1	Table of heterogenous electron transfer rate constant (k^0) for glassy carbon, SPCE-Acheson, SPCE-Ercon and SPGE.	58
2.2	A (cm^2) values calculated and measured for each kind of carbon-based screen-printed electrodes. Standard deviations ($\pm \text{cm}^2$) is calculated over 10 electrodes recording the current during a two minutes period at constant potential (+800 mV vs on-board Ag/AgCl) in 0.5 mM $\text{K}_4\text{Fe}(\text{CN})_6$ solution with 0.1 M KCl.	61

3.1	Examples of published work on the applications of SPCEs as electrochemical metal sensors.	72
3.2	Comparing results for the determination of metal ions in waste-water samples using square-wave anodic stripping voltammetry (SWSASV)	89
3.3	Comparing results for the determination of metal ions in aqua regia and acetic acid extracted waste-water samples using square-wave anodic stripping voltammetry (SWSASV).	89
4.1	Examples of metal ions detection with peptides-modified electrodes	94
4.2	Measurements of cadmium (II) in soil samples extract.	117
4.3	Measurements of cadmium (II) in waste-water samples.	118
4.4	Results for Lead (II) Analysis using chronopotentiometric technique compared to ICPMS.	121
4.5	Copper contents obtained for soil extracts with cysteine modified screen-printed electrodes.	129
5.1	Relation of metals presented in the wastewater samples and the results found for lead by square-wave analysis.	153
5.2	Determination of lead (II) in acetic acid and aqua regia extracted soil samples.	154
5.3	Results for Lead (II) in 0.05 M HCl analysis using square wave voltammetry technique. The analysis was done at SENSPOL Technical Meeting on Problems related to Diffuse Pollution Sources: Characterization of Sediment, Dredged Material and Groundwater Koblenz, Germany, 28 - 31 October 2003.	157
6.1	Summary for arsenic detection on gold or gold film electrode using stripping voltammetry and potentiometry.	167

NOTATION

Symbols (units):

C	Concentration (Mol dm^{-3} , M)
D	Diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$)
e	Electron

E	Potential (volts, V)
F	Faraday's constant (96,485 C)
I	Current (Ampere, A)
j_0	Current density (A, cm^{-2})
k^0	Heterogeneous rate constant ($cm\ s^{-1}$)
Q	Charge (Coulombs, C)
α	Transfer coefficient
τ	Transition time (s)
r^2	Correlation coefficient
R	Resistance (Ω)
R	Universal gas constant ($8.314\ JK\ mol^{-1}$)
T	Absolute temperature (K)
t	Time (second, s)
μ	Micro
ψ	Kinetic parameter

Abbreviations:

AAS	Atomic Absorption Spectrometry
AdCSV	Adsorptive cathodic stripping voltammetry
AdSV	Adsorptive stripping voltammetry
Ag/AgCl	Silver/silver chloride
AgSPEs	Screen-printed silver electrodes
ASV	Anodic stripping voltammetry
BCR	Community Bureau of Reference
C.O.S.H.H	Control of substances hazardous to health
CadSV	Catalytic adsorptive stripping voltammetry
CCSCP	Constant current stripping chronopotentiometry
Cd	Cadmium
CE	Counter electrode
CME	Chemically modified electrode
CMSPE	Copper/mercury-plated screen-printed electrode
CPE	Carbon paste electrode

CRM	Certified reference material
CSV	Cathodic stripping voltammetry
Cu	Copper
CV	Cyclic voltammetry
ΔE_p	Peak separation (V)
DPASV	Differential pulse anodic stripping voltammetry
DPV	Differential pulse voltammetry
dt/dE	Inverse potential derivative with time
EC	European Community
E_{dep}	Deposition potential (V)
emf	Electromotive force
E_p	Peak potential (V)
EPA	Environmental Protection Agency
EU	European Union
FAAS	Flame atomic absorption spectrometry
GCE	Glassy carbon electrode
GDE	Gold disc electrode
GFAAS	Graphite furnace atomic absorption spectrometry
HCl	Hydrochloric acid
Hg^{2+}	Mercury
HgO	mercury oxide
HMDE	Hanging mercury drop electrode
HNO_3	Nitric acid
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma- Mass Spectrometry
ISE	Ion-selective electrode
KCl	potassium chloride
LSV	Linear sweep voltammetry
M	Moles per litre
MFSPCE	Mercury film screen-printed carbon electrode
$mg\ l^{-1}$	Milligram per litre
mM	Millimolar
ms	Millisecond

mV	Millivolt
NAA	Neutron Activation Analysis
nC	Nanocoulombs
ng l ⁻¹	Nanogram per litre
Pb	Lead
PSA	Potentiometric stripping analysis
RE	Reference electrode
RSD	Relative standard deviation
S/N	Signal to noise ratio
SCE	Saturated calomel electrode
SMDE	Static mercury drop electrode
SPCE	Screen-printed carbon electrode
SPE	Screen-printed electrode
SPGE	Screen-printed gold electrode
SWASV	Square-wave square-wave anodic stripping voltammetry
SWV	Square-wave voltammetry
TMF	Thin mercury film
μg l ⁻¹	Microgram per litre
UK	United Kingdom
UV	Ultraviolet
w/v	Weight per volume
w/w	Weight per weight
WE	Working electrode
WHO	World Health Organisation
XRF	X-Ray Fluorescence

CHAPTER 1

1.0 GENERAL INTRODUCTION

1.1 OUTLINE

In this chapter, the problems of exposure to heavy metals in the environment and their toxicity to human beings are presented. Moreover, the analytical methods used to detect the metals in various samples are also outlined. Lastly, the aims and objectives of the projects are also stated.

1.2 BACKGROUND

While many of the world's people are enjoying the benefits of technological and economic expansion and higher living standards, there are others who perceived that the extraordinary developments are not without cost. Indeed, the impact of global environmental changes on human has become of great concern. As early as the 1950s and 1960s, many urban dwellers and residents in the vicinity of industrial plants began to recognize undesirable changes in the environment, particularly a general deterioration of air and water quality. A great deal of field and laboratory research was conducted, revealing the seriousness of the problem. Subsequently, it was widely recognized that there is an urgent need to protect living systems and the environment against further environmental deterioration caused by numerous pollutants generated from anthropogenic sources like heavy metals (Yu, 2001).

Many metals are naturally abundant, and, as such, occur in forms largely inoffensive to the ecosystem. Extraction, processing and use by man often cause metals to be reintroduced to the environment in a manner potentially toxic to both man and that environment. At least 20 metals are known to be toxic and these, include metals such as arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver and zinc. Metals are classified as persistent environmental toxins because they cannot be rendered harmless by chemical or biological remediation processes (Blake *et al.*, 1998; Lloyd & Lovley, 2001).

“Heavy metals” are a group of metallic elements that exhibit certain chemical and electrical properties and are generally those having the atomic density greater than 6.0 g cm^{-3} , thus

adding zinc, chromium, tin, indium and manganese to the group. These metals exceed the atomic mass of calcium. Some attention has been paid to the heavier metalloids selenium (4.79 g cm^{-3}), arsenic (5.72 g cm^{-3}) and tellurium (6.4 g cm^{-3}) (Williams *et al.*, 2000; Yu, 2001).

Heavy metals have a strong affinity for sulphur, and their effect on biological systems is mainly via interactions with thiol groups in proteins. Cysteinyll residues have essential roles in the function of many enzymes, particularly those involved in hydrolytic and redox catalysis, e.g. glutathione reductase. Therefore, metals interacting with the essential thiol groups of protein can be highly detrimental, or even lethal, to biological species. It must be noted also that some metals, e.g. copper and zinc, are essential to many organisms at trace levels, but toxic at elevated concentrations.

The effects of toxic heavy metals on living organisms have for a long time been considered almost exclusively a problem of exposed industrial workers and of accidental childhood poisonings. Much of the literature concerning the subject, therefore, deals with experiments regarding children's exposure to lead paint. Although much improvement has been made in reducing the level of general environmental pollution, problems with several heavy metals, such as lead, cadmium, mercury, and arsenic, persist in part of the world (Yu, 2001).

A range of metal ions are essential as trace elements but at higher concentrations they become toxic. Heavy metals are difficult to remove from the environment and unlike many other pollutants cannot be chemically or biologically degraded and are retained in the environment and in the food chains. Today, many heavy metals constitute a global environmental hazard (Mejare & Bulow, 2001). Mining, pouring, casting, processing and using metals have led to their dispersion into the general environment and impaired health of the general population by the ingestion of food and beverages containing or contaminated by metals (Apostoli, 2002).

There are published examples of cases of mining spills due to dam failures such as at Baia Borsa (Romania) in 2000 and Aznalcollar in south west Spain in 1998. At Baia Borsa, Romania, two accident spills in two months carried hundreds of tons of toxic heavy metals and cyanide that travelled downstream and flowed into the Danube and affected four countries: Romania, Hungary, Yugoslavia and Bulgaria (Fleit and Lakatos, 2003). The

Aznalcaollar accident happened when a tailings dam at Boliden Apirsa's mine was breached, flooding nearby rivers with approximately 5.5 million m³ of acidic water and 1.3 million m³ of heavy metal-bearing tailings. This spill took place in the surroundings of Doñana Park, the largest reserve of bird species in Europe (Hudson-Edwards *et al.*, 2003; Grimalt *et al.*, 1999). The impact of the disaster, which affected a vast region, revealed how easily environmental damages can expend to larger scales. Due to the bioaccumulation of heavy metals, such accidents can have long-term consequences in the environment, and continuous monitoring of metal content in the area even after clean up is therefore essential. Some of these metals are further explored here since they form part of this investigation.

1.2.1 Cadmium (Cd)

Cadmium (Cd) is a silver-white metal with an atomic weight of 112.4 and a low melting point of 321°C. Cd is used in plating, in nickel-cadmium batteries as a component of various alloys and solders, as a PVC stabilizer, and is contained in some pigments, paints, and pottery (Landis & Yu, 1998; Aw & Vale, 1996). General sources of exposure to Cd include air, water, and food. Exposure also occurs in areas around non-ferrous mines and smelters. Tobacco is an important source of Cd uptake in smokers (up to several hundred µg/day) whereas in non-smokers the major route of exposure is via food (Aw & Vale, 1996). Waterborne Cd is probably the largest problem because it is common in the aquatic environment. Many Cd-containing wastes end up in lakes and marine water (Landis & Yu, 1998).

Cd compounds are poorly absorbed orally but are well absorbed through the lungs. Cd is deposited in the liver and kidneys as a complex with metallothionein. Cd is known to cause toxicity to the kidney after long-term exposure to inorganic cadmium compounds, i.e. the chemical species Cd (II) (Nordberg, 1998). Cadmium is excreted primarily in the urine but only very slowly so that the half-life of Cd is some 10 to 30 years (Aw & Vale, 1996).

1.2.2 Copper (Cu)

Copper is a reddish transition metal that is stable in its metallic state and form monovalent (cuprous) and divalent (cupric) cations. Metallic copper is malleable, ductile and good thermal and electrical conductor. It has many commercial uses because of its versatility.

Copper is used to make electrical wiring, pipes, valves, fittings, coins, cooking utensils and building materials. It is present in ammunition, alloys (brass, bronze) and coatings. Copper compounds are used as or in fungicides, algicides, insecticides and wood preservatives and in wood electroplating, azo dye manufacturer, engraving, lithography, petroleum refining and pyrotechniques. Copper compounds can be added to fertilizers and animal feeds as a nutrient to support plant and animal growth (WHO 1998). Copper is present in the atmosphere from wind dispersion of particulate geological materials and particulate matter from smokestack emissions. Copper is found in surface water, air, sea water and drinking water, but it is primarily present as complexes or as particulate matter (ASTDR, 2004). Food is a principal source of copper exposure for humans. Liver and other organ meats, seafood, nuts and seeds (including whole grains) are good sources of dietary copper. Food and water are the primary sources of copper exposure in developed countries. In general, dietary copper intakes for adults range from 1 to 3 mg/day (WHO, 1998). Copper is an essential nutrient and is required for the proper functioning of many enzymes. It only exerts a toxic action at high concentrations (recommended dose 10 mg/day, IOM, 2001). At high concentrations, copper is responsible for the Wilson's disease, which includes hepatic cirrhosis, neurologic degeneration, and copper deposits in the eye cornea (WHO, 1998).

1.2.3 Lead (Pb)

Lead (Pb) represents a good example of heavy metal emitted into the atmosphere and adsorbed by soils, plants and animals. It has a variety of use in manufacturing ammunition, solders, metal alloys, ceramic glazes, storage batteries and shielding from radiation sources. Increasing amounts of Pb in the body, as benchmarked by blood Pb levels can cause impaired neurobehavioral development in children, increased blood pressure, kidney injury, and anemia (CDC, 2003).

Lead (Pb) has a low melting point (326°C). It is a soft, malleable metal and it can be easily formed into a variety of shapes (Landis & Yu, 1998). Many industrial activities, and particularly its use in petrol (gasoline), have led to its wide distribution in human bodies. Recent studies suggest that much of the existing soil contamination is probably a result of deposition from exhaust from cars that used leaded gasoline, in addition to leaded paint used on the exterior of buildings (Mielke, 1999). Pb from these sources exists as or evolves into a variety of Pb (II) compounds, which are remarkably persistent in the environment.

Unfortunately, these sources of exposure are often expensive to remediate, and the politics surrounding this issue is complex, suggesting that the legacy of lead poisoning will continue to plague mankind for many years to come (Lanphear, 1998).

Once Pb is absorbed into the body either via ingestion (approximately 10-15 per cent is absorbed) or inhalation (almost 100 per cent is absorbed) it is mainly (95%) deposited in the bones and teeth. Of the Pb in the blood, 99% is associated with erythrocytes. As the body accumulates Pb over many years and releases it into the urine only slowly, even small doses can in time lead to intoxication (Aw & Vale, 1996). Manifested effects of Pb poisoning include nausea, anorexia, severe abdominal cramps, weight loss, anemia, renal tubular dysfunction, muscles aches, and joint pains. Pb can pass the placental barrier and may reach the fetus, resulting in miscarriages, abortions, and stillbirths (Landis & Yu, 1998).

1.2.4 Mercury (Hg)

Mercury (atomic number 80, atomic weight 200.59) has a high specific gravity, 13.6 times that of water. Its boiling point is 357°C, which is relatively low, and this property leads to easy separation from its ores and amalgams (Landis & Yu, 1998). Mercury (Hg) exists in three forms, metallic (Hg^0), mercurous (Hg_2^{2+}) and mercuric (Hg^{2+}). Hg enters the environment from a large number of miscellaneous sources related to human use of the element. These include discarded laboratory chemicals, batteries, broken thermometers, amalgam tooth fillings, and formerly lawn fungicides and pharmaceutical products (Manahan, 2000).

Many foodstuffs contain small amounts of inorganic Hg but organic Hg compounds accumulate in the aquatic food chain so that certain fish contain significant amounts of methyl Hg. The absorption of Hg depends on its chemical form. Inhaled Hg vapour is absorbed rapidly and oxidized to Hg^{2+} in erythrocytes and other tissues. Prior to oxidation, absorbed Hg vapour can cross the blood-brain barrier but the divalent ion oxidation product serves to trap Hg in the brain. Organic mercuric salts are better absorbed following ingestion than are inorganic mercuric salts. Organic Hg compounds cross the blood-brain barrier readily to accumulate in the brain. In contrast the kidney is the main storage organ for inorganic Hg compounds. In vivo, Hg is bound to metallothionein, which serves a protective role since renal damage is caused only by the unbound metal. Hg is excreted mainly in urine

and faeces although a small amount of absorbed inorganic Hg is exhaled as Hg vapor. The half life of most body Hg is 1 to 2 months but a small fraction has a half-life of several years (Aw & Vale, 1996).

1.2.5 Arsenic (As)

Arsenic (As) is termed a metalloid as it has properties of both metals and non-metals. It forms both trivalent (e.g. As trioxide, arsenious acid, and arsenites) and pentavalent (e.g. As pentoxide, As acid, and arsenates) derivatives. Inorganic arsenical compounds may generate arsine gas when in contact with acids and reducing metals (e.g. iron and zinc) or with sodium hydroxide and aluminium. As is used in the electronic industry, in the production of special types of crystal and optical glass, in hardening lead and copper alloys, in the manufacturer of fireworks, and as a wood preservative and pesticide. It is a by product of copper smelting. Some 90% of an ingested dose of most inorganic trivalent and pentavalent arsenicals are absorbed, the exception being some insoluble compounds such as As selenide. Soluble arsenical compounds can be absorbed by inhalation but skin absorption is generally poor. In exposed individuals high concentrations of As are present in bone, hair, and nails. The half-life is in the range of 1 to 3 days. Excretion is predominantly in the urine as mono and dimethyl-derivatives (Aw & Vale, 1996; Yu, 2001).

1.3 LEGISLATION CONCERNING HEAVY METALS

Environmental action by the European Community (EC) began in 1972 with four successive action programmes, based on a vertical and sectoral approach to ecological problems. During this period, the Community adopted some 200 pieces of legislation, chiefly concerned with limiting pollution by introducing minimum standards, notably for waste management, water pollution and air pollution. For example, in case of water pollution, a number of Directives have been adopted by the Member States to introduce water quality standards: drinking water, bathing water, water for fish farms and shellfish culture (EU, 2004). One of the key EC directives was the dangerous substances in Water Framework Directive (76/464). The Directive introduced the concept of list I and list II substances. The purpose of the Directive is to eliminate pollution from list I substances and to reduce pollution from list II substances. Cadmium and mercury and its compounds are the only two heavy metals on the List I due to their toxicity, persistence and bioaccumulation in the environment. Zinc, selenium, copper,

arsenic, chromium and are some of the heavy metals listed on the List II due to deleterious effect on the aquatic environment, which can, however, be confined to a given area and which depend on the characteristics and location of the water into which they are discharged (EU, 2004). All the listed directives are implemented as a variety of legislative instruments in the EU countries and many non EU countries. Together with its equivalent in the USA, the framework provided by the Directives is regarded as a model for environmental legislation in the rest of the world (Gopel *et al.*, 1993).

The maximum allowable concentration of metals in water and soil samples by environmental bodies and world organisation are presented in Table 1.1 and 1.2.

Table 1.1: Guidelines and standards for drinking water quality (WHO, 1998; US EPA, 2004; EU, 2004; Baird, 1999).

METAL	EU DRINKING WATER	US EPA DRINKING WATER	WHO DRINKING WATER
	mg l ⁻¹	mg l ⁻¹	mg l ⁻¹
Zinc	1	-	3
Cadmium	0.005	0.005	0.003
Lead	0.01	0.015	0.01
Copper	2	1.3	-
Arsenic	0.05	0.01	0.01
Mercury	0.001	0.002	0.001
Chromium	0.05	0.1	-
Nickel	0.05	0.1	-

UK: United Kingdom, **EU:** European Union, **US EPA:** United States Environmental Protection Agency, **WHO:** World Health Organisation.

Table 1.2: Maximum admissible concentration values for heavy metals in soil for United Kingdom, European Union and Japan (Wild, 1993).

METAL	UK	EU	JAPAN
	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹
Zinc	300	300	250
Lead	100	300	400
Nickel	50	75	100
Copper	100	140	125
Chromium	50	-	-
Cadmium	3	3	-

The need for continuing monitoring of the environment to follow the EU legislation would require suitable analytical methods for rapid quantification, assessment and remediation of contaminated sites, for the enforcement of the regulations on heavy metals discharge from many industrial, agricultural and domestic sources. Section 1.4 details the analytical methods used for heavy metals analysis.

1.4 ANALYTICAL METHODS FOR HEAVY METAL ANALYSIS

In the field of trace metal analysis there are some typical determination methods which have become the most important over the last few years. Metals can be determined with accuracy down to milligram per litre (mg l⁻¹) level or below by Atomic spectrometry, Visible spectrophotometry, Instrumental Neutron Activation Analysis (INAA), Immunoassay, Biosensors (Electrochemical, Optical, Pizelectrical and Thermal), X-ray fluorescence (XRF) spectroscopy and Electrochemical technique (e.g., potentiometry, electrochemical stripping analysis. etc).

1.4.1 Atomic Spectrometry

Atomic absorption spectrometry (AAS) is based on light absorption by free atoms. While passing a sample of gaseous atoms, light of defined wavelengths interacts with outer electrons of the elements to be determined and excites them to higher energy levels. This

energy absorption results in a decrease of the light intensity at this wavelength, which is directly proportional to the concentration of analyte in the flame/graphite furnace and is derived from the Beer's Law: $A = \epsilon bC$, where A is absorbance; ϵ is molar absorptivity; b is the path length of the light through the absorbing solution and C is the concentration of absorbing substance (Christian, 1994; Cammann *et al.*, 2000). The linear relationship between the measured absorbance and the concentration of analyte atoms in the flame/graphite, which is proportional to the concentration of atoms in sample solution, holds in practice, over two orders of magnitude. A series of standard solutions with known analyte concentrations covering the range required or, in some cases, by the method of additions are used to establish the precise relationship between absorbance and solution concentration of the analyte.

1.4.1.1 Flame AAS

This technique is based on the introduction of sample solution into the flame, which is generated from a liquid by a nebulizer producing an aerosol (Cammann *et al.*, 2000). Most modern systems use either air/acetylene or N_2O /acetylene as the fuel mixture for the flame, giving temperature up to about 2500 and 3000 K, respectively. A narrow burner slot (5-10 cm in length) aligns a flat flame with the optical beam (Currel, 2000). Flame systems have advantages of low capital costs, rapid and can easily be automated, simple method for routine use, good precision and accuracy, good detection limits in the range $0.05 - 1 \text{ mg l}^{-1}$ for most metals with a precision of 2 - 5% RSD, standard procedures are available for all metals and the analyses are generally free from interferences. Known interferences can easily be overcome. Little or no sample preparation is needed for aqueous environmental samples (Reeve, 1994; Currel, 2000). However, there are some disadvantages of this technique; time consuming, the sensitivity may still be insufficient for low-concentration metal ions and the risk of sample contamination is considerably increased (Reeve, 1994; Christian, 1994). Some refractory elements cannot be determined with good sensitivity because flame temperatures are often not hot enough to induce complete atomization. This problem means that trace levels of B, W, Ta, Zr, As and Sn may not be determined by FAAS (Brown & Milton, 2005).

1.4.1.2 Graphite furnace or Flameless AAS (GF-AAS)

GF-AAS is another technique of atomization where the introduction of gaseous, liquid or solid samples into a graphite furnace and is heated electrothermally to temperatures up to 2500°C and the metals can be detected very fast. It is possible to heat the furnace according to a special temperature programs in order to achieve the following phases: drying and ashing, allowing the careful vaporization of e.g. solvents or the mineralization of organic matrices before the atomization phase (Currel, 2000; Cammann *et al.*, 2000). To avoid losses of volatile analytes (e.g. Pb, Cd) special modifiers like magnesium nitrate are added to the furnace as a thermochemical reagent. Another advantageous effect of these modifiers is the accelerated destruction of the matrix by oxidation. The graphite furnace AAS technique permits very good limits of detection and is 2-3 orders of magnitude better than those of AAS and ICP-AES (Bersier *et al.*, 1994) and is widespread in chemical laboratories for routine analytical applications. Its limit of detection exceed those of the flame AAS by a factor of 10^3 . One great advantage of the GF-AAS is the very small sample volume. Only microlitres need to be injected into the furnaced. Interferences are now largely controlled by a combination of high-quality graphite material, platform technology and Zeeman background correction with matrix matching and standard additions.

1.4.1.3 Hydride generation AAS

Some elements especially Sn, Pb, As, Sb, Se and Te show the tendency to form volatile hydrides, eg. SbH_3 , by reaction with sodium borohydride in an acid medium. Typically a quartz cell is used as an atomization unit. The generated hydrides are introduced in the cell and atomized at temperature of up to 1100°C. A further development is the use of a graphite furnace as atomization unit. Even extreme traces of these elements can be matrix separated by this technique and excellent limits of detection are reached (Cammann *et al.*, 2000). The newest AAS can determine six elements at one time. However, the technique could become slow and tedious for multi-element analysis. The variations in concentrations of the samples can be problematic as the linear range of AAS is very limited. The development of Inductively coupled plasma techniques can be seen as an attempt to overcome these problems.

1.4.1.4 Inductively coupled plasma optical emission spectrometry (ICP-OES)

The basic functional principle of ICP-OES is when an electron of an atom is located on an upper energy level and descends to a lower one, the energy difference between those two levels is emitted as electromagnetic radiation which is characteristic for each single element. In a plasma, those element species with electrons on upper energy levels are generated (Cammann *et al.*, 2000).

In common with other emission techniques, there is the problem of spectral overlap from different elements, an element producing many more lines in its emission spectrum than in its corresponding absorption spectrum. The choice of the analytical wavelength is based on freedom from interference and the sensitivity. For routine water analysis this problem has been largely overcome, sensitive and interference-free lines being well documented (Reeve, 1994).

1.4.1.5 Inductively coupled plasma mass spectrometry (ICP-MS)

The physical principal of mass spectrometry is based on the generation of analyte ions by electrical discharge followed by separation of the ions due to their mass-to-charge ratio caused by the influence of electric or magnetic fields on their way of flight (Cammann *et al.*, 2000). The mass spectra of inorganic mixtures are simple in comparison with the more familiar organic compound spectra and few interferences occur in metal analysis. These include the following: isobaric ions, e.g. tin and cadmium at 114DA, polyatomic ions arising from the atomization process within the argon torch, with these mainly involving argon and oxygen and lastly, multiply charged ions. (Currel, 2000). Although the technique is not strictly simultaneous, the ions being determined sequentially, determination of 20 elements is possible within 4s. The sensitivity is slightly lower than that of graphite furnace AAS but is still sufficient to determine trace metal ions at below $1 \mu\text{g l}^{-1}$ in aqueous samples (Reeve, 1994). The best technique for metals analysis could be an isotope dilution (ID) procedure when coupled with ICP-MS (ID-ICP-MS) because of its inherently greater accuracy than other techniques. The isotope of the analyte of interest is added to the sample preparation. The amount of the analyte in the sample can be calculated when comparing the known original isotopes ratios and the measured altered isotope ratio.

Each development in atomic spectroscopy has brought with it significant increase in instrument capital cost. The cost of instrumentation is generally flame AAS<GF-AAS<ICP-OES<ICP-MS. In addition, ICP techniques have significantly higher running costs owing to the consumption of argon necessary to generate the plasma. The advantages of ICP techniques are, however, so great that ICP-OES has for several years been the preferred technique for the high-concentration metal analyses in major water analysis laboratories, with ICP-MS rapidly becoming established for lower concentration metals. AAS methods find a role in smaller laboratories where the sample throughput is insufficient to justify the additional capital and running costs of ICP techniques.

1.4.2 Visible Spectrophotometry

Visible spectrophotometry is not commonly used technique for heavy metal analysis due to the widespread use of atomic spectrometry techniques. There are two difference ways used for selectivity of specific compounds. Firstly, solvent extraction with precise control of pH and the use of masking agent and secondly, colour forming complexing agent for aqueous samples without extraction. The compounds of interests are detected by the colorimetric reaction with absorbance in the visible or ultra-violet region used for quantification (Reeve, 2002). This technique is simple and inexpensive. There are commercially available spectrophotometric test kits for heavy metals based on colorimetric reagent from the Hach company (USA), Aquatic Life Ltd (Canada) and LaMotte Company (USA). However, these spectrophotometric techniques are not sensitive for detecting heavy metals at very low concentration.

1.4.3 X-ray Fluorescence Spectrometry (XRF)

X-ray fluorescence spectrometry (XRF) is a multielement technique based on the irradiation of an atom with X-rays leading to the ejection of an electron from an inner shell. Outer-shell electrons cascade to the inner shell to fill the vacancy, emitting X-rays. The wavelength of this radiation is related to the atomic number of the nucleus (Reeve, 2002). XRF can be used to analyse routinely for almost any element from Na to U, including non-metals. The sensitivity of the XRF depends on the energy of the incident radiation, the geometry of the instrument used and the efficiency of the detector. The limit of detection achievable depends

on the sensitivity of the instrument and the background level of the sample matrix. State of the art instrumentation for XRF exhibits precision better than 0.1% (Brown & Milton, 2005).

1.4.4 Instrumental Neutron Activation Analysis (INAA)

The term activation analysis refers to identification and quantitative determination by use of radionuclides produced from a target element. Instrumental neutron activation analysis is the most common variants in which neutrons are used to irradiate and to activate the sample. INAA is a multi-element, solid sample technique for the analysis of soil. The sample is irradiated with neutrons to produce nucleotides of the elements of interest. As the radioactive nucleus decays, it emits gamma rays. The intensity of the gamma ray spectrum can be related to the original concentration in the sample (Reeve, 2002; Brown & Milton, 2005). The advantages of this technique are firstly, highly sensitive, detection limits for elements in airborne can be as low as $2 \times 10^{-5} \text{ ug m}^{-3}$ and secondly, no chemical pre-treatment required. The disadvantage of this technique is the need of a neutron source.

1.4.5 Immunoassays

Immunoassays technology which been mostly used for organic environmental analysis, has now been extended to heavy metal analysis. This technology has advantages for characterisation, inexpensive, quick and easy to perform, offer highly selective and sensitive measurements and reasonably portable for field screening (Blake *et al.*, 1998; Khosraviani *et al.*, 1998; Blake *et al.*, 2001). This technology relies on suitable antibody that is developed to have a high degree of sensitivity and specificity to the target metal and coupled with a sensitive colorimetric reaction which will provide a visual result. There are few examples in the literature of immunoassays selectively developed for the determination of metal ions. An immunoassay method to measure chelated metal ions has been reported for the determination of indium (Chakrabarti *et al.*, 1994). The assay utilised a monoclonal antibody that bound specifically to In-EDTA complexes in an antigen-inhibition format. The range of the assay was from 0.6 to 320 mg l^{-1} , 0.1 to 120 mg l^{-1} , or 0.005 to 2000 $\mu\text{g l}^{-1}$ in different solution of soluble inhibiting reagent. A monoclonal antibody specific to recognise Cd-EDTA complexes was reported by Blake *et al.* (1996). The developed antibody was later applied for the optimisation and validation of a portable assay (Khosraviani *et al.*, 1998). The Cd

immunoassay detected the metal in ambient water samples at level from approximately 10-2000 $\mu\text{g l}^{-1}$ in 1-2 hours. There are no interferences from cations commonly found in water samples. The same authors also developed antibodies that recognise chelated forms of Cd (II), Hg (II), Pb (II) and Ni (II) to construct immunoassays for these metals (Blake *et al.*, 1998). Khosraviani *et al.* (2000) reported the same procedure to obtain a monoclonal antibody which has the ability to interact with Pb (II) at high affinity. Darwish & Blake (2001) further developed an immunoassay previously developed for Cd-EDTA complexes by producing a one-step immunoassay for the determination of this metal in human serum. The assay showed a working range between 0.24-100 $\mu\text{g l}^{-1}$ Cd (II), with a detection limit of 0.24 $\mu\text{g l}^{-1}$.

Competitive immunoassays for Cd (II), Co (II), Pb (II) and U (VI) were developed in two different assay formats using an identical reagent, a competitive microwell format and an immunosensor format with a KinExATM 3000 automated immunoassay system (Blake *et al.*, 2001). The KinExATM assays yielded a sensitivity 10-100 fold greater than the microwell format assay. The limit of detection for the metals range from 0.03 to 2.4 $\mu\text{g l}^{-1}$. Doyle *et al.* (2004) reported electrochemical immunosensors based on the electrochemical detection of a metal chelate labelled protein model. Human serum albumin was coupled with indium via a bifunctional chelating agent. Following a competitive heterogeneous equilibrium with a specific immunoabsorbent, the indium was released by acidification and determined by differential pulse anodic stripping voltammetry with detection limit of 5.0 $\mu\text{g ml}^{-1}$. Marx and Hock, (1998) developed a monoclonal antibody for an immunoassay for the determination of Hg (II). A detection limit of 2.8 $\mu\text{g l}^{-1}$ HgCl_2 was achieved with a non-competitive enzyme immunoassay (EIA) with cross-reactivities from other metals was below 1%. Measurement of spiked water samples with this EIA was possible and showed good correlation with results given by ICP-MS. Marx *et al.* (1998) had optimised the previous developed heterogeneous non-competitive EIA. The sensitivity of the system was improved by an avidin-biotin-complex (ABC) amplification system with detection limit of 1.1 $\mu\text{g l}^{-1}$ could be achieved for Hg (II). A conventional competitive EIA with the competition reaction between bound and free Hg (II) for antibody sites yielded a detection limit of 1.0 $\mu\text{g l}^{-1}$ Hg (II). The last improvement was achieved with a competitive displacement EIA. In this case, antibody molecules bound to immobilised haptens were displaced in the next step by free Hg (II), resulting a detection limit of 0.4 $\mu\text{g l}^{-1}$ Hg (II).

1.4.6 Biosensors

A biosensor was defined by IUPAC as a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is in direct spatial contact with a transducer element. A biosensor should be clearly distinguished from a bioanalytical system, which requires additional processing steps, such as reagent addition. Furthermore, a biosensor which can rapidly, reproducibly and a non-regenerable device should be named single use biosensor (Thevenot *et al.*, 2001). The biochemical receptors that have been applied to biosensors include enzymes, antibodies, receptors, membranes, tissue, cells and organelles and can be classified in three mechanisms: biocatalytic, bioaffinity and microbe-based systems. The biochemical receptors have been linked to electrochemical, optical, mass (piezoelectric), fluorescence and luminescence for detection of analyte. (Rogers & Mascini, 1998; Tothill and Turner, 1998). The advantages of biosensors are miniaturisation, versatility, low cost and possibility of mass production especially from the biosensors that make use of electrochemical transducers and coupled to screen-printed electrodes (SPE) (Alberta-Sirvent *et al.*, 2000). The details about the use of different biochemical receptors for biosensors construction are described as follows:

1.4.6.1 Cell based biosensors

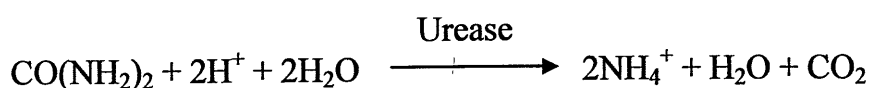
Biosensors based on the use of organic cells or organisms have been used for metal ions detection by many authors. Gardea-Torresday *et al.* (1988) studied the bioaccumulation and measurement of copper (II) at an alga-modified carbon paste electrode using differential pulse voltammetry. Several parameters such as pre-concentration time, pH, ionic strength, Cu (II) concentration, electrode composition and voltammetric waveform were evaluated. The peak height increased with the percentage of alga in the carbon paste matrix and no major interference from other metals. The limit of detection was 2×10^{-6} M ($127 \mu\text{g l}^{-1}$). Lichen-modified carbon paste electrode was developed for Pb (II) and Cu (II) using differential pulse anodic stripping voltammetry (Connor *et al.*, 1991). It was found a linear response for the accumulation of Pb (II) with increasing lichen loading in the carbon paste. A calibration curve was constructed for Pb (II) and Cu (II) in the concentration range 0-1 mM, which yielded a detection limit of $20 \mu\text{M}$ (4 mg l^{-1}) for Pb (II). Two biosensors were described by

Cobisier *et al.* (1999) for the detection of bioavailable heavy metals in environmental samples. The first biosensor was a whole cell bacterial biosensor containing metal-induced *lux* reporter system that emitted a bioluminescent or fluorescent signal in the presence of biologically available heavy metals. The second type of sensor was based on the direct interaction between metal-binding proteins and heavy metal ions. In this case, the capacitance changes of the protein, GST-SmtA fusion protein and MerR, were detected in the presence of femtomolar to millimolar metal ion concentrations. Bontidean *et al.* (1998) had utilised the proteins GST-SmtA and MerR to detect heavy metal ions. The proteins were immobilised to a self-assembled thiol layer on a gold working electrode in a potentiometric arrangement in a flow analysis system. A capacitive signal transducer was used to measure the conformational change following binding. The metal ions studied were Cu, Cd, Hg and Zn ions. GST-SmtA electrodes displayed a broader selectivity compared to the MerR-based ones, which showed accentuated selectivity for mercury ions. Metal ions could be detected with both electrode types down to femtomolar concentration. The electrodes could be regenerated with EDTA.

1.4.6.2 Enzyme-based biosensors for the determination of metal ions

Enzymes are high-molecular weight proteins with an active centre where the binding of the substrate and its chemical transformation take place (Srinivasan *et al.*, 1985). The introduction of inhibitor into the enzyme system alters the balance of the chemical and functional activities of the enzyme, normally in detriment of the organism. Therefore, enzymes or living organisms can be used for toxicity determination provided that the activity of such biological systems can be measured (Junter, 1988). The inhibition of enzymatic reactions has been used very often to determine environmental pollutants (Krawczyk, 1998). Enzymes can be immobilized onto the surface of electrodes by simple procedures such as adsorption, covalent attachment with cross-linking compounds, or film deposition of proteins in polymers enable a fast production of stable enzymatic biosensors. The application of such immobilization procedures to screen-printed electrodes can result in the production of large quantities of disposable biosensors (Tu *et al.*, 2001). For the determination of heavy metals, various enzymes such as urease, invertase, xanthine oxidase, peroxidase, glucose oxidase or alkaline phosphatase have been used (Shekhovtsova *et al.*, 1994). The detection of enzymes inhibitor can be performed because the interaction of a single inhibitor molecule with an enzyme can result in reduction of the enzyme activity. Metal ions normally combine with a functional group of the enzyme. The inhibitor destroys the catalytic activity of the enzyme

either by binding to the catalytic site or as a result of a conformational change affecting the catalytic centre but not affecting the substrate binding (Krawczyk, 1998). The toxic effect of metal ions on enzymes usually due to their binding to thiol groups present in, or near the active centre (Webb, 1966) and producing a non-competitive inhibition. An inhibition biosensor is more sensitive than a substrate biosensor. The detection limit is much lower than the maximum permissible concentrations in environmental samples (Bontidean *et al.*, 1998; Evtugyn *et al.*, 1998). Therefore the use of the inhibitory effect of metals on enzyme activity would provide a sensitive biosensor for the screening of toxic heavy metals. There are many papers report the use of different enzymes and proteins for the analysis of metal ions, a summary of which is shown in Table 1.3. Among the enzymes used to determine metal ions, urease has been the most popular because its activity inhibit by many metals. Urease catalyses the breakdown of urea to ammonia and carbon dioxide according to the reaction shown in below:



Sulfhydryl groups presence within the urease structure play an essential role in which bind with metals to form metal-sulfides (Webb, 1966; Wittekindt *et al.*, 1996). The inhibition of urease by metal ions has been reported as non-competitive (Preininger & Wolfbeis, 1996), or partially competitive (Oehlschlager *et al.*, 1998), competitive (Zaborka *et al.*, 2001) and mixed (Antonelli *et al.*, 1987). Most studies on urease inhibition use the determination of pH changes as an indirect indicator of enzyme activity (Shi *et al.*, 1997; Kormos & Langauer, 2000; Krawczyk *et al.*, 2000). Urease acitivity can also be followed by measuring the production of ammonia (Gil & Piedade, 1992; Soldatkin *et al.*, 2000;). A significant shortcoming of urease inhibition tests base on measurement for ammonia evolution is the limitation to environmental samples with low content of ammonia (Jung *et al.*, 1995). The increase in pH is used as an indicator of ammonia production. A comparison of environmental samples is only possible if they have a uniform buffer capacity, which is mainly achieved by titration of the sample after incubation with urea and urease to the starting pH (Brack *et al.*, 2000). On the other hand, potentiometric sensors based on the determination of protons show the disadvantage of deactivating urease due to increase in pH value around the electrode when it is operated during long term. Strong buffers are usually

Table 1.3 : Enzyme-based biosensors developed for heavy metals determinations.

ENZYME	SUPPORT/ELECTRODE	METHOD OF ANALYSIS	ANALYTES	REFERENCES
Carbonic anhydrase	-	Spectrophotometric	Zn (II), Cu (II), Cd (II), Co (II)	Thompson and Jones (1993-1999)
Oxidases	Polypropylene membrane on O ₂ electrode	Amperometry	Hg (II), Ag (I), Cd (II), Zn (II)	Gayet <i>et al.</i> (1993)
	Platinum based H ₂ O ₂ probe	Amperometry	Hg (II), Cu (II), V (V) and Ni (II)	Compagnone <i>et al.</i> (2001)
	Clarke-type O ₂ electrode		Co (II), Zn (II), Cd (II)	Beard <i>et al.</i> (1995)
GOX	Nylon Mesh membrane	Amperometry	Cu (II), Hg (II), Ag (I)	Donlan <i>et al.</i> (1989)
Invertase	Immobilon affinity membrane	Amperometry	Hg (II)	Bertocchi <i>et al.</i> (1999)
Alkaline Phosphatase	Cellulose acetate	Potentiometric	Hg (II), Cu (II)	Danzer and Schwedt (1996)
	GA cross-linking	Chemiluminescent	Zn (II), Be (II), Bi (III)	Kamtekar <i>et al.</i> (1995).
Peroxidase	GA cross-linking	Amperometry	Hg (II), Hg (I)	Han <i>et al.</i> (2001)
		Spectrophotometric	Cd (I)	Keyhani <i>et al.</i> (2003)
LDH	Polysulfone membrane on O ₂ electrode	Amperometry	Hg (II), Ag (I), Pb (II), Cu (II), Zn (II)	Fennouh <i>et al.</i> 1998
Urease		Spectrophotometric	Cd (II), As (III), Zn (II), Pb (II)	Oehlschläger <i>et al.</i> (1998)
	Free	Spectrophotometric	Hg (II), Co (II), Cr (III), Zn (II), Cd (II), Pb (II)	Wittekindt <i>et al.</i> (1996)
	-	Spectrophotometry	Cu ,Hg ions	Prellswaid & Schwedt (1994)
	-	Flow Injection/Fluorimetry	Hg (II), Ag (I)	Narinesingh <i>et al.</i> (1994)
	Polyacrylamide gel	Potentiometric	Hg (II), Ag (I), Hg (II)	Shi <i>et al.</i> (1997)
	pH electrode	Potentiometry	Pb (II), Fe (II), Sn (II), Zn (II), Hg (II)	Kormos & Lengauer (1999)
	Wall cuvette covered with ammonia sensor	Spectrophotometric	Ag (I), Hg (II), Cu (II), Ni (II), Co (II), Cd (II), Fe (III), Zn (II), Pb (II)	Preininger & Wolfbeis (1996)
	Nafion film	ISFE/Potentiometric	Ag (I), Hg (II), Cu (II)	Volotovskiy <i>et al.</i> (1997)
	PVC	ISFE/Potentiometric	Cu (II), Hg (II)	Sennillou <i>et al.</i> (1999)
	Nafion film	Potentiometric	Ag (I), Ni (II), Cu (II)	Soldatkin <i>et al.</i> (1999)
	Polypyrrole	Potentiometric	Ag (I)	Komaba <i>et al.</i> (1998)
	Cellulose acetate	Optical	Hg (II)	Shi & Jiang (2002)

LDH=Lactate dehydrogenase; GOX=glucose oxidase; GA=glutaraldehyde; ISFE= Ion sensitive field effect transistor.

required in these cases, making the sensor response dependent on buffer concentration (Yoneyama *et al.*, 2001). An amperometric assay was studied in order to minimize the buffer dependence inherent in potentiometric readings, however proved difficult because urea (urease substrate) is electrochemically inert. However, Bello Rodriguez *et al.* (2004a,b) reported that the coupling of urease to glutamic dehydrogenase allowed the optimization of an amperometric procedure that showed a remarkable sensitivity to metals inactivation. The sensor shows low detection limits for metals such as Hg, Cu and Cd along with reproducible and accurate results obtained for the analysis of environmental samples.

1.4.7 Electrochemical techniques

1.4.7.1 Potentiometry – Ion-selective electrode

Ion-selective electrodes (ISEs) are used in a wide variety of applications for determining the concentrations of various ions in aqueous solutions. For example, they have been used in pollution monitoring, agriculture, food processing, salt content of meat, fish, dairy products, fruit juices, brewing solutions, F in drinking water and other drinks, Ca in dairy products and beer, K in fruit juices and wine making, corrosive effect of NO_3 in canned foods, detergent manufacture, paper manufacture, explosives, electroplating, biomedical laboratories, F in skeletal and dental studies. When compared to many other analytical techniques, ISEs are relatively inexpensive and simple to use and have an extremely wide range of applications, wide concentration range, very robust, durable and ideal for use in either field or laboratory environments. The pH electrode is the most well known and simplest member of this group and can be used to illustrate the basic principles of ISEs (Rundle, 2004). The functioning of the ISEs is based on the selective passage of charged species from one phase to another leading to the creation of a potential difference. The use of an ISE system involves dipping the electrodes in the test solution and reading a signal from the voltmeter (Prichard *et al.*, 1996). The three main problems with ISEs measurements are the effect of interference from other ions in solution, the effect of the ionic strength of the solution reducing the measured activity relative to the true concentration at high concentrations, and the drift in electrode potential during a sequence of measurements.

1.4.7.2 Stripping analysis

Stripping analysis (stripping voltammetry and stripping potentiometry) is widely used for the detection of heavy metals in various environmental samples. The term electrochemical stripping analysis is a two step technique. The first or deposition step involves the electrolytic deposition of a small portion of the metal ions in solution into the working electrode to preconcentrate the metals. This is followed by the stripping step (the measurement step), which involves the dissolution (stripping) of the deposit. Different versions of stripping analysis can be employed, depending upon the nature of the deposition and measurement steps (Cassidy *et al.*, 1998; Wang, 2000).

There are many advantages of stripping analysis as compared to conventional methods for heavy metal detection (Wang 1994; Palchetti, 1998; Wang *et al.*, 1999). There are:

- The ability to measure simultaneously four to six trace metals at concentration levels down to sub microgram per litre.
- The equipment is relatively cheap.
- Advantage of species characterization.
- Suitability for automatic on-line monitoring and for in-situ measurements.

However, the disadvantages for this technique might be the problems with the stability of the electrodes as well as fouling and interferences when the electrodes are in physical contact with sample solutions (Prichard *et al.*, 1996). However, continuing developments in the field of microelectronics and microsensor technology are addressing these problems. Examples of environmental applications of stripping analysis are shown in Table 1.4 for heavy metal analysis.

Table 1.4: Examples of Environmental Applications of Stripping Analysis

METAL	ELECTRODE	MATRIX	METHOD	DETECTION LIMT	REFERENCE
Lead	Silver electrode	River water and drinking water	ASV	0.5 nM (0.1 $\mu\text{g l}^{-1}$)	Kirowa-Eisner <i>et al.</i> , 1999
Mercury	Gold wire electrode	Sea water	CCSCP	0.3 $\mu\text{g l}^{-1}$	Wang <i>et al.</i> , 1998a
Chromium	Bismuth plated GCE	Tap water and soil	AdSV	100 ng l^{-1}	Chatzitheodorou <i>et al.</i> , 2004
Cadmium, lead	Mercury film plated GCE	Lake waters & certified seawaters	ASV	5pM (Cd) 8 pM (Pb)	Fisher & van den Berg, 1999
Mercury Selenium Copper Lead	Gold film on GCE	River water and sea water	PSA	15 ng l^{-1} (Hg) 0.1 $\mu\text{g l}^{-1}$ (Selenium)	Gil & Ostapczuk, 1994
Lead Thallium	Bismuth plated GCE	Sea water	PSA	10 ng ml^{-1} (Pb & Th)	Jaya <i>et al.</i> , 1985
Arsenic	HMDE	Stream sediment and water samples	CSV	0.6 nM (4.4 $\mu\text{g l}^{-1}$)	Greulach & Henze, 1995
Chromium	HMDE	Tap and river water samples	CAdSV	0.2 nM (10.4 ng l^{-1})	Grabarczyk & Korolczuk, 2003
Cadmium Chromium Copper Lead Zinc	SMDE Mercury plated GCE	Soil and sediment samples	ASV PSA AdSV	1 $\mu\text{g l}^{-1}$	Olsen <i>et al.</i> , 1994
Arsenic Selenium Copper Lead Zinc Cadmium	Stationary mercury electrode	Estuarine and river sediments	CSV ASV	Around 10^{-9} M	Locatelli and Torsi, 1999
Cadmium Lead	CPE bulk modified with bismuth oxide	Drinking water and mineral water	ASV	5 $\mu\text{g l}^{-1}$ (Cd) 5 $\mu\text{g l}^{-1}$ (Pb)	Pauliukaite <i>et al.</i> , 2002

ASV: Anodic stripping voltammetry, AdSV: adsorptive stripping voltammetry, AdCSV: Adsorptive cathodic stripping voltammetry, CAdSV: Catalytic Adsorptive stripping voltammetry, CCSCP: Constant current stripping shronopotentiometry, CSV: Cathodic stripping voltammetry, PSA: Potentiometry stripping analysis, SW: Square-Wave, CPE: Carbon paste electrode, HMDE: Hanging mercury drop electrode, GCE: Glassy carbon electrode, SMDE: Static mercury drop electrode.

1.5 ELECTROCHEMICAL METHODS

The fundamental process in electrochemistry is the transfer of electrons between the electrode surface and molecules in the solution adjacent to the electrode surface which, it is a heterogenous process. One quantity that is important in electrochemical experiments is current (I or i), and the current per/unit area is called current density (amps/cm^2). Current density is the more fundamental quantity, since it is independent of the electrode surface area, whilst current is proportional to the electrode surface area. Since current results from a chemical substance dissolved in the solution adjacent to the electrode surface, the probability of a molecule or ion reacting at the electrode surface is directly related to the concentration. Therefore, current density is directly proportional to the concentration, provided the electrode reaction does not produce unusual features, such as adsorption and dimerisation (Kissinger, 2002). Current is expressed in amperes (A); that is coulombs/second. It implies quantitative expression how fast something is happening. Voltage is the driving force behind an electrical reaction and it is sometimes referred to as electromotive force (emf) or potential difference (pd). It has units of volts (V) and the symbol of E . Resistance is the opposition to the voltage, preventing a flow of current. It has units of ohms (Ω) and the symbol R . The coulomb (C) is the unit of charge. An electron carries a charge of 1.60210×10^{-19} C. Current can be related to the charge by:

$$I = Q/t \quad (1.01)$$

Faraday's constant (F) is the charge carried by a mole of electrons. This has a value of

$$\begin{aligned} F &= 6.022552 \times 10^{23} \text{ electrons mol}^{-1} \times 1.60210 \times 10^{-19} \text{ C/electron} \\ &= 96487 \text{ C mol}^{-1} \end{aligned} \quad (1.02)$$

1.5.1 Potentiometry

Potentiometry is a static (zero current) technique in which the information about the sample composition is obtained from measurement of the potential established across a membrane (Wang, 2000). In the ISE, the relationship between the ionic concentration (activity) and the electrode potential is given by the Nernst equation:

$$E = E^0 + (2.303RT/nF) \times \text{Log}(A) \quad (1.03)$$

Where E = the total potential (in mV) developed between the sensing and reference electrodes, E^0 is a constant which is characteristic of the particular ISE/reference pair., the 2.303 is the conversion factor from natural to base10 logarithm, R is the Gas Constant (8.314 joules/degree/mole), T is the Absolute Temperature, n is the charge on the ion (with sign), F is the Faraday Constant (96,500 coulombs) and $\text{Log}(A)$ = the logarithm of the activity of the measured ion. The $2.303RT/nF$ is the slope of the line (from the straight line plot of E versus $\text{log}(A)$ which is the basis of ISE calibration graphs) and this is an important diagnostic characteristic of the electrode - generally the slope gets lower as the electrode gets old or contaminated, and the lower the slope the higher the errors on the sample measurements.

1.5.2 Voltammetry

Voltammetry refers to the measurement of current that result from the application of potential. The current arises from transfer of electrons between the energy levels of the working electrode and the molecular energy levels of the system under study. The electron transfer between a metal and species in solution near the electrode depend on the energy of electrons in the electrode and the energy of the lowest unoccupied orbitals or the highest occupied orbitals or the species in the solution. The energy level of electrons in the metal is referred to as the Fermi level. As the charge on the electrode becomes more negative the Fermi level increases. When the energy of electrons in the metal equals or exceeds that of the lowest unoccupied orbitals, electrons can transfer from the electrode to the species in the solution. The species is reduced in the process. If the potential of the electrode is made more positive it will reach the point where the energy of electrons in the metal is lower than that of electrons in the highest occupied orbitals of the dissolved species. In this case, electrons can transfer from the species in solution to the metal (McCarron, 1999).

For voltammetry experiments, a potentiostat is required for controlling the applied potential, and a current-to-voltage converter is required for measuring the current. All chemical cells require at least two electrodes, since the potential of a given electrode can only be measured relative to another electrode, the potential of which must be constant. The three electrode cell is one of the most common configurations used to study electrochemical reactions. It consists of a counter electrode (CE), which serves to complete the circuit for carrying the current generated at the working electrode (WE). In order that the potential (voltage) on the working electrode is precisely known, as a third electrode known as the reference electrode (RE) is held close to the surface of the WE and the potential difference measured. This is because the

RE has no current passing through it, but merely maintains an invariant constant potential, no matter what is happening around it (Gunzler and Williams, 2001; Higson, 2003).

1.5.3 Processes at Electrode – Solution Interface

There are two types of processes occur at the electrode-solution interfaces during voltammetric experiments, faradaic and non-faradaic processes. Both these processes occur when electrode reactions take place (Bard and Faulkner, 2001).

1.5.3.1 Non-Faradaic processes

A non-faradaic process involves the accumulation of charges at the metal/solution interface. The structure formed in this process is called the electric double layer. The double layer resembles a capacitor. The double layer capacitance depends on the solution composition and also depends somewhat that on potential applied to the electrode. The specific capacitance of typical electrodes is usually in the range of 5 to 50 $\mu\text{F}/\text{cm}^2$. Non-faradaic processes occurring at electrodes cause a flow of non-faradaic currents. Since all species present in solution may affect the double layer capacitance, the non-faradaic currents are usually non-specific and they are rarely used to provide analytical signals; however non-faradaic currents contribute to the background noise (inf $\uparrow$, noise $\uparrow$, analytical sensitivity $\downarrow$). Non-faradaic currents are particularly large when surface of the electrode changes with time (e.g. in case of the dropping mercury electrode) (Bard and Faulkner, 2001; University of Saskatchewan, 2004).

1.5.3.2 Faradaic processes

Faradaic processes are associated with electron transfer across the interface. A faradaic process lead to reduction or oxidation of species present at the interface. Electron transfer may occur via overlapping of orbitals (in the case of adsorption), via bridging ligands or via electron tunnelling; in any case, a molecule undergoing the electron transfer process must be in near proximity of the electrode surface (usually $< 1 \text{ nm}$). Species present in bulk solution may be reduced or oxidised only if they are brought to the electrode surface via a mass transport process. The objective of controlled-potential experiments is to obtain a current response that is related to the concentration of the target analyte. This objective is accomplished by monitoring the transfer of electron(s) during the redox process of the analyte (Bard and Faulkner, 2001; University of Saskatchewan, 2004):



where O and R are the oxidised and reduced forms, respectively, of the redox couple.

The Nerst equation relates the concentrations of ions taking part in an electrochemical reaction at equilibrium with the potential that the electrode takes up relative to the standard electrode potential of the system:

$$E = E^0 + \frac{2.3 RT}{nF} \log \frac{C_O}{C_R} \quad (1.05)$$

Where E^0 is the standard potential for the redox reaction, R is the universal gas constant ($8.314 \text{ JK}^{-1}\text{mol}^{-1}$), T is the Kelvin temperature, n is the number of electrons transferred in the reaction, and F is the Faraday constant (96,487 coulombs).

1.5.4 The Rate of Electrode Reaction

The exact shape and magnitude of the voltammetric response is governed by the processes involved in the electrode reaction. The total current is the summation of the faradaic currents for the sample and blank solutions, as well as the non-faradaic charging background current. The pathway of the electrode reaction can be quite complicated, and takes place in a sequence that involves several steps. Simple reactions involves only mass transport of the electroactive species to the electrode surface, the electron transfer across the interface, and the transport of product back to the bulk solution. More complex reactions include additional chemical and surface processes that precede or follow the actual electron transfer. The net rate of the reaction, and hence the measured current, may be limited either by mass transport of the reactant or by the rate of electron transfer. The more sluggish process will be the rate-determining step. Whether a given reaction is controlled by the mass transport or electron transfer is usually determined by the type of compound being measured and by various experimental conditions (electrode material, media, operating potential, mode of mass transport, time scale etc.). For a given system, the rate-determining step may thus depend on the potential range under investigation. When the overall reaction is controlled solely by the rate at which the electroactive species reach the surface, the current is said to be mass transport-limited. Such reactions are called *nernstian* or *reversible*, because they obey thermodynamic relationships (Wang, 2000).

1.5.4.1 Mass Transport

There are essentially three mechanisms for transporting electroactive species to the electrode surface (Wang, 2000):

- *Diffusion* is the movement of molecules along a concentration gradient, from an area of high concentration to an area of low concentration.
- *Migration* is the transport of a charged species under the influence of an electric field.
- *Convection* is the transport of species by hydrodynamic transport (e.g. natural thermal motion and/or stirring).

1.5.5 Linear Sweep and Cyclic Voltammetry

1.5.5.1 Linear Sweep Voltammetry

The simplest technique that uses a simple potential wave form is linear sweep voltammetry (LSV). The potential range is scanned in one direction at a scan rate (v), starting at the initial potential, V_1 (no electrode reaction) and finishing at the final potential, V_2 and measure the current (Figure 1.1).

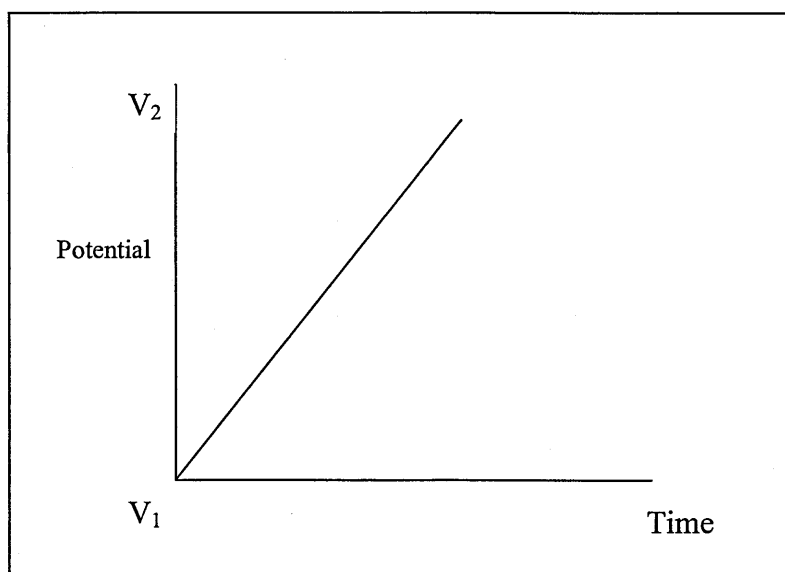


Figure 1.1: Time-potential excitation signal in a linear sweep voltammetry experiment.

Figure 1.2 shows the current response vs. voltage. For example, if consider the $\text{Fe}^{3+}/\text{Fe}^{2+}$ system

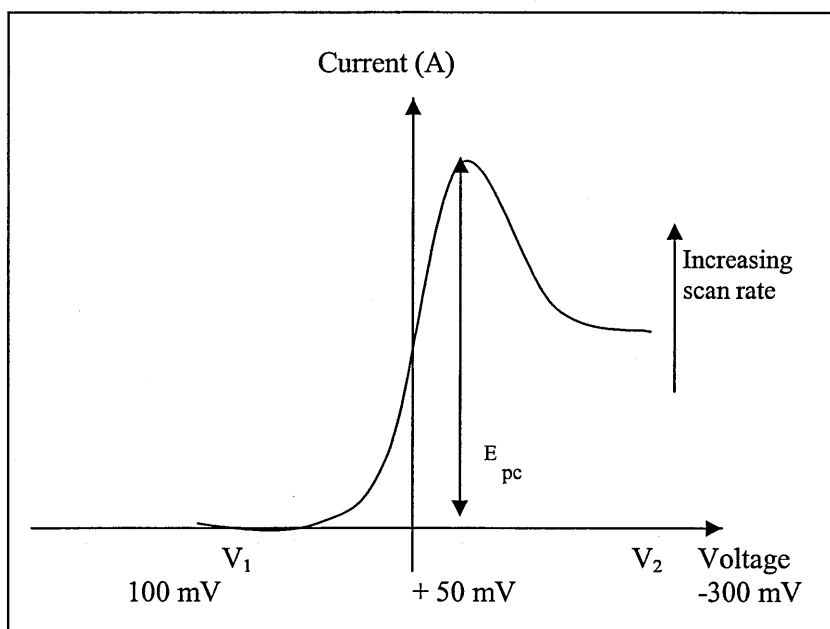
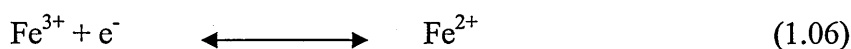


Figure 1.2: Typical linear sweep voltammogram showing the resulting current-potential curve.

The following voltammogram (Figure 1.2) is observed for a single potential scan using an electrolyte solution containing only Fe^{3+} . The scan begins from the left hand side of the current/voltage plot where no current flows. As the voltage is swept further to the right (to more reductive value) a current begins to flow and eventually reaches a peak before dropping. To rationalise this behaviour we have to consider the influence of voltage on the equilibrium established at the electrode surface. If we consider the electrochemical reduction of Fe^{3+} to Fe^{2+} , the rate of electron transfer is fast in comparison to the voltage sweep rate. Therefore at the electrode surface an equilibrium is established identical to that predicted by thermodynamics. From equilibrium electrochemistry that the Nernst equation (equation 1.05) predicts the relationship between concentration and voltage (potential difference). So as the voltage is swept from V_1 to V_2 the equilibrium position shifts from no conversion at V_1 to full conversion at V_2 of the reactant at the electrode surface. This can be shown clearly in Table 1.5 (University of Bath, 2004).

Table 1.5 : The ratio of Fe³⁺ to Fe²⁺ changes.

Fe ³⁺ + e ⁻ ←	Fe ²⁺	V1
Fe ³⁺ + e ⁻ ⇌	Fe ²⁺	
Fe ³⁺ + e ⁻ ⇌	Fe ²⁺	~ E _p
Fe ³⁺ + e ⁻ ⇌	Fe ²⁺	
Fe ³⁺ + e ⁻ →	Fe ²⁺	V2

The exact form of the voltammogram can be rationalised by considering the voltage and mass transport effect. As the voltage is initially swept from V₁, the equilibrium at the surface begins to alter and the current begins to flow: the current rises as the voltage is swept further from its initial value and the equilibrium position is shifted further towards products (Fe²⁺), thus converting more reactant. The peak occurs, since at some point the diffusion layer has grown sufficiently above the electrode so that the flux of reactant to the electrode is not fast enough to satisfy that required by Nernst equation. In this situation the current begins to drop. In fact the drop in current follows the same behaviour as that predicted by the Cottrell equation. If the scan rate is altered the current response also changes. A series of linear sweep voltammograms can be recorded at different scan rate for the same solution of Fe³⁺.

Each curve has the same form but it is apparent that the total current increases with increasing scan rate. This can be explained by considering the size of the diffusion layer and the time taken to record the scan. The linear sweep voltammogram will take longer to record as the scan rate is decreased. Therefore the size of the diffusion layer above the electrode surface will be different depending upon the voltage scan rate used. In a slow voltage scan the diffusion layer will grow much further from the electrode in comparison to a fast scan. Consequently the flux to the electrode surface is considerably smaller at slow scan rates than it is at faster rates. As the current is proportional to the flux towards the electrode the magnitude of the current will be lower at slow scan rates and higher at high scan rates. The important point when examining LSV and Cyclic voltammerty (CV), although there is no time axis on the graph, the potential scan rate (and therefore the time taken to record the voltammogram) do strongly effect the behaviour seen (University of Bath, 2004).

It is clear to note that the position of the current maximum, peak occurs at the same voltage and this is the characteristic of electrode reactions which have rapid electron transfer kinetics. These rapid processes are often referred to as reversible electron transfer reactions. For the reactions that are slow (so called quasi-reversible or irreversible electron transfer reactions) the voltage applied will not result in the generation of the concentrations at the electrode surface predicted by the Nernst equation. This happens due to the kinetics of the reaction are slow and thus the equilibria are not established rapidly (in comparison to the voltage scan).

1.5.5.2 Cyclic Voltammetry

Cyclic voltammetry is the most widely used technique for acquiring qualitative information about electrochemical reactions. Cyclic voltammetry (CV) is closely related to LSV, consists of scanning the potential of a stationary working electrode in an unstirred solution so that the measured current is limited by analyte diffusion at the electrode surface. The electrode potential is swept between two values at a fixed rate, when the potential reaches the second values, V_2 , the scan reverse back to the starting voltage, V_1 (Figure 1.3). A basic shape of the current response for a cyclic voltammetry experiment is shown in Figure 1.4. For a fully reversible reaction, on the forward sweep, where electrons are put into the electrode (the cathodic sweep) the curve is identical to that seen in LSV. On reversing the scan direction, electrons are withdrawn from electrode and the material reduced in the forward sweep are oxidised back. The cyclic voltammogram is characterised by several important parameters. Two peak currents and two peak potentials provide the basis for the diagnostics developed for analysing the cyclic voltammetric response (New Mexico State University, 2003, Higson, 2003; Wang, 2000).

A CV of a fully reversible system will display the following characteristics (Wang, 2000; University of Bath, 2004):

- The voltage separation between the current peaks is

$$\Delta E = E_{pa} - E_{pc} = 59 \text{ mV}/n \quad (1.07)$$

- The positions of the peak voltage do not alter as a function of voltage scan rate
- The ration of the peak currents is equal to one

$$\frac{i_{pa}}{i_{pc}} = 1 \quad (1.08)$$

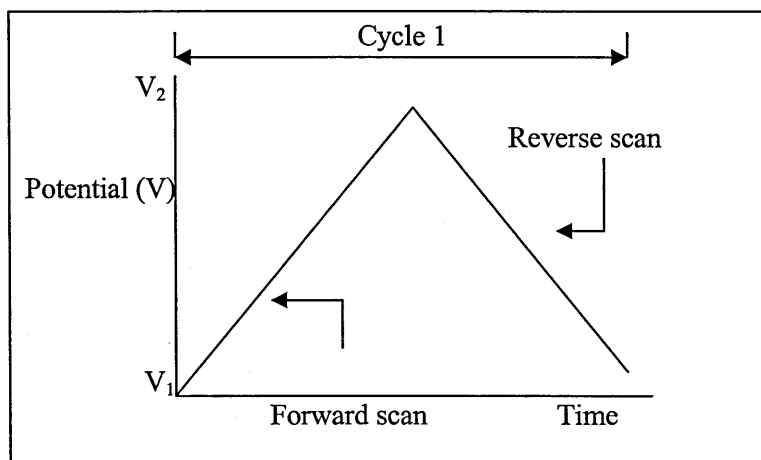


Figure 1.3 : Potential-time excitation signal in cyclic voltammetric experiment (adapted from Wang, 2000)

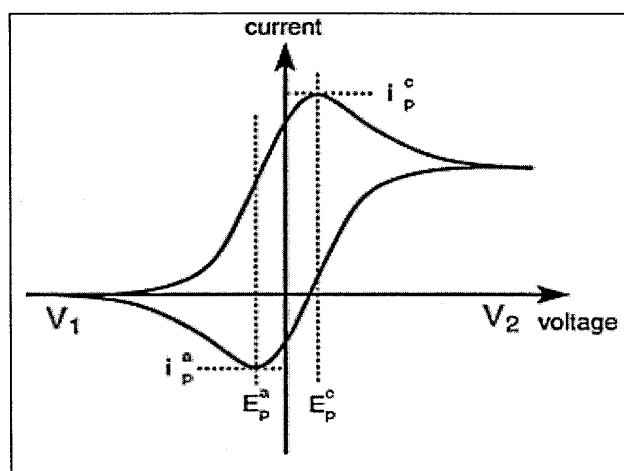


Figure 1.4 : Cyclic voltammogram recorded for a reversible redox couple during a single potential cycle (adapted from University of Bath, 2004).

- The peak currents are proportional to the square root of the scan rate

$$i_{pa} \text{ and } i_{pc} \propto \sqrt{v} \quad (1.09)$$

- The electron kinetics are very fast-that is the rate limiting step of the electrode reaction is influenced purely by the ease of getting material to the electrode to react – mass transport.
- The peak current (at 25°C) is given by Randles-Sevcik equation:

$$i_p = (2.69 \times 10^5) n^{3/2} A D^{1/2} C^* v^{1/2} \quad (1.10)$$

Where n is the number of electrons, A the electrode area (in cm^2), C the concentration (in mol/cm^3), D the diffusion coefficient (in cm^2/s), and v the scan rate (in V/s).

For irreversible processes (those with sluggish electron exchange), the individual peaks are reduced in size and widely separated. Totally irreversible systems are characterised by :

- a shift of the peak potential with the scan rate

$$E_p = E^0 - \frac{RT}{\alpha n_a F} \left[0.78 - \ln \frac{k^0}{D^{1/2}} + \ln \left\{ \frac{\alpha n_a F v}{RT} \right\}^{1/2} \right] \quad (1.11)$$

where α is the transfer coefficient and n_a is the number of electrons involved in the charge-transfer step. Thus E_p occurs at potentials higher than E_0 , with the overpotential related to k_0 and a . Independent of the value of k_0 , such peak displacement can be compensated by an appropriate change of the scan rate. The peak potential and the half-peak potential (at 25°C) will differ by $48/\alpha n$ mV. Hence, the voltammograms becomes more drawn-out as αn decreases.

- The peak current, given by:

$$i_p = (2.99 \times 10^5) n(\alpha n_a)^{1/2} A C D^{1/2} v^{1/2} \quad (1.12)$$

is still proportional to the bulk concentration, but will be lower in height (depending upon the value of α).

- For quasi-reversible systems

$$10^{-1} \text{ cm s}^{-1} > k^0 > 10^{-5} \text{ cm s}^{-1} \quad (1.13)$$

The current is controlled both the charge transfer and mass transport.

1.5.6 Pulsed Voltammetry

Pulsed voltammetric techniques are aimed at lowering the detection limits of voltammetric measurements by increasing the ratio between the faradaic and non-faradaic currents. The various pulse technique (Normal pulse voltammetry, Differential pulse voltammetry and Square-Wave voltammetry) are all based on a sampled current potential-step (chronoamperometric experiment). A sequence of such potential steps, each with a duration of about 50 ms, is applied to the working electrode. After the potential is stepped, the charging current decays rapidly (exponentially) to a negligible value, while the faradaic current decays more slowly. Thus, by sampling the current late in the pulse life, an effective discrimination against the charging current is achieved (Wang, 2000). Square-wave voltammetry (SWV) is a large-amplitude differential technique in which a waveform composed of a symmetrical square-wave, superimposed on a base staircase potential, is applied to the working electrode. The current is sampled twice during each square-wave cycle, once at the end of the forward pulse (at t_1) and once at the end of the reverse pulse (at t_2). The difference between the two measurements is plotted against the base staircase potential. The SWV technique is more rapid and sensitive as compared to differential pulse voltammetry.

1.5.7 Chronopotentiometry

The basis of control current experiments is that a redox (electron transfer) reaction must occur at the surface of the working electrode in order to support the applied current. Chronopotentiometry is the most basic constant current experiment where a current step is applied across an electrochemical cell (without stirring) and the current excitation signal and potential response are shown as a function of time (Figure 1.5).

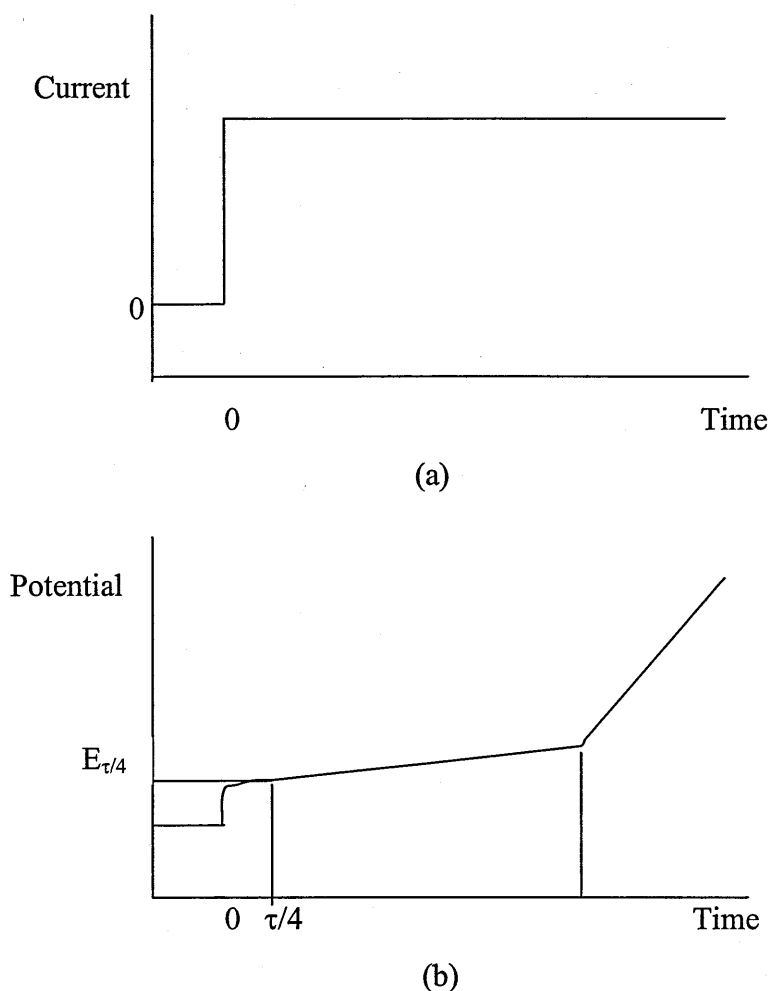


Figure 1.5: Current excitation (a) and potential response (b) for chronopotentiometry

The time between the steep sections of the curve is called the transition time τ , which, in diffusion-controlled reversible electrode processes, is described by the Sand equation (Gunzler & Williams, 2001).

$$\tau^{1/2} = \frac{\pi^{1/2} n F D^{1/2}}{2 j_0} C \quad (1.14)$$

where j_0 is the current density (A cm^{-2}), and $\tau^{1/2}$ corresponds to the limiting diffusion current in DC polarography. At constant j_0 , the potential-time function is:

$$E = E_{\tau/4} + \frac{RT}{nF} \ln \frac{\tau^{1/2} - t^{1/2}}{t^{1/2}} \quad (1.15)$$

with $E_{\tau/4}=E_{1/2}$ for a reversible reaction.

Normal chronopotentiometry has little importance for analysis. Determinations are possible in the range 10^{-4} - 10^{-5} mol/l at best. However, the technique is of use in understanding the methods of stripping chronopotentiometry and potentiometric stripping analysis, which are important in trace analysis.

1.5.8 Chronoamperometry

Chronoamperometry involves stepping the potential of the stationary working electrode from a value at which no faradaic reaction occurs to a potential at which the surface concentration of the electroactive species is effectively zero. The resulting current-time dependence is monitored. As mass transport under these conditions is solely by diffusion, the current-time curve reflects the change in the concentration gradient in the vicinity of the surface. This involves a gradual expansion of the diffusion layer associated with the depletion of the reactant, and hence decrease slope of the concentration profile as time progresses. The current decays with time as given by Cottrell equation:

$$I(t) = \frac{nFACD^{1/2}}{\pi^{1/2} t^{1/2}} \quad (1.16)$$

The current is proportional to $t^{-1/2}$, which is termed Cottrell behaviour. Chronoamperometry is often used for measuring the diffusion coefficient of electroactive species or the surface area of the working electrode. Analytical applications of chronoamperometry are many in biosensor area which relies on pulsing of the potential of the working electrode repetitively at fixed time intervals. Chronoamperometry can also be applied to the study of mechanisms of electrode processes (Wang, 2000).

1.5.9 Stripping Analysis

The term electrochemical stripping analysis is applied to a family of procedures involving a preconcentration of determinand (or a salt or derivative of the determinand) onto the working electrode or indicator electrode, prior to its direct or indirect determination by means of an electroanalytical technique (Fogg & Wang, 1999). In Table 1.6, a tentative classification of different stripping electroanalytical analysis schemes is given. There are two major groups differentiated, either electrolytic or non-electrolytic preconcentration. In Table 1.7, more detailed information about the basis of the different experimental approaches in stripping voltammetry is summarised.

Table 1.6 : A tentative classification of stripping electroanalytical schemes (Esteban & Casassas, 1994).

ELECTROLYTIC PRECONCENTRATION	SCHEME
By formation of amalgams.	Anodic stripping voltammetry Stripping potentiometry
The formation of sparingly soluble species.	Cathodic stripping voltammetry Anodic stripping voltammetry
By formation of sparingly soluble compounds with metal ions from the electrode.	Cathodic stripping voltammetry Stripping potentiometry
By formation of sparingly soluble compounds by co-electrolysis with another species.	Cathodic stripping voltammetry
NON-ELECTROLYTIC PRECONCENTRATION	SCHEME
Adsorptive stripping voltammetry.	Non-faradaic measurements/ faradaic measurements
Preconcentration on chemically modified electrodes.	By complexation with specific groups By ion-exchange reactions (ion-exchange voltammetry)

Table 1.7 : Different techniques of stripping analysis (Esteban and Casassas, 1994).

PRECONCENTRATION STEP	MEASURING STEP
Schemes of electrolytic preconcentration	
Anodic stripping voltammetry (ASV) $M^{n+} + ne \rightarrow M(Hg)$	$M(Hg) \rightarrow M^{n+} + ne$
Cathodic stripping voltammetry (CSV) $M + X^{m-} \rightarrow M_mX_n + ne$	$M_mX_n + ne \rightarrow M + X^{m-}$
Potentiometric stripping analysis (PSA) $M^{n+} + ne \rightarrow M(Hg)$ $M + X^{m-} \rightarrow M_mX_n + ne$ Constant current stripping chronopotentiometry (CCSCP) $M^{n+} + ne \rightarrow M(Hg)$ $M + X^{m-} \rightarrow M_mX_n + ne$	$M(Hg) + \text{Oxid.} \rightarrow M^{n+} + ne$ $M_mX_n + \text{Red.} \rightarrow M + X^{m-}$ $M(Hg) + \text{Oxid. Current} \rightarrow M^{n+} + ne$ $M_mX_n + \text{Red. Current} \rightarrow M + X^{m-}$
ASV or CSV with formation of sparingly soluble species, without reagent addition $M^{n+} + (n+1)OH^- \rightarrow M(OH)_{n+1} + e$ $M^{n+} + (n-1)OH^- + e \rightarrow M(OH)_{n-1}$	$M(OH)_{n+1} + e \rightarrow M^{n+} + (n+1)OH^-$ $M(OH)_{n-1} \rightarrow M^{n+} + (n-1)OH^- + e$
ASV or CSV with formation of sparingly soluble species, with reagent addition $M^{n+} + (n+1)L^- \rightarrow ML_{n+1} + e$ $M^{n+} + (n-1)L^- + e \rightarrow ML_{n-1}$	$ML_{n+1} + e \rightarrow M^{n+} + (n+1)L^-$ $ML_{n-1} \rightarrow M^{n+} + (n-1)L^- + e$
CSV with formation of sparingly soluble species by co-electrolysis of another species $M_1^{n+}, M_2^{n+} + e \rightarrow M_1M_2$	$M_1M_2 + e \rightarrow M_1^{n+}M_2^{n+}$
Schemes of non-electrolytic preconcentration	
Adsorptive stripping voltammetry (AdSV) onto conventional electrodes $A \rightarrow A_{ads}$ $M^{n+} + L^{m-} \rightarrow (M_mL_n)_{ads}$	$A_{ads} \rightarrow A$ $A_{ads} \rightarrow A^* + ne$ $(M_mL_n)_{ads} \rightarrow M^{n+} + L^{m-}$ $(M_mL_n)_{ads} \rightarrow M^{n+} + L^{p-} + (m-p)e$ $(M_mL_n)_{ads} \rightarrow M + L^{m-} + ne$
Stripping voltammetry with preconcentration on chemically modified electrodes $M^{n+} + R(LH)_n \rightarrow R(LM)_n + nH^+$ $Org^{n+} + R(LH)_n \rightarrow R(LOrg)_n + nH^+$	$R(LM)_n + me \rightarrow M^{(n-m)+}$ $R(LOrg)_n + me \rightarrow Org^{(n-p)+}$
Abrasive stripping voltammetry (AbrSV) Mechanical transfer of A	$A + e \rightarrow A^-$

M = Metal, **L** = Ligand, **Oxid.** = Oxidising, **Red.** = Reducing, **R(LH)_n** = Ion exchangers, **Orgⁿ⁺** = Organic cation, **A** = Absorbable species

Stripping voltammetry involves the determination of an accumulated determinand by monitoring the faradaic current during a potential scan (Fogg & Wang, 1999). Different versions of stripping analysis can be employed, depending upon the nature of the deposition and measurements step. These include Anodic stripping, potentiometric stripping, adsorptive stripping and cathodic stripping voltammetry. Anodic stripping voltammetry (ASV) is the most widely used form of stripping analysis. The metals are preconcentrated by electrodeposition into a small-volume mercury electrode. The preconcentration is done by cathodic deposition at a controlled time and potential. The deposition is usually 0.3-0.5 V more negative than E^0 for the least easily reduced metal ion to be determined. The metal ions reach the mercury electrode by diffusion and convection, where they are reduced and concentrated as amalgams. Following the preselected time of the deposition, the potential is scanned anodically, either linearly or in a more sensitive potential-time (pulse) waveform that discriminates against the charging background current (usually square-wave or differential-pulse ramps). During this anodic scan, the amalgamated metals are reoxidised, stripped out of the electrode and a current flows. The potential-time sequence used in ASV, along with the resulting stripping voltammogram, is shown in Figure 1.6 (Wang, 2000).

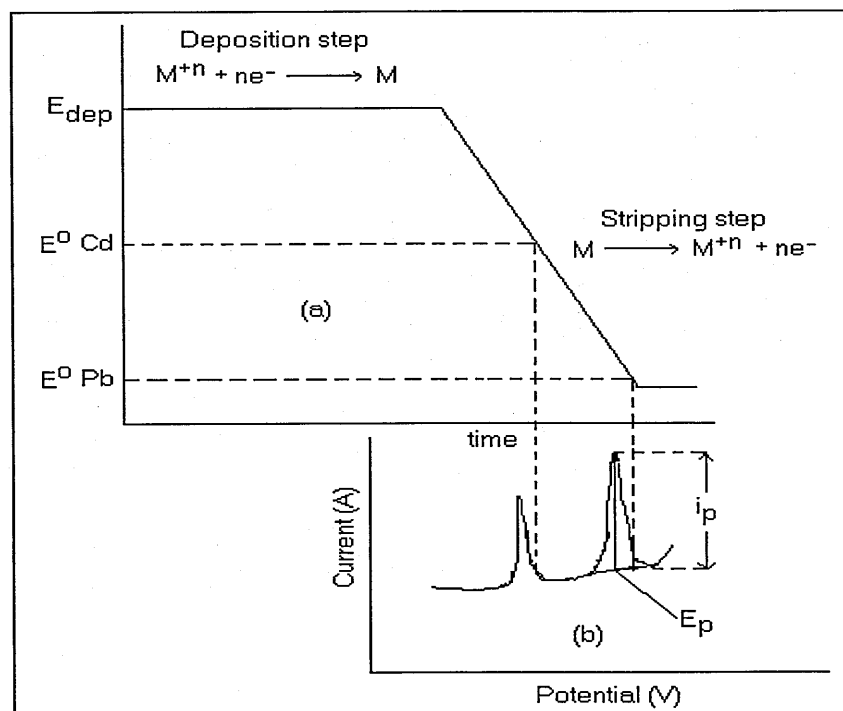


Figure 1.6: Anodic stripping voltammetry: the potential-time waveform along with the resulting voltammogram (adapted from Wang, 2000).

The major types of interferences in ASV procedures are overlapping stripping peaks caused by a similarity in the oxidation potentials (e.g., of the Pb, Tl, Cd, Sn, or Bi, Cu, Sb groups), the presence of surface-active organic compounds that adsorbed on the mercury electrode and inhibit the metal deposition and the formation of intermetallic compounds (e.g., Cu-Zn) which affects the peak size and position. Improved signal-to-background characteristics can be achieved using dual-working-electrode techniques, such as ASV with collection or subtractive ASV.

Stripping potentiometry is based on the potentiostatic enrichment of analytes on an electrode surface and the subsequent re-oxidation or re-reduction of the analytes by means of either spontaneous chemical reactions or by combination of such reactions and an applied constant current (Jagner *et al.*, 1996), and the change of electrode potential with time is monitored. No control is made of the potential of the working electrode during stripping because the potentiostat switches off once the deposition has taken place (Estela *et al.*, 1995; Wang, 2000). When the oxidation potential of a given metal is reached, the potential scan is slowed down as the oxidant or current is used for its stripping. A sharp potential step thus accompanies the depletion of each metal from the electrode. The resulting potentiogram consists of stripping plateaus, as in a redox titration curve. The transition time needed for the oxidation of a given metal, τ , is a quantitative measure of the sample concentration of the metal. Modern electrochemical analysers use microcomputers to register fast stripping events and to convert the wave-shaped response to a more convenient peak over a flat baseline. The most important difference between potentiometric and voltammetric stripping is that in potentiometric no current passes through the working electrode during stripping (Munoz & Palmero, 2004).

Cathodic stripping voltammetry (CSV) involves the electrochemical reductive determination of a determinand by stripping voltammetry. The determinand is accumulated anodically (e.g. as a mercury salt) and is then determined cathodically (Fogg & Wang, 1999). Quantification is accomplished by measuring the height of the resulting reduction peak. The deposited analyte forms an insoluble film of the mercury salt on the mercury surface and result for the calibration curves to display non-linearity at high concentrations due to saturation of the electrode surface (Esteban & Casassas, 1994).

Adsorptive stripping voltammetry (AdSV) is applied to stripping voltammetric techniques in which accumulation is affected by adsorption of (mainly) organic determinands. This technique has been used to enhance the sensitivity and selectivity of some voltammetric measurements. A wide range of surface-active organic and inorganic species that cannot be preconcentrated electrolytically, the adsorption approach serves as an effective alternative approach. Measurements of aluminium, chromium, nickel, vanadium, uranium, iron and thallium as their surface-active complexes, based on adding a suitable chelator on electrodes has been shown to be extremely useful for metals analysis. The analyte is allowed to accumulate interfacially on the electrode surface for a specified length of time, under conditions of maximum adsorption (optimise for electrolyte, pH, potential, mass transport, etc.); then the adsorbed materials are determined by applying a negative- or positive-going potential scan (for reducible or oxidisable species, respectively) (Ewing, 1994).

1.6 SURFACE ANALYTICAL TECHNIQUES

Many important chemical processes occur at the surface of a solid. Some examples include catalysis, electrochemistry and adhesion. Understanding these processes requires an understanding of the composition and structure of the surfaces. The analytical techniques that can selectively characterise solid surfaces can be divided into two categories: techniques that are primarily used for elemental analysis at surfaces, and techniques primarily used for surface imaging.

1.6.1 Electron Microscopy

Electron microscopy is an imaging technique that used an electron beam to probe a material. Since the wavelength of an electron is much smaller than the wavelength of visible light, diffraction effects occur at much smaller physical dimensions. The imaging resolution in electron microscopy is on the order of 1 nm, which is much better than the μm resolution of light microscopy. When electrons penetrate a sample, they are diffracted to form a diffraction pattern. This diffraction pattern can be transformed with a lens to obtain the sample image. Electron microscopy finds its widest use in materials science and microbiology.

The use of electron beams requires that the sample be placed in a vacuum chamber for analysis. An electron beam is produced by applying a high voltage to a hot tungsten filament, and accelerating the emitted electrons through a high electric field, typically 10-100 keV. The

electron beam is then focused with magnetic field lenses to a typical spot diameter of 1-100 nm on the sample (Virginia Tech, 2004).

1.6.1.1 Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) uses the secondary electrons that are ejected from a sample to image a surface. These images are useful for studying surface morphology or measuring particle sizes. The electron beam is rastered across the sample by ramping the voltages on x- and y-deflection plates through which the electron beam passes (the z axis is the electron-beam direction). A detector above the sample detects the secondary electrons to produce an intensity map as function of electron-beam position, which is displayed on a video or computer screen. Nonconductive samples require an evaporated gold or graphite coating over the sample to prevent charging effects that would distort the electric fields in the electron microscope. A recent innovation in scanning electron microscopy is to use several stages of differential pumping between the electron gun and the sample, which is placed in a vacuum of a few torr. This method is called environmental scanning electron microscopy (E-SEM) and allows imaging of samples that would quickly vaporise in a high-vacuum environment (Virginia Tech, 2004).

1.7 PROJECT AIMS AND OBJECTIVES

The objective of this research was to develop disposable sensors for rapid toxic heavy metal detection in environmental samples. The aim of the thesis was also to reduce or eliminate the use of mercury in the assay procedure for metal detection. Screen-printed three electrodes configurations based on carbon and gold working electrode were developed and characterised. The use of cysteine which is able to bind metal ions was investigated as a mean to improve the detection of metals on screen-printed carbon electrode. Constant current chronopotentiometric stripping analysis was used for the quantitative determination of cadmium (II), lead (II) and copper (II) after modification with cysteine. The next stage was to use the screen-printed gold electrode for determination of lead (II), cadmium (II) and arsenic (III). Square-wave stripping voltammetry was used for lead (II) and Cd (II) analysis whilst linear sweep voltammetry for As (III) analysis.

The required stages and objectives of the thesis progression were carried out as follows:

1.7.1 Fabrication and characterisation of screen-printed electrodes

- Fabrication of screen-printed carbon/gold electrode using screen-printing techniques.
- Characterisation of screen-printed carbon/gold working electrode with electrochemical methods.
- Surface characterisation of the screen-printed carbon/gold working electrode with SEM.

1.7.2 Heavy metal ions detection on mercury film screen-printed carbon electrode (MFSPCE)

- Development and optimisation of the square-wave anodic stripping voltammetry technique.
- Determine sensor calibration curves and sensitivity.
- Apply the sensor to measure environmental samples.
- Compare the sensor performance to a conventional analytical reference technique, ICP-MS.

1.7.3 Modification of mercury film screen-printed carbon electrodes with cysteine for the sensitive determination of metal ions

- Modification of the chemical sensor with cysteine.
- Development and optimisation of the chronopotentiometric stripping analysis method.
- Determine sensor calibration curve and sensitivity.
- Validation of developed sensors under laboratory and field conditions for heavy metal ion detection in waste-water and extracted soil samples.

1.7.4 Heavy metal ions detection with mercury free screen-printed gold electrode

- Selection of electrochemical technique for the most sensitive determination of metal ions.
- Development and optimisation of the stripping analysis method.
- Evaluate sensor characteristics, response, sensitivity, etc. for different metal ions.
- Compare the sensor performance to mercury film screen-printed electrode (1.7.2) and ICP-MS.

The outline of the different stages undertaken in this thesis is illustrated in the flowchart below:

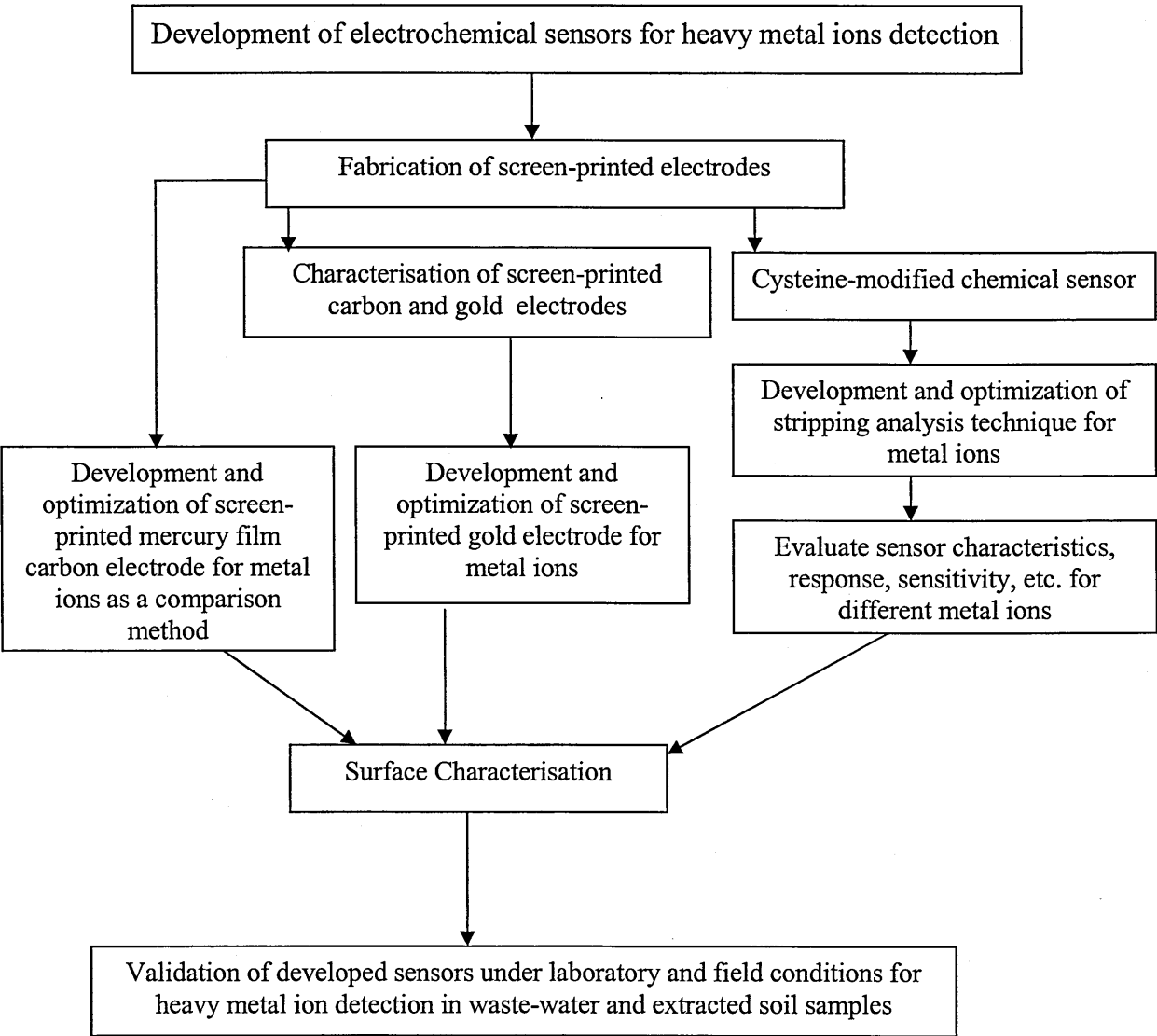


Figure 1.7 : Flowchart outlining the different stages of the work in this thesis.

CHAPTER 2

2.0 FABRICATION AND CHARACTERISATION OF SCREEN-PRINTED ELECTRODES

2.1 OUTLINE

The aim of this chapter is to describe the use of thick-film technology for the fabrication of disposable electrochemical sensors using screen-printing technique. The characterization of screen-printed carbon electrode (SPCE) and screen-printed gold electrode using voltammetric and amperometric techniques is also covered in this chapter.

2.2 INTRODUCTION

In recent years, due to high demand for on-site monitoring of heavy metals, some innovative techniques for sensor preparation have been proposed; particular attention has been given to the replacement of bulky electrodes and cumbersome cells with self-contained test-strips. The advantages of a single-use disposable sensor include elimination of problems associated with carryover of contamination and resterilisation and also eliminate the fear of expensive damage often associated with a re-usable sensor (Desmond *et al.*, 1998). Sensor microfabrication technologies such as thick film screen-printing, thin-film lithography, silicon technology, optical sensor technology can provide a pathway for the simple and inexpensive mass production of disposable electrodes. Screen-printed electrodes (SPEs) rely on planar carbon or gold working electrodes and a silver reference electrode which are printed on plastic or ceramic support. Hence, each strip can be considered as a disposable electrochemical cell onto which the sample droplet is placed. Different electrode geometries can be fabricated using suitable patterned photomasks or stencils. Electrode structures with 0.5 μm dimensions can be produced with modern microlithographic processes, while larger (75-100 μm) structures can be achieved using screen-printing fabrication (Wang, 1994; Wang, 1999).

There are several significant advantages of thick film technology which have attractive option for the fabrication of sensors and biosensors. These include flexibility of design and choice of materials, easy integration with electronic circuits, low cost in infrastructure and mass production, the possibility of automation of the fabrication process, a low barrier for technological transfer (from thick-film to other technologies, and vice versa). Miniaturisation is feasible, along with a high degree of electrochemical activity of the devices deriving from their high specific area and microporous structure. However, thick-film technology also has its own drawbacks. This technology mostly has problems related to compatibility between the different materials, and between the materials and the manufacturing process. Other shortcomings are related to sensor-fabrication processes, such as the need of complicated curing cycles with high temperatures and long times, and low reproducibility of the constructed devices resulting, for example, from evaporation of solvents in the paste formulation (Galan-Vidal *et al.*, 1995).

Thick-film technology (screen-printing) is a set of procedures where a paste (dielectric or conductive materials) is applied to a substrate materials following a design defined by a suitable mask. The deposition is attained by pressing the paste through a silkscreen mask, followed by a thermal curing. The paste-films have a typical thickness between 10 and 50 μm . The performance of chemical sensors fabricated by thick-film technology depends critically on the applied materials and the manufacture. The model, theory and realisation of a sensor have to be precisely designed, taking into account the group of materials that has been chosen to form part of the technology. There is a wide range of inks and substrate materials that can be used to mass-produce low cost sensor strips. Commercial carbon and metal (Pt or Au) inks formulations are commonly used for printing the working electrodes, whereas silver-based inks are used for obtaining the reference electrode (Wang *et al.*, 1998).

Carbon inks are particularly attractive for sensing applications because they are relatively inexpensive and lead to low background currents and a wide potential range. Such inks are composed of graphite particles, a polymeric binder (for these particles) and other

additives (essential for the dispersion, printing and adhesion processes). The exact ink formulation is regarded by the manufacturer as proprietary information. Such differences in the ink composition (e.g., type, size or loading of graphite particles), and in the printing and curing conditions, may strongly affect the electron transfer reactivity and overall analytical performance of the resulting carbon sensors (Wang *et al.*, 1996). There are many publications reported about the electrochemical characteristics of thick-film carbon electrode (Osborne *et al.*, 1996; Wang *et al.*, 1996b, 1998b; Cui *et al.*, 2001; Grennan *et al.*, 2001; Morrin *et al.*, 2003). Different types of commercially available carbon inks from Acheson, Ercon, Gwent and Dupont were tested and compared for electrochemical performance of SPCE (Wang *et al.*, 1998). The SPCE sensor based on different inks will exhibit a wide range of electrochemical reactivities (peak separation, hydrogen overvoltage and oxygen overvoltage) for benchmark redox systems. The results obtained show that the different compositions and preparations of carbon strip electrodes have profound effect upon their electrochemical behaviour and electroanalytical utility. Grennan *et al.* (2001) reported that peak separations (ΔE_p) obtained with SPCE were higher than those obtained by glassy carbon. The heterogeneous electron transfer rate (k^0) for the ferrocyanide redox couple at the SPCE was much lower than that of GCE. This was attributed to the presence of the polymer binder in the carbon ink, which serves to inhibit electron transfer rates.

In this chapter, work carried out in the development of an electrochemical sensor fit for heavy metal ion detection is described. Electrochemical methods were used to characterise the carbon and gold – working electrode fabricated using screen-printing techniques.

2.3 EXPERIMENTAL

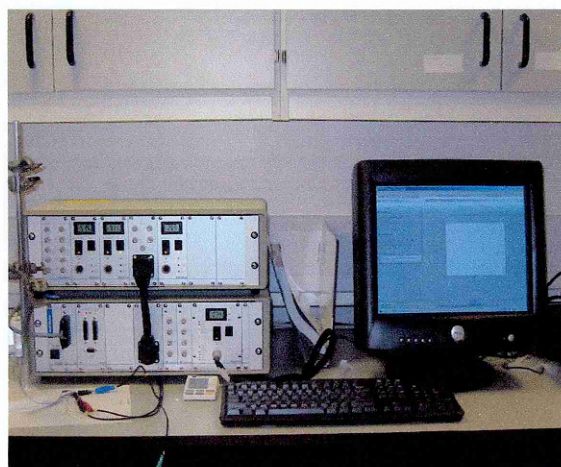
2.3.1 Printing Materials/Reagents

The printing inks for screen printed carbon electrodes were E423-SS graphite-based ink from Acheson Colloids (U.K.), a 15% silver/silver chloride in silver paste ink from MCA Services (Cambridgeshire, U.K.) and insulating ink from Agnet ESL (Reading, UK). For

screen-printed gold electrodes, the printing inks used were graphite based ink G449 (I), gold ink R-464(DPM-78) and silver/silver chloride in silver paste R-414(DPM-68)1.25) from Ercon (USA).

2.3.2 Equipments

A computer-controlled PGSTAT10, the Autolab Electrochemical Analyser with general-purpose electrochemical software operating system version 4.7 (Eco Chemie, Utrecht, The Netherlands) was used for electrochemical measurements (linear sweep voltammetric, cyclic voltammetric and chronoamperometric experiments) (Plate 2.1 (a)). A hand-held, battery-powered PalmSens potentiostat/galvanostat (Palm Instruments BV, The Netherlands) interfaced to a Compaq iPAQ pocket PC was used for field monitoring and will be described in next chapter (Plate 2.1 (b)). A specially adapted electrical edge connector obtained from Maplin (Milton Keynes, U.K.) was used for connection of electrodes to the electrochemical analyser/portable potentiostat. Conventional electrodes (glassy carbon/gold disc electrode, standard calomel electrode, silver/silver chloride reference electrode and platinum wire electrode) were obtained from Bioanalytical System Ltd. (Cheshire, U.K.).



(a)



(b)

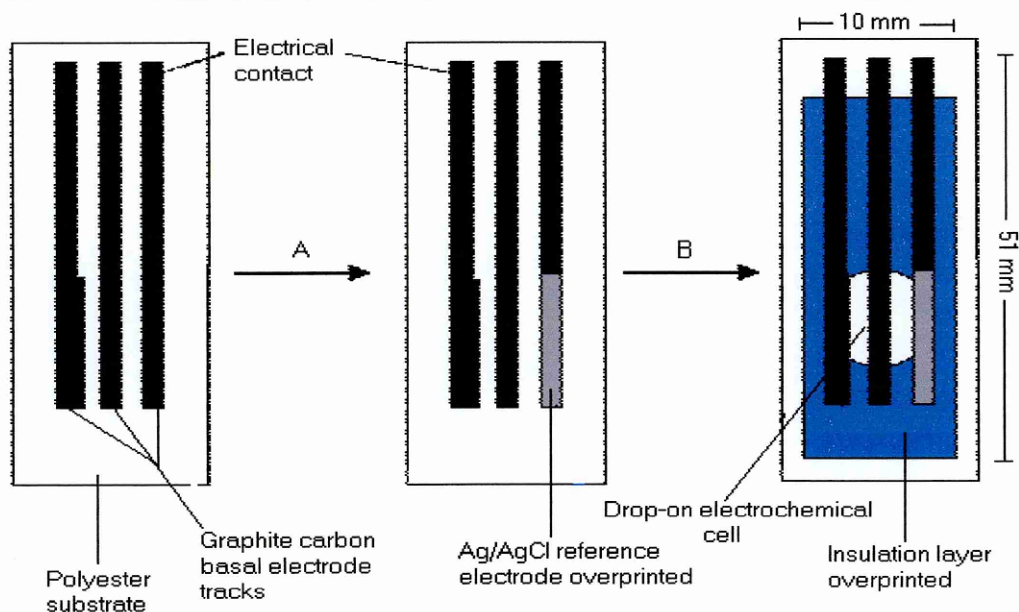
Plate 2.1: An Autolab (Electrochemical Analyser) (a) and Palmsens (b).

2.3.3 Safety

COSHH (control of substances hazardous to health) form was filled up and follows all the time; these include identified hazards, disposal requirements, control and emergency procedures.

2.3.4 Fabrication of electrodes

The screen-printed carbon/gold electrode (SPE, Figure 2.1 and 2.2) consisting of a working electrode (1.3 mm^2 planar area), counter electrode and reference electrode were mass fabricated in-house by a multi-stage screen-printing process using a DEK model 248 machine (DEK, Weymouth, UK) (Plate 2.2). The electrodes were printed onto $250 \mu\text{m}$ thick 'Melinex' ST725 polyester sheet obtained from Cadillac Plastic (Swindon, UK) using different printing inks. The printer parameters were set to print at a 4 psi squeegee pressure at a 50 mm sec^{-1} carriage speed with a 2.5 mm print gap. The fabrication cycle started with the placement of graphite based ink E423-SS obtained from Acheson Colloids (UK) or graphite based ink G449 (I) from Ercon (USA) onto the patterned screen, followed by it being forced through the screen mesh onto the polyester sheet with the aid of squeegee as illustrated in Figure 2.3, and dried in an oven at 60°C for 2 hours. For screen-printed gold electrode construction, the basal tracks previously printed with graphite based ink from Ercon (USA) were used to print the gold ink R-464 (DPM-78) obtained from Ercon (USA) (Figure 2.2). In the second step, the reference electrode ink containing 15% silver chloride in silver paste (MCA Services, Cambridge, UK) or silver/silver chloride in silver paste R-414(DPM-68)1.25) from Ercon (USA) was printed onto one of the basal tracks and left to dry. In the last step, the basal tracks were insulated with an insulating ink (242-SB epoxy-based protective coating ink), which was obtained from Agment ESL (Reading, UK), leaving a defined circular shaped area (electrochemical cell with an area of 3.2 mm^2) for measurements. The insulation layer was cured at 120°C for 2 hours and later left to dry. About 100 electrodes can be printed per sheet at the same time. The sheet will be cut into individual electrode in order to connect it to the potentiostat.



A=addition of reference layer B=addition of insulating layer

Figure 2.1: Schematic representation of the fabrication steps for the screen-printed electrode system prepared for this work (adapted from Kadara, 2003).

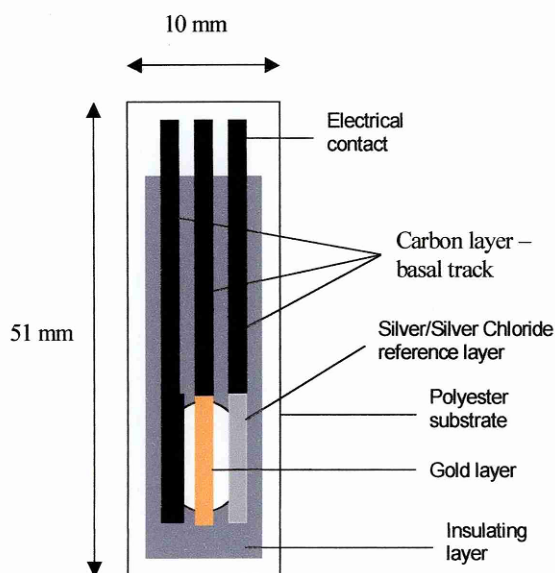


Figure 2.2: Screen-printed gold electrode used in this work.



Plate 2.2: A DEK 248 semi-automatic precision screen-printer.

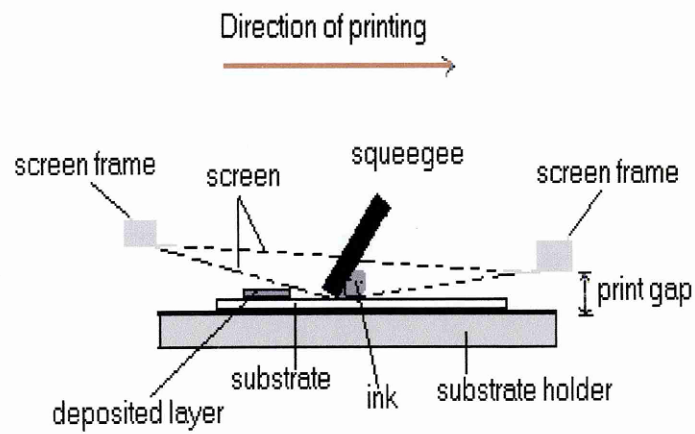


Figure 2.3: Schematic representation of the screen-printing process (adapted from Bachmann, 1999).

2.3.5 Electrochemical measurements

2.3.5.1 Voltammetric studies

Evaluation of the polarization range on screen-printed carbon electrode (SPCE), screen-printed gold electrode (SPGE), glassy carbon electrode (GCE) and gold disc electrode (GDE) was obtained by recording the linear sweep voltammetric curve in a mixed medium solution of 0.1 M HCl + 0.1 M ammonium acetate and 0.025 M Tris-HCl/0.1 M KCl, pH 7.4. Before each experiment, the GCE and GDE were polished on slurries of alumina powder (0.3 μm) on a polishing cloth, sonicated for few minutes to remove any remaining alumina particles and then cleaned with deionised water. The GDE was electrochemically cycled in 0.1 M H_2SO_4 between 0.0 and 1.5 V at 200 mV s^{-1} until a stable and reproducible voltammogram for gold oxide formation and reduction was obtained. The LSV measurements were performed from -1.5 V to 1.5 V relative to Ag/AgCl reference electrode using a scan of 50 mV s^{-1} . All cyclic voltammetry experiments were carried out in unstirred and non-deaerated solutions.

The electrochemistry of the screen-printed carbon and gold working electrode surface were investigated using CV and benchmark compound, potassium ferrocyanide. CV was employed to determine the electroactive surface area of the working electrode (WE) as well as the electrochemical reversibility of potassium ferrocyanide on the electrode. Cyclic voltammetric measurements of potassium ferrocyanide were carried out by scanning over a pre-set potential range (-0.6 V to 0.8 V) relative to onboard Ag/AgCl reference electrode or saturated calomel electrode. Stock solution of potassium ferrocyanide (5 mM) was prepared in 0.1 M KCl and diluted to any appropriate concentration with 0.1 M KCl. The electrochemical behaviour of potassium ferrocyanide (0.5 mM) on the SPCEs was studied by varying the scan rate between 10 to 75 mV s^{-1} . Different concentrations of potassium ferrocyanide (0.5, 0.8, 1.3 and 5 mM) were also studied at fixed scan rate of 50 mV s^{-1} .

2.3.5.2 Chronoamperometric measurement

Chronoamperometric measurement of potassium ferrocyanide on SPCE and SPGE was used for calculating the surface area of the WE. The measurements were carried out at an appropriate fixed potential (+ 0.8 V) over a period in unstirred solutions. The screen-printed carbon-working electrode was dipped into an electrochemical cell containing 10 ml of 0.1 M KCl (blank) and other electrodes (saturated calomel reference electrode (SCE) and platinum wire counter electrode). The chronoamperometric measurements involved taking out 1 ml of the 0.1 M KCl solution from the electrochemical cell after a steady state has been obtained for the response and then replacing it with 1 ml of 5 mM potassium ferrocyanide. The response is recorded over 2 minutes at constant potential.

2.3.6 Data Analysis

All experimental analysis carried out in this work, was repeated three times with different screen-printed electrodes. Each response data point represents the mean value of three measurements and the error bar represents the standard deviation. The mean, standard deviation and relative standard deviation (RSD) were calculated for the measurements using Microsoft Excel 2000 and SPSS version 10.

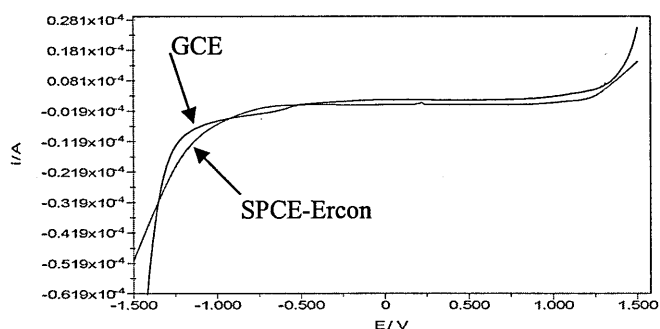
2.4 RESULTS

2.4.1 Voltammetric characterisation of the working electrode

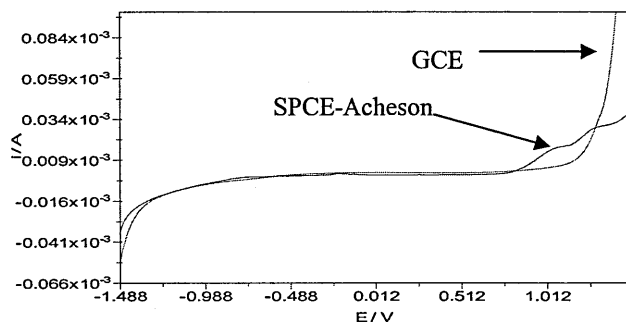
The background current of thick-film carbon electrodes is strongly affected by the carbon ink employed. In order to assess the useful potential window for the screen-printed carbon- and gold- working electrode, linear sweep voltammetry (LSV) and cyclic voltammetry (CV) were used to characterise the working electrode surface. Comparison was made with GCE and GDE, a common working electrode with high hydrogen overpotential and wide potential window. The current response for two blank solutions of 0.1 M HCl + 0.5 M ammonium acetate and Tris-HCl/0.1M KCl, pH 7.4 was examined. The polarization range for the SPCE and SPGE were examined since this parameter defines an electrode's negative and positive potential limits of operation.

When the SPCE using Ercon ink was tested in 0.1 M HCl + 0.5 M ammonium acetate (pH 4.6), a smooth background response obtained with a negative potential limit of approximately ($\sim$) -1.1 V and a positive potential limit of $\sim$ + 1.1 V (Figure 2.4 (a)). Figure 2.4 (b) shows that in solution of 0.025 M Tris-HCl/0.1 M KCl, pH 7.4, SPCE-Acheson potential windows shifted to more positive value. This is due to the increase in the pH of the medium. There is no interfering peak observed for SPCE-Ercon in 0.1 M HCl + 0.5 M ammonium acetate. There is also no interfering peak observed for SPCE-Acheson in 0.025 M Tris-HCl/0.1 M KCl, pH 7.4. Figure 2.4 (c) shows that the potential windows for SPGE are slightly more negative value than GDE whilst GDE has more positive potential limit ($\sim$ - 0.45 V (GDE), $\sim$ -0.5 V (SPGE) and + 0.8 V (GDE), $\sim$ + 0.7 V (SPCE)). The polarisation range obtained for two different carbon ink used and gold ink were sufficient for electrochemical detection of electroactive species on the working electrode surface.

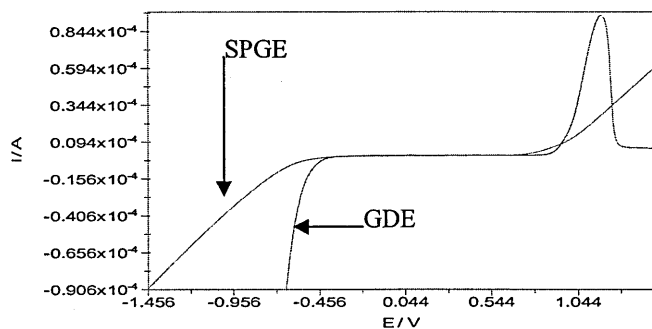
Due to its versatility and relatively easy measurement, cyclic voltammetry (CV) was the initial electroanalytical technique performed for the electrochemical characterization of the SPCE and SPGE surfaces. By utilizing a well characterized redox couple such as ferrocyanide, exploration of the electrochemical nature of the electrode surface was accomplished. Figure 2.5 and 2.6 show cyclic voltammograms obtained at the working electrode for the screen-printed carbon (SPCE-Acheson and SPCE-Ercon), screen-printed gold electrode immersed in 0.5 mM potassium ferrocyanide, GCE and GDE (for comparison purpose). At a low scan rate of 20 mV s^{-1} , the peak-to-peak separation (ΔE_p) obtained for SPCE-Acheson was 286 mV, whilst SPCE-Ercon was 330 mV. A peak separation value of 86 mV was obtained using the GCE in this work. For SPGE, at a scan rate of 20 mV s^{-1} , ΔE_p obtained was 250 mV and 71 mV for gold disc electrode (Figure 2.6). Figure 2.7 and 2.8 show voltammograms obtained on SPCE-Acheson and SPGEs for potassium ferrocyanide at four different scan rates. The position of the oxidation peak potential (E_{pa}) in the forward sweep shifts slightly to the right with each increasing scan rate. This indicates that the redox reaction at the electrode is quasireversible.



(a)



(b)



(c)

Figure 2.4: Linear sweep voltammograms of (a) 0.1 M HCl + ammonium acetate electrolyte solution on SPCE-Ercon and GCE (b) 0.025 M Tris-HCl/0.1M KCl for SPCE-Acheson and GCE (c) 0.05 M HCl on gold disc electrode and SPGE. Scan range: -1.5 V to 1.5 V, scan rate: 50 mV s⁻¹. Reference electrode: Ag/AgCl.

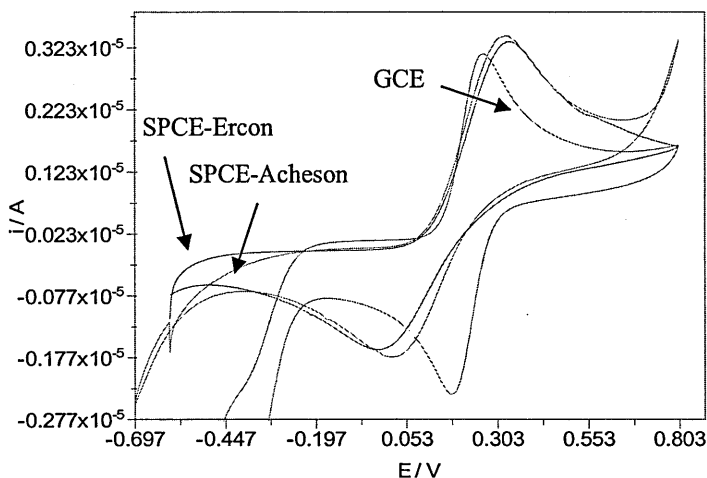


Figure 2.5: Cyclic voltammograms of 0.5 mM potassium ferrocyanide in 0.1 M KCl. SPCE (Acheson and Ercon)(vs. screen-printed Ag/AgCl reference electrode) and GCE (vs. SCE, 3 M KCl). Scan range: -0.6 V to 0.8 V, scan rate: 20 mV/s.

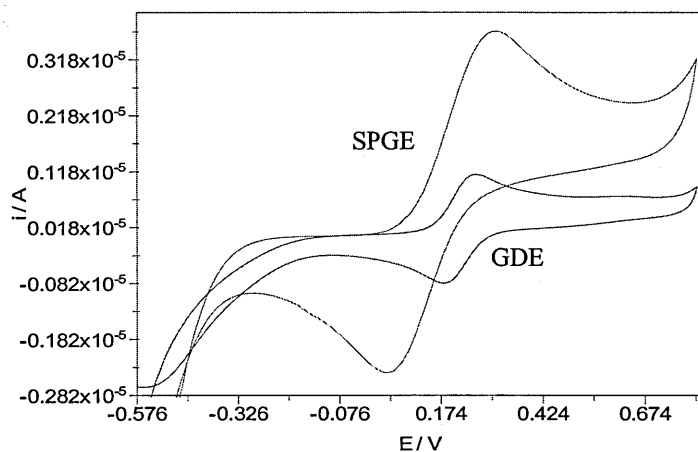


Figure 2.6: Cyclic voltammograms of 0.5 mM potassium ferrocyanide in 0.1 M KCl. SPGE (vs. screen-printed Ag/AgCl reference electrode) and GDE (vs. SCE, 3 M KCl). Scan range: -0.6 V to 0.8 V, scan rate: 20 mV/s.

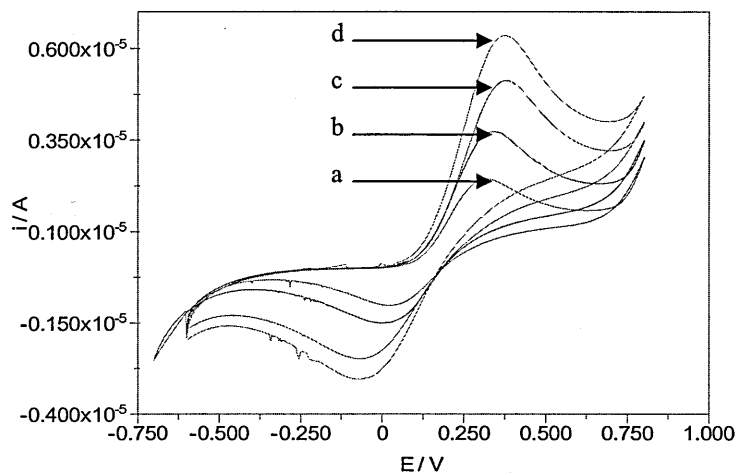


Figure 2.7: Cyclic voltammograms showing the variation in scan rates for 0.5 mM potassium ferrocyanide in 0.1 M KCl on SPCE-Acheson (a) 10, (b) 25, (c) 50, (d) 75 mV s^{-1} . Scan range -0.6 V to 0.8 V (vs. screen-printed Ag/AgCl reference electrode).

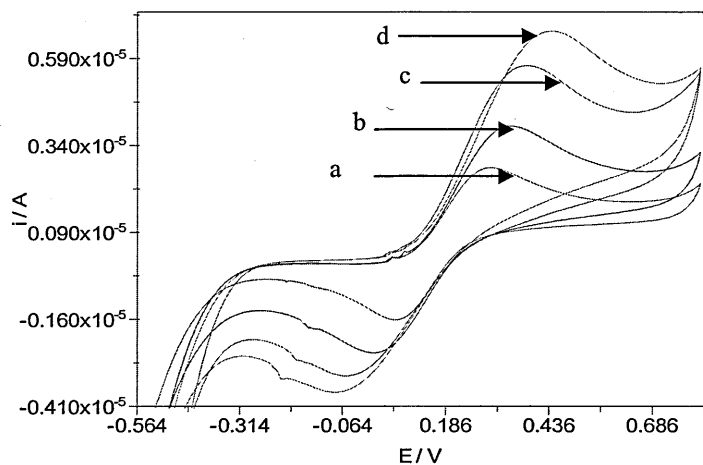


Figure 2.8: Cyclic voltammograms showing the variation in scan rates for 0.5 mM potassium ferrocyanide in 0.1 M KCl on SPGE (a) 10, (b) 25, (c) 50, (d) 75 mV s^{-1} . Scan range -0.6 V to 0.8 V (vs. screen-printed Ag/AgCl reference electrode).

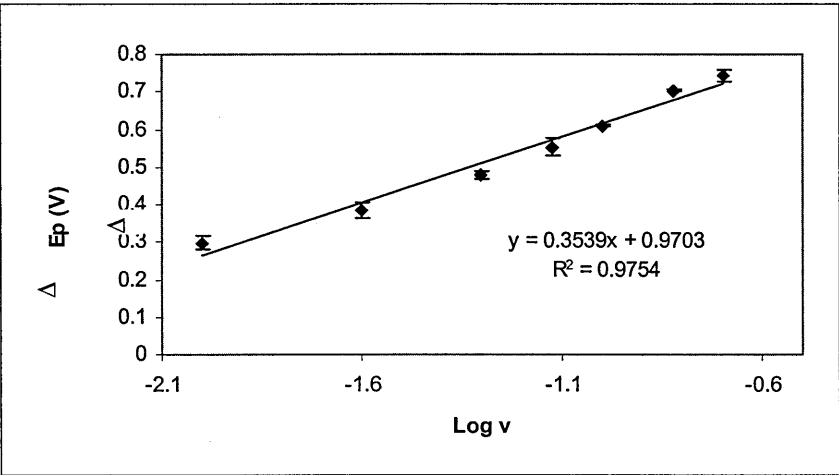
Heterogenous electron transfer rate constant (k^0) for the hexacyanoferrate (III)-(II) redox couple were assessed from the current-potential curve and calculated using the method of Nicholson (1965), according to equation ,

$$k^0 = \psi \left(D_O \pi \nu \left(\frac{nF}{RT} \right) \right)^{1/2} \left(\frac{D_R}{D_O} \right)^{\alpha/2}$$

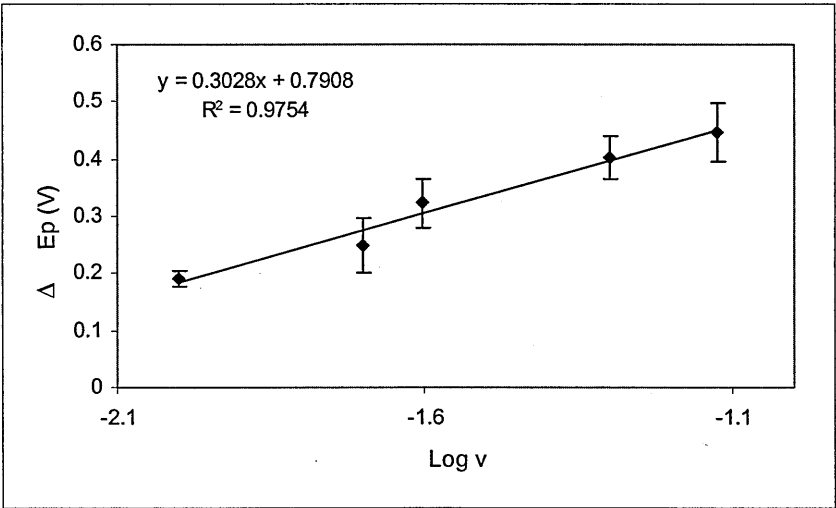
where ψ refers to a kinetic parameter, D_O is the diffusion coefficient for ferricyanide ($7.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$), D_R is the diffusion coefficient for the ferrocyanide ($6.3 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$), and α is the transfer coefficient, R is the universal gas constant ($8.314 \text{ JK mol}^{-1}$), T is the absolute temperature (K), n is the number of electrons transferred, and F is Faraday's constant ($96,485 \text{ C}$). The α value of 0.31 ± 0.002 , 0.35 ± 0.001 , 0.30 ± 0.007 were obtained from plotting ΔE_p versus $\log \nu$ for 5 mM ferrocyanide in 0.1 M KCl for SPCE-Acheson, SPCE-Ercon and SPGE (Figure 2.9 (a) and (b)). Using the α value obtained, k^0 values were calculate for SPCE-Acheson, SPCE-Ercon, SPGE and glassy carbon and shown in Table 2.1.

Table 2.1: Table of heterogenous electron transfer rate constant (k^0) for glassy carbon, SPCE-Acheson, SPCE-Ercon and SPGE.

Electrode	$k^0 \text{ (cm s}^{-1}\text{)}$
Glassy Carbon	6.3×10^{-2}
SPCE-Acheson	9.4×10^{-5}
SPCE-Ercon	3.7×10^{-5}
SPGE	1.41×10^{-4}



(a)



(b)

Figure 2.9: Plot of peak-peak separation (ΔE_p) versus $\log v$ for determining the transfer coefficient (α) of 5 mM ferrocyanide in 0.1 M KCl on (a) SPCE-Ercon and (b) SPGE. CV scan range = -0.6 V to 0.8 V (vs. screen-printed Ag/AgCl reference electrode).

The recorded current for the anodic peak increased linearly with the square root of the scan rate ($r^2=0.9973$), ($r^2=0.995$) and ($r^2=0.9983$) for SPCE-Acheson, SPCE-Ercon and SPGE, respectively, and the obtained slope for this relationship was employed in the calculation of the electrochemical active area for the ferrocyanide redox reaction on the SPCE and SPGE surface. Such calculation was performed using the Randles equation modified for a quasi-reversible reaction, as observed by the peak-peak separation and was deducted using the equation:

$$i_p = (2.99 \times 10^5) n(\alpha n_a)^{1/2} A C D^{1/2} v^{1/2}$$

where i_p is the peak current (A), A refers to electrode area (cm^2), D_o is the diffusion coefficient for ferrocyanide ($6.3 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$), C is concentration of analyte in the bulk solution (mol dm^{-3}), n_a is number of exchanged electrons, v is sweep rate (Vs^{-1}) and 2.99×10^5 is Randles-Sevcik-constant.

Using the α value of 0.31 ± 0.002 , 0.35 ± 0.001 , 0.30 ± 0.007 for SPCE-Acheson, SPCE-Ercon and SPGE and the known diffusion coefficient (D) of 6.3×10^{-6} for the ferrocyanide ion (Morris *et al.*, 2003), values of 0.102 ± 0.007 , 0.093 ± 0.009 , $0.108 \pm 0.01 \text{ cm s}^{-1}$ were calculated from plot of anodic peak currents of 0.5 mM ferrocyanide versus square root of scan rates ($10\text{-}75 \text{ mV s}^{-1}$, Figure 2.10), respectively.

The planar working surface area (cm^2) of the electrodes (Table 2.2) can be calculated from the Cottrell equation after rearranging:

$$A = \frac{it^{1/2} \pi^{1/2}}{nFCD^{1/2}}$$

Where i is the current (A), t is the time (s), F the Faraday constant ($9.65 \times 10^4 \text{ C mol}^{-1}$), C the concentration (mol/cm^3) and D the diffusion coefficient of potassium ferrocyanide (II) ($6.3 \times 10^{-6} \text{ cm}^2 \text{ sec}^{-1}$). The result obtained is shown in Table 2.2.

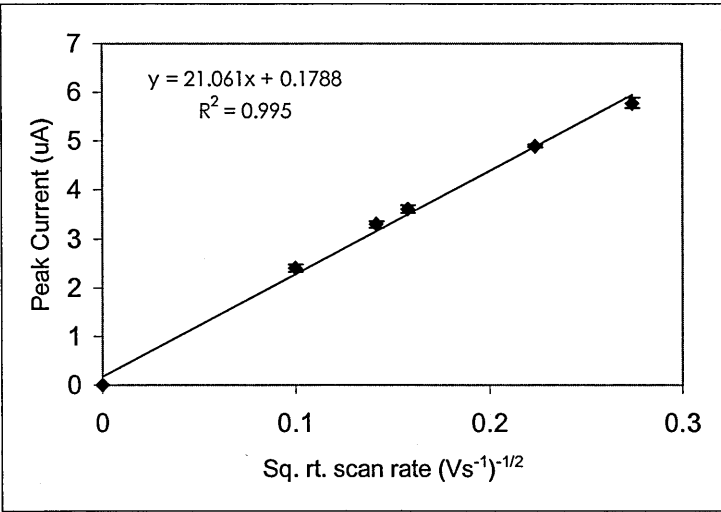


Figure 2.10: Plot of anodic peak currents versus square root of scan rates for the determination of the electrochemical active area of SPGE. CV scan range = -0.6 V to 0.8 V (vs. screen-printed Ag/AgCl reference electrode).

Table 2.2: A (cm²) values calculated and measured for each kind of carbon-based screen-printed electrodes. Standard deviations ($\pm$ cm²) is calculated over 10 electrodes recording the current during a two minutes period at constant potential (+ 800 mV vs on-board Ag/AgCl) in 0.5 mM K₄Fe(CN)₆ solution with 0.1 M KCl.

ELECTRODES	A (cm ²) MEASURED	A (cm ²) CALCULATED (standard deviation, $\pm$ cm ²)
SPCE-Acheson	0.13	0.067 $\pm$ 0.009
SPCE-Ercon	0.13	0.059 $\pm$ 0.007
SPGE	0.13	0.072 $\pm$ 0.004

The screen-printed carbon electrodes (SPEs) characterized here in this chapter would be employed in the subsequent Chapters (3 (SPCEs-Ercon), 4 (SPCEs-Acheson), 5 and 6 (SPGE-Ercon) for the development of heavy metal ion sensors for use in synthetic aqueous solutions and in environmental samples.

2.5 CONCLUSION

In this chapter, the design and the production of three configuration electrode system (working, reference and counter electrode) by screen-printing technology was described. The electrochemical behaviour of screen-printed carbon using two different inks and gold electrodes were investigated. The characterization of the screen-printed electrode showed that its surface structure and electrochemical activity is dependent on the electrode material. The screen-printed carbon and gold electrode did not display the ideal reversible behaviour that might be expected for potassium ferrocyanide. This unattractive behaviour suggests a low graphite-carbon and gold loading.

The SPCE was not ideally suited for the study of electrochemical kinetics as compared to glassy carbon or gold disc electrode. In alkaline solution, SPCE-Acheson shows potential window in the range -1.2 to $\sim +0.7$ V, while in slightly acidic solution in 0.1 M HCl + 0.5 M ammonium acetate, the SPCE-Ercon operable in the potential range ~ -1.1 to $\sim +1.1$ V. Screen-printed gold electrode has shorter potential window in 0.05 M HCl (acidic solution) from ~ -0.5 to $\sim +0.7$ V.

However, SPE can still be used for the voltammetric determination of toxic heavy metals such as cadmium, lead and arsenic without any pre-treatment of the working electrode surface since the potential window is wide enough in which the half-wave potential of these metals occurs.

The integrated drop on screen-printed electrode (SPE) sensor characterized in this chapter would be used in the subsequent chapters (3,4,5, and 6) for the development of toxic heavy metal ion sensors for use in synthetic aqueous solutions and in various environmental matrices.

CHAPTER 3

3.0 HEAVY METAL IONS DETECTION ON MERCURY FILM SCREEN-PRINTED CARBON ELECTRODES

3.1 OUTLINE

This chapter reviews the use of screen-printed electrodes for heavy metals detection. The stripping analysis for cadmium (II), lead (II) and copper (II) on mercury film screen-printed carbon electrodes using square-wave stripping voltammetry (SWASV) is described. The aim of the work was to optimise the method for the analysis of waste-water and soil samples. The results obtained will then be used to compare to those obtained using screen-printed gold electrode (chapter 5).

3.2 INTRODUCTION

The high demand for on-site monitoring of heavy metals using self-contained test-strips to replace bulky electrodes has attracted much attention from researchers. Different types of electrodes such as hanging mercury electrodes and mercury plated thin film glassy carbon electrode have been used in field experiments (Williams & D'Silva, 1994). However, due to the difficulties in using the electrodes for field analysis (Bond *et al.*, 1997), the development and application of single-use screen-printed electrodes have been widely reported (Wang & Tian, 1992; 1993a, 1993b, Wang *et al.*, 1993; Desmond *et al.*, 1998; Reeder & Heineman, 1998; Palchetti *et al.*, 1999; Honeychurch *et al.*, 2000; Zen *et al.*, 2002; Masawat *et al.*, 2003; etc). Disposable screen-printed electrodes are designed for one use and therefore have the advantages of not being affected by the problems associated with carryover of contaminants and this minimise the damage often associated with a re-usable sensors (Desmond *et al.*, 1998). The use of screen-printing technology also provides a pathway for a simple and inexpensive mass production of disposable electrodes.

Screen-printed electrodes (SPEs) are printed on an inexpensive plastic or ceramic support which consists of planar carbon, gold or silver working electrodes and a silver reference electrode. The strip which the sample droplet is placed on is called electrochemical cell and can be considered as disposable electrode. The stripping performance of the strips is comparable with traditional electrodes which provide

well-defined peaks with reproducible measurements, low background signals and could be used for laboratory and field applications (Wang, 1994; Wang & Tian, 1992). The presence of oxygen in the solution does not effect the measurement significantly and makes the SPEs even a stronger competitor for standard electrochemical approach (Wang *et al.*, 1993). The electrodes can also be coupled to a compact and user-friendly electrochemical analyser (Wang, 1994). Moreover, the SPEs can be electrochemically activated with simple procedures (Wang *et al.*, 1996) and the surface can easily be modified using enzymes, redox compounds (Kroger & Turner, 1997) or catalytic particles (Newman *et al.*, 1995) thus increase the range of compounds detectable. There are also many choices of carbon inks for the working electrode, which results in different electrochemical behaviour. Electroanalytical performance can be applied for specific electroanalytical application using different techniques (Wang *et al.*, 1998).

Screen-printed electrodes provide a highly stable response are very attractive for routine use, low cost and can be designed for single-use decentralised operations and have been applied widely for the stripping analysis of metal ions in recent years (Wang *et al.*, 1992). Lead is the most common studied metal by stripping analysis and produces a well-defined and sharp peaks with very low detection limit in nM concentrations ($0.2 \mu\text{g l}^{-1}$) when mercury coated SPCE is used (Wang & Tian, 1992; Wang *et al.*, 1993; Reeder & Heineman, 1998). The most widely used electrode material in electrochemical stripping analysis is mercury. This is due to its many advantageous features with respect to stripping applications such as a readily renewable and reproducible surface, a high hydrogen overpotential and the ability to dissolve many metals that aids the preconcentration process (Khoo & Guo, 2002). In the following sub sections (3.2.1, 3.2.2 and 3.2.3), a review of published work on the applications of SPCEs as an electrochemical metal sensor is presented. Detection on different electrode surfaces, i.e., mercury film screen-printed carbon electrodes, unmodified bare screen-printed carbon electrodes (SPCEs) and other metallic film coated screen-printed electrodes are highlighted.

3.2.1 Detection with mercury film-screen-printed carbon sensors

Wang & Tian (1992) reported the use of screen-printed ExacTech glucose carbon electrode strips (MediSense Inc) for the determination of lead using anodic stripping voltammetry (ASV) and potentiometric stripping analysis (PSA). The carbon strip was pre-plated with 80 mg l^{-1} mercury in 0.02 M HCL to form the working mercury film electrode. The stripping performances (sensitivity and signal to background) were not compromised by those recorded with mercury-coated glassy carbon electrode and hanging mercury drop electrode. The use of differential pulse stripping voltammetry (DPASV) provides optimum sensitivity after 10 minutes preconcentration from stirred solution with detection limits of 0.03 and $0.05 \text{ } \mu\text{g l}^{-1}$ for lead and cadmium, respectively. Wang *et al.* (1993) also described a disposable composite screen-printed electrode arrays for on-site analysis of lead, cadmium and copper. The authors found that placing a non-deaerated and unstirred samples drop on the strip, as desired for field monitoring of trace metals did not compromise the stripping performances. The calibration range obtained with SWASV for lead and cadmium after two minutes deposition time was $10\text{-}80 \text{ } \mu\text{g l}^{-1}$. The detection limit calculated was $0.1 \text{ } \mu\text{g l}^{-1}$ when $0.5 \text{ } \mu\text{g l}^{-1}$ lead analysed following 4 minutes deposition time was used.

Desmond *et al.* (1996) reported a simultaneous detection of copper, cadmium, lead and zinc by ASV with SPEs in a manner comparable to bulky electrodes. The use of SPEs on silicon substrates with an application specific integrated circuit (ASIC) and software control offered excellent performance and allowed the authors to obtain calibration plots for all four metals in the $\mu\text{g l}^{-1}$ range. A portable environmental monitoring system based on stripping voltammetry and SPEs was described by Desmond *et al.* (1998) for trace metal analysis. These SPEs consisted of working electrode, reference electrode and counter electrode. The mercury film was plated *in situ* onto the working electrode surface. Concentration ranges of between 0 and $1000 \text{ } \mu\text{g l}^{-1}$ gave reasonably linear calibration curves with limits of detection being 55 , 71 , 64 and $123 \text{ } \mu\text{g l}^{-1}$ for zinc, cadmium, lead and copper, respectively, using a 300 s preconcentration time.

Palchetti *et al.* (1999) described the characterisation of disposable SPEs for the detection of heavy metals. A thin mercury film was pre-plated on a graphite working

electrode. With the measurement parameters optimised for electrochemical preconcentration of heavy metals, detection limit of 0.4, 1 and 8 $\mu\text{g l}^{-1}$ were obtained from the analysis of 10 $\mu\text{g l}^{-1}$ of cadmium (II), lead (II) and copper (II), respectively. Square wave anodic stripping voltammetry was used for 3 minutes deposition time.

Yarnitsky *et al.* (2000) developed a hand-held electrochemical analyser for field screening of lead (II). The instrument was designed to meet the requirements for decentralised stripping lead analysis such as size, weight and power-consumption while maintaining high sensitivity and reliability. The technique was based on potentiometric stripping measurement of trace lead. A 300 μl of sample drop was placed on the SPE strip which was then inserted into the meter and the *in situ* plating of the mercury film took place. It was reported that the analyser response varied linearly with the lead concentration between 20 – 300 $\mu\text{g l}^{-1}$ (50 s deposition) using 100 $\mu\text{g l}^{-1}$ cadmium as a pilot ion. The analyser was evaluated for on-site assays of drinking water. The data obtained was compared favourably with an established laboratory based stripping instrument.

A copper/mercury plated screen-printed electrode (CMSPE) was described by Zen *et al.* (2000) for trace lead (II) determination by SWASV. The intermetallic property of lead with copper was exploited to increase the sensitivity of Pb (II) detection. The calibration curve was linear in the range 0-100 Pb (II) with a detection limit of 0.81 $\mu\text{g l}^{-1}$ for 60 seconds pre-concentration time. Bismuth (III), thallium (III) and tin (III) were reported to have an adverse effect on the lead (II) response. The analytical utility of the CMSPE was demonstrated by applying to various water samples.

Choi *et al.*, 2001 described a disposable sensor for the ASV analysis of heavy metals which can be prepared by integrating a HgO-modified working electrode, thus eliminating the pre-plating step. The use of a HgO-carbon mixture as ink allows the mass production of disposable mercury electrodes. The mercuric oxides particles are reduced to fine mercury droplets by *in situ* or pre-cathodic conditioning. Simultaneous detection of cadmium, lead and copper by linear sweep ASV was accomplished in the $\mu\text{g l}^{-1}$ range, although the linearity for the latter was poor. The stability of the sensor was disturbed by hydrogen evolution at highly negative potentials, thus limiting its

applicability to the analysis of metals with high reduction potential. The analytical utility of the developed sensor was investigated by applying to lake water samples.

Analysis of copper by *in situ* co-deposition of Cu (II) ions with mercury film on SPEs was achieved by CCSCP after appropriate choice of experimental conditions and electrolyte (Kadara *et al.*, 2003). The stripping response for copper was linear in the range 10-2000 $\mu\text{g l}^{-1}$ following 2 minutes deposition time. The detection limit was 6 $\mu\text{g l}^{-1}$, and the proposed method was successfully applied to the determination of copper in acetic-acid bioavailable fraction of a certified reference material and soil samples from a mining site.

Palchetti *et al.* (2005) reported a miniaturised electrochemical screen-printed carbon electrode modified with cellulose-derivative mercury coating for on-site stripping analysis of trace heavy metals. The cellulose-derivative was used as mercury entrapping system in order to overcome problems of sensitivity encountered with perfluorosulfonate polymers (e.g. nafion) due to changes in morphology and geometrical parameters of the polymers when highly concentrated acid has to be used. Coupling this sensor with SWASV, deposition potential of -1.1V was applied for 120s, detection limit of 0.3, 1 and 0.5 $\mu\text{g l}^{-1}$ were found for lead, cadmium and copper in 0.1 M HCl, respectively. The method was evaluated by determining lead, cadmium and copper in soil and waste-water samples from a mining area.

3.2.2 Detection with unmodified bare screen-printed carbon sensors

Reeder & Heineman (1998) reported the use of a thick-film carbon sensor for trace determination of lead. The sensor consisted of a graphite carbon black working electrode and Ag/AgCl reference electrode printed onto a polycarbonate surface. Linear sweep anodic stripping voltammetry (LSASV) was used with 90 s preconcentration time which resulted a linear concentration range of 2.5 – 20 μM (0.5 – 4 mg l^{-1}). The LSASV technique was compared with SWASV and the latter was found to offer the advantage of rapid determination without the need to remove the oxygen. Bare carbon sensor was reported to be comparable to that of mercury film electrode for the determination of lead. Both sensors show good precision with

sensor/sensor variability (5.8 %) and repetitive analysis upon a single carbon sensor (5.1 %).

Honeychurch *et al.* (2000) and 2002, reported the use of SPCEs as disposable sensor for the measurement of trace levels of lead and copper without any chemical modification of the sensor surface, respectively. For the lead measurement, DPASV was employed with -1.4 V deposition potential. With a deposition time of 150s, the calibration graphs were linear in the range $6.3 - 24 \mu\text{g l}^{-1}$ and $24 - 50 \mu\text{g l}^{-1}$. Detection limit calculated was $2.5 \mu\text{g l}^{-1}$ with coefficient of variation (CV) at a concentration of $12.6 \mu\text{g l}^{-1}$ was calculated to be 2.4 % (n=5) on one single electrode. There was no interference from other metals such as copper, cadmium and zinc on the lead stripping peak. The proposed sensors were examined by analysing spiked and unspiked pond water. The recovery obtained to be 97.4 % and CV was 3.2 % at a concentration of $12.6 \mu\text{g l}^{-1}$. For the copper measurement, DPASV result showed that copper response is linear from 8 to $450 \mu\text{g l}^{-1}$ using a deposition potential of -1.0 V and deposition time 150 s with detection limit of $8.2 \mu\text{g l}^{-1}$. Mercury was found to have significant effect on the copper response as compared to other metals tested such as bismuth, cadmium, iron, lead, antimony and zinc. The sensor was examined by analysing copper in bovine serum, tap water, river and sea water.

3.2.3 Detection with other metallic-film screen-printed electrodes

Due to the high toxicity of mercury, alternative approaches have been investigated for the fabrication of mercury-free electrodes. Wang & Tian (1993) reported a mercury-free disposable lead sensor based on PSA at gold coated SPEs. The electrodes used were from ExacTech blood glucose strips (MediSense Inc.). The electrodes were pre-plated with non-deaerated 50 mg l^{-1} gold solution in 0.25 wt % HCl by holding the carbon strip electrode at -0.40 V for a 20 minutes period. The potential was then switched to +0.70 V for 5 minutes. The gold film was also prepared *in-situ*, by co-deposition with the target lead ions, however, while yielding a well-defined lead response, the peak could only be stabilised after 5 to 6 deposition and stripping cycles. The pre-plated gold film was used using acetate buffer (pH 4.5). Three different detection limits were reported, 0.1 and $0.6 \mu\text{g l}^{-1}$ for 20 and 4 minute deposition time in stirred solution, whilst $3 \mu\text{g l}^{-1}$ was obtained in unstirred solution for deposition

time of 10 minutes, respectively. The same authors (Wang & Tian, 1993b) also used the same screen-printed carbon strip coated with a thin gold film for potentiometric stripping analysis of trace levels of mercury. A detection limit of $0.5 \mu\text{g l}^{-1}$ was obtained for 4 minutes deposition time. The precision was good with 20 repetitive measurements of $25 \mu\text{g l}^{-1}$ of mercury yielding a relative standard deviation of 2.5%.

The use of bismuth-coated SPEs for the measurements of lead was described by Wang *et al.* (2001). It offered a reliable quantification of trace lead with ASV. The bismuth film was preplated onto the electrode surface using a solution containing 100 mg l^{-1} bismuth (III). The stripping response was linear over the $10\text{-}100 \mu\text{g l}^{-1}$ lead range when deposition time of 120s is used and a detection limit of $0.3 \mu\text{g l}^{-1}$ achieved for 600s deposition time and a good precision (RSD=7.4% at $20 \mu\text{g l}^{-1}$ lead).

Screen-printed silver electrodes have also been reported for the determination of lead (II) (Zen *et al.*, 2002). A conducting silver-gel was printed on a flexible polypropylene film followed by coating of a thin silver gel layer. The unit was then cured under UV irradiation for 1 hour to finalise the electrode fabrication. Square wave anodic stripping voltammetric analysis of lead yielded a linear range of $5 - 80 \mu\text{g l}^{-1}$, with a detection limit of $0.46 \mu\text{g l}^{-1}$, respectively. The interference effect was thoroughly studied with various metals and no significant change in current was found in the determination of $5 \mu\text{g l}^{-1}$ lead. The electrode also proved to be stable for repetitive measurements, and its practical applications were demonstrated by analysing lead in pond and ground water samples.

Masawat *et al.* (2003) reported the use of a disposable sputtered gold sensor for undepotential lead preconcentration in combination with a specially fabricated thin-layer flow cell for square wave anodic stripping analysis. The flow injection (FI) system was optimised and allowed the monitoring of lead with detection limit of $0.8 \mu\text{g l}^{-1}$ for 120 s preconcentration. The method was tested by determining lead in spiked drinking and tap water samples; the recoveries of lead were 103 % (RSD 2.8 %) and 97.9 % (RSD 7.1 %), $n = 5$, respectively.

Kadara & Tothill (2004) described the use of screen-printed working electrode as a substrate for in situ deposition of a metallic film of bismuth, which allows the electrochemical preconcentration of metal ions. Lead and cadmium were simultaneously detected using stripping chronopotentiometry at the bismuth film electrode. A bismuth (III) concentration of $500 \mu\text{g l}^{-1}$ was chosen for co-deposition onto the bare screen-printed carbon electrode. A linear range of $20 - 300 \mu\text{g l}^{-1}$ from the calibration curves were obtained for both metal ions. Using deposition potential of -1 V for a deposition time of 120 s , the detection limits of 8 and $10 \mu\text{g l}^{-1}$ were obtained for cadmium (II) and lead (II), respectively. The developed method was applied to the determination of lead and cadmium in soils extracts and wastewaters obtained from polluted sites. For comparison purposes, a mercury film electrode and ICP-MS were also used for validation. The same authors reported that copper (II) present at significant level in samples was found to affect the sensitivity of the bismuth film screen-printed carbon electrode (BFSPCE) by reducing the constant current stripping chronopotentiometric (CCSCP) response of lead (II). However, recovery of the lead stripping response at the BFSPCE in the presence of copper was obtained when ferricyanide was added to the test solution. The ferricyanide was found to circumvent the detrimental effect of copper (II) on lead (II) stripping response by masking the copper ions by forming a complex (Kadara & Tothill, 2005).

Table 3.1, summarises some of the published work on the applications of SPCEs as electrochemical metal sensors.

Table 3.1: Examples of published work on the applications of SPCEs as electrochemical metal sensors.

Detection with mercury film-screen-printed carbon sensors				
Metal	Sample	Method	Detection limit	Reference
Lead	Urine drinking water	DPASV PSA	0.05µg l ⁻¹ -Cd 0.03µg l ⁻¹ -Pb 0.4µg l ⁻¹ -Cd 0.3µg l ⁻¹ -Pb	Wang & Tian, 1992
Cadmium Lead Copper	Drinking water	SWASV PSA	0.1 µg l ⁻¹ -Lead 0.3 µg l ⁻¹ -Lead	Wang <i>et al.</i> , 1993
Zinc Cadmium Lead Copper	-	ASV	-	Desmond <i>et al.</i> , 1996
Zinc Cadmium Lead Copper	-	ASV	55 µg l ⁻¹ -zinc 71 µg l ⁻¹ -cadmium 64 µg l ⁻¹ -lead 123 µg l ⁻¹ -copper	Desmond <i>et al.</i> , 1998
Cadmium Lead Copper	Tap water	SWASV	0.6 µg l ⁻¹ -lead 0.4 µg l ⁻¹ -cadmium 0.8 µg l ⁻¹ -copper	Pachetti <i>et al.</i> , 1999
Lead	Drinking water	PSA	-	Yarnitsky <i>et al.</i> , 2000
Lead	Water	SWASV	0.81 µg l ⁻¹	Zen <i>et al.</i> 2000
Cadmium Lead	Certified Reference Material Lake water	ASV	-	Choi <i>et al.</i> , 2001
Copper	CRM 601 lake sediment Extracted soil Samples	CCSCP	6 µg l ⁻¹	Kadara <i>et al.</i> , 2003
Cadmium Lead Copper	Waste water Extracted soil sample	SWASV	0.3 µg l ⁻¹ -lead 1 µg l ⁻¹ -cadmium 0.5 µg l ⁻¹ -copper	Palchetti <i>et al.</i> , 2004
Detection with unmodified bare screen-printed carbon sensors				
Lead	-	LSASV SWASV	-	Reeder & Heineman, 1998
Lead	Pond water	DPASV	2.5 µg l ⁻¹	Honeychurch <i>et al.</i> 2000
Copper	Bovine liver, tap water, river water, sea water	DPASV	8.2 µg l ⁻¹	Honeychurch <i>et al.</i> , 2002a
Detection with other metallic-film screen-printed electrodes				
	Electrode	Matrix		
Lead	Gold coated	Drinking water Urine Samples	PSA 0.1-20 min, stirred 0.6- 4 min, stirred 3-10, unstirred	Wang & Tian, 1993
Mercury	Gold coated	-	PSA 0.5 µg l ⁻¹	Wang & Tian, 1993b
Lead	Bismuth	Drinking water	SWASV 0.3 µg l ⁻¹	Wang <i>et al.</i> , 2001a
Lead	Silver	Pond water Ground water	SWASV 5 µg l ⁻¹	Zen <i>et al.</i> , 2002
Lead	Sputtered gold	Drinking water Tap water	SWASV 0.8 µg l ⁻¹	Masawat <i>et al.</i> , 2003
Cadmium Lead	Bismuth	Extracted soil , Waste water	CCSCP 8 µg l ⁻¹ -cadmium 10 µg l ⁻¹ -lead	Kadara & Tothill, 2004

CCSCP: Constant current Stripping Chronopotentiometry, SWASV: Square wave anodic stripping voltammetry, PSA: Potentiometric Stripping Analysis, DPASV: Differential pulse anodic stripping voltammetry.

3.3 EXPERIMENTAL

3.3.1 Apparatus and reagents

A computer-controlled electrochemical Analyser (Autolab PGSTAT10) with general purpose electrochemical software operating system GPES version 4.7 or 4.8 (Eco Chemie B.V., Urecht, The Netherlands) was used for square-wave stripping voltammetric measurements. The screen-printed electrodes were fabricated in-house (see section in Chapter 2) for the electrochemical measurements. A specially adapted electrical edge connector from Maplin (Milton Keynes, UK) was used to connect with electrochemical analyser.

All chemicals received were of analytical grade. Hydrochloric acid (HCl) and potassium chloride (KCl) were obtained from BDH Ltd. (Poole, England). Ammonium acetate was purchased from Merck (Darmstadt, Germany). Potassium hexacyanoferrate (III), atomic spectroscopic standard stock solutions of 1000 mg l⁻¹ in 1 wt % HNO₃ of cadmium (II), lead (II), copper (II), mercury (II) were obtained from Aldrich (Gillingham, Dorset, UK).

3.3.2 Risk assessments and code of practice

Experiments were carried out in compliance with Departmental code of practice and COSHH regulations; waste was disposed by approved routes.

3.3.3 Soil/sediment sample treatment

The sampling protocols and sample treatments using conventional methods were carried out by Grup de Techniques de Separació (Universitat Autònoma Barcelona, GTS-UAB, Spain) and by Técnicas de Protección Ambiental S.A. (TPA, Madrid, Spain). The description of the extraction process applied to soil and sediment samples can be explained in three steps. The first step (water extraction) is carried out as a compulsory test in EU regulations to evaluate the type of contaminated soil and can also be applied to evaluate the metal release under rain condition: 1 g of the solid sample was placed in a 65 ml stopper extraction tube (polyurethane centrifuge tubes, ALCO Industrias, S.A, Spain) where 40 ml of HPLC grade water was added and then shaken for 16 hours in a rotary rack shaker (Horizontal Shaker SBS ABT-4) at a speed of 30 ± 10 rpm at room temperature 22 ± 3 . The mixture was then centrifuged

(using DIGICEN CE 007) at 3000 g for 20 minutes for phase separation and the aqueous phase filtered with a 0.22 μm pore size filters (Millex r-GS, Millipore Corporation, Bedford MA).

For the second step, the acetic acid extraction constitutes the mobile fraction of heavy metals in soils (ion exchangeable and bound to carbonates): the solid remaining from step 1 was shaken as above for 16 hours with 40 ml of 0.11 M acetic acid (pH 2.85). Centrifugation and filtration was carried out as in step 1. In the last step, aqua regia extraction will provide the total content of heavy metals in soil samples. In fact, it is the content of heavy metals not absorbed in the silicate matrix, also known as pseudo-total content: 0.25 g portion of the remaining solid sample from step two was placed in a microwave vessel and 9 ml of aqua regia (6 ml HCl, 2 ml HNO₃ and 1 ml HPLC grade water) were added and then left for 10 hours to predigest before microwave treatment. A total digestion preceded using Analytical Microwave (MARS 5, CEM Corporation, USA) by first ramping to 200 °C for 4 minutes and then maintaining for 6 minutes using pressure between 160-180 psi. The vessel was cooled down to room temperature before opening and the remaining solution brought up to a final volume of 100 ml. The solution was filtered with Millipore filters of 0.22 μm pore size. All the extracts, the treated soils and the aqueous fractions from the three steps, were kept refrigerated at 4°C prior to the analysis being carried out in the laboratory.

3.3.4 Water sample treatment

Aliquots of water samples were filtered with a 0.22 μm pore size filters and used for the analysis.

3.3.5 Procedure

Electrochemical measurements were carried out in triplicate by placing a 100 μl sample drop on a new three-electrode strip in non-deaerated and unstirred solution. Metal standard solutions were prepared by diluting the standard stock solution of 1000 mg l⁻¹ with deionised water or supporting electrolyte (0.1 M HCl/0.5 M NH₄Ac). Other reagents were prepared by dissolving it in deionised water.

Square-wave anodic stripping voltammetry (SWASV) measurements were carried out by placing the samples (metal ions of cadmium, lead and copper) on the screen-printed carbon electrode surfaces plated *in situ* with metallic films of mercury. A deposition potential of -1 V (vs screen-printed Ag/AgCl) reference electrode) was applied to preconcentrate the metals for 120s onto the surface of working electrode. After the deposition period, the preconcentrated metals are stripped from the surface of the working electrode until a limit of 0 V. A SW frequency of 50 Hz, a step potential of 5 mV and square-wave amplitude of 25 mV were used for the measurements. The background signal for the supporting electrolyte was also measured in the same manner. The analytical signal is subtracted from the background signal.

3.3.6 Real sample analysis

The concentrations for cadmium (II), lead (II) and copper (II) in the waste-water samples, acetic acid soil or sediments and soil or sediment aqua regia extracts were quantified by the use of standard additions method following an initial screening of the target species in the samples. SWASV was conducted by diluting the waste-water samples, soil and sediment extracts in the supporting electrolyte. The diluted samples were then analysed by spiking with appropriate concentrations of standard metal ion solutions for standard addition experiments.

The voltammetric measurements of the sample solutions were carried out by placing a 100 μ l sample drop on the three-electrode strip. Each electrochemical measurement was carried out in triplicates with a new electrode strip in non-deaerated for the stripping measurements.

3.3.7 Data Analysis

All experiments carried out in these studies were repeated three to five times with different SPEs. The data represent the mean value of three measurements and the error bar represents the standard deviation.

3.4 RESULTS

3.4.1 Mercury film screen-printed carbon electrode – Optimisation of experimental variables for stripping voltammetric detection of cadmium, lead and copper.

Square-wave anodic stripping voltammetry studies were carried out with 0.1 M HCl + 0.5 M ammonium acetate. The effect of experimental variables such as mercury (II) concentration on the stripping responses of cadmium, lead and copper, the effect of deposition potential, the effect of deposition time and the concentration dependence for each of the metal ions were investigated.

3.4.1.1 Influence of Mercury (II)

As part of the work carried out the influence of mercury (II) concentration on the stripping responses of lead (II), cadmium (II) and copper (II), the metals were examined in the mercury ion concentration range of 0 to 100 mg l⁻¹. Figure 3.1, 3.2 and 3.3 show the influence of mercury (II) concentration on the square-wave stripping voltammetry response of cadmium (II), lead (II) and copper (II). Cadmium, lead and copper stripping response levelled off above a mercury concentration of about 60, 40 and 60 mg l⁻¹, respectively after increasing rapidly at lower mercury (II) concentration. Hence a mercury concentration of 80 was used for subsequent stripping voltammetric work.

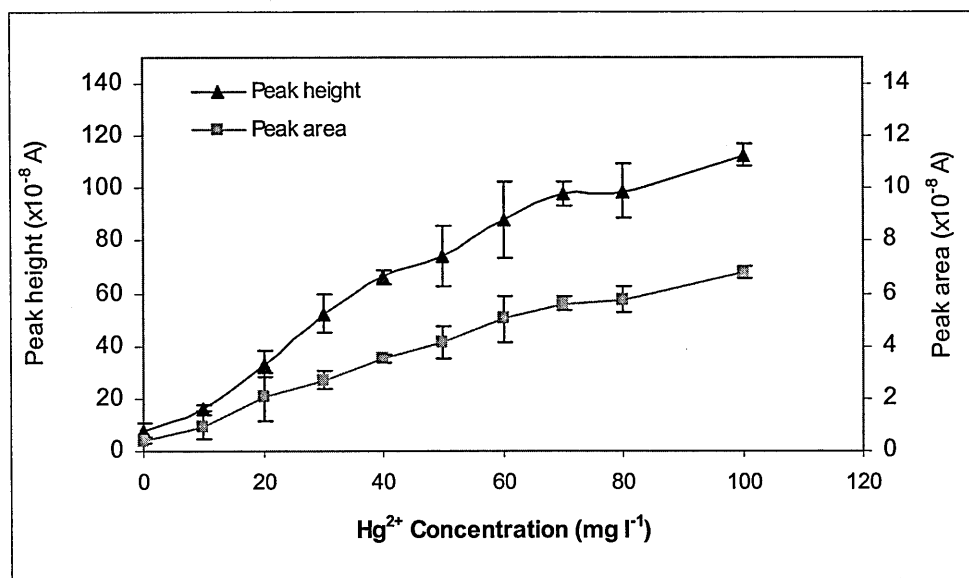


Figure 3.1: Effect of mercury concentration on the stripping voltammetric response of 50 $\mu\text{g l}^{-1}$ cadmium (II) in 0.1 M HCl/0.5 M ammonium acetate solution. Experimental conditions: deposition potential = -1.1 V, final potential = -0.05 V, deposition time = 120 s, SW frequency = 25 Hz, step potential = 5 mV, SW amplitude = 0.025 V.

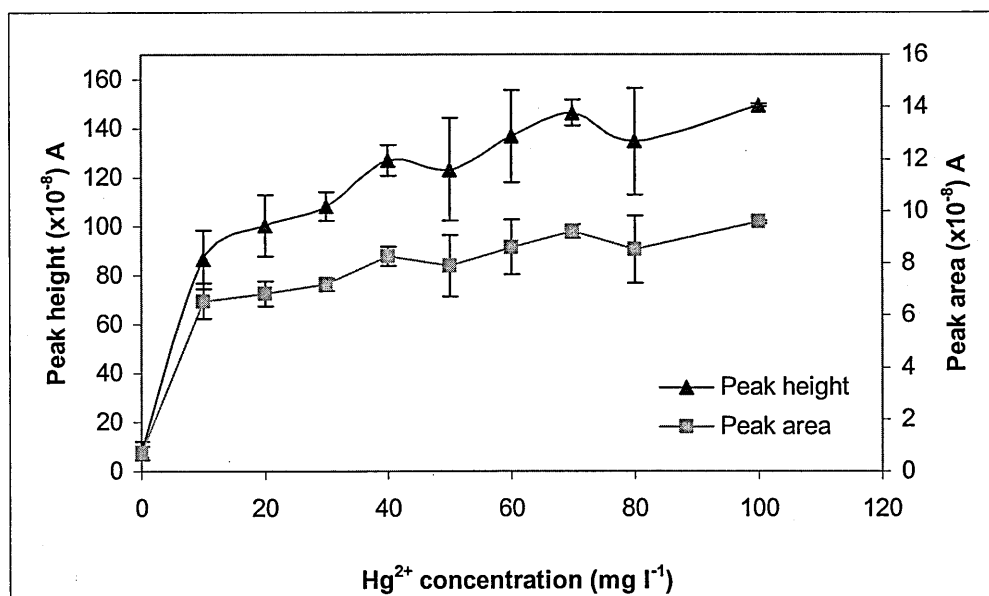


Figure 3.2: Effect of mercury concentration on the stripping voltammetric response of 50 $\mu\text{g l}^{-1}$ lead (II) in 0.1 M HCl/0.5 M ammonium acetate solution. Experimental conditions: deposition potential = -1.1 V, final potential = -0.05 V, deposition time = 120 s, SW frequency = 25 Hz, step potential = 5 mV, SW amplitude = 0.025 V.

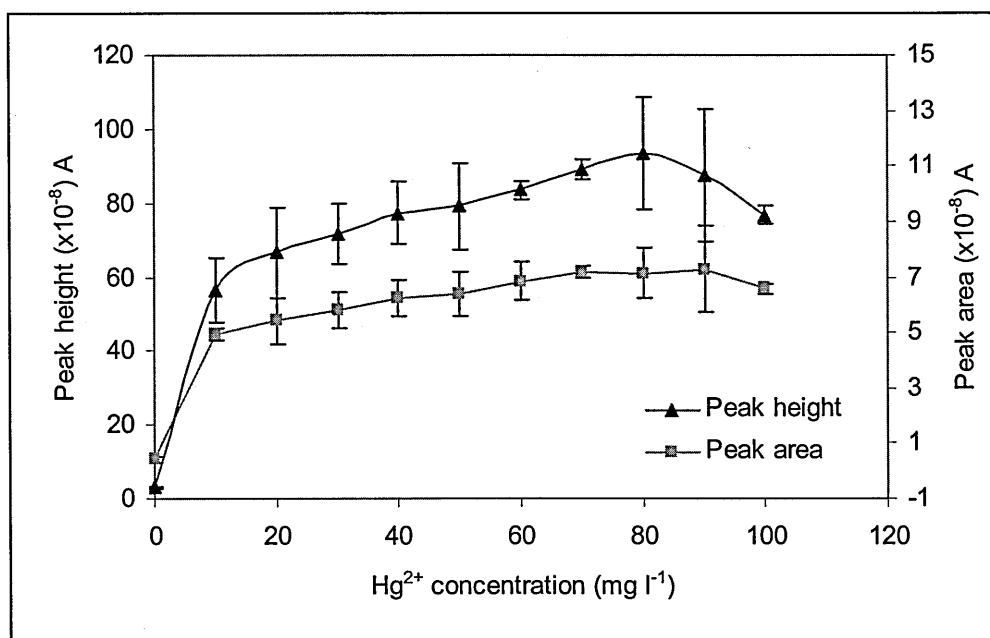
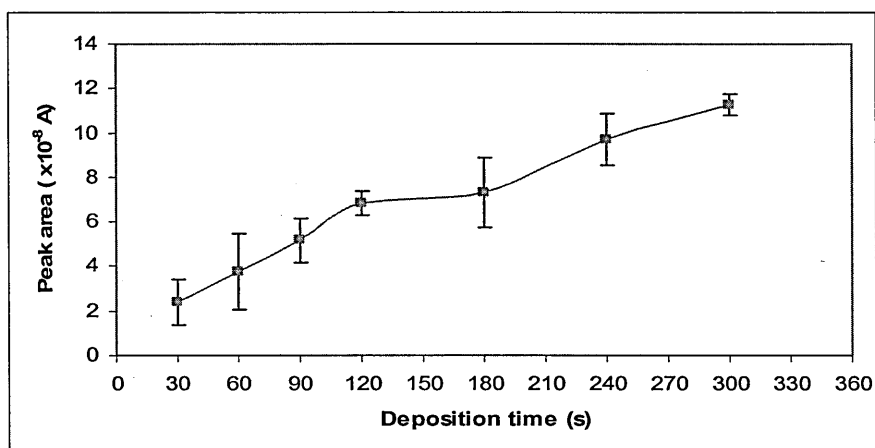


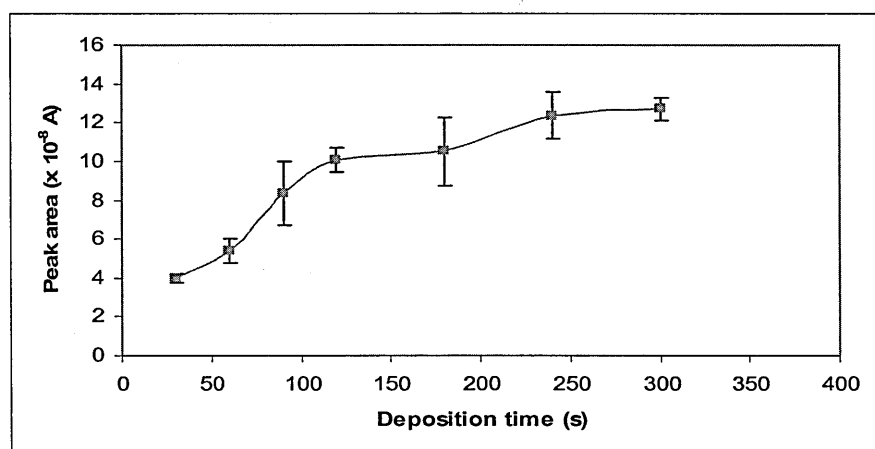
Figure 3.3: Effect of mercury concentration on the stripping voltammetric response of 50 $\mu\text{g l}^{-1}$ copper (II) in 0.1 M HCl/0.5 M ammonium acetate solution. Experimental conditions: deposition potential = -1.1 V, final potential = -0.05 V, deposition time = 120 s, SW frequency = 25 Hz, step potential = 5 mV, SW amplitude = 0.025 V.

3.4.1.2 Effect of deposition time

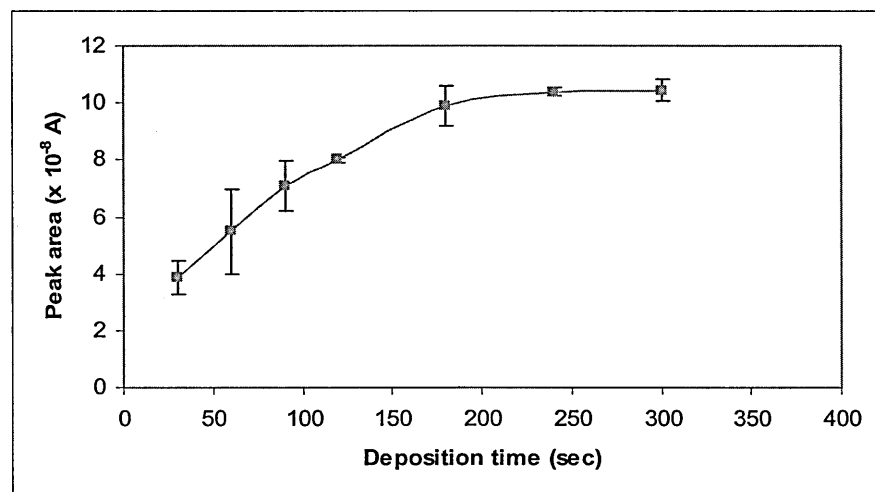
Effect of deposition time on stripping voltammetric of cadmium (II), lead (II) and copper (II) are shown in Figure 3.4 (a), (b) and (c). As can be seen, the stripping response of cadmium (II) increased rapidly with deposition time from 30 to 300 s. However, lead (II) and copper (II) stripping voltammetry peak show that at 250 s and 200 s, respectively, the peak start to level off and these were due to the saturation of the mercury coated SPE surface. A deposition of 120 s was used for subsequent experiments, as this is sufficient to obtained well-defined stripping peaks for cadmium, lead and copper at microgram per liter concentrations.



(a)



(b)



(c)

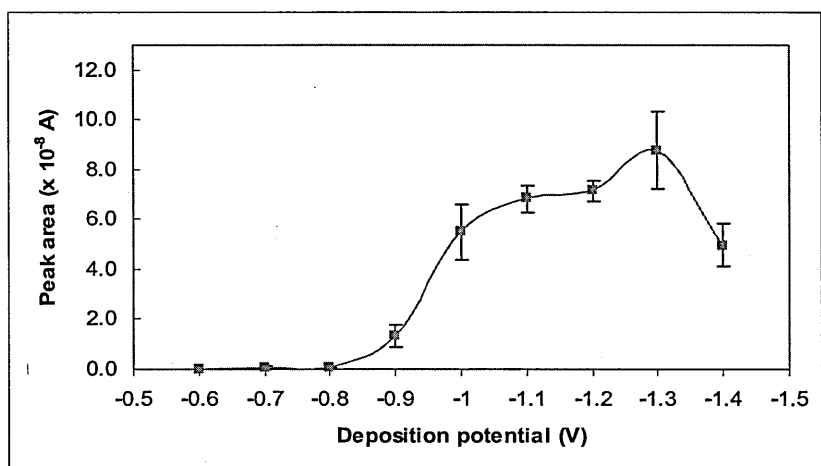
Figure 3.4: Effect of deposition time on the stripping voltammetric response of $50 \mu\text{g l}^{-1}$ (a) cadmium (II), (b) lead (II) and (c) copper (II). Deposition potential = -1.1 V, SW frequency = 25 Hz, step potential = 5 mV, amplitude = 25 mV, $\text{Hg}^{2+} = 80 \text{ mg l}^{-1}$.

3.4.1.3 Effect of deposition potential

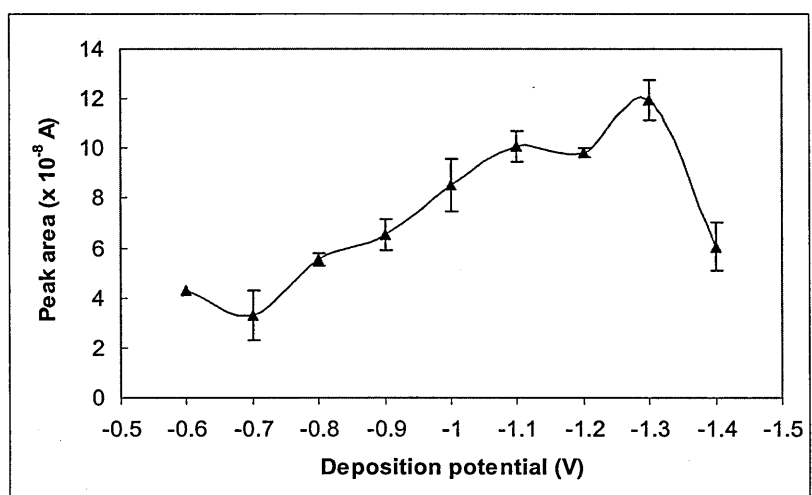
Figure 3.5 (a), (b) and (c) show the effect of deposition potential on the stripping response. For cadmium (II), lead (II) and copper (II), the SWASV signal increased when the deposition potential proceeded to more negative potentials. However, at potentials more negative than -1.1 V, the background response start to increase, thus affect the reproducibility of the stripping responses. A potential of -1.1 or -1.0 V were employed for simultaneous detection of cadmium, lead and copper because it provide the best compromise between sensitivity of the main peak and the background response.

3.4.1.4 Effect of Frequency

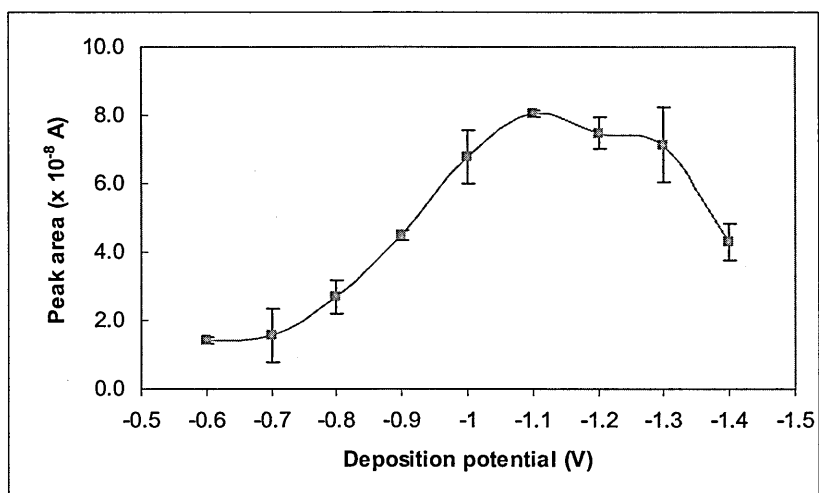
For the effect of frequency on the stripping response, the SWASV signals for cadmium, lead and copper decreased when the frequency proceeded to higher value (Figure 3.6 (a), (b) and (c)). For compromise among the three metals analysed, frequency of 20 Hz was used as it provide the most stable peak shape for the simultaneous analysis.



(a)

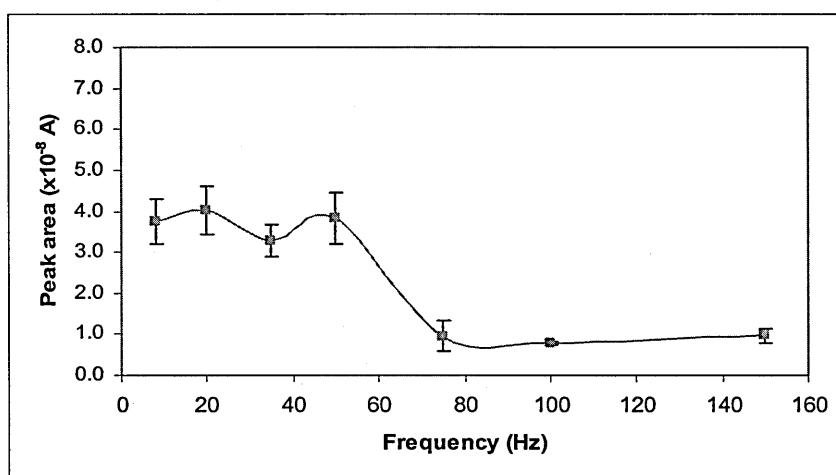


(b)

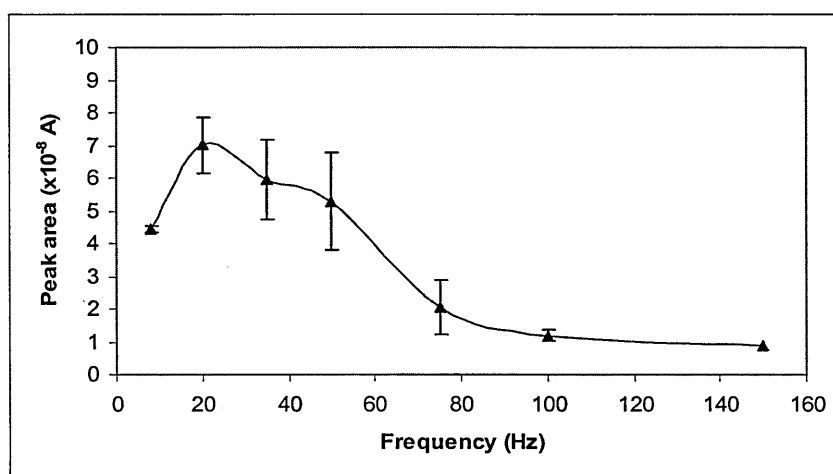


(c)

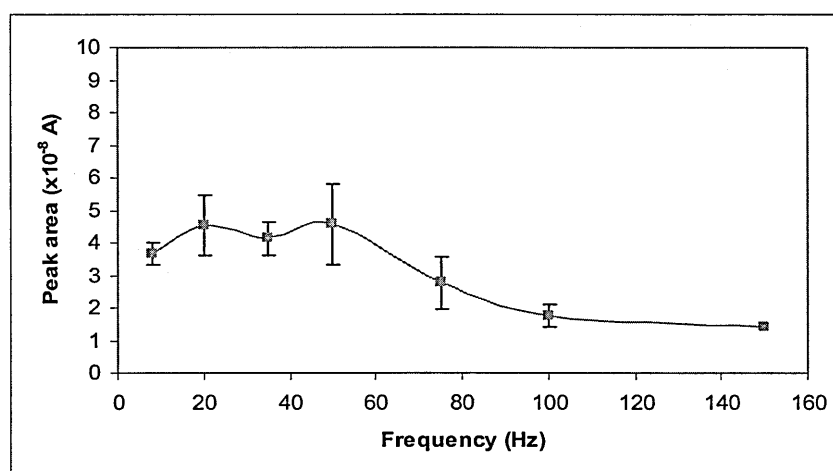
Figure 3.5 : Effect of deposition potential on the stripping voltammetric response of $50 \mu\text{g l}^{-1}$ (a) cadmium (II), (b) lead (II) and (c) copper (II). Deposition time = 120 s, SW frequency = 25 Hz, step potential = 5 mV, amplitude = 25 mV, $\text{Hg}^{2+} = 80 \text{ mg l}^{-1}$.



(a)



(b)



(c)

Figure 3.6 : Effect of frequency on the stripping voltammetric response of $50 \mu\text{g l}^{-1}$ (a) cadmium (II), (b) lead (II) and (c) copper (II). Deposition time = 120 s, SW frequency = 25 Hz, step potential = 5 mV, amplitude = 25 mV, $\text{Hg}^{2+} = 80 \text{ mg l}^{-1}$.

3.4.1.5 Calibration curve for cadmium (II), lead (II) and copper (II)

Figure 3.7 shows the stripping voltammograms obtained for increasing concentration of cadmium (II), lead (II) and copper (II) in the range 0 to 200 $\mu\text{g l}^{-1}$.

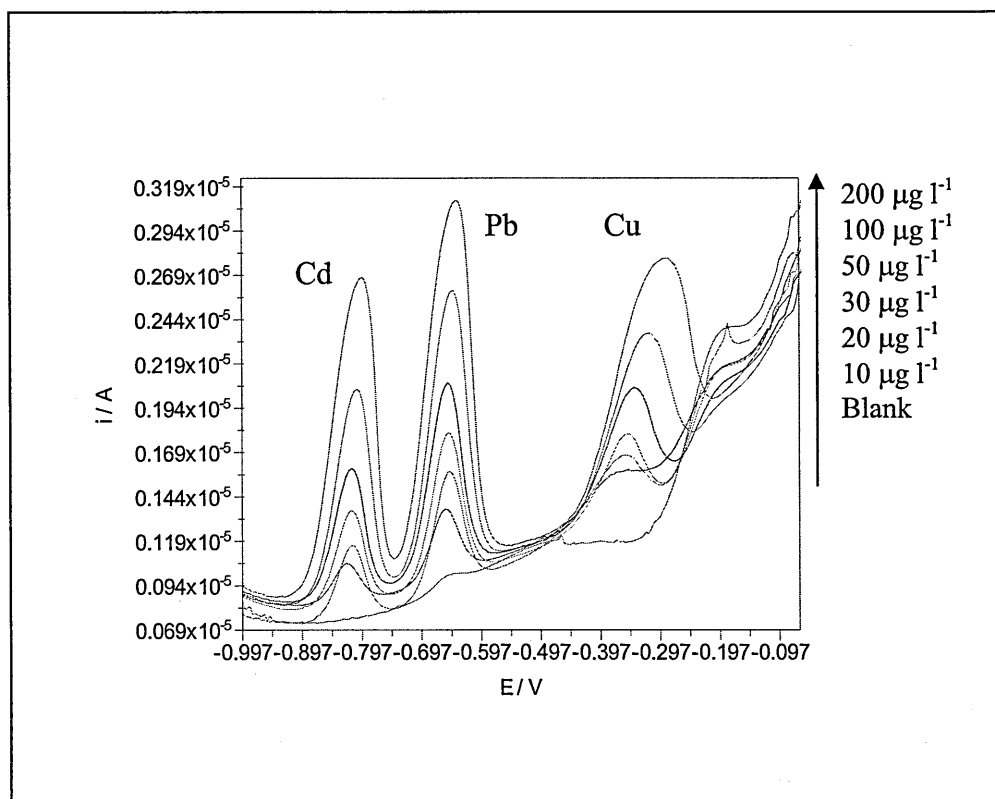
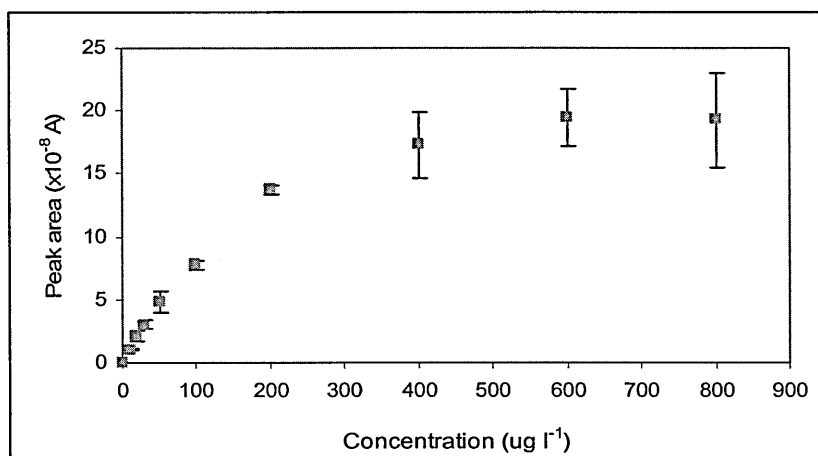


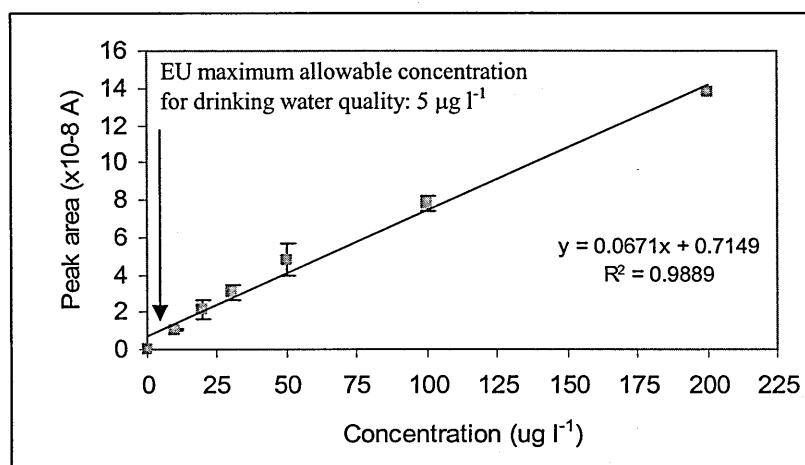
Figure 3.7: Stripping voltammograms for increasing concentration of cadmium (II), lead (II) and copper (II) in 0.1 M HCl + 0.5 M ammonium acetate along with the blank response. $\text{Hg}^{2+} = 80 \text{ mg l}^{-1}$, deposition time = 120s, deposition potential = -1 V, final potential = 0.05 V, frequency = 20 Hz, SW amplitude = 25 mV, step potential = 5 mV.

(a) Cadmium (II)

The calibration plot obtained for cadmium (II) was found to be bimodal with linear ranges between 0 and 200 $\mu\text{g l}^{-1}$ (correlation coefficient for Cd, $(r^2)=0.9889$) (Figure 3.8). The precision (relative standard deviation) of the measurements determined by performing 6 replicate measurements of 5 $\mu\text{g l}^{-1}$ cadmium was 6.5 %. A detection limit of 2 $\mu\text{g l}^{-1}$ was estimated for 120 s deposition using 3 x standard deviation of the blank.



(a)



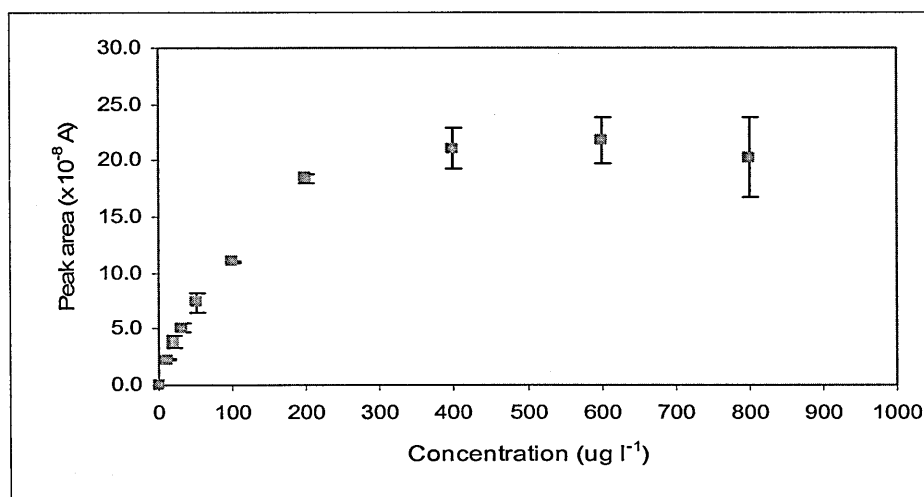
(b)

Figure 3.8: Calibration curve for cadmium (II) in 0.1 M HCl + 0.5 M ammonium acetate a) and (b) linear ranges for cadmium (II) at mercury coated SPCE. $\text{Hg}^{2+} = 80 \text{ mg l}^{-1}$, deposition time = 120s, deposition potential = -1 V, final potential = 0.05 V, frequency = 20 Hz, SW amplitude = 25 mV, step potential = 5 mV.

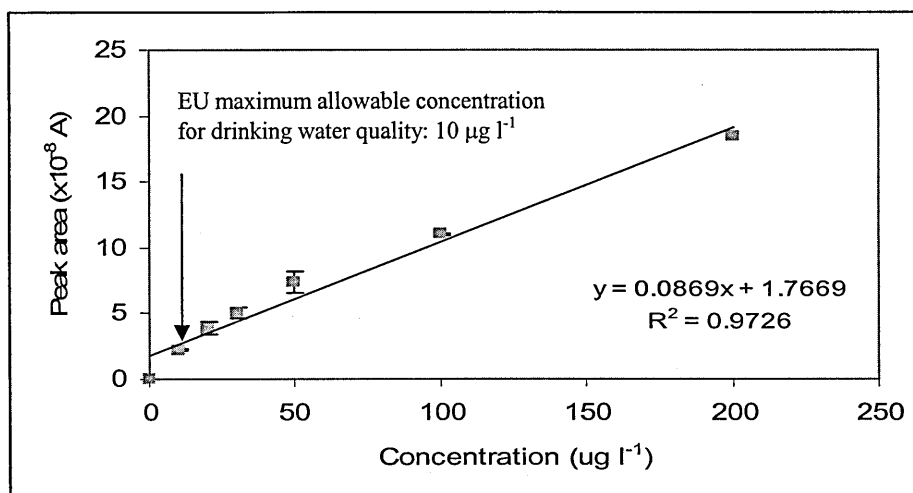
(b) Lead (II)

The stripping voltammograms obtained for increasing concentration of lead (II) in the range of 0 to $100 \mu\text{g l}^{-1}$ is depicted in Figure 3.7. For the calibration graph presented in Figure 3.9 (b), a linear concentration was obtained between the stripping response (peak area) and lead (II) concentration in the range 0 to $200 \mu\text{g l}^{-1}$ (correlation coefficient, $r^2 = 0.9726$) for 120 s deposition. Above concentration level of $100 \mu\text{g l}^{-1}$, there was deviation in the linearity of the calibration plot. The precision measurement

(R.S.D.) determined by performing 6 replicate measurements lead (II) on solutions containing 50 and 100 $\mu\text{g l}^{-1}$ with the calculated relative standard deviation values of 6.9 % and 9.1 %, respectively. A detection limit of 1 $\mu\text{g l}^{-1}$ was estimated using a deposition time of 120s.



(a)

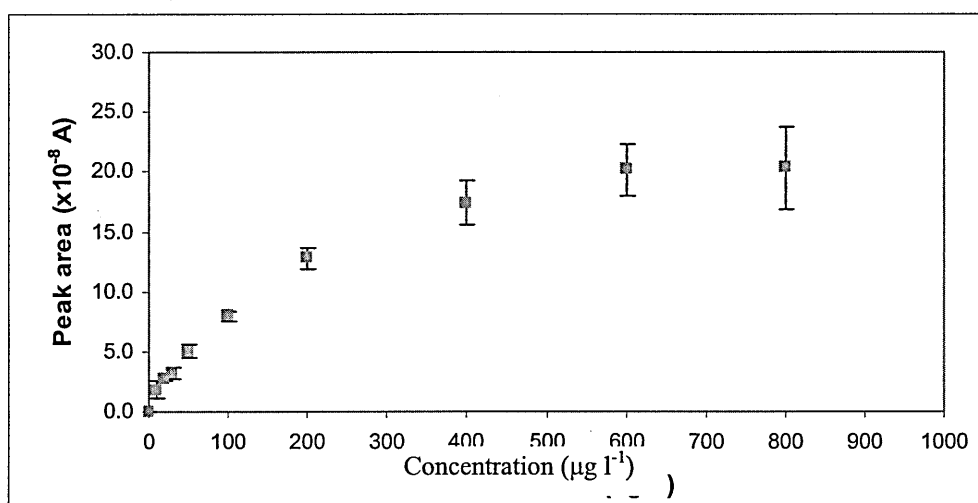


(b)

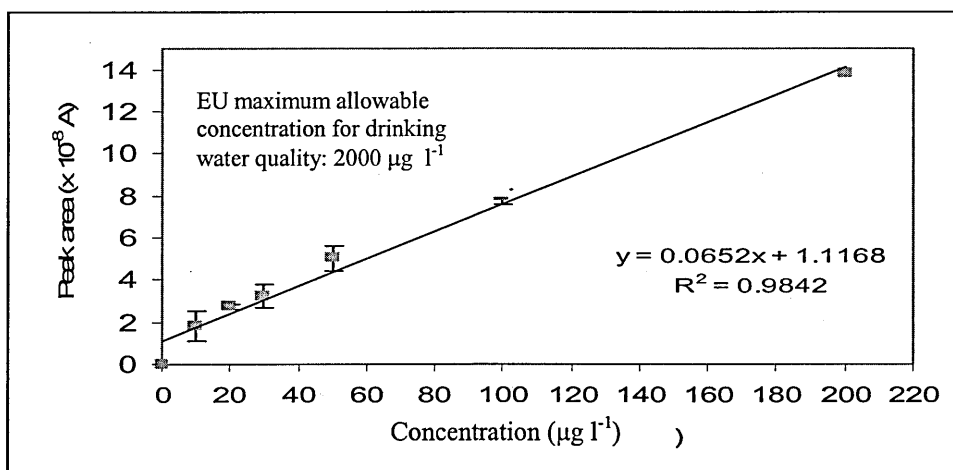
Figure 3.9 Calibration curve for lead (II) 0.1 M HCl + 0.5 M ammonium acetate a) and (b) linear ranges for cadmium (II) at mercury coated SPCE. $\text{Hg}^{2+} = 80 \text{ mg l}^{-1}$, deposition time = 120s, deposition potential = -1 V, final potential = 0.05 V, frequency = 20 Hz, SW amplitude = 25 mV, step potential = 5 mV.

(c) Copper (II)

The stripping voltammograms and calibration plot obtained for increasing concentration of copper are depicted in Figure 3.7 and 3.10. There was a linear correlation between the stripping peak area and copper concentration in the range 0 to 200 $\mu\text{g l}^{-1}$ ($r^2 = 0.9842$) for 120 s deposition time. There was a leveling off of the calibration curve at higher than 200 $\mu\text{g l}^{-1}$. This was due to the saturation of the *in situ* plated mercury film SPCE. The precision of the measurements (R.S.D.) determined by performing 6 replicate measurements of 10 $\mu\text{g l}^{-1}$ copper was 7.5 %. A detection limit of 5 $\mu\text{g l}^{-1}$ was estimated for 120 s deposition.



(a)



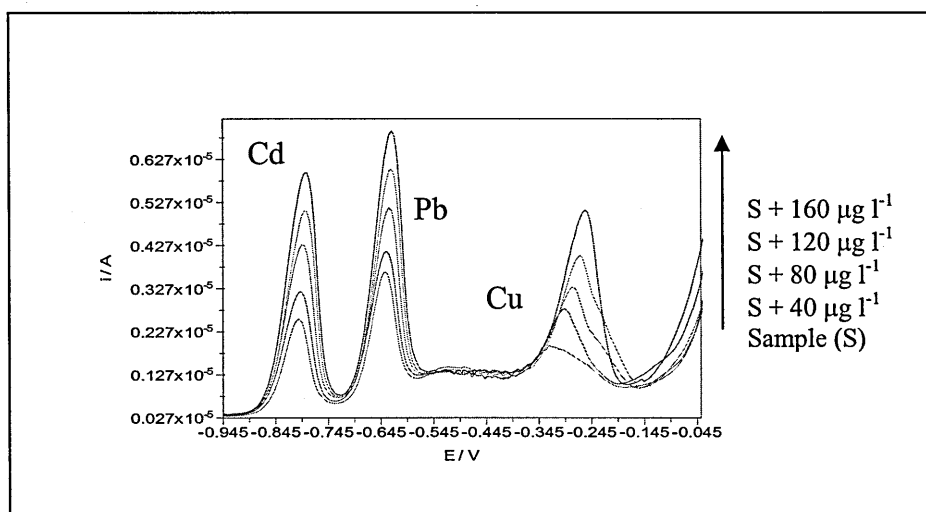
(b)

Figure 3.10: Calibration curve for copper (II) 0.1 M HCl + 0.5 M ammonium acetate a) and (b) linear ranges for copper (II) at mercury coated SPCE. $\text{Hg}^{2+} = 80 \text{ mg l}^{-1}$, deposition time = 120s, deposition potential = -1 V, final potential = 0.05 V, frequency = 20 Hz, SW amplitude = 25 mV, step potential = 5 mV.

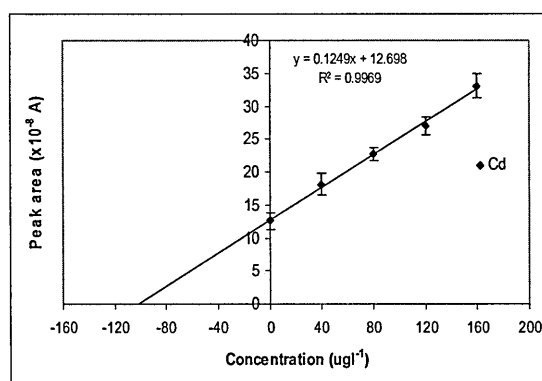
3.4.1.6 Sample Evaluations

The concentrations of metal ions in the waste-water and extracted water samples obtained from Aznalcollar mining area were determined by the extrapolation of the standard addition plot constructed using the standard addition method. Figure 3.11 shows typical stripping voltammograms and standard addition plots obtained for one of the acetic acid extracted sample and subsequent standard additions of different concentrations of cadmium (II), lead (II) and copper (II). The results in Table 3.2 and 3.3 are for the square-wave stripping voltammetric (SWASV) determination of cadmium, lead and copper in waste-water and soil acetic-acid extracted at MFSPCE. These measurements were carried out with the aim to comparing with the data obtained for screen-printed gold electrode in chapter 5. The results of SWASV determination are verified by using the data obtained for ICP-MS.

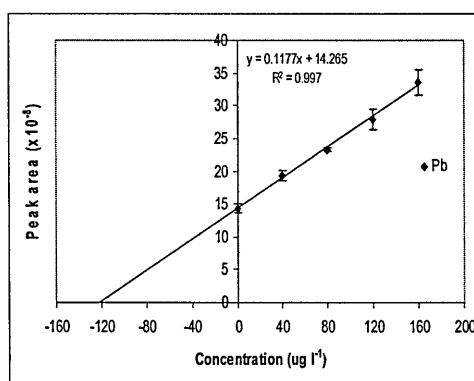
Comparison between concentration data obtained for different acetic acid and aqua regia extracted soil samples using SWASV coupled to the integrated mercury film screen-printed carbon three-electrode device and ICP-MS (the reference method) is presented in Figure 3.12. There is fairly good agreement between some of the results obtained with ICP-MS and SWASV. Cadmium and copper generally shows higher results using SWASV as compared to ICP-MS. A relative high correlation coefficient value obtained between the two methods of determination ($r^2=0.9442$) confirms the validity of the proposed method (SWASV coupled to MFSPCE) for lead detection in environmental soil samples using 0.1M HCl/0.5 M NH₄Ac as supporting medium.



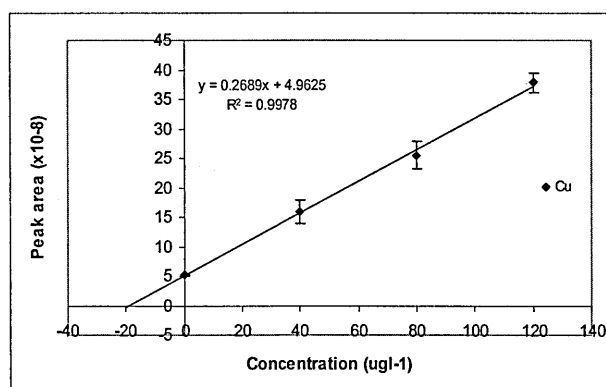
(a)



(b)



(c)



(d)

Figure 3.11: (a) Stripping voltammograms and (b), (c), (d) standard addition plot for the determination of cadmium (II), lead (II) and copper (II) on mercury coated SPCE in one of the extracted soil samples. Depending on the real concentrations, standard solutions of cadmium (II), lead (II) and copper (II) were added in 40, 80 and 120 and 160 $\mu\text{g l}^{-1}$ increments to the sample. $\text{Hg}^{2+} = 80 \text{ mg l}^{-1}$, deposition time = 120s, deposition potential = -1 V, final potential = 0.05 V, frequency = 20 Hz, SW amplitude = 25 mV, step potential = 5 mV.

Table 3.2: Comparing results for the determination of metal ions in waste-water samples using square-wave anodic stripping voltammetry (SWSASV).

Waste-water Samples	Sample 1 ICP-MS ($\mu\text{g l}^{-1}$)	Sample 1 SWSV ($\mu\text{g l}^{-1}$) SPMFE	Sample 2 ICP-MS ($\mu\text{g l}^{-1}$)	Sample 2 SWSV ($\mu\text{g l}^{-1}$) SPMFE
Lead (Pb)	890 ± 0.02	955.4 ± 0.70	740 ± 0.09	862.6 ± 0.18
Cadmium (Cd)	620 ± 0.04	739.8 ± 1.21	630 ± 0.009	517.1 ± 0.5
Copper (Cu)	180 ± 0.01	155.9 ± 0.92	180 ± 0.01	-

Table 3.3: Comparing results for the determination of metal ions in aqua regia and acetic acid extracted waste-water samples using square-wave anodic stripping voltammetry (SWSASV).

Technique	ICP-MS ($\mu\text{g l}^{-1}$)			SWSV ($\mu\text{g l}^{-1}$) SPMFE		
	Pb	Cd	Cu	Pb	Cd	Cu
Metals						
S3b	2853 ± 35.4	195 ± 2.10	1882 ± 37.8	2220.36 ± 0.65	216 ± 0.2	2439.4 ± 1.3
S4b	1537 ± 27.1	17 ± 2.08	262 ± 40.9	1171.3 ± 0.67	30.7 ± 0.5	357 ± 0.5
S5c	664	44	540	636.5 ± 0.5	101.7 ± 0.6	981.7 ± 2
S6c	5294	35	1197	5743.8 ± 0.64	80.5 ± 0.8	2233.7 ± 0.9
S7c	2423 ± 20.8	12 ± 3.7	1853 ± 3.5	1981.6 ± 0.4	30 ± 0.05	2009 ± 1.5
S8c	3046 ± 26.2	< 5	1087 ± 2.8	2284.7 ± 0.1	nq	1153.4 ± 0.2
S9c	2799 ± 44	50 ± 34.60	1298 ± 42.2	2765.2 ± 0.7	76.3 ± 0.3	1367 ± 0.8

b – acetic acid extract; c – aqua regia extract; S – sample; nc – not certified; nq – not quantified; ^aValue is the mean of triplicate determination $\pm$ standard deviation.

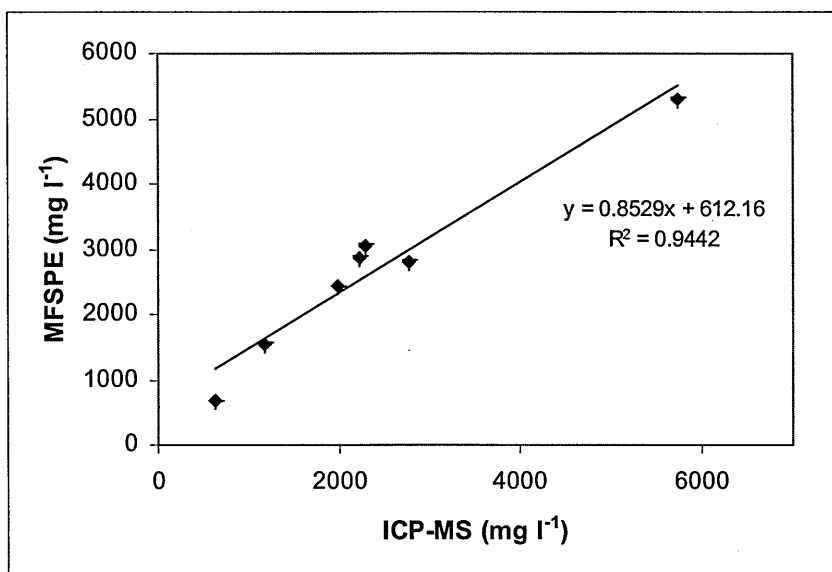


Figure 3.12: Correlation plot between SWASV-MFSPCE and ICP-MS for the determination of lead in extracted soil samples.

3.5 CONCLUSION

This chapter presented the results obtained for electrochemical stripping voltammetric analysis of cadmium, lead and copper using in-house fabricated electrodes. The three-electrode configuration system coupled with SWASV has provided a means of a relatively inexpensive on-site detector for trace levels of trace metals. Detection of these metals was carried out on MFSPCE using the optimized procedures developed for measurements in this work. The bare SPCE acted as supports for the *in situ* plating of mercury film. With the optimized working conditions, the results obtained indicate that the screen-printed electrochemical sensors are sensitive and reproducible enough for the SWASV determination of cadmium, lead and copper in the parts per billion range.

Although the linear concentration range obtainable with SWASV is narrow (0 - 200 $\mu\text{g l}^{-1}$), it is still can be used for determining cadmium, lead and copper in synthetic and waste-water and extracted soil samples. The preconcentration of cadmium, lead and copper metallic phase from aqueous solutions was favoured when co-depositing with mercury onto the bare electrode surface. These approaches allowed the trace

metal determination of cadmium, lead and copper with limit of detection below $10 \mu\text{g l}^{-1}$. The reproducibility of the measurements was below 15 % for MFSPCE.

For environmental samples, the usefulness of SWASV coupled to screen-printed electrodes for the determination of cadmium, lead and copper in waste-water and extracted water samples was evaluated. Overall, the results show that the developed screen-printed electrochemical sensors are capable of measuring cadmium, lead and copper in waste-water and extracted water samples. After comparison to ICP-MS, the data generated with the electrochemical sensors revealed fairly accurate measures of cadmium, lead and copper in waste-water and extracted water samples. The mobile cadmium, lead and copper ions in the soils or sediments were determined after sequential extraction has been carried out with water (free copper), acetic acid (exchangeable cadmium, lead and copper) and aqua regia (pseudo total lead, copper). Result obtained for samples analysed with SWASV was comparable to one obtained with ICP-MS.

CHAPTER 4

4.0 HEAVY METAL IONS DETECTION ON CYSTEINE MODIFIED MERCURY FILM SCREEN-PRINTED CARBON ELECTRODES

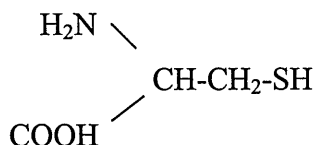
4.1 OUTLINE

In this chapter, a review on the determination of metals with peptides modified electrode is presented. The modification of screen-printed carbon electrodes with cysteine for the determination of cadmium, lead and copper using screen-printed carbon electrode will be described.

4.2 INTRODUCTION

There are many compounds most widely employed for the analysis of metal ions that can produce improved sensitivity in the stripping analysis by coupling this technique to catalytic reactions such as formation or interfacial accumulation of appropriate metal chelates (Wang, 1994). Metal-binding proteins (metalloproteins) are produced by many cells in response to the presence of specific metals and are important for the detoxification mechanisms. These proteins and peptides are known to bind metal ions and can be used as receptors for the concentration and quantification of metal ions in environmental samples and biological fluids (Tanaka & Yoshida 1982; Bai *et al.* 1998; Liu *et al.* 1999; Gooding *et al.* 2001; Yang *et al.* 2001). Metalloproteins usually contain high levels of the amino acid cysteine (up to 30 %) in their amino acid sequence. Their structure contains a great number of potential donor atoms through the peptide backbone and amino acids side chains (Kozłowski *et al.*, 1999). Peptides containing sulfurhydryl groups in cysteine residues display a higher affinity toward metal chelating, which results in the formation of strong complexes directly on the electrode surface (Gooding *et al.*, 2001).

Cysteine or 2-Amino-3-mercaptopropanoic acid (HS-CH₂-CH (NH₂)-COOH) is shown below:



This is a sulfur-containing amino acid that has been used in copper ion analysis (Arrigan & Bihan. 1999; Kozlowski *et al.* 1999). The amino acid complexes the copper ions (Cu (II)) at the mercury electrode resulting in a low detection limits for copper using anodic stripping voltammetry (ASV, 0.5 nM) and potentiometric stripping analysis (PSA, 0.6 nM) respectively (Tanaka & Yoshida 1982; Bai *et al.* 1998). Increase in sensitivity has also been observed for the determination of mercury with metallothioneins (Ju & Leech, 2000). Table 4.1 shows a few examples of the use of peptides for the electrochemical determination of toxic metals.

Table 4.1: Examples of metals ion detection with peptides-modified electrodes

AMINO ACIDS AND PROTEINS	METAL	ELECTRODE	TECHNIQUE	DETECTION LIMIT (µg l ⁻¹)	REFERENCE
Cysteine	Cu (II)	HMDE	DPASV	0.038	Tanaka & Yoshida, 1982
Cysteine	Cu (II)	MFE	PSA	0.032	Bai <i>et al.</i> , 1998
Cysteine	Cu (II)	Au	SWASV	5	Yang <i>et al.</i> , 2001
Metallothionein	Hg (II)	Au	DPCSV	16	Ju & Leech, 2000
L-tyrosine	Hg (II)	Pt	Amperometry	0.014	Majid <i>et al.</i> , 2002
Gly-Gly-His	Cu (II)	Au	SWASV	0.0002	Yang <i>et al.</i> , 2003

Abbreviations: HMDE=hanging mercury drop electrode; MFE=mercury film electrode; DPASV=differential pulse anodic stripping voltammetry; SWASV=square-wave stripping anodic stripping voltammetry; PSA=potentiometric stripping analysis; DPCSV=differential pulse cathodic stripping voltammetry.

The anodic stripping voltammetric behaviour of Cu in the presence of various compounds with -SH groups with a hanging mercury-drop electrode (HMDE) was investigated by Tanaka & Yoshida (1982). In the presence of these compounds, the copper stripping peak at 0.0 V vs. SCE was observed to decreased, whereas a new sharp wave appeared at 0.06 V vs SCE, whose height increased with the ligand concentration. This wave also proportional to the copper concentration, and thus it was chosen for the determination of this metal. The biggest enhancement was observed in the presence of cysteine. The appearance of the sensitive wave was explained by the decomposition of a copper cysteine-complex at the oxidation step to originate cysteine and cupric ions. The detection limit calculated for Cu determination was 6×10^{-10} ($0.038 \mu\text{g l}^{-1}$). Bai *et al.* (1998) reported the enhancement in copper ion detection when cysteine modified mercury film electrodes were prepared by electrochemical *in-situ* co-deposition of mercury and cysteine on glassy carbon using potentiometric stripping analysis. Copper was deposited at -0.9V and the subsequent oxidation yielded two enhanced stripping signals at 0.05 and -0.16 V vs Ag/AgCl. The proposed mechanism displayed the oxidation of a cuprous cysteinate to copper (II) ions at -0.16 V, and the formation of a mercuric cysteinate complex at 0.05 V. Copper was determined in the range of 3.9×10^{-8} - 4.3×10^{-7} M (2.5 - $27.3 \mu\text{g l}^{-1}$) with detection limit of $0.032 \mu\text{g l}^{-1}$. The comparison with unmodified electrodes suggested that this technique can achieve 100-fold increase in the detection limit of copper (II).

Most of the published literature report on the use of self-assembly of sulfur containing molecules such as cysteine on gold electrode for the detection of copper. L-cysteine modified gold electrodes have also been successfully demonstrated for the determination of Cu (II) at ppb level concentrations using square wave voltammetry (Liu *et al.* 1999; Yang *et al.* 2001). Cysteine was adsorbed on the surface of gold electrodes via the thiol side-chain of the amino acid. The presence of copper yielded the formation of a cupric thiolate after breakdown of part of the cysteine monolayer. The SWASV determination of copper (II) after electrode modification yielded a linear calibration plot between $0.08 - 10 \mu\text{M}$ ($5 - 635 \mu\text{g l}^{-1}$). Detection limit of $5 \mu\text{g l}^{-1}$ was achieved. The presence of other metals at concentration below $1 \mu\text{M}$ such as zinc, lead, manganese and silver did not interfere with the result significantly. Cysteine can also complex with other metals such as cadmium, lead and copper, however, no

literature have been found regarding the use of cysteine-modified electrode for cadmium (II) and lead (II) analysis.

In this chapter, the fabrication of cysteine modified mercury film screen-printed electrode and its application to the determination of cadmium (II), lead (II) and Cu (II) using constant current stripping chronopotentiometry (CCSCP) is presented. CCSCP is an electroanalytical technique that is readily amendable for decentralized screening, providing accurate measurements of low concentrations with rapid analysis times (Kadara *et al.* 2003). Just like ASV, CCSCP exploit *in situ* formation of a mercury film and comprises two-steps, a preconcentration and a measurement step. The preconcentration step involves metal ions in the solution being reduced and simultaneously amalgamated on the working electrode at constant potential. In the subsequent determination step, the potentiostat is transformed into a galvanostat and the reduced and amalgamated analytes are re-oxidised electrochemically by means of an applied constant oxidative current. The change of potential at the working electrode with time during the stripping of the deposited analyte is recorded and processed. The response is digitally processed into dt/dE (the inverse potential derivative with time) versus potential (E), with the amount of deposited analyte determined by integrating the corresponding stripping peak. In order to avoid the fouling problem, which is a characteristic of many electroanalytical methods, low cost, disposable screen-printed electrodes were used in this investigation. The electrodes were produced in-house and were capable of handling small volumes of samples. The electrodes were used with a portable potentiostat, PalmSens (Palm Instruments BV, The Netherlands) for field analysis. Water and soil samples extracted with acetic acid from a site contaminated with high levels of heavy metals, were tested using these electrodes.

4.3 EXPERIMENTAL

4.3.1 Chemicals

Experimental solutions were prepared using purified water of HPLC grade from BDH (Poole, England). All glassware were cleaned with 1% HNO_3 and carefully rinsed with reversed osmosis water to avoid contamination. All chemicals were of analytical grade. L-cysteine (Cys), Tris(hydroxymethyl)methylamine (Tris), Hydrochloric acid

(HCl), potassium chloride (KCl) and mercury (II) atomic absorption standard solution (1000 mg l⁻¹ in 1% nitric acid) were obtained from BDH (Poole, England). The cadmium (II) and lead (II) atomic absorption standard stock solution (994 µg ml⁻¹ in 1% HNO₃) was from Sigma (St. Louis, U.S.A). The buffer used in the experiments was 0.025 M Tris-HCl containing 0.1 M KCl, pH 7.4 for cadmium (II) and pH 6.4 for lead (II). For copper (II), buffer used for the analysis was 2 M HCl. Leachate solutions from extracted soil samples were supplied by Tecnicas de Proteccion Ambiental (TPA), S.A., Madrid (Spain) and Grup de Techniques de Separació (Universitat Autònoma Barcelona, GTS-UAB, Spain).

4.3.2 Surface Characterisation of Screen-printed carbon electrode with SEM

Different mixtures (100 µl) including: (1) 10 mg l⁻¹ Hg (II) + 100 µg l⁻¹ Cd (II); (2) 10 mg l⁻¹ Hg (II) + 40 mM Cys; (3) 10 mg l⁻¹ Hg (II) + 40 mM Cys + 100 µg l⁻¹ Cd (II) in 0.025M Tris-HCl/0.1M KCl were preconcentrated on three different SPCE using Chronopotentiometry technique at -1V for 120s. The analysis was stopped without the performance of the stripping step. The left over samples were then discarded and the electrodes were washed carefully with reversed osmosis water to remove any residual buffer from the electrode surface which can mask the electrode during SEM analysis. The electrodes were then left to dry at room temperature. Silver in methyl isobutyl ketone (Acheson Electrode, Essex) was used to connect carbon working electrode with metal base of the sample plate. The XL30 SFEG Scanning electron microscope (SEM) (FEI company, Holland) was used to characterise the surface of the screen printed electrodes. The analysis was done based on the XLFEF/SFEG Scanning Electron Microscope operating instruction manual.

4.3.3 Measurement Procedure

Electrochemical measurements were carried out using a computer controlled potentiostat-10, the Autolab Electrochemical Analyser with general purpose electrochemical software operating system GPES version 4.8 (Ecochemie, Utrecht, The Netherlands). The electrodes were also used with a portable potentiostat, PalmSens (Palm Instruments BV, The Netherlands) for field analysis using a specially adapted electrical edge connector (Maplin, Milton Keynes, UK) (Plate 4.1). The in-house fabricated screen-printed electrodes (chapter 2, section 2.3.4) were used for the

electrochemical measurements with connection made to the electrochemical analyzer and portable potentiostat using a specially adapted electrical edge connector from Maplin (Milton Keynes, UK). Tests were carried out by placing a 100 μ l sample on a new three-electrode strip for each experiment in unstirred and non-deaerated solution. Experiments were carried out using the CCSCP technique by *in situ* deposition of 10 mg l^{-1} Hg (II), 40 mM Cys (for cadmium (II)), 20 mM Cys (for lead (II) and Cu (II), the target cadmium (II) and lead (II) at a potential of -1 V and Cu (II) at a potential of -0.9 V (vs. screen-printed Ag/AgCl) for 120s, respectively. A stripping current of 1 μ A was applied during the measurement step and the process terminated at 0 V (vs. screen-printed Ag/AgCl). Comparison was made with the use of 10 mg l^{-1} Hg (II) for the formation of mercury film electrode. Screen-printed electrodes were used without any prior cleaning performed. All dilution was made using 0.025 M Tris-HCl containing 0.1 M KCl, pH 7.4 (for cadmium (II) and lead (II)) and 2 M HCl (for copper (II)). The pH of the mixed solution was adjusted if required (for cadmium (II) and lead (II)) using more concentrated buffer (0.4 M).

For sample analysis, appropriate dilution of extract solutions (BCR acetic acid bioavailable fraction) from soil samples were modified with 10 mg l^{-1} Hg (II), 40 mM Cys and analysed by spiking with appropriate concentrations of standard cadmium (II) or lead (II) solution in 0.025 M Tris-HCl buffer containing 0.1 M KCl, pH 7.4. Direct calibration method and method of standard addition was applied. The results were compared with those obtained independently by Atomic Absorption Spectrometry (AAS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

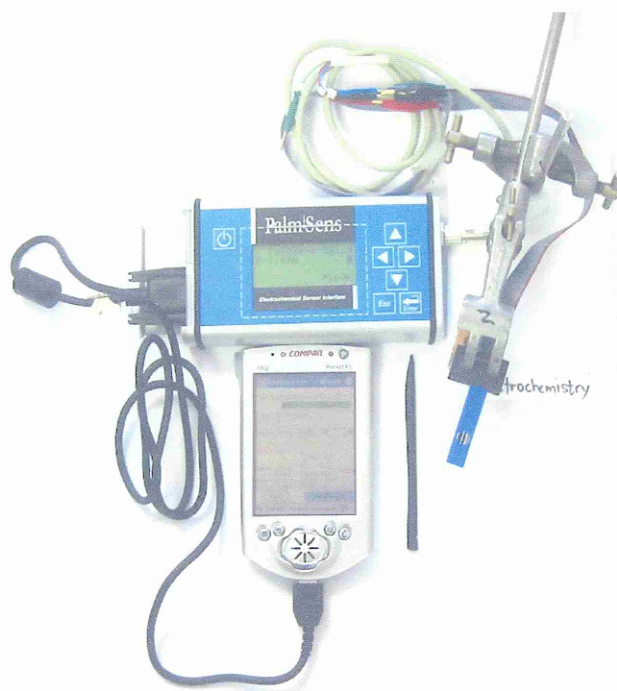


Plate 4.1: PalmSens with screen-printed carbon electrode.

4.4 RESULTS

4.4.1 Selection of optimum measurement conditions for CCSCP analysis of cadmium (II) and lead (II)

Optimization of the sensor's response relies on several measurement variables. These variables include the effect of Cys concentration, mercury concentration, deposition time, modification (electrolysis) potential and stripping current. A potassium chloride concentration of 0.1 M was used in the buffer solution due to the chloride dependency of the screen-printed Ag/AgCl reference electrode. The chloride level should be controlled for every analysis in order to maintain the reference electrode potential (Palchetti *et al.*, 1999; Choi *et al.*, 2001; Kadara *et al.*, 2003). Stripping time peak area was used throughout the analysis. The stripping area should be proportional to the amount of metal reduced during the deposition step, i.e. the free metal ion plus that dissociated from the complex during the time scale of the experiment (Town., 1999).

4.4.2 Cysteine concentration and reaction mechanism

The effect of Cys on the sensitivity of the resulting modified electrodes for cadmium (II), lead (II) and copper (II) analysis was investigated. Cysteine solution was prepared in 0.025 M Tris-HCl, 0.1M KCl, pH 7.4 as supporting electrolyte with 10 mg l⁻¹ Hg and 50 µg l⁻¹ cadmium (II) and lead (II), respectively. Various concentration of Cys from 0.5 mM to 80 mM were investigated. The cadmium (II) signal (peak area) increased more than ~ 60% upon raising the Cys concentration from 5 to 10 mM and in average remained at that level until 80 mM (Figure 4.1). For lead (II), the peak area increased more than ~ 80 % at 10 mM and ~ 100 % at 30 mM cysteine (Figure 4.1). The lead (II) peak doesn't increase significantly after 30 mM cysteine used. Figure 4.2, shows a sharp cadmium (II) peak (Hg-Cys-Cd-SPE) is detected at a potential near to cadmium (II) peak potential (-0.75 V) using mercury film electrode. This peak (Hg-Cd-SPE) is due to the oxidation of Cd (0) from the mercury film electrode. Significant increase in stripping potentiogram of cadmium (II) and lead (II) with 10 mM Cys modified electrode are observed compared to the use of mercury film alone.

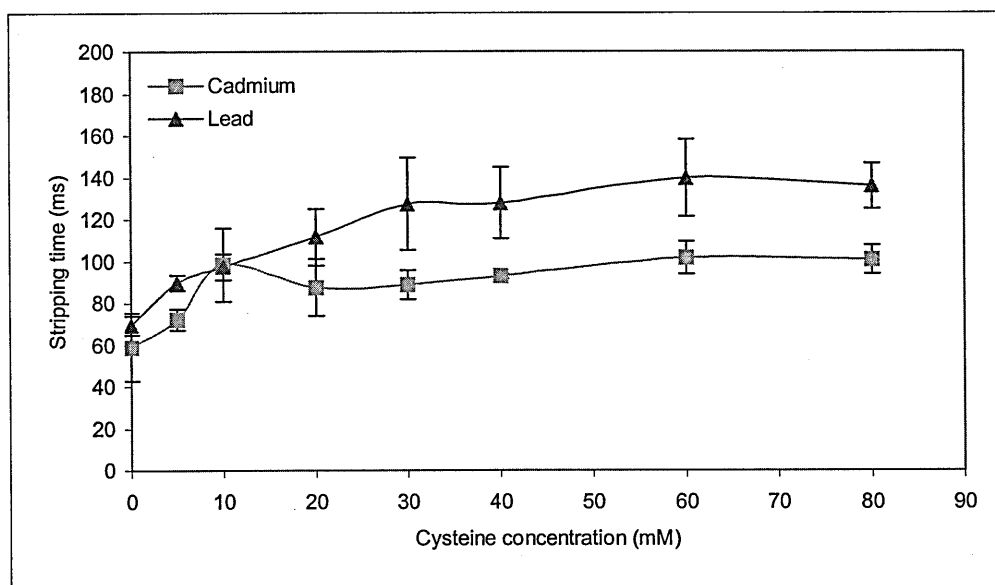


Figure 4.1. Effect of cysteine concentration on the constant current stripping chronopotentiometric of cadmium (II) and lead (II). Experimental condition: 50 µg l⁻¹ cadmium (II) and lead (II), 10 mg l⁻¹ Hg (II), 0.025 M Tris-HCl/0.1M KCl. Deposition potential = -1 V, deposition time = 2 min, constant current = 1 µA.

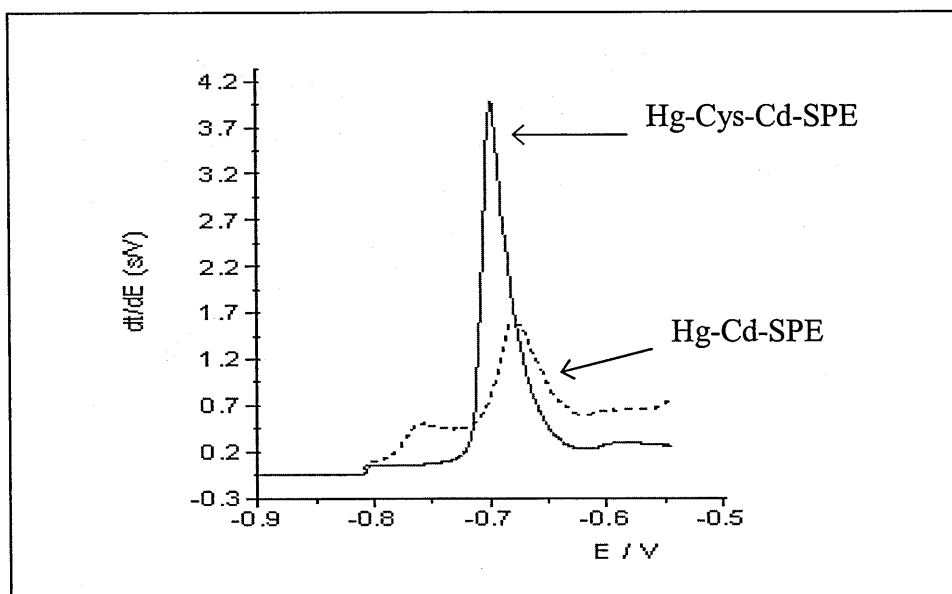
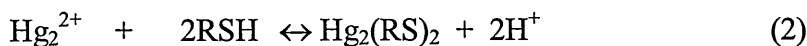


Figure 4.2. Stripping chronopotentiograms of cadmium (II) with and without addition of 10 mM cysteine. Experimental condition: $50 \mu\text{g l}^{-1}$ cadmium (II), 10 mg l^{-1} Hg, 0.025 M Tris-HCl/0.1M KCl. Deposition potential = -1V, deposition time = 2 min, constant current = $1 \mu\text{A}$. Hg-Cd-SPE : Cadmium (II) with mercury on screen-printed electrode. Hg-Cys-Cd-SPE : Cadmium (II) with mercury and addition of cysteine on screen-printed electrode.

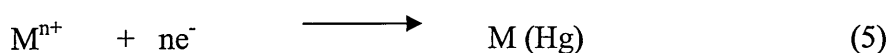
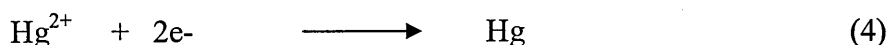
However, to achieve optimal sensitivity and reproducibility, 40 mM and 20 mM of Cys was used for further analysis of cadmium (II) and lead (II), respectively. The use of more concentrated Cys is not recommended because it can create high background interference. The enhancement in sensitivity has also been reported for Cu (II) peak at 10-35 mM cysteine when anodic stripping voltammetry and potentiometric stripping analysis were used (Bai *et al.*, 1998; Tanaka & Yoshida, 1982). Tanaka and Yoshida (1982) suggested that the complex-forming reaction between the mercapto-group and cupric ion is involved in the enhancement effect.

The electrochemical response of -SH containing compounds on mercury electrodes is still controversial in some respects, but it is generally agreed that it involves a strong adsorption step, and a predominantly diffusion-controlled mechanism. Such a mechanism involves the electrochemical oxidation of the mercury from the electrode to produce mercuric/mercurous thiolate (Mendieta *et al.*, 1996). The complex

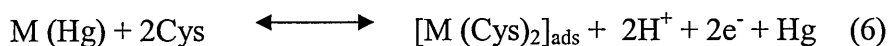
interactions of Cys with mercury has been studied and analyzed in details by many researchers and the anodic reaction is as follows (Heyrovsky *et al.*, 1994;1997):



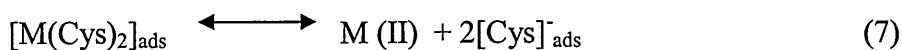
If mercury on the electrode is in the form of Hg_2^{2+} (Eq. (1)), it will subsequently complex with Cys in the solution (Eq. (2)) where the chelating occurs mainly via the sulfur atom (Stankovich & Bard 1977; Cakir *et al.* 1999, Kozlowski *et al.*, 1990). This complex creates a reversible process where the anodic peak corresponds to the oxidation of mercuric Cys thiolate, RS-Hg-SR at -0.02 V in cyclic voltammetry. In the solution, the preconcentration step in CCSCP involves metal ions in the solution being reduced and simultaneously amalgamated (cathodic deposition) on the working electrode at constant potential (-1 V) according to reactions:

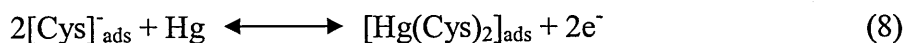


The following reaction is proposed for binding of the metal (M) by the cysteine which has two stages according to Tanaka & Yoshida (1982):



The metal in the amalgam is oxidized to form the adsorbing complex with Cys diffused from the solution. The complex subsequently decomposes to metallic ion and Cys^- at potential more positive than the deposition value. The released Cys^- oxidizes mercury to form the mercuric complex, as described in the equation below:





This reaction was proposed base to the evident in the case of copper, as observed by ASV, since the Cu (I) produced in the reaction is stabilized by the presence of both chloride and cysteine in the solution (Van den Berg *et al.*, 1988). The formation of a Cu (I) complex with the ligand may also leave a spare chelating group for the mercury to bind, hence yielding the formation of an intermetallic complex between Hg (I), Cu (I) and the –SH group, which will then favour copper deposition on the surface of the mercury electrode (Bai *et al.*, 1998). Cadmium may also be able to reach valency I, thus having the same suppression effect on the mercuric complex as copper [Miller & Teva, 1972]. The possibility of enhancing the response by CCSCP suggests that the technique somehow enables Cd (II) to either react with the mercuric complex adsorbed on the electrode surface or substitute the mercury in the thiolate. In CCSCP, the use of lower amounts of mercury (II) allows cadmium (II) to bind the added cysteine. A current is applied for reoxidation of the deposited metals, but unlike ASV, the mercury present in the solution also acts as oxidant, thus producing more dramatic stripping of the amalgamated metals. This effect is translated into the enhanced sensitivity. At the experimental pH (7.4), all the groups present in the ligand molecules are unprotonated. Among the metals present in the solution, Hg (II) has the strongest affinity towards the –SH group and is well known to bind to it and form linear complexes (Dolphin *et al.*, 1989). Therefore, the most likely electrode conformation might be characterised by the adsorption of a mercuric thiolate in which the hydrophilic end Hg-S-R is anchored at the mercury surface and the hydrophobic part –CH(NH₂)COO[–] is turned towards the solution (Heyrovsky *et al.*, 1997). The unprotonated amino and carboxyl groups may therefore be able to bind with other metals in the solution (Bello Rodriguez, 2004).

4.4.3 Mercury concentration

The influence of mercury on the size of the stripping peak area during analysis of cadmium (II) and lead (II) were investigated using 50 µg l^{–1} cadmium (II) and lead (II) with 40 and 20 mM Cys, respectively (Figure 4.3). For cadmium (II) and lead (II), no peaks were observed when 1 and 5 mg l^{–1} mercury were used. This was due to insufficient concentration of mercury for the Cys-Cd complex to adsorb on the mercury surface. The optimum peak was obtained at 10 mg l^{–1} mercury and starts to

level off above this level. Selection of appropriate concentration of mercury is important to achieve optimum stripping time. Our results indicate that 10 mg l^{-1} is sufficient to achieved reproducible peak area (R.S.D. 6%) and lower mercury content is important to minimise mercury waste. The strategy in developing this sensor is to use lower concentration of mercury by adding 40 mM cys (for cadmium (II)) and 20 mM Cys (for lead (II)) in the system for the formation of mercuric cysteinate film on the electrode at deposition potential -1 V. Other researchers use higher mercury concentration (80 mg l^{-1}) (Wang & Tian 1992; Choi *et al.* 2001) for preplating on the screen-printed electrode. The reduction in mercury concentration from 80 mg l^{-1} to 10 mg l^{-1} only is highly beneficial in reducing mercury use, but the use of 10 mg l^{-1} is essential to achieve a reproducible signal. Therefore, all subsequent work employed a mercury concentration of 10 mg l^{-1} .

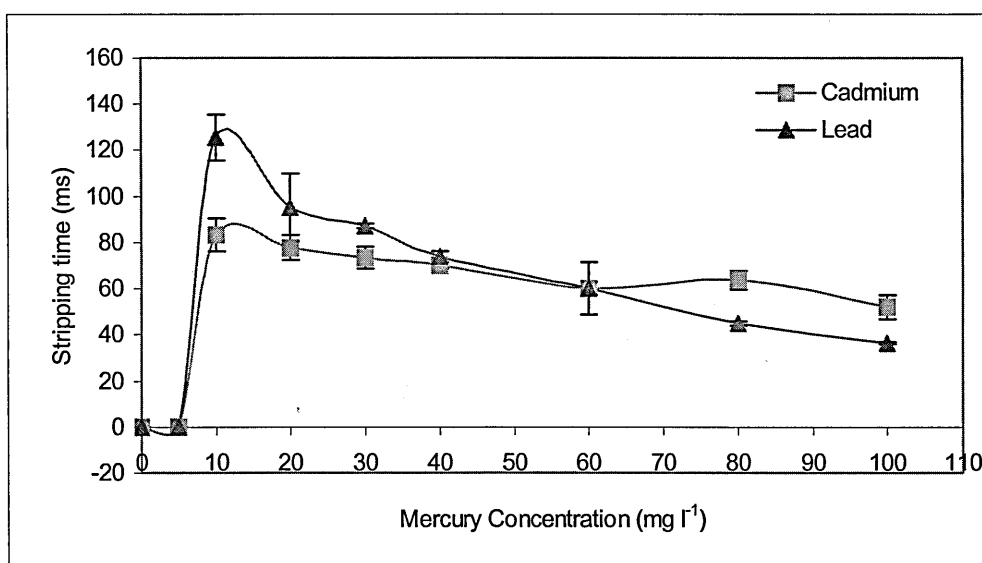


Figure 4.3. Effect of mercury (II) concentration on stripping peak area of $50 \text{ } \mu\text{g l}^{-1}$ cadmium (II) and lead (II). Experimental condition: 10 mg l^{-1} Hg, 0.025 M Tris-HCl/0.1M KCl, pH 7.4 & 6.4, respectively. Deposition potential = -1V, deposition time = 2 min, constant current = $1 \text{ } \mu\text{A}$.

4.4.4 Deposition potential.

A further parameter that can influence the stripping time (peak area) is the deposition potential. The effect of the deposition potential was evaluated over the -0.6 V to -1.3 V range ($50 \mu\text{g l}^{-1}$, 2 min deposition for unstirred nondeaerated solution) for cadmium (II) and lead (II), respectively (Figure 4.4). For cadmium (II), the results showed that no signal was observed at deposition potential of -0.6 V to -0.8 V, which indicate that the potential is not sufficient to reduce Hg-Cys-Cd (II) on the working electrode. The stripping time increased gradually with the potential between -0.9 and -1.1 V, and then it started to level off. Further reduction of the deposition potential (to -1.3 V) resulted in diminished stripping peak heights, the formation of multiple stripping peaks and increased background currents. These negative responses, which were most likely caused by hydrogen evolution and subsequent destruction of the mercury-cysteine film, were avoided by keeping the deposition potential more positive. For lead (II), the peak started to appear at -0.7 V and remain at the same levels until -0.9 V. The lead (II) peak area started to increase at -1 V, level off at -1.2 V and decreased after this potential. Experimental results indicated that the modification (deposition) potential of -1 V produced the best sensitivity with lower background interferences and hence, was used in all subsequent CCSCP determination of cadmium (II) ion.

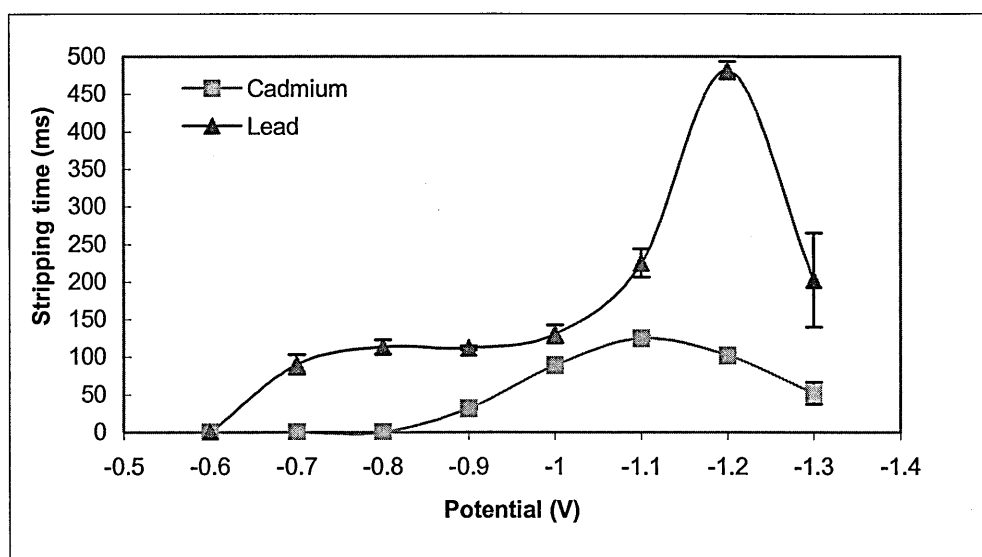


Figure 4.4: Effect of deposition potential on stripping response of $50 \mu\text{g l}^{-1}$ cadmium (II) and lead (II) in 0.025 M Tris-HCl/ 0.1 M KCl. Other of experimental conditions are as Figure 4.2.

4.4.5 Deposition time.

As expected for a stripping operation, the response of the screen-printed electrodes is strongly dependent upon the deposition time. For the selected deposition potential of -1.0 V, Figure 4.5 displays plot for 25 and $50 \mu\text{g l}^{-1}$ cadmium (II) and Figure 4.7 shows $50 \mu\text{g l}^{-1}$ lead (II) after different deposition periods (0-360 s), respectively. For lead (II), the same samples were kept for two days and rerun for lead (II) response. Increasing the deposition time resulted in increased signals (peak stripping heights and area) due to the increased amount of cadmium (II) and lead (II) ion reduced at the working electrode surface. A very short accumulation times (e.g. 30s) are sufficient for obtaining favorable cadmium (II) and lead (II) signals. In the case of cadmium (II), the resulting plot of stripping time versus deposition time is characterized with a linear relationship at $25 \mu\text{g l}^{-1}$ but not linear at $50 \mu\text{g l}^{-1}$ between peak area and deposition time up to 360 s. The responses do not level off within 5 minutes. The deposition time was set to 2 minutes. By employing a deposition time shorter than that of the saturation level, the dynamic range can be improved to 2 orders of magnitude, despite a sacrifice in sensitivity (Liu *et al.* 1999). Longer pre-concentration periods allow convenient quantitation of sub-ppb concentrations of cadmium (II). Freshly prepared samples with cysteine have to be used all the time to keep the optimum signal. This is due to decrease stripping signal for lead (II) for samples that were kept for two days (Figure 4.6).

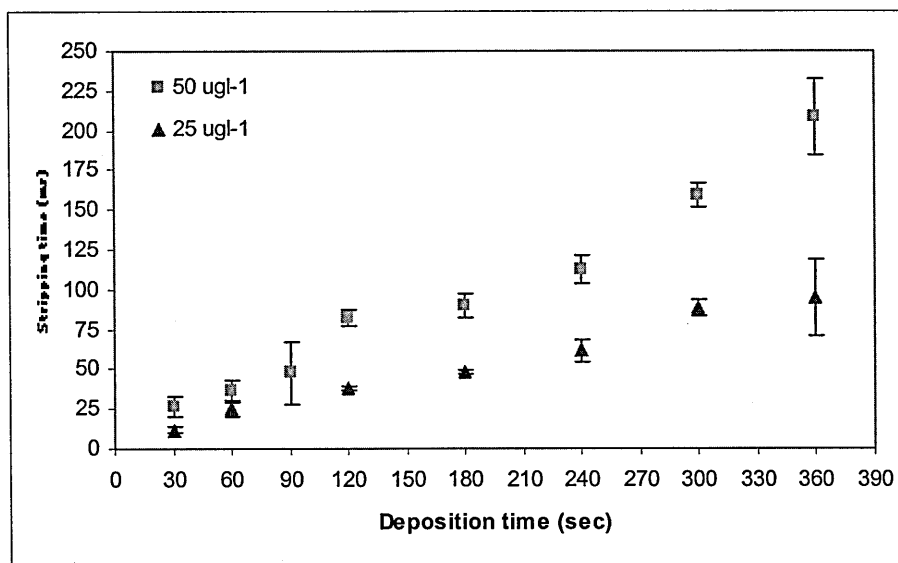


Figure 4.5. Effect of deposition time on the constant current stripping chronopotentiometric of 25 and 50 $\mu\text{g l}^{-1}$ cadmium (II) using 40 mM cysteine. Other experimental conditions as for Figure 4.2.

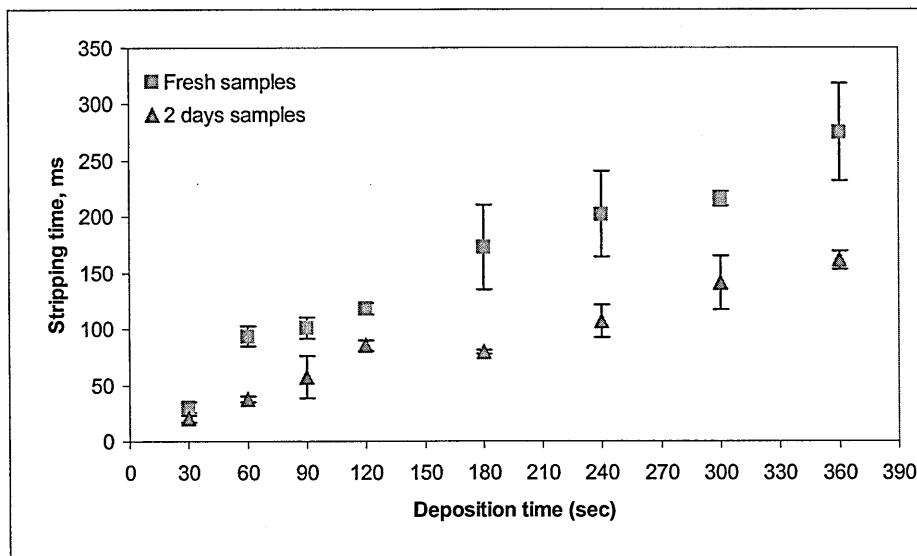


Figure. 4.6: Effect of deposition time on the constant current stripping chronopotentiometric of 50 $\mu\text{g l}^{-1}$ lead (II) in 0.025 M Tris-HCl/0.1M KCl for fresh samples and 2 days samples. Other experimental conditions as in Figure 4.2.

4.4.6 Stripping current

The effect of the stripping current on the cadmium (II) and lead (II) peak ($50 \mu\text{g l}^{-1}$) were also investigated (Figure 4.7). The stripping current was found to have a profound effect upon the cadmium (II) and lead (II) response and peak potential (E_p). A nearly exponential decrease in the stripping time was observed upon increasing the stripping current between 0.1 and 10 μA . Such profile reflects the faster oxidation (i.e., shorter stripping) of the deposited cadmium (II) and lead (II) at larger currents and the response decreased. The E_p also shifts to more positive values as the stripping current increases. This shift is due to an ohmic potential (iR) drop between the working and reference electrode (Beinrohr *et al.* 1994; 1996; 1999). From the studies carried out, higher response was obtained with a lower stripping current, 0.1 μA . However, stripping current of 1 μA was chosen for all subsequent work as compromise between signal reproducibility and sensitivity.

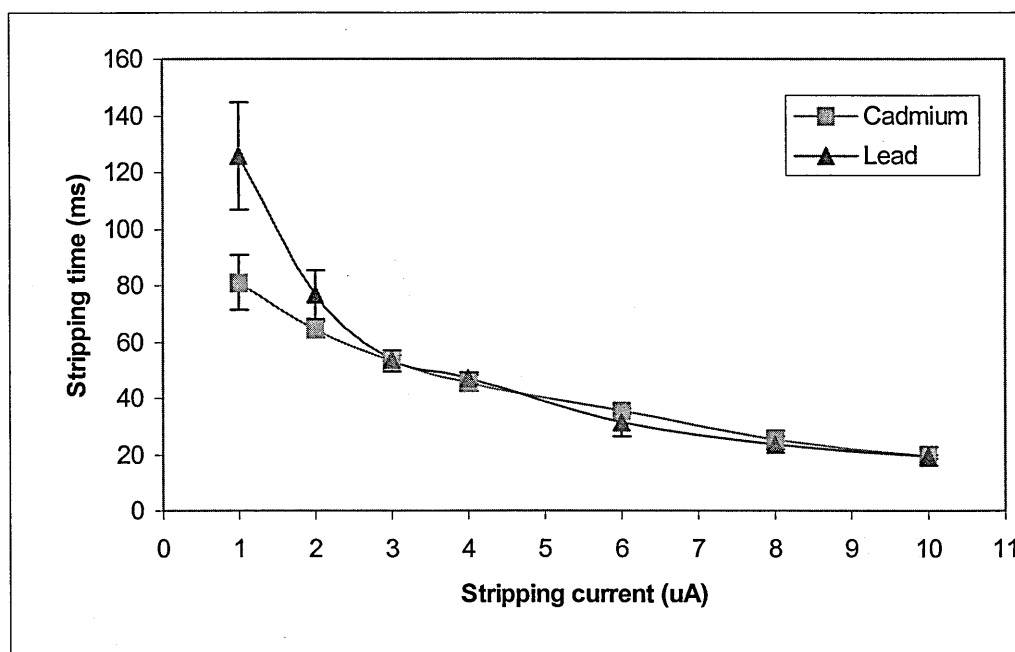
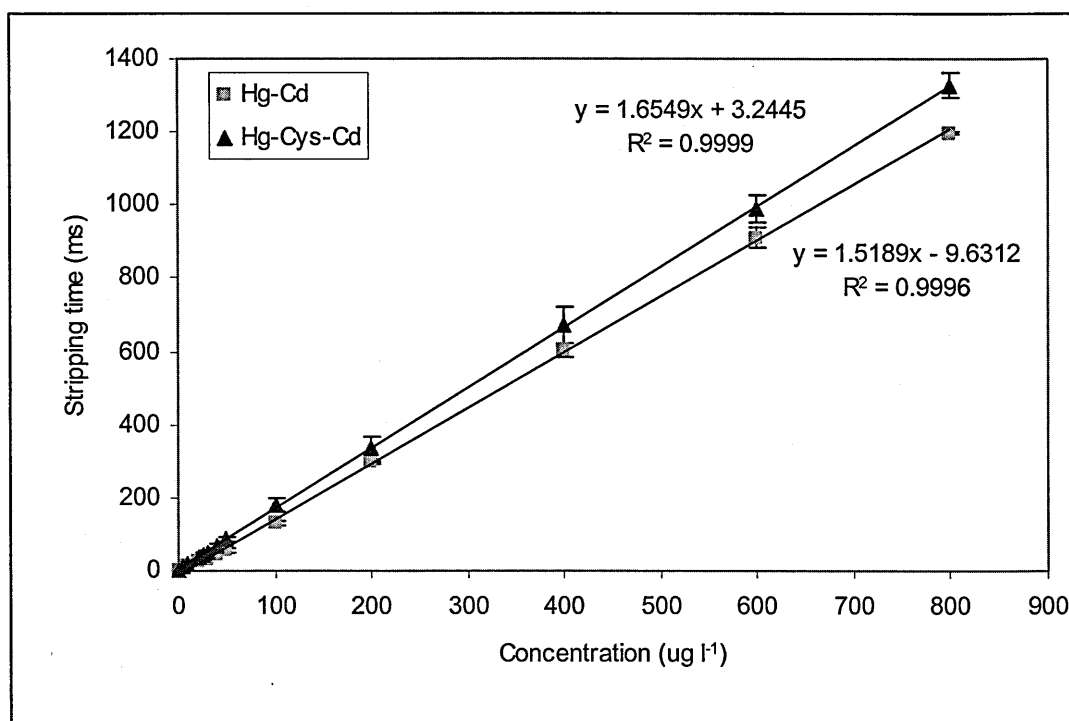


Figure 4.7: Effect of stripping current on the chronopotentiometric stripping response of $50 \mu\text{g l}^{-1}$ cadmium (II) and lead (II) in 0.025 M Tris-HCl/0.1 M KCl.

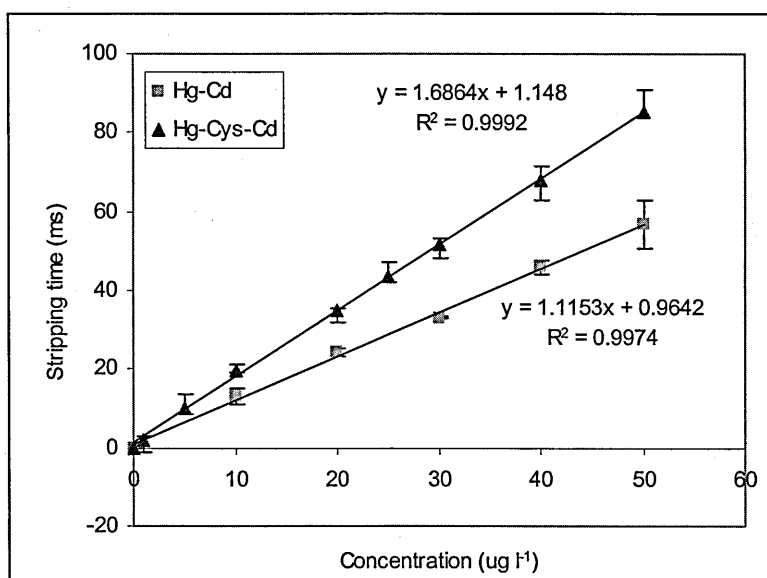
4.4.7 Calibration plot, detection limit and precision

Figure 4.8 (a), displays the comparison between two-calibration plot obtained from 0 to 800 $\mu\text{g l}^{-1}$ of cadmium (II) when 40 mM cysteine (Hg-Cys) and mercury film (Hg) electrodes are used. Hg-Cys electrode provides better stripping time (area) and lower concentration of cadmium (II) down to 0.4 $\mu\text{g l}^{-1}$ can be measured. The Hg film electrode is less sensitive and 10 $\mu\text{g l}^{-1}$ cadmium (II) is the lowest concentration that can be used for reproducible measurement. The difference on stripping time between the two techniques is bigger at higher concentration of cadmium (II) measured. The Hg-Cys electrode can still show good sensitivity at high concentration of cadmium (II). Well-defined peaks are observed following a 2 min deposition period for both electrodes. The peak area of the dt/dE versus E curve was used for the determination of cadmium (II). For Hg-Cys-Cd electrode, the stripping time increases proportionally with the metal concentration to yield a linear calibration plot in the range examined, with a slope of 1.6549 $\mu\text{A/ppb}$ (0.4-800 $\mu\text{g l}^{-1}$, correlation coefficient, $r^2=0.9999$, $n=13$) and 1.6864 $\mu\text{A/ppb}$ (0.4-50 $\mu\text{g l}^{-1}$, correlation coefficient, $r^2=0.9999$, $n=8$), respectively. For Hg-Cd, a slope of 1.5189 $\mu\text{A/ppb}$ was obtained from range 2-800 $\mu\text{g l}^{-1}$, correlation coefficient, $r^2=0.9996$, $n=11$) and 1.1153 $\mu\text{A/ppb}$ (2-50 $\mu\text{g l}^{-1}$, correlation coefficient, $r^2=0.9642$, $n=6$), respectively. Each calibration point was an average of 3 readings.

For Hg-Cys-Pb, a linear calibration plot obtained with a slope of 1.8264 $\mu\text{A/ppb}$ (0.7-1000 $\mu\text{g l}^{-1}$, correlation coefficient, $r^2=0.9991$, $n=12$) (Figure 4.9). The detection limit was evaluated with the criterion $3 \times S_b/m$, where S_b is the standard deviation of the intercept and m is the slope of the linear plot (Kadara & Tothill, 2004). The lowest limit of detection for cadmium (II) and lead (II) were 0.4 $\mu\text{g l}^{-1}$ in 0.025 M Tris-HCl/0.1M KCl, pH 7.4 and 0.7 $\mu\text{g l}^{-1}$ in 0.025 M Tris-HCl/0.1M KCl, pH 6.4 with a deposition time of 2 min. As comparison, the Hg film electrode produced a detection limit of 2 and 3 $\mu\text{g l}^{-1}$ for cadmium (II) and lead (II) which is about five times higher than the Hg-Cys electrode.



(a)



(b)

Figure 4.8. Calibration plot (a) from 0 to 800 $\mu\text{g l}^{-1}$ and (b) from 0 to 50 $\mu\text{g l}^{-1}$ for increasing concentration of cadmium (II) with 40 mM cysteine (Hg-Cys-Cd) and without using cysteine (Hg-Cd), respectively. Other experimental condition as for 4.2.

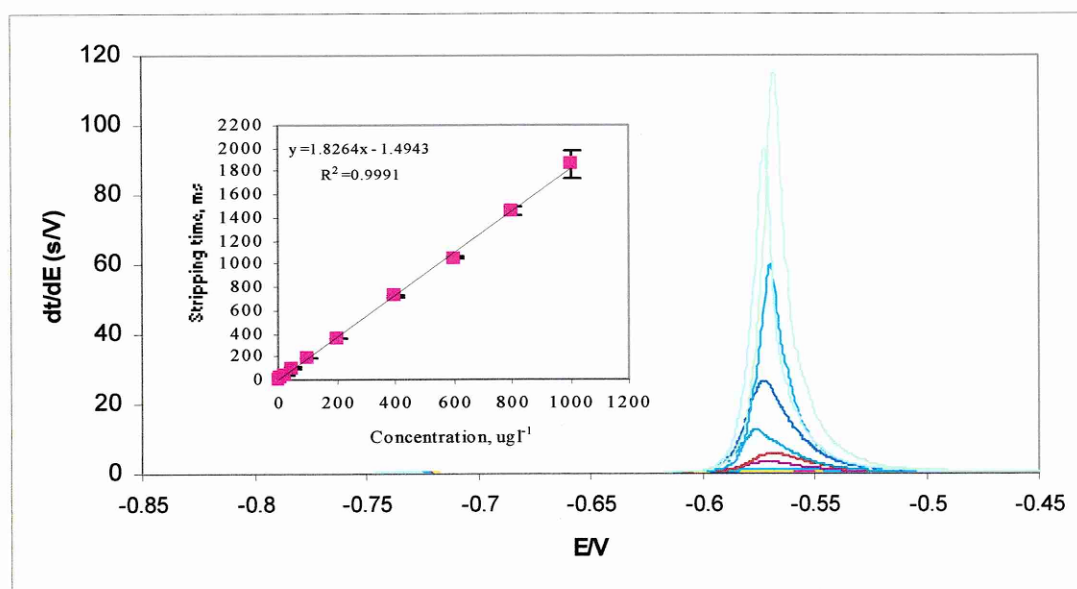


Figure 4.9. Calibration plot for lead (II) from 0.7 to 1000 $\mu\text{g l}^{-1}$ in 0.025M Tris-HCl/0.1M KCl, pH 6.4 with 20 mM cysteine.

For accurate determination of an element, the reproducibility of the analytical signal is an important parameter, which can influence the analytical signal. The sensitive response of the cadmium (II) and lead (II) measurement is highly reproducible, as was indicated by the relative standard deviation (5.9% and 6.5%) obtained for a series of 8 measurements of 10 $\mu\text{g l}^{-1}$ cadmium (II) and lead (II) standard solution (2 min deposition), respectively. The low detection limit and simplicity of the techniques suggested that cysteine modified screen-printed electrodes could be used for the analysis of cadmium (II) and lead (II) in environmental samples.

4.4.8 Effect of lead and Copper on the cadmium Stripping peak

To detect the selectivity and stability of Hg-Cys-Cd-SPE electrodes, the influence of Pb (II) and Cu (II) on the modified electrodes were examined. Lead (II) and Cu (II) at different concentrations were added to the solution containing 50 $\mu\text{g l}^{-1}$ Cd (II). The results show that the peak of 50 $\mu\text{g l}^{-1}$ Cd (II) was not significantly influenced by excess of added Pb (II) (50 and 150 $\mu\text{g l}^{-1}$) and Cu (II) (25 and 50 $\mu\text{g l}^{-1}$) and was in the range of $\pm 5\%$ (Figure 4.10). The stripping peak for Cu were poorly defined and not stable even at higher concentration (150 $\mu\text{g l}^{-1}$) and probably due to surface reactions when the electrode is anodize or exposed to dissolved oxygen. By

appropriate choice of supporting medium, well-resolved and reproducible response for copper could be obtained (Kadara *et al.* 2003). Therefore, we could conclude that the Hg-Cys-Cd-SPE modified electrodes shows excellent selectivity against the influence of Pb (II) and Cu (II) in the samples.

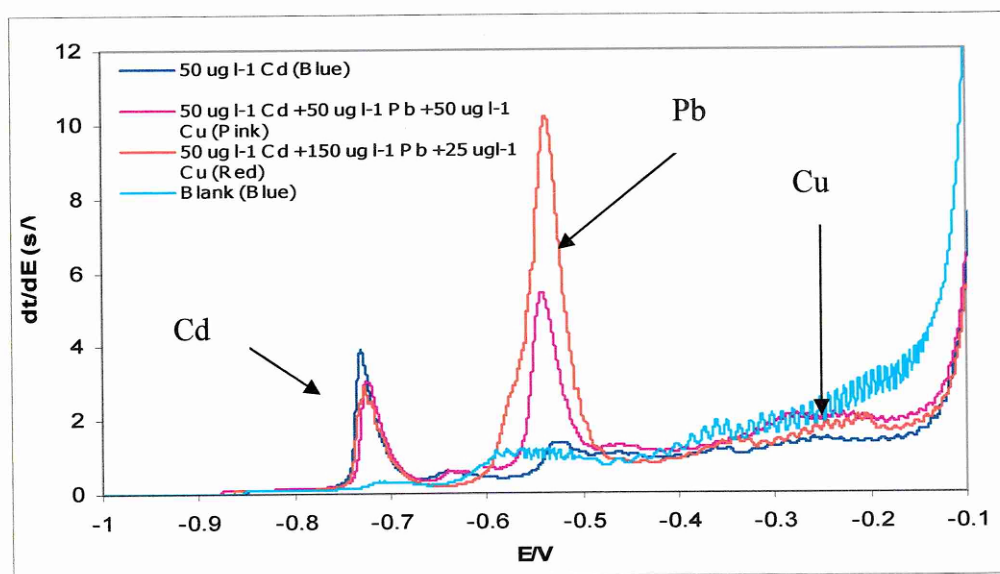
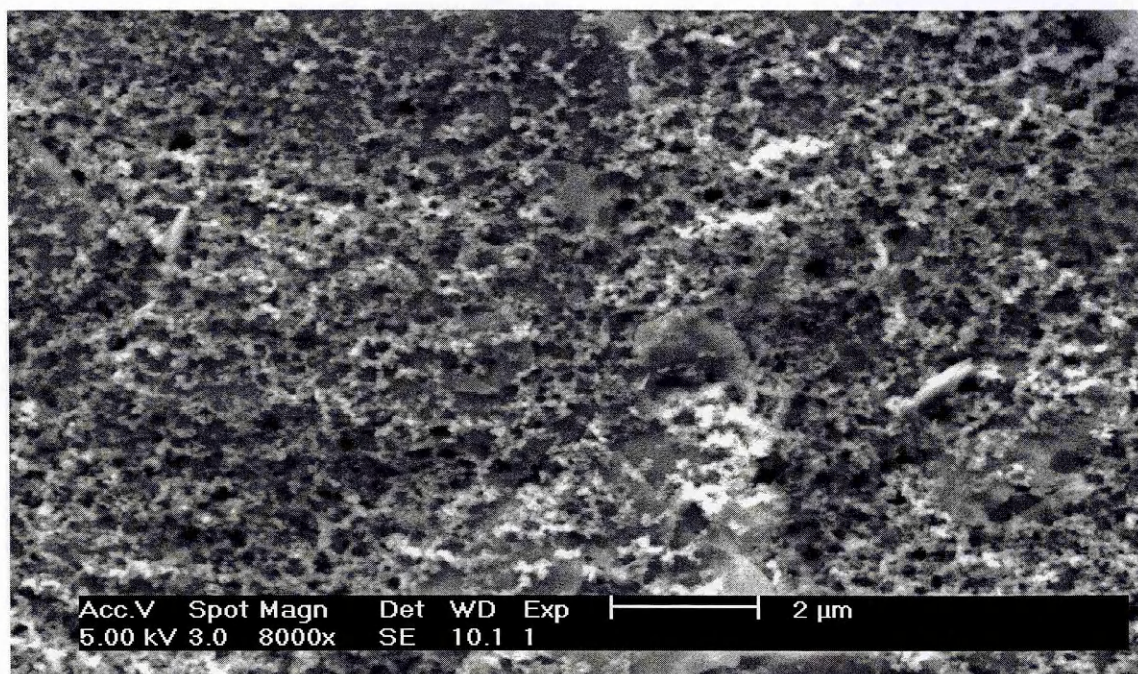


Figure 4.10: Stripping chronopotentiograms of 50 $\mu\text{g l}^{-1}$ Cd (II) (blue line), 50 $\mu\text{g l}^{-1}$ Cd + 50 $\mu\text{g l}^{-1}$ Pb + 50 $\mu\text{g l}^{-1}$ Cu (pink line) and 50 $\mu\text{g l}^{-1}$ Cd + 150 $\mu\text{g l}^{-1}$ Pb + 25 $\mu\text{g l}^{-1}$ Cu (red line). Experimental condition: 10 mg l^{-1} Hg (II), 40 mM Cys in 0.025 M Tris-HCl/0.1M KCl. Deposition potential = -1V, deposition time = 2 min, constant current = 1 μA .

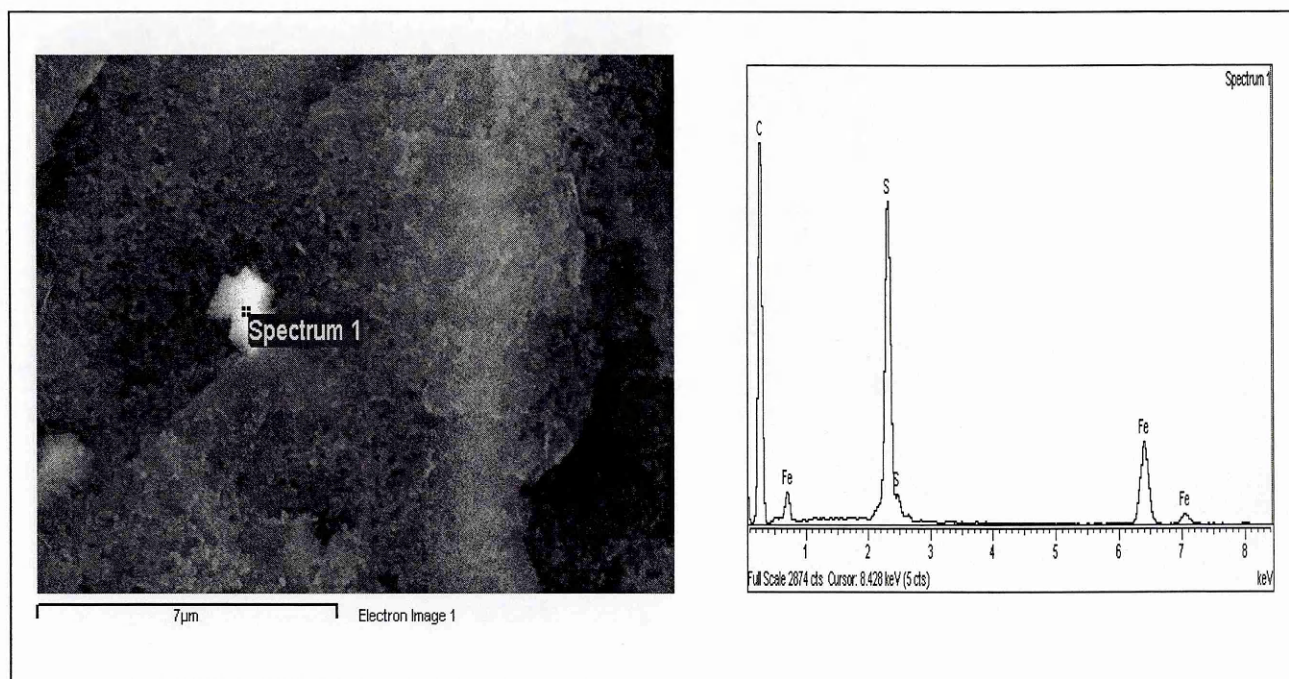
4.4.9 Surface characterisation of screen-printed carbon electrode by SEM

The physical form in which the metals, both Hg-Cys and analyte cations, are deposited by reduction on the electrode surface is important in determining the quality of the chronopotentiometric signal (Maccà *et al.*, 2004). The surface of SPCE was observed by Scanning Electron Microscopy (SEM). Figure 4.11 (a), shows a typical surface of SPCE with carbon working electrode fabricated in house at 1000 magnification. As observed in these images, the surface of SPCE appears to be relatively rough. This is due to graphite particles randomly distributed and oriented on the polyester sheet. The roughness and presence of clusters on the electrode surface have been reported to results in an increase in its active surface area (Moreno-Baron *et al.*, 2003). About 40 mM Cysteine in 0.025M Tris-HCl/0.1M KCl was

preconcentrated without stripping for 2 minutes on SPCE. The electrode was then rinsed with 1 mL reversed osmosis water to minimize the buffer effect on the electrode surface. Figure 4.11 (b) (left) shows the Cys on the SPCE and analysis result of its content is shown in Figure 4.11 (b) (right). The analysis proves that the spectrum is cysteine due to high concentration of sulfur peak at 2.3 keV. The preconcentration of Hg (II) for 120s at -1 V create Hg film and these can be clearly seen as numerous individual spherical micro droplets of $< 1 \mu\text{m}$ (Figure 4.11 (c) left). About $< 1 \%$ of the geometric area is covered by the Hg. The analysis shows that the micro droplet is Hg (Figure 11(c) right). Wang & Tian (1992) reported bigger shape of micro droplets (of 1-2 μm) and more than 20% of the geometric area covered by the mercury on SPCE. The bigger shape of droplet diameter is due to increasing the plating time (Maccà *et al.*, 2004) and bigger covered geometric area is probably due to the use of more concentrated Hg of 800 mg l^{-1} (Wang & Tian, 1992; Maccà *et al.*, 2004) for preplating the SPCE. Figure 4.11 (d) shows the formation of Cys-Hg film on the SPCE. The presence of Cys with only 10 mg l^{-1} Hg to form Cys-Hg film seems to give same diameter of droplets as Hg alone. As can be seen from Figure 4.11 (d) left, more geometric areas on SPCE was covered by Hg-Cys film as compared to Hg film alone. The results show that the formation of amalgamates between the Hg-Cys on the surface of the carbon electrode could not be clearly confirmed because the sulfur peak appears to overlap with the Hg peak on the analysis spectra. However, from the analysis Figure it can be seen that the Hg peak area has increased considerably when Cys was used an indication of the Hg-Cys amalgamate formation. It was also noted that when Cys was used the mercury-Cys micro droplets number increased on the surface of the electrode which can indicate the enhancement effect Cys has on the detection of metal ions. Since Cys-Cd and Hg-Cys-Cd SEM scans look similar to Hg-Cys, the Figures are not shown.

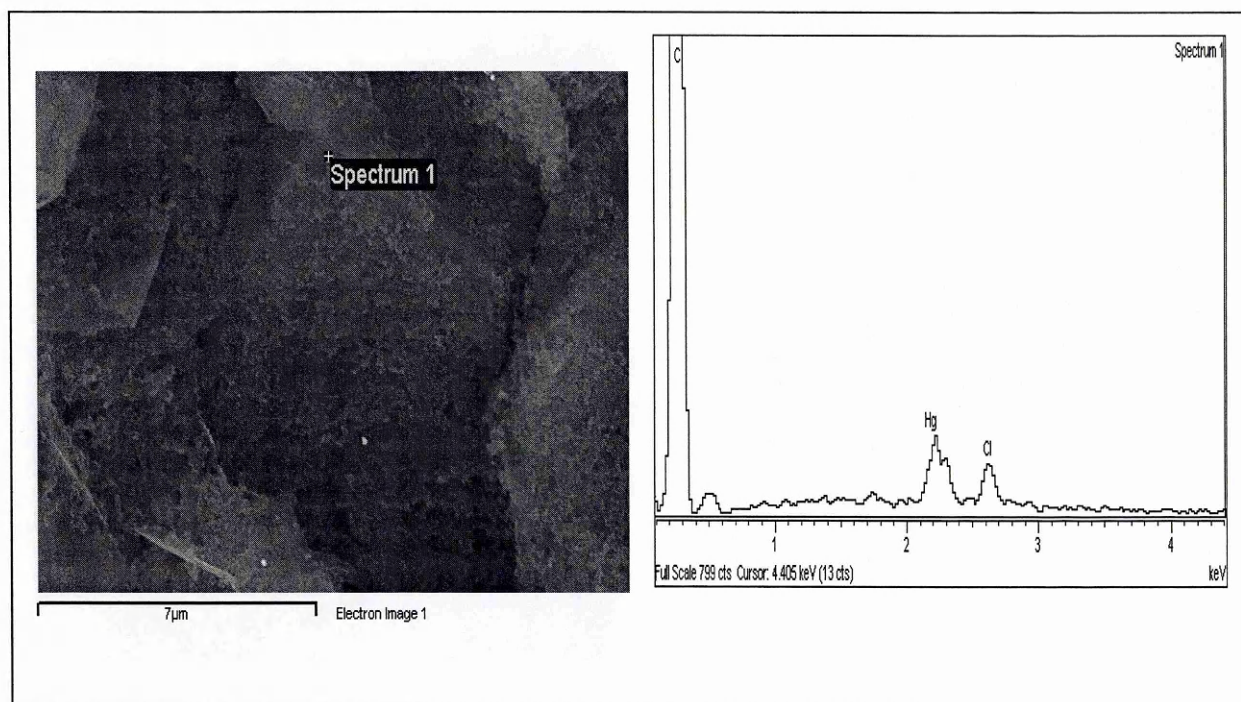


(a)

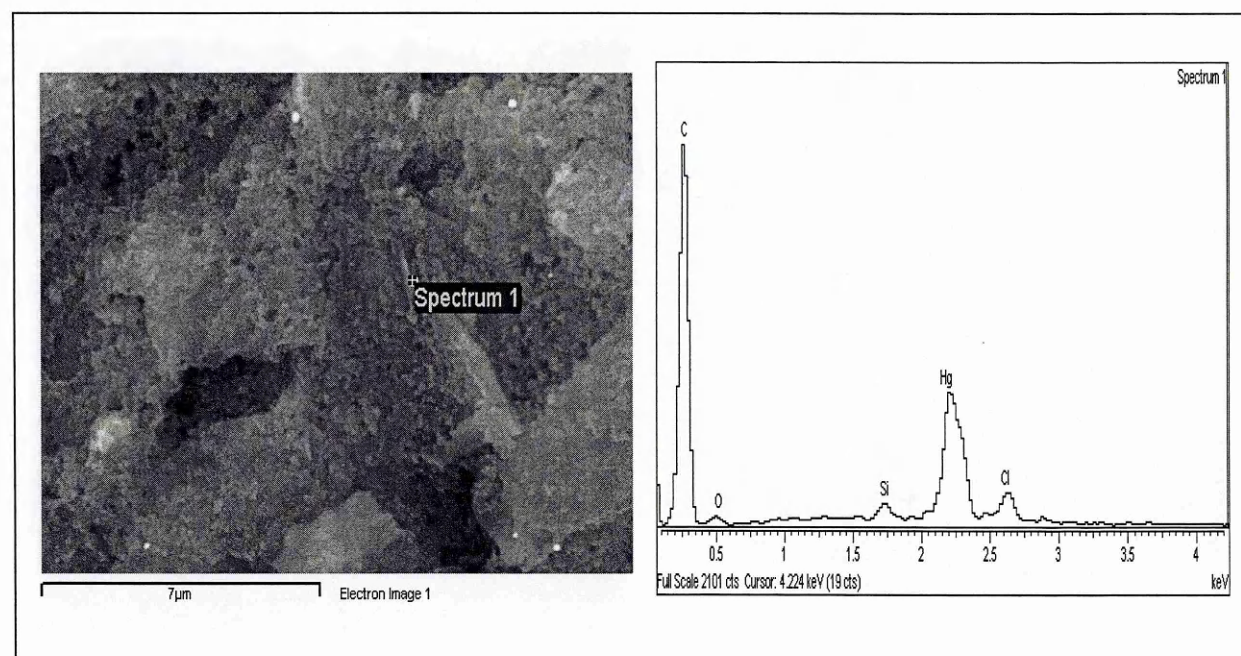


(b)

to be continued on next page.



(c)



(d)

Figure 4.11: SEM micrographs showing the surface features of various complexes formed on the surface of SPCE at 8000 magnification a) SPCE without amalgam b) $10 \text{ mg l}^{-1} \text{ Hg} + 100 \text{ μg l}^{-1} \text{ Cd}$ c) $10 \text{ mg l}^{-1} \text{ Hg} + 40 \text{ mM Cys}$ and d) $10 \text{ mg l}^{-1} \text{ Hg} + 40 \text{ mM Cys} + 100 \text{ μg l}^{-1} \text{ Cd}$.

4.4.10 Application to Water and Soil Samples Extracts

The analytical application of the cysteine-modified screen-printed electrode is demonstrated for the determination of trace cadmium (II) level in six soil samples extracted with acetic acid. The concentrations of cadmium (II) in the samples were determined by direct calibration and standard of addition method. Normally, the method of standard addition is used if matrix effect is observed through low recovery obtained from direct measurement of the samples and this is due to the chemicals used to extract the metals from the soil.

Four standard additions (of 50, 100, 200 and 300 $\mu\text{g l}^{-1}$) to the extracted soil samples resulted in well-defined stripping peaks. There were no interferences observed from other metals such as lead (II) and copper (II) in the samples analysed. The Cd (II) peak was well separated from lead (II) and copper (II) which was also contained in the extracted soil samples. The Cd (II) peak for the original sample can thus be quantified by means of the resulting standard addition plot (Figure 4.12). Summarized in Table 4.2 are the measured values obtained from 4 analysis from two different calibration methods. Comparison was made with the reference method, i.e. AAS and ICP-MS and the developed method can also be seen in Table 4.2. There is an agreement between the results for the AAS or ICP-MS and the modified electrode coupled to CCSCP for both methods. A relative high correlation coefficient value obtained between the two methods of determination ($r^2=0.9957$) confirms the validity of the proposed method (CCSCP coupled to MFESPCE). However, results obtained using the calibration plot is lower compared to standard addition method for all samples analysed. This is expected due to some matrix effect in the sample solution compared to the aqueous standard solution used for calibration. The recovery of cadmium (II) was determined by adding cadmium (II) solutions with known concentrations to the extracted samples. The range of recoveries was from 97% to 102%, suggesting that the Cys modified electrode is effective for the determination of trace levels of cadmium (II). The results show that acetic acid (0.11 M) extract of soil samples could provide good result using both calibration techniques.

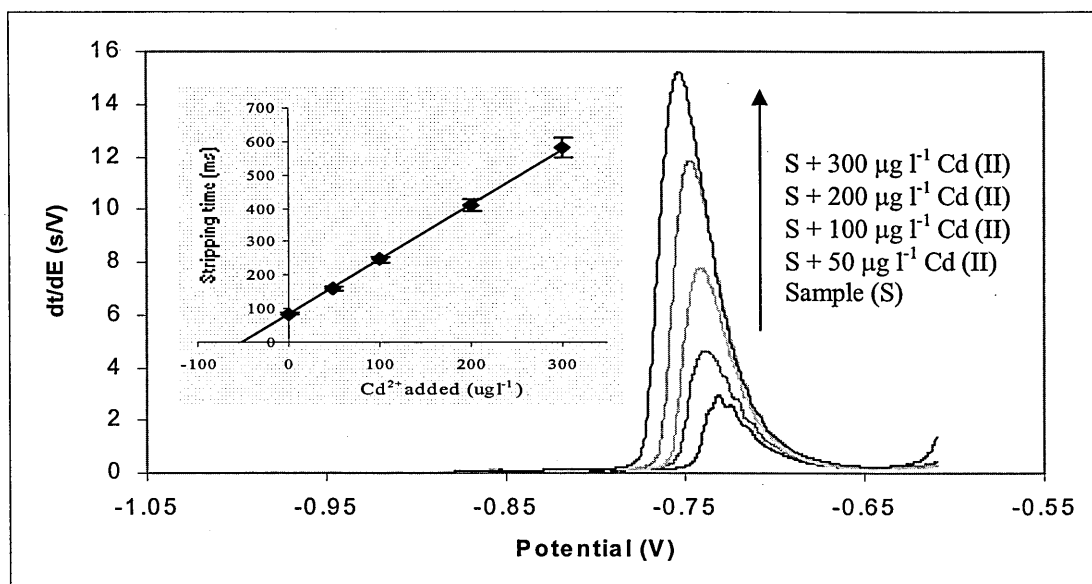


Figure 4.12: Stripping chronopotentiograms and standard addition plot (inset) for the determination of cadmium (II) in real soil sample using 0.025M Tris-HCl/0.1M KCl, pH 7.4 as buffer with 40 mM cysteine. Other experimental conditions are as for Figure 4.2.

Table 4.2. Measurements of cadmium (II) in soil samples extract.

Soil Samples Extracts	^a AAS ($\mu\text{g l}^{-1}$) / ^b ICP-MS ($\mu\text{g l}^{-1}$)	^c CCSCP ($\mu\text{g l}^{-1}$) Standard of addition	^c CCSCP ($\mu\text{g l}^{-1}$) Direct Calibration
^a No.1	49.72 ± 0.01	$48.95^{\text{d}} \pm 2.16$	$44.18^{\text{d}} \pm 2.16$
^a No.2	28.6 ± 1.0	$27.2^{\text{d}} \pm 2.64$	$21.66^{\text{d}} \pm 2.65$
^a No.3	29.7 ± 1.0	$25.4^{\text{d}} \pm 1.0$	$22.06^{\text{d}} \pm 3.9$
^b No.4	62 ± 2.03	77 ± 1.19	47.6 ± 1.2
^b No.5	195 ± 2.10	229 ± 2.67	109 ± 2.7
^b No.6	106 ± 26	127 ± 0.48	88 ± 0.5

^cexperiment carried out with 10 mg l^{-1} mercury, 40 mM Cysteine, 0.025 M Tris-HCl/0.1M KCl, pH 7.4 *in situ* plated, 120s, $1 \mu\text{A}$.

^dmean of 4 replicate measurements.

$\pm$ Standard Deviation

This sensor performed well when applied for water and soil samples solution analysis collected from contaminated site. The sensor was also tested for field analysis at a site contaminated by metal mining activities using a portable potentiostat, PalmSens (Palm Instruments BV, The Netherlands) in an European workshop (Senspol Technical Meeting, Sevilla) (Md Noh *et al.* 2004). Waste-water and soil samples were analysed for cadmium (II) and lead (II). The same samples were also analyzed in the laboratory using Autolab Electrochemical Analyser and the results for two water samples are shown in Table 4.3. The results achieved indicated that there was no need to use the standard addition method for the analysis of these water samples, as the direct calibration method was found to be good and agreed with the result obtained by ICP-MS.

Table 4.3. Measurements of cadmium (II) in waste-water samples.

Waste-water Samples	ICP-MS ($\mu\text{g l}^{-1}$)	^a CCSCP ($\mu\text{g l}^{-1}$) Direct Calibration
No. 1	620 $\pm$ 0.04	620 ^b $\pm$ 10
No. 2	630 $\pm$ 0.01	657 ^b $\pm$ 22

^aexperiment carried out with 10 mg l⁻¹ mercury, 40 mM Cysteine, 0.025 M Tris-HCl/0.1M KCl, pH 7.4 *in situ* plated, 120s, 1 μA .

^bmean of 3 replicate measurements.

$\pm$ Standard Deviation

Figure 4.13 shows calibration graph and corresponding stripping peak for lead (II) prepared at Senspol Technical Meeting, Sevilla) (Md Noh *et al.* 2004) using Palmsens. Figure 4.14 shows typical stripping chronopotentiograms and standard addition plot for the determination of lead (II) in the extracted soil samples on cysteine modified mercury film screen-printed carbon electrode. The results obtained for various soil samples are shown in Table 4.4 using standard addition and direct calibration method. Table 4.4 also shown results for the same samples when analysis done in the laboratory using the Autolab instrument. Comparison between two

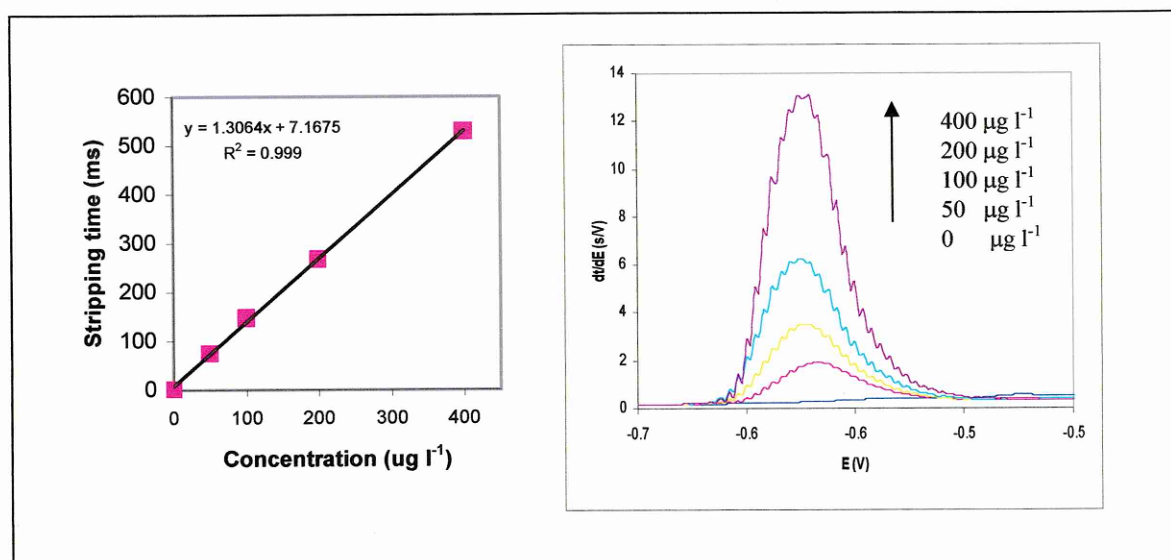


Figure 4.13: Calibration graph (left) and stripping chronopotentiograms of lead (II) standards from 0 – 400 $\mu\text{g l}^{-1}$ in 0.025 M Tris-HCl/0.1M KCl, pH 6.4. The standards were prepared using Palmsens at Senspol Technical Meeting, Seville, 2002.

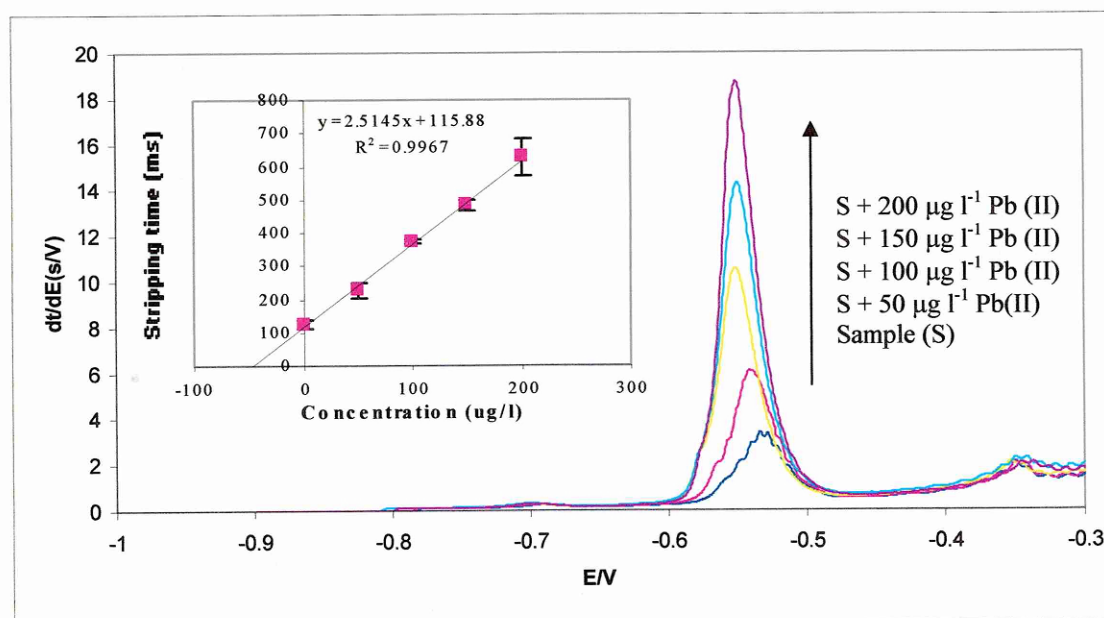


Figure 4.14: Stripping chronopotentiograms and standard addition plot (inset) for the determination of lead (II) in real soil sample using 0.025M Tris-HCl/0.1M KCl, pH 6.4 as buffer with 20 mM cysteine. Other experimental conditions are as for Figure 4.2.

different calibration methods was done. Lead (II) is easier to analyse due to its higher concentration in most of the samples. For acetic acid extract soil and sediment samples, results obtained with standard of addition method are better than direct calibration method. This is because the results obtained by method of standard addition are closer to the results provided by ICP-MS, a conventional analytical reference technique. It is suggested that method of standard addition should be a method of choice for acetic acid extract soil samples analysis. Table 4.4 also shows result for aqua regia extract of soil sample. The analysis with autolab shows that method of standard addition performs better than direct calibration method.

Lead (II) in waste-water samples (GT693 and GT694) showed good results with direct calibration compare to standard of addition (Table 4.4). Therefore, water samples can be analysed using direct calibration. Allowable lead (II) level for drinking water as regulated by EPA and EU are 15 ppb and 10 ppb, respectively. The detection limit of the sensor for lead (II) is < 1 ppb which is lower than EPA and EU allowable level and this would make the sensor very sensitive for measuring very low level of lead (II) in water sample. The reference sample (721 and 722) shows lower lead (II) contents compared to ICP-MS results using direct calibration method. However, the method of addition gives more accurate result.

Table 4.4: Results for Lead (II) Analysis using chronopotentiometric technique compared to ICPMS.

Sample		Aznalcollar	Post-Aznalcollar		ICPMS ($\mu\text{g l}^{-1}$)
		Direct Calibration (CCSCP) ($\mu\text{g l}^{-1}$)	Direct Calibration (CCSCP) ($\mu\text{g l}^{-1}$)	Method of Addition (CCSCP) ($\mu\text{g l}^{-1}$)	
GTS683	AA	-	2752 ± 5	2984 ± 5	3153 ± 38
	AR	3010	$2060 \pm$	-	2423 ± 21
GT684	AA	-	1418 ± 15	1422 ± 15	1537 ± 27
	AR	3090	$3040 \pm$		3046 ± 26
GT685	AA	-	136 ± 2	174 ± 2	146 ± 32
	AR	730	580 ± 3	784 ± 3	819 ± 13
GT686	AA	-		-	39 ± 32
	AR	73	17.1 ± 1	-	141 ± 15
GT687	AA	-		-	30 ± 32
	AR	260	232 ± 4	426 ± 4	446 ± 14
GT688	AA	-	175 ± 2	-	176 ± 8
	AR	470	248 ± 3	478 ± 3	530 ± 14
GT689	AA	-	-	-	<10
	AR	-	0.78 ± 1	-	38 ± 16
GT690	AA	-	-	-	<10
	AR	33	24 ± 1	-	111 ± 15
GT691	AA	-	2623 ± 2	3183 ± 2	2853 ± 35
	AR	3760	3256 ± 22	-	2799 ± 44
GT692	AA	-	205 ± 5	-	357 ± 7
	AR	4600	3453 ± 30	-	3155 ± 50
GT693		-	1038 ± 48	1207 ± 8	890 ± 20
GT694		-	1026 ± 1	1119 ± 1	740 ± 40
GT721c		-	405.2 ± 14	700 ± 14	664
GT722c		-	6099 ± 7	6864 ± 7	5294

AA-Acetic acid
extract

AR-Aqua regia
extract

(-) Analysis was not carried
out /limitation

4.5 OPTIMISATION OF EXPERIMENTAL VARIABLES FOR STRIPPING CHRONOPOTENTIOMETRIC MEASUREMENT OF COPPER

For the analysis of copper (II), the stripping peak obtained was obscured by the background signal from the supporting electrolyte solution, which is stripped at the potential range in which copper is detected (-0.4 V to -0.1 V). Such a great background signal could be due to surface reactions when the electrode material is anodized or exposed to dissolved oxygen (Kadara *et al.*, 2003). To solve this problem of the large background signal and ill-defined copper stripping peak obtained on the MFSPCE (10 mg l⁻¹ mercury plated *in situ*), the effect of increasing concentrations of HCl on the copper stripping chronopotentiometric response was investigated instead of increasing the concentration of mercury (II) plated *in situ*. The overlaid stripping chronopotentiograms obtained in Figure 4.15 shows that the copper-stripping peaks become more defined as the concentration of the chloride ion mediums exceed 1 M even though the magnitude of the stripping signal decreases as well. Hence, a chloride concentration of 2 M was used for subsequent experiments with HCl as the choice, because it is normally free from metal ion impurities.

4.5.1 Effect of mercury (II) concentration

Figure 4.16 shows the effect of mercury (II) concentration on the stripping-peak of copper (II) in 2 M HCl. In contrast to the results obtained with cadmium (II) and lead (II) where their peaks reach plateau at 10 mg l⁻¹, the peak for copper (II) increases as the mercury (II) concentration increases and reach plateau at 30 mg l⁻¹ Hg. However, due to the intention of the analysis was to reduce the amount of mercury used, 10 mg l⁻¹ Hg (II) has been used for the rest of the analysis.

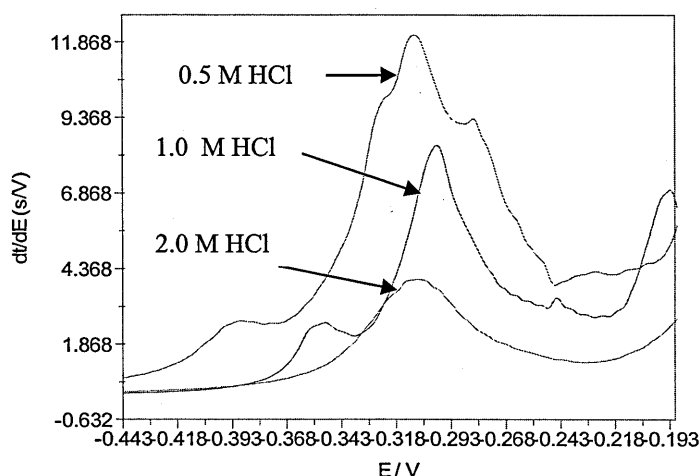


Figure 4.15: Stripping chronopotentiograms for (a) 50 µg l⁻¹ copper (II) in solutions containing HCl at different concentration using MFSPCE. Hg²⁺ = 10 mg l⁻¹, deposition time = 120 s, Cys = 20 mM, deposition potential = -0.9 V, constant current = 1 µA.

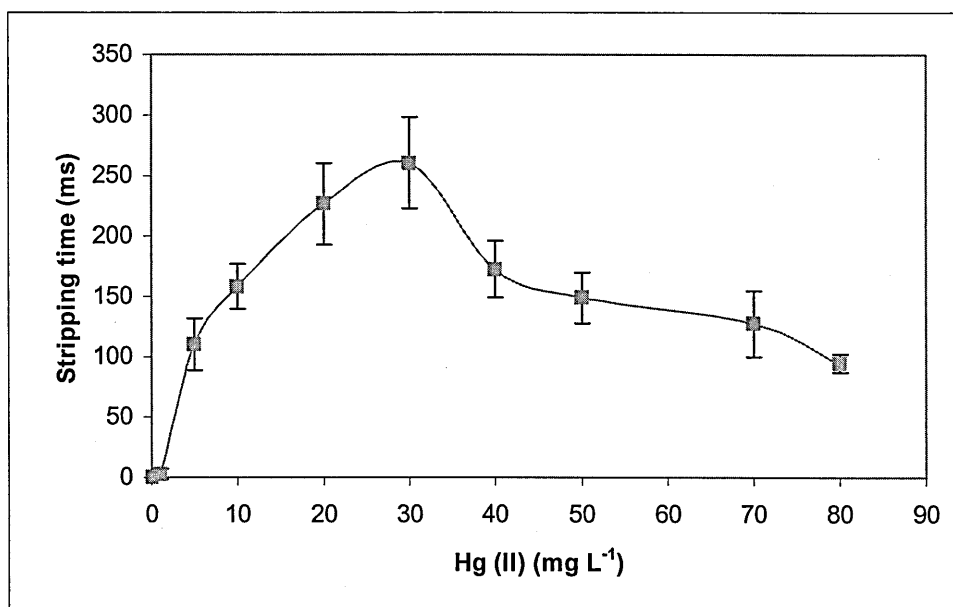
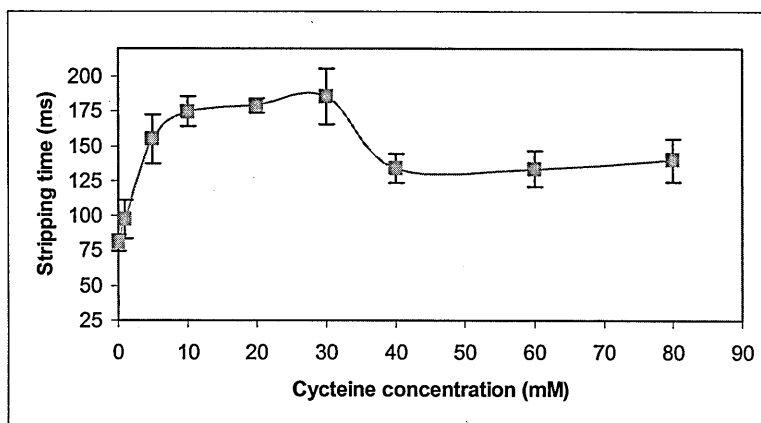


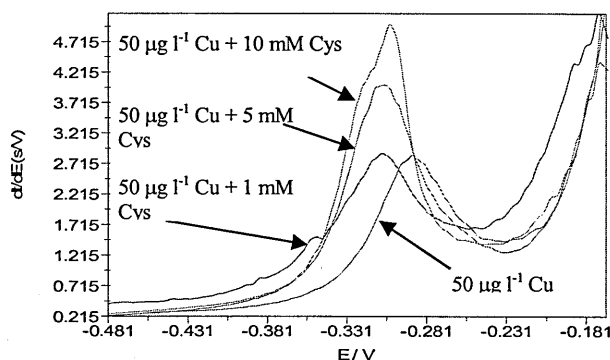
Figure 4.16: Effect of mercury concentration on stripping chronopotentiometric stripping of 50 µg l⁻¹ Cu in 2M HCl.

4.5.2 Effect of cysteine concentration

The use of lower mercury (II) concentration in CCSCP along with the higher response of the technique was now exploited for the analysis of copper (II) in the presence of cysteine. The cysteine concentration in the range of 0 – 80 mM were added to solutions containing $50 \mu\text{g l}^{-1}$ Cu in 2 M HCl and the stripping response obtained for Cu (II) metal recorded and plotted versus cysteine concentration (Figure 4.17 (a)). Figure 4.17 (b) shows the stripping chronopotentiograms of $50 \mu\text{g l}^{-1}$ Cu (II) alone and when increase concentration of cysteine added into the solution from 1 mM to 10 mM in 2 M HCl. The Cu (II) response increase steadily with cysteine concentrations and reaches plateau at 20 mM cysteine. Higher cysteine concentrations than 30 mM decreases the peak response. The optimal cysteine concentration of 20 mM was therefore chosen as a compromise between signal response and reproducibility.



(a)

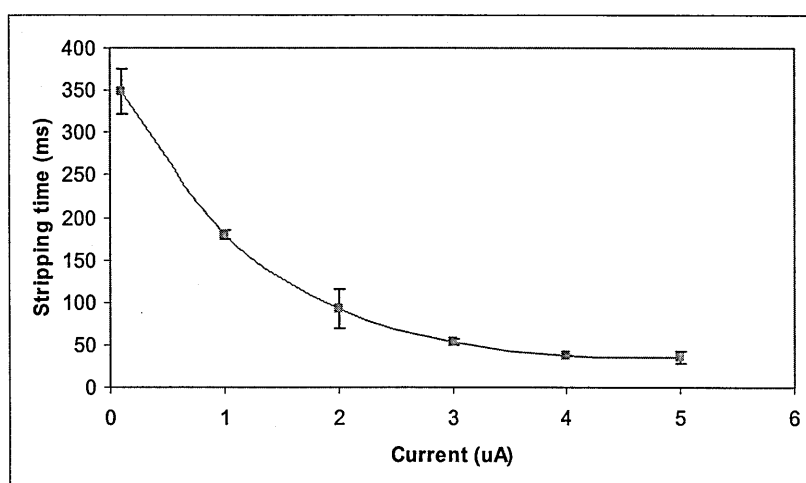


(b)

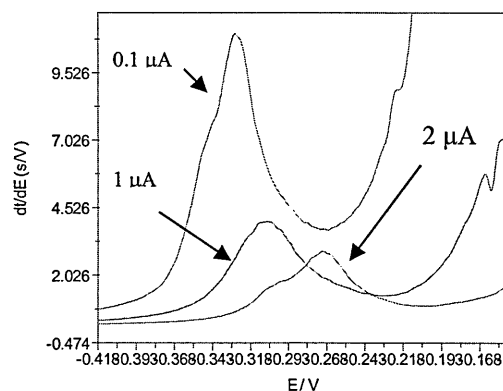
Figure 4.17: Effect of cysteine concentration on the chronopotentiometric stripping of $50 \mu\text{g l}^{-1}$ Cu in 2M HCl.

4.5.3 Effect of constant current

Another parameter of significant importance is the current applied for the oxidation of the reduced and amalgamated metals. Such current must be high enough to completely oxidize the metals of interest without oxidation of other potential interferents. The stripping time recorded for $50 \mu\text{g l}^{-1}$ Cu (II) in 2 M HCl when oxidizing current in the range of 0.1 to 5 μA were applied showed a dramatic decrease for current values $> 0.1 \mu\text{A}$ (Figure 4.18 (a)). Also, a significant shift towards more positive potentials was observed when stripping currents increase. Further experiments therefore made use of 1 μA oxidizing current (Figure 4.18 (b)).



(a)



(b)

Figure 4.18: Effect of constant current on the stripping chronopotentiometric of $50 \mu\text{g l}^{-1}$ Cu (II) in 2 M HCl (a) and stripping voltammograms (b).

4.5.4 Effect of deposition time

The deposition time was also examined in order to determine the optimal value for the parameter. As observed in Figure 4.19, the study of deposition time showed that maximal results were achieved after 300 s accumulation period for 1 μA current. It is observed that for longer period of analysis, lower current should be used for good copper (II) response. Therefore, for future tests, 120s deposition time was employed.

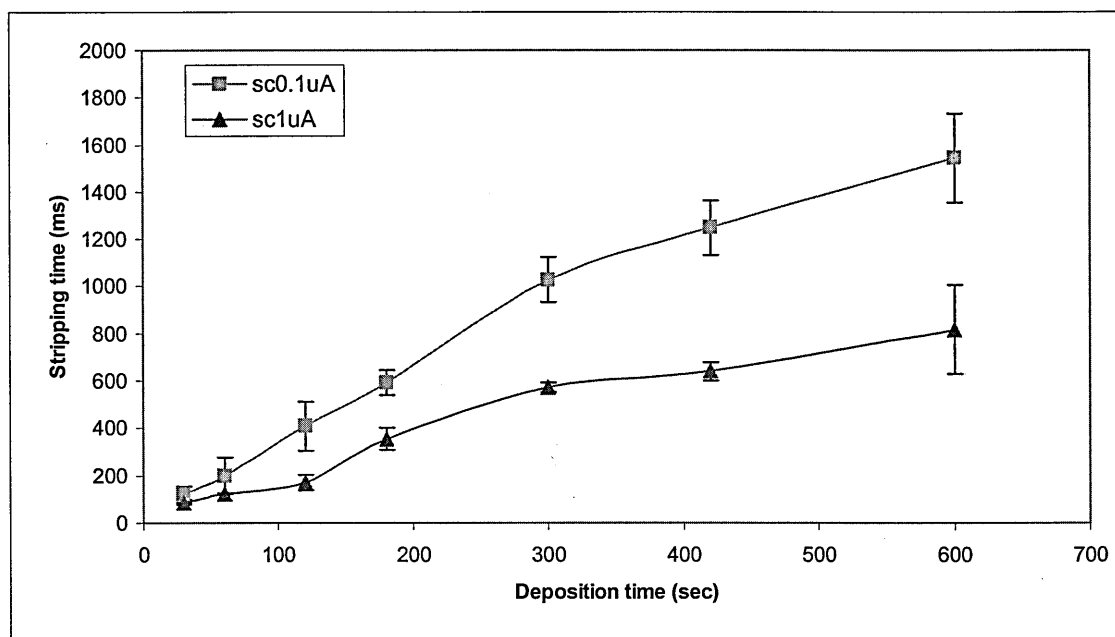


Figure 4.19: Effect of constant current on stripping chronopotentiometric of 50 $\mu\text{g l}^{-1}$ Cu in 2M HCl.

4.5.5 Effect of deposition potential

The study of the effect of deposition potential shows an optimum deposition potential achieved at -0.7 V (Figure 4.20). There is a rapid change of signal when the potential shift from -0.6 to -0.7 V. Deposition potential more negative than -0.7 V produced a decrease in the copper (II) stripping peak. A more constant stripping peak obtained after -1 V deposition times; however, it produced more error due to high standard deviations.

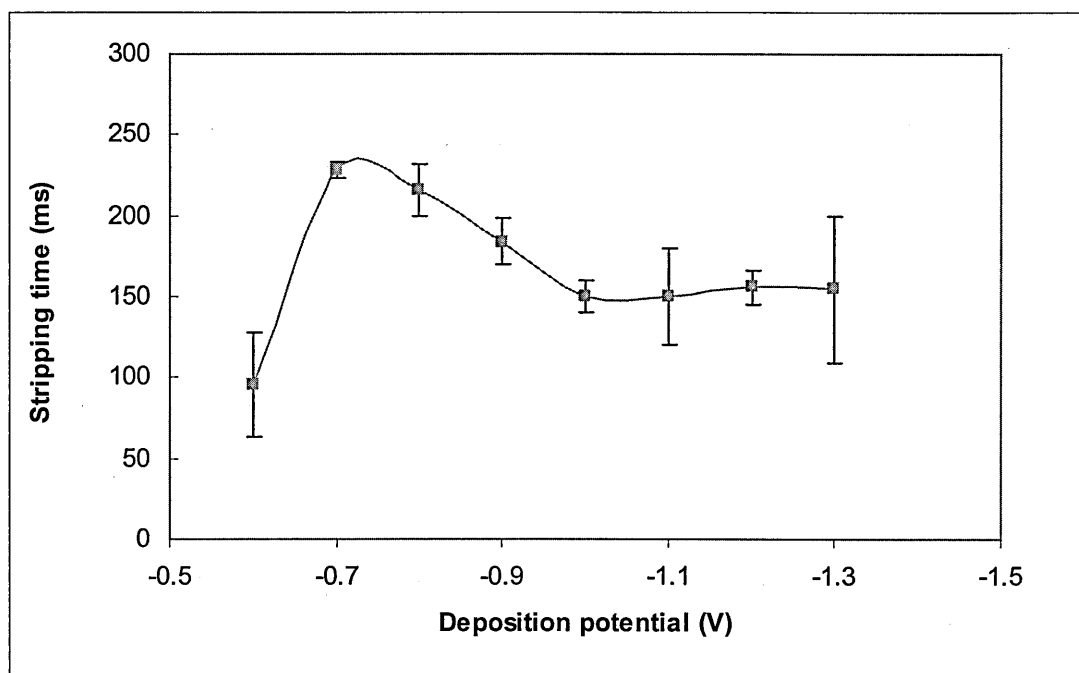
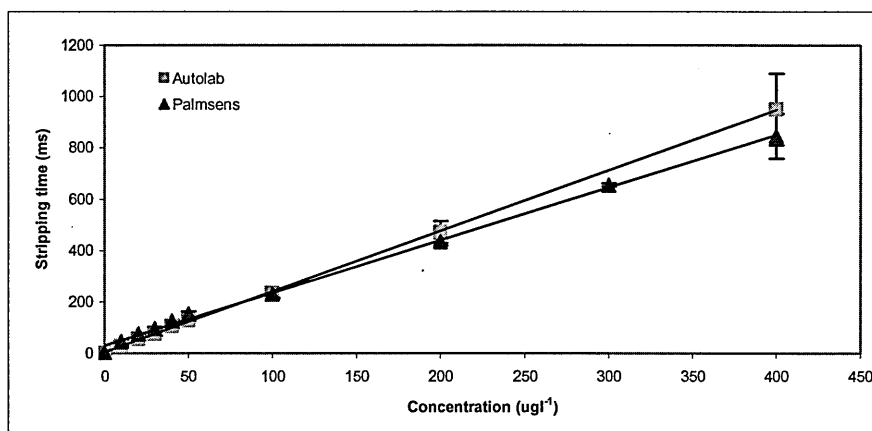


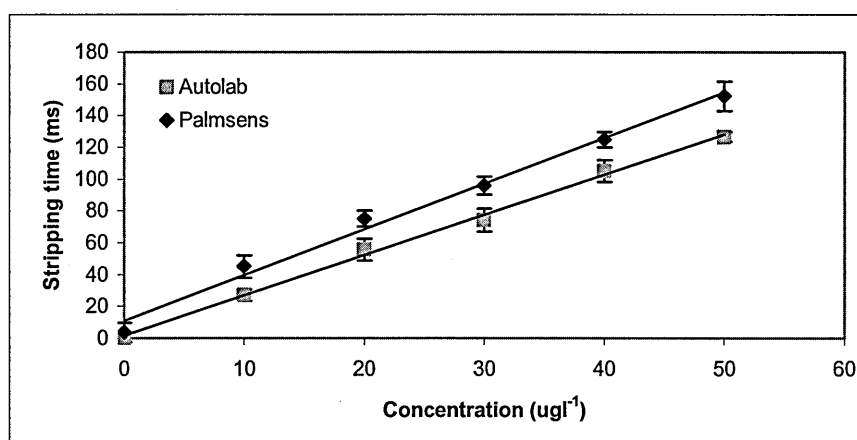
Figure 4.20: Effect of deposition potential on stripping chronopotentiometric of $50 \mu\text{g l}^{-1}$ Cu (II) in 2 M HCl.

4.5.6 Calibration Graph

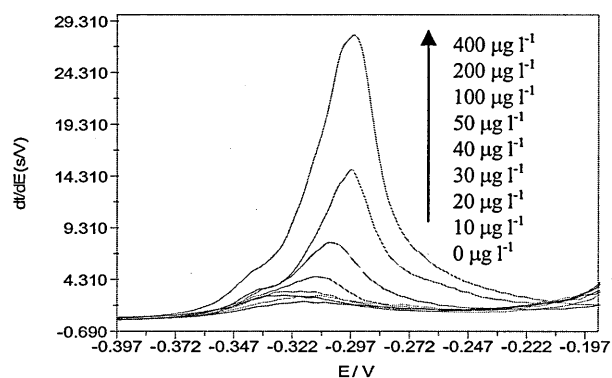
Figure 4.21 (a) displays the calibration plot for increasing concentration of copper (II) in 2 M HCl using Autolab and Palmsens. The calibration plot was linear in the range examined, $10 - 400 \mu\text{g l}^{-1}$, with sensitivity of $2.3587 \pm 0.001 \text{ ms } 1 \mu\text{g}^{-1}$ ($r^2 = 0.9998$) for analysis using Autolab and $2.0527 \pm 0.01 \text{ ms } 1 \mu\text{g}^{-1}$ ($r^2 = 0.9979$). For calibration plot linear in the range examined $0 - 50 \mu\text{g l}^{-1}$, sensitivity recorded for Autolab was $2.8689 \pm \text{ms } 0.002 \mu\text{g}^{-1}$ ($r^2 = 0.9908$) and $2.5318 \pm \text{ms } 0.02 \mu\text{g}^{-1}$ ($r^2 = 0.997$) for Palmsens. A detection limit ($S/N = 3$) of $3 \mu\text{g l}^{-1}$ was estimated following 120 s deposition. The stripping response was also reproducible with a series of eight repetitive measurements of 10 copper (120 s deposition) yielding a relative standard deviation of 8 %.



(a)



(b)



(c)

Figure 4.21: Calibration graph of Cu (II) in 2M HCl (a) from 10 - 400 $\mu\text{g l}^{-1}$ (b) from 0 - 50 $\mu\text{g l}^{-1}$) using Autolab and Palmsens, respectively. The analysis was done using different set of standards prepared at different time and (c) Stripping chronopotentiograms for increasing concentrations of copper (II) in 2 M HCl from 0 – 400 $\mu\text{g l}^{-1}$ using Autolab.

4.5.7 Real Samples Analysis

Extracts from samples collected in mining areas in Seville, Spain, were analysed using CCSCP with the cysteine modified electrodes. As seen in Table 4.5, the values obtained for the acetic acid and aqua regia extracted copper was close to ICP-MS analysis result, with recoveries close to 100 % in most cases. A correlation plot of the results obtained for different soil samples using both the modified electrode and ICP-MS methods showed a relatively high correlation coefficient ($r^2=0.9552$, $n=7$), along with a slope close to 1, which confirms the validity of the sensor developed for copper determination in environmental samples (Figure 4.22).

Table 4.5: Copper contents obtained for soil extracts with cysteine modified screen-printed electrodes.

SAMPLE	CONTENT BY ICP-MS $\mu\text{g l}^{-1}$	CONTENT BY CCSCP WITH 20 mM CYSTEINE ($\mu\text{g l}^{-1}$)	RECOVERY (%)
GT683(AA)	1240 ± 36	1320 ± 39	103
GT683(AR)	1853 ± 4	1873 ± 17	108
GT684 (AR)	1087 ± 3	1124 ± 25	97
GT685(AR)	711 ± 3	775 ± 4	103
GT691(AA)	1882 ± 38	2166 ± 42	81
GT691(AR)	1298 ± 42	1232 ± 26	84
GT692(AR)	1443 ± 44	1582 ± 18	85

AA- Acetic acid extract

AR-Aqua regia extract

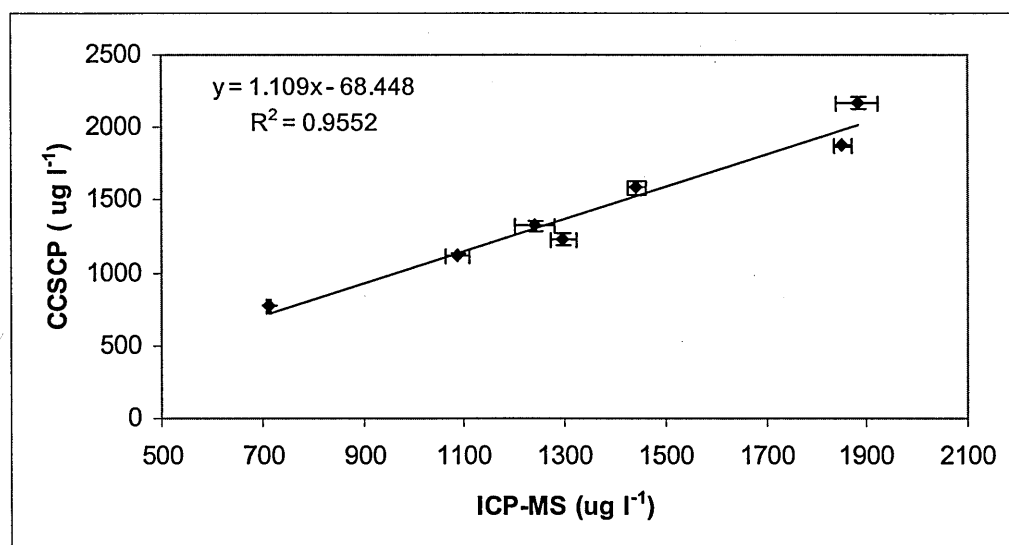


Figure 4.22: Correlation between results obtained by CCSCP and ICP-MS for copper (II) analysis in 2 M HCl.

The method was tested for field analysis at SENSPOL Technical Meeting on Problems related to Diffuse Pollution Sources: Characterization of Sediment, Dredged Material and Groundwater, Koblenz, Germany, 28 - 31 October 2003. The analysis was done using Palmsens. The correlation result was obtained and presented in Figure 4.23. There was good correlation ($r^2=0.9997$) between two method tested for copper (II) analysis in spiked water samples, i.e. modified cysteine-screen-printed carbon electrode and GFAAS (results no. 24 - Cranfield University). The correlation results prove that the method suitable to be used for copper (II) analysis in surface and ground water samples. Results no. 22 was obtained by other agency and included in the graph for comparison with results no. 24 from Cranfield University. Results from no. 24 show better correlation to GFAAS as compared to results obtained by no. 22.

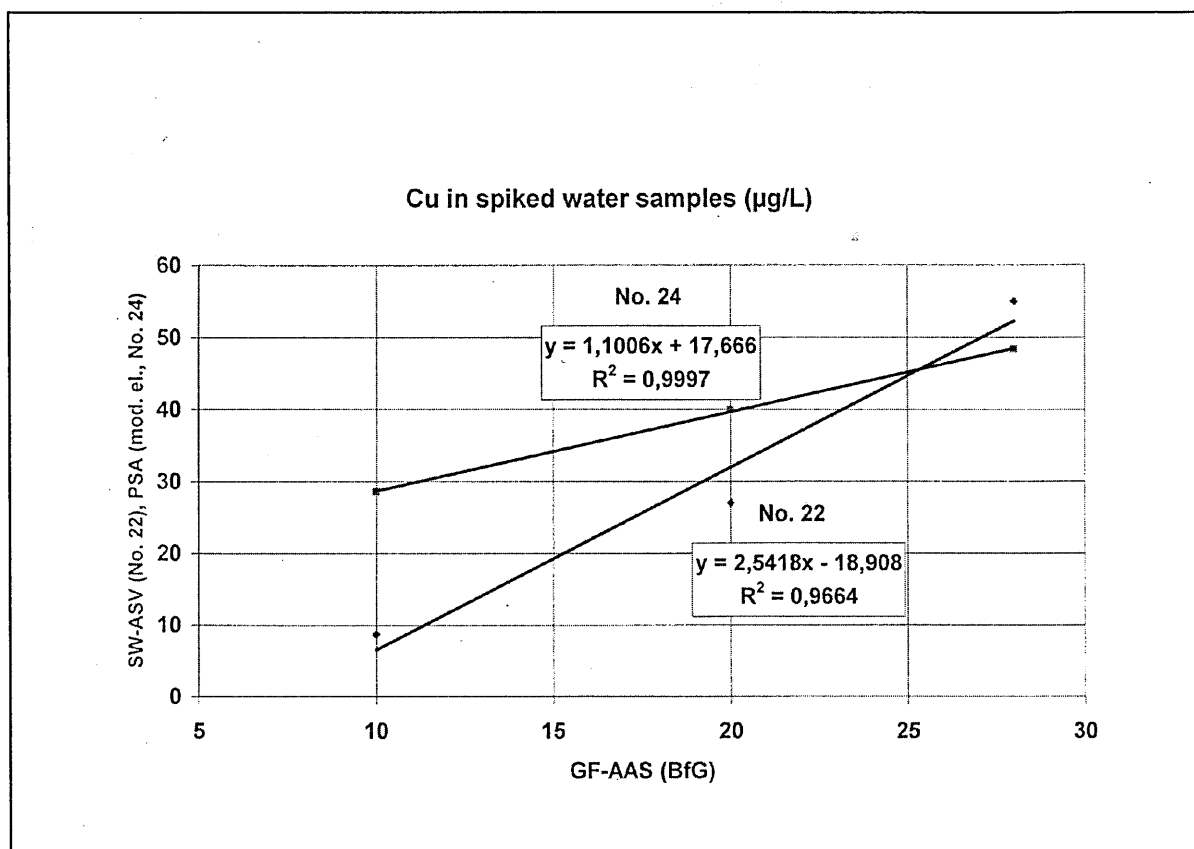


Figure 4.23: Results (No. 24 - Cranfield University) shows correlation between Cu^{2+} level using cysteine modified electrodes and GF-AAS. There is another result (No. 22) from other agency. Results were produced by BfG, Germany.

4.6 CONCLUSION

Disposable, low cost Cys modified mercury film electrode prepared *in situ* by co-depositing of mercury and Cys, offers an enhanced sensitivity and excellent reproducibility for its application in chronopotentiometric stripping analysis (CCSCP) of cadmium (II), lead (II) and copper (II). The improvement in sensitivity over unmodified mercury film electrode ($10 \text{ mg l}^{-1} \text{ Hg}$) is attributed to the formation of complex and adsorption of cadmium-cysteinate, lead-cysteinate and copper-cysteinate with the mercury film on the carbon-working electrode. Several experimental variables affecting the sensor stripping response were characterized and optimized. These include cysteine and mercury concentration, deposition time, deposition potential and stripping current. Using a deposition time of 2 minute, the stripping chronopotentiometric response for cadmium (II), lead (II) and copper (II) were linear

in the concentration range 0.4 - 800, 0.7 - 800 and 3 - 400 $\mu\text{g l}^{-1}$, respectively. Detection limit of 0.4, 0.7 and 3 $\mu\text{g l}^{-1}$ was derived for cadmium (II), lead (II) and copper (II) using Tris-HCl/KCl and 2 M HCl as the supporting electrolyte. The sensor were applied to the determination of cadmium (II), lead (II) and copper (II) in waste-water and soil samples extracted with acetic acid and aqua regia. The results obtained using the developed electrodes agreed satisfactorily with the values achieved using AAS and ICP-MS analysis. The achieved results gave sufficient evidence for the feasibility of using the disposable cysteine-modified screen-printed electrode for the determination of cadmium (II), lead (II) and copper (II) in water and soil samples at site contaminated with heavy metals. This indicates the feasibility of using this type of portable and cheap sensors for field analysis.

CHAPTER 5

5.0 DETECTION OF LEAD (II) AND CADMIUM (II) USING SCREEN-PRINTED GOLD ELECTRODES

5.1 OUTLINE

In this chapter, reviews and analysis of lead (II) and cadmium (II) with screen-printed gold electrodes using square-wave stripping voltammetry are described.

5.2 INTRODUCTION

Nowadays, there are many conventional methods available that have been used to detect lead (Pb) in environmental and biological samples, such as atomic absorption spectrometry (AAS), inductively coupled plasma-atomic emission spectrometry (ICP-AES) and inductively coupled plasma-mass spectrometry (ICP-MS) (Popek, 2003). However, most of these methods are not suitable for on-site screening due to their size, cost and the time required to carry out the analysis. Therefore, there is a pressing need for a simple, relatively cheap, sensitive and reliable method for on-site measurement of toxic heavy metals in many application areas such as biomedical, environmental, and industrial samples. To meet these criteria, electroanalytical methods, especially stripping analysis, have been presented as the most promising approach to fulfill this analytical need. The recent emergence of pocket size portable electrochemical analyzers which can be coupled to disposable sensors can provide a low power, low cost, rapid and sensitive field analysis..

Screen-printing technology is widely used for the mass-production of disposable electrochemical sensor. The most known is the glucose sensor which still dominate the blood glucose market. Screen-printed electrode (SPE) has low unit costs, with a high degree of precision and a wide range of geometries (either as arrays or single electrodes) and can be used as whole electrode systems (reference, working and counter electrode), while maintaining adequate levels of reproducibility. The main advantages of SPE over conventional electrodes is that the problems of carry over and surface fouling are alleviated, as they are normally used only once and then discarded (Honeychurch & Hart, 2003, Morrin *et. al.*, 2003).

Stripping analysis is a powerful electrochemical technique for measuring trace metals. This technique is used when metal levels are very low in solution; and in-situ enrichment is necessary to accumulate metals that form amalgam with mercury (Hg) such as Pb (II) by cathodic deposition in a (drop or film) Hg electrode. Subsequently the amalgam is anodically oxidised in the determination stage. The mercury drop electrode can be applied down to approximately $0.1 \mu\text{g l}^{-1}$ (Apostoli, 2002).

For trace determination, the use of the Hg film electrode is necessary to achieve detection limits at the sub $\mu\text{g l}^{-1}$ level. This is due to many metals of interest have fast electrode kinetics at mercury and form amalgams. Amalgam formation provides an excellent approach for the preconcentration of metals into the working electrode of the cell prior to the determination of the metals by their stripping (removal) from the mercury phase (Herzog & Arrigan, 2005). However, as Hg is toxic, its incorporation into sensors poses problems, particularly from the viewpoint of disposability (Honeychurch & Hart, 2003). The use of non-toxic materials such as gold and bismuth electrode can provide an alternative approach to the Hg electrode for electrochemical detection of heavy metal. Both gold and bismuth electrodes are considered safe and environmental friendly technique.

A range of published papers have reported the use of screen-printed electrodes with stripping analysis techniques for Pb measurements (Wang, 1992; Wang, 1993; Desmond, 1996; Palchetti *et al.* 1999; Honeychurch, 2000). However, most of the techniques use screen-printed carbon electrodes preplated with mercury film. Gold electrodes have been applied for metals analysis, but, most are either based on the use of conventional gold electrodes (Bonfil *et al.* 1999, 2000; Richter *et al.*, 2001; Bonfil *et al.* 2002; Beni *et al.*, 2004), modified or chemically modified gold electrodes (Turyan & Mandler, 1994; Arrigan *et al.*, 1999; Herzog & Arrigan, 2003; Herzog & Arrigan, 2003; Herzog *et al.*, 2004). Papers describing the use of screen-printed gold electrode (SPGE) have emerged but they only been applied for clean water and urine samples analysis. Lead and mercury in urine and water samples were analysed by Wang & Tian (1993a, b) using gold-coated screen-printed electrode coupled with stripping potentiometric analysis. Detection limits of 0.1 and $0.6 \mu\text{g l}^{-1}$ lead were estimated following 20 and 4 minutes deposition, respectively, from stirred solutions,

while a $3 \mu\text{g l}^{-1}$ value was calculated for the use of unstirred solution for 10 minutes. However, such measurements were applied in cases where the screen-printed gold electrode was placed alongside conventional silver/silver chloride (Ag/AgCl) reference electrode and platinum counter electrode in a conventional electrochemical cell. Masawat et al. (2003) reported the use of disposable gold-sputtered SPE for lead detection in spiked drinking and tap water samples using flow injection measurement. With 2 minutes deposition times, detection limit of $0.8 \mu\text{g l}^{-1}$ was achieved.

The fundamental basis of the accumulation of Pb^{2+} on gold electrode is underpotential deposition (UPD). UPD is the phenomenon of depositing a monolayer or submonolayer of a metal onto foreign substrates at potentials more positive than the thermodynamic reversible potential that expected for bulk metal deposition. Normally, electrochemical behaviour for UPD corresponds at low concentration range (Bonfil et al., 2002, Beni et al., 2004). This phenomenon occurred due to stronger interaction between the Pb atoms and the gold substrate than between adjacent atoms of the deposited metal itself. Thus, the monolayer of lead on gold had a stronger interaction with the gold than with other lead atoms and so requires higher energy (higher applied potential) in order to strip it from the surface. The bulk deposited leads were stripped at more negative potentials due to a weaker interaction between layers of lead atoms than between a layer of lead atoms on the gold surface (Herzog & Arrigan, 2003; Masawat et al., 2003).

The use of UPD in conjunction with stripping voltammetry (SV) at a number of different solid electrodes has proven to be quite analytically useful in recent years. Kirowa-Eisner and colleagues (1997) have pioneered the use of the UPD-SV for analytical applications. The UPD-SV offers several advantages, such as the use of short deposition times. The results obtained can be repeated, as the electrode-surface structure remains undisturbed by repeated use of the electrode. This is because the metallic deposit covers a very low portion of the working electrode surface. Hence, the structure of the electrode surface remains mainly unchanged, resulting in a good repeatability of the analytical response. This advantage could avoid the need for mechanical and electrochemical treatment of the electrode between series of experiments (Herzog & Arrigan, 2005). There is also possibility of working without

removing dissolved oxygen based on the differences in oxygen reduction at solid electrodes compared to mercury electrode and the use of less negative reductive potential (Kirowa-Eisner et al, 1997).

Analytical capabilities of UPD-SV were demonstrated for different metal ions such as Pb^{2+} , Cd^{2+} , Hg^{2+} and Cu^{2+} at Au and Ag rotating disk electrodes. Detection limit range from part perbillion and sub-part perbillion level were reported with relatively short deposition times (between 60 and 120s). For examples, for the determination of lead, Kirowa-Eisner groups reported the use of Ag electrode (vs Agquasi-reference electrode) in HNO_3 (10 mM) + KCl (10 mM). At deposition potential of -0.7 V, detection limit of 0.5 nM ($0.1 \mu\text{g l}^{-1}$) was achieved in 120 s. A dynamic range of 2 - 10 nM was reported (Brand et al., 1997). An improved detection limit of 0.05 nM ($0.01 \mu\text{g l}^{-1}$) in 60s for lead was revealed by Kirowa-Eisner et al. (1999) using Ag electrode (vs. Ag/AgCl/KCl, 1 M). When deposition potential of -0.7 V was used, two dynamic range were reported (0 - 6 nM, deposition time = 60s) and (0 - 0.7 nM, deposition time = 120s). In 2002, Bonfil et al. described the use of Ag electrode (vs. Ag/AgCl/KCl, 1 M) for UPD-SV of lead. Detection limit of 0.04 nM ($0.008 \mu\text{g l}^{-1}$) was obtained with 120s deposition time using deposition potential of -0.4 V. Linear range of 15 - 170 nM achieved when 15 s deposition time was applied to the electrode. Bonfil and Kirowa-Eisner (2002) also described lead determination using UPD-SV with Ag electrode (vs. Ag Quasi-reference electrode). With only short deposition time of 90s using -0.8 V deposition potential, detection limit of 0.5 nM ($0.1 \mu\text{g l}^{-1}$) and dynamic range 0.4 - 3 nM (deposition time=90s) and 10 - 60 nM (deposition time = 30 s) were reported. The use of other kinds of electrodes substrate for the detection of lead was also reported.

Zen et al. (2002) reached detection limit of $0.5 \mu\text{g l}^{-1}$ (2.2 nM) using screen-printed silver electrodes (vs. Ag/AgCl/NaCl, 3 M). Only short deposition time applied (60s) using deposition potential of -0.5 V, a dynamic linear range 20 - 390 nM was achieved. Shih et al. (2004) reported a disposable-type screen-printed non-single crystal silver electrode, which demonstrated sensitive and selective quantification of lead in cosmetic hair-dye formulations using its UPD-Pb process stripping

voltammetry. Detection limit of 0.31 nm ($0.06 \mu\text{g l}^{-1}$) reached for 60 s deposition time.

This chapter describes the development of a disposable screen-printed gold sensor comprising a three- electrode configuration system. The developed sensor was applied to lead analysis in wastewater and soil samples isolated from a heavy metal contaminated site. Details of the voltammetric behaviour of copper and lead in soil samples were reported by Beni et al. (2004) using conventional gold electrode. The authors found that it is possible to detect copper and lead ions by stripping voltammetry in three BCR sequential extraction media without altering the matrix. However, we report the first use of SPGE as the whole electrode system (reference, working and counter electrode) for the determination of Pb (II) in extracted wastewater and soil samples.

The Pb (II) levels in soil samples are normally present at part per million levels, hence, there is no need to achieve very low detection limits as described in many published papers for drinking water. The accuracy of the results obtained with the SPGE were compared to mercury film screen-printed electrodes (MFSPE) since these electrodes are still considered the electrode of choice for stripping analysis due to their outstanding electrochemical characteristics (Kadara & Tothill, 2003, 2004). ICP-MS was used for verification of the analysis results. In the following sections, details of the construction of the SPGE, the advantages obtained and the results are discussed.

5.3 DETERMINATION OF LEAD (II)

5.3.1 Experimental

5.3.1.1 Chemicals

Solutions were prepared with purified water of HPLC grade from BDH (Poole, England). All glassware were cleaned with 1% HNO_3 and carefully rinsed with reversed osmosis water to avoid contamination. All chemicals were of analytical grade. Hydrochloric acid (HCl), potassium chloride (KCl), lead (II), cadmium (II), calcium (II), copper (II), zinc (II), Iron (II), arsenic (III), mercury (II) and chromium standard solution were obtained from BDH (Poole, England). The electrolyte used in

the experiments was 0.05 M HCl for SPGE and 0.1M HCl in 0.5M NH₄Ac (Merck, Darmstadt, Germany) (pH 4.6) for SPMFE.

5.3.1.2 *Electrode fabrication*

The details on the screen-printed electrodes (SPEs) fabrication techniques were described in chapter 2.

5.3.1.3 *Procedure*

Electrochemical measurements were carried out on a computer controlled potentiostat-10, the Autolab Electrochemical Analyser with general purpose electrochemical software operating system GPES version 4.8 (Windsor Scientific Limited, Slough, Berkshire, UK) and a hand-held, battery-powered PalmSens potentiostat/galvanostat (Palm Instruments BV, The Netherlands) interfaced to a Compaq iPAQ pocket PC. Voltammetric measurements were carried out by placing a 100 μ l sample on a three-electrode strip for each experiment in unstirred and non-deaerated solution. Experiments were carried out using Square Wave technique by deposition of the target lead (II) or cadmium (II) at a potential of -0.7 V (vs. screen-printed Ag/AgCl) for 120s. Subsequently, stripping step was used and the amalgam was anodically oxidised and the process terminated at 0.25 V (vs. screen-printed Ag/AgCl). Every new electrode was cleaned prior to analysis by cyclic voltammetry, from 0 to 1.5 V for 10 times at 100 mV in 0.5 M H₂SO₄/0.01M KCl. The SPGE can be reused for twenty times before disposing. The electrode was cleaned at conditioning potential of 0.8 V for 90s with 0.5 M H₂SO₄ prior to the next measurement cycle and was sufficient to remove the deposited Pb. Method of standard addition was used for real soil sample analysis by spiking with appropriate concentrations of standard lead (II) solution. The results were compared with those obtained by inductively coupled plasma-mass spectrometer (ICP-MS) and screen-printed mercury film electrode (MFSPCE). For experiments using MFSPCE, deposition potential of -1 V (vs screen-printed Ag/AgCl) for 120s was used. The frequency was set at 20 Hz and the process terminated at 0 V (vs screen-printed Ag/AgCl). The rest of the parameters are the same for lead (II) analysis. All data were obtained at room temperature.

5.3.1.4 Soil/Sediment sample treatment

The sampling and sample treatment using conventional methods were carried out by Grup de Techniques de Separació (Universitat Autònoma Barcelona, GTS-UAB, Spain) and by Técnicas de Protección Ambiental S.A. (TPA, Madrid, Spain). The description of the extraction process applied to soil and sediment samples were explained in chapter 3.

5.3.1.5 Surface Characterisation of Screen-printed gold electrode with SEM

The working electrode of the screen-printed carbon and gold electrode were used for surface characterisation. The working electrode was connected with metal base of a round sample plate using silver in methyl isobutyl ketone (Acheson Electrode, Essex). The XL30 SFEG Scanning electron microscope (SEM) (FEI company, Holland) was used to characterise the surface of the screen printed electrodes. The analysis was done based on the XLFEG/SFEG Scanning Electron Microscope operating instruction manual. An AnalySIS[®] 3.0 software (Soft Imaging System, Germany) was used for the analysis of metals content on the electrode surface.

5.3.2 Results

5.3.2.1 Surface Characterisation of Screen-printed Gold electrode by Scanning Electron Microscope

The physical form in which the metals, analyte cations are deposited by reduction on the electrode surface is important in determining the quality of the square wave signal (Macca et al., 2004). A typical surface of SPGE with gold working electrode at 1000 magnification is shown in Figure 5.1. The surface structure shows a group of micro granule combined with each other and each granule has diameter $< 1 \mu\text{m}$. The micro granule groups are not uniformly distributed with pin holes shown on the electrode surface. Cleaning with 0.5M H_2SO_4 by means of cycling 10 times from 0 to 1.5 V doesn't change the morphology of the surface. The small granule shape could significantly contribute to an increase in active surface area of the gold electrode. The analysis of the SPGE shows that the main contents of the surface are gold (Au), with a small amount of carbon and oxygen (Figure 5.1).

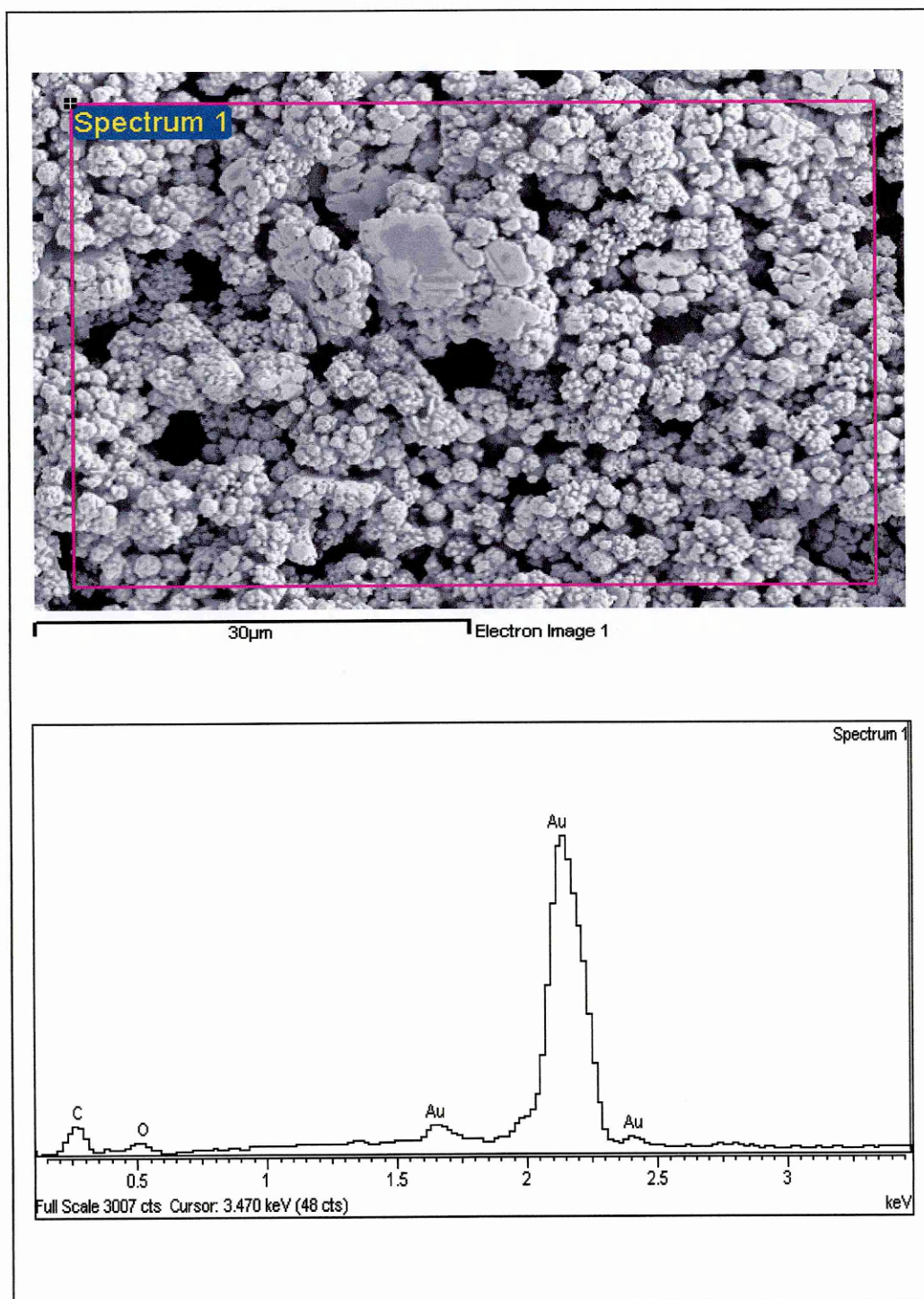


Figure 5.1 Typical scanning electron micrographs (1000X magnification) showing the surface features of a screen-printed gold electrode and analysis results.

5.3.2.2 Linear Sweep Voltammetry of Lead (II) on Screen-printed gold electrode

A typical linear sweep voltammograms of Pb^{2+} (10 mg l^{-1}) on the SPGE under various deposition time are shown in Figure 5.2. At deposition time of 15 s, a peak at -0.09 V and one ill defined peak at -0.35 V were observed. The ill defined peak would start to clearly appear when increasing deposition times from 15 s to 90 s. The response at the most positive potentials (-0.09 V) was due to the stripping of UPD1 lead monolayer from the gold surface, follow by UPD2 at -0.35 , while the peak at the most negative potential (-0.47) appeared at deposition time of 45 s, results from stripping of lead bulk deposits. The presence of four peaks for lead stripping from SPGE at longer deposition and/or higher concentration of lead were almost similar as obtained using gold disc working electrode and these can be attributed to the sensitivity of the lead voltammetry to surface crystallinity (Beni et al., 2004).

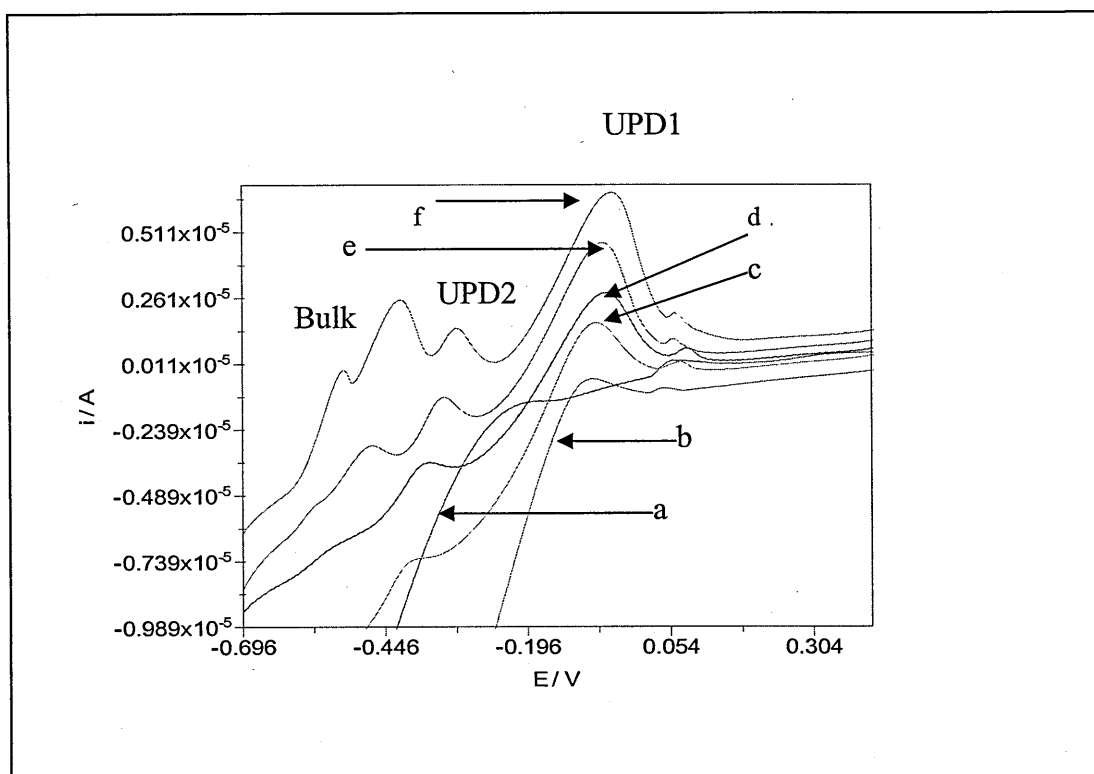


Figure 5.2: Linear sweep voltammetry behaviour of lead (10 mg l^{-1}) at SPGE in 0.05 M HCl . (a) no lead (b) 0 s, (c) 15 s, (d) 30 s, (e) 45 s and (f) 90 s. The electrodes were cleaned electrochemically with $0.5 \text{ M H}_2\text{SO}_4/0.01 \text{ M KCl}$ prior to LSV analysis. Deposition was in unstirred solution at -0.7 V deposition potential. Scan rate $= 100 \text{ mV s}^{-1}$, step potential $= 2.44 \text{ mV}$.

5.3.2.3 Optimisation of measurement parameters

Analysis with the SPGE was performed by square wave stripping voltammetry (SWSV) in order to achieve peak responses which are relatively simple to evaluate in a very short time. In order to perform the determination of lead (II) in wastewater, acetic acid and aqua regia extracts of soils, the parameters which most influence the measurement process were optimized: these were the deposition time (t_{dep}), deposition potential (E_{dep}), frequency, effect of HCl and concentration dependence of the target metal. The effect of interfering ions on the stripping responses was also examined and a comparison made between the performance of the bulk sized laboratory electrochemical analyzer and the portable electrochemical analyzer coupled to screen-printed gold electrode. Baseline correction was performed because of the sloping baseline caused by oxygen reduction and the gold surface reactions as reported by Cserey (2004) and Masawat *et al.* (2004).

In Figure 5.3, the signal for deposition time from 60s to 420s is shown for 50 and 100 $\mu\text{g l}^{-1}$ Pb using Palmsens and Autolab, respectively. For the effect of deposition time, the longer the deposition time, the more lead is collected. For longer times than 420s, the deposition potential is less effective, which is due to the saturation of the surface of the gold electrode. For higher concentrations of Pb (II), the saturation of the surface could be achieved in shorter time. For lower concentrations, an almost linear increase in the signal can be expected for longer deposition time. A time of 2 min was chosen for deposition of lead (II), as this is a compromise between length of analysis and sensitivity for routine analysis.

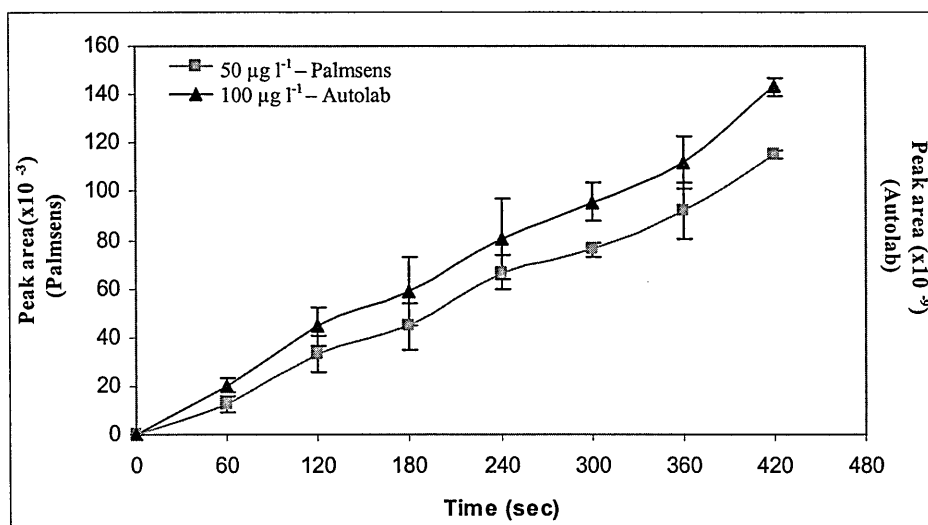


Figure 5.3 Optimisation of deposition time parameters for square-wave analysis of 100 and 50 $\mu\text{g l}^{-1}$ lead using Autolab and Palmsens, respectively. Experiments carried out with in 0.05 M HCl. Deposition potential = -0.7 V, deposition time = 120 s; frequency = 8 Hz; amplitude = 0.025 V and step amplitude = 0.05 V.

Figure 5.4, illustrates the influence of the deposition potential for 50 and 100 $\mu\text{g l}^{-1}$ lead in the range of -0.1 V to -1.1 V using Palmsens and -0.4 to -1.2 V using Autolab, respectively. Both equipments show different trends for the effect of deposition potential. The Palmsens shows almost linear trends of increase on lead (II) peak area when deposition potential change from -0.1 V to -0.9 V. However, more errors were encountered at deposition more negative than -0.9 V. In the case of Autolab, when the deposition potential was adjusted at -0.4 to -0.6 V, the amount of lead deposited was low. However, there was significant improvement of lead deposition at -0.7 V.

Although more lead can be collected at more electronegative potential than -0.7 V, on the basis of sensitivity, resolution and reproducibility, an E_{dep} of -0.7 V was employed for lead measurements using both equipments. This is because at more negative than -0.9V, we observed interference from H_2 generation. The gas generated could cause some irregularity to the signals and the baseline was less reproducible. The same finding was also reported by Richter et al. (2003).

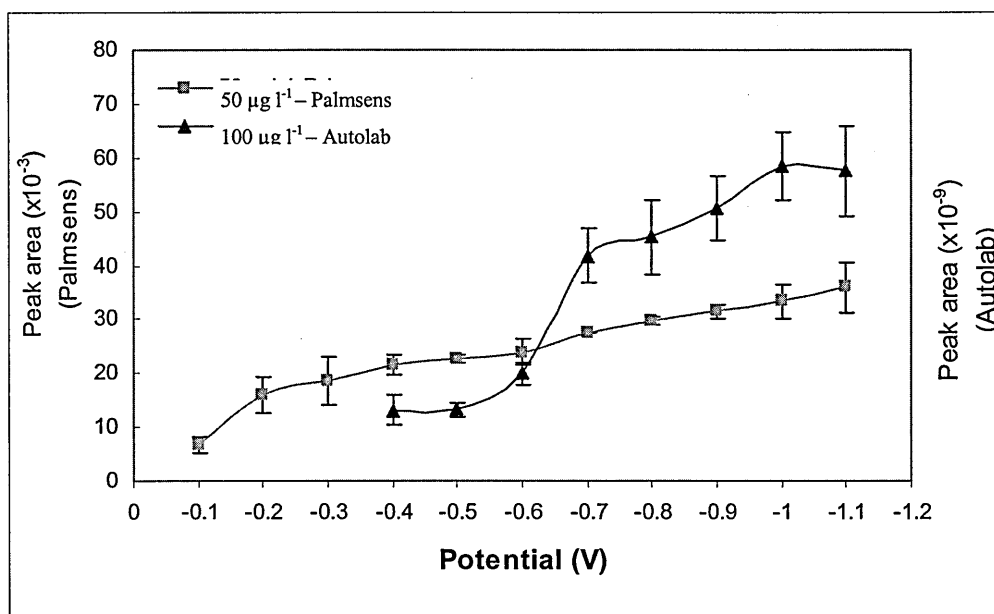


Figure 5.4 Optimisation of deposition potential parameters for square-wave analysis of 100 and 50 $\mu\text{g l}^{-1}$ lead using Autolab and Palmsens, respectively. Experiments carried out with in 0.05 M HCl. Other conditions as in Figure 5.3.

Figure 5.5 shows the relationship between the peak current and the frequency for 50 and 100 $\mu\text{g l}^{-1}$ Pb using Palmsens and Autolab, respectively. Due to different setting used in both equipments, 8 and 1 are the lowest frequency available for testing using Autolab and Palmsens. These results indicate that the lead signal decreases with the frequency. The higher the frequency, the peak becomes broader and less sensitive. Looking at these aspects, due to favorable condition, the ratio between signal and noise, the frequency selected for the following experiments was 8 Hz.

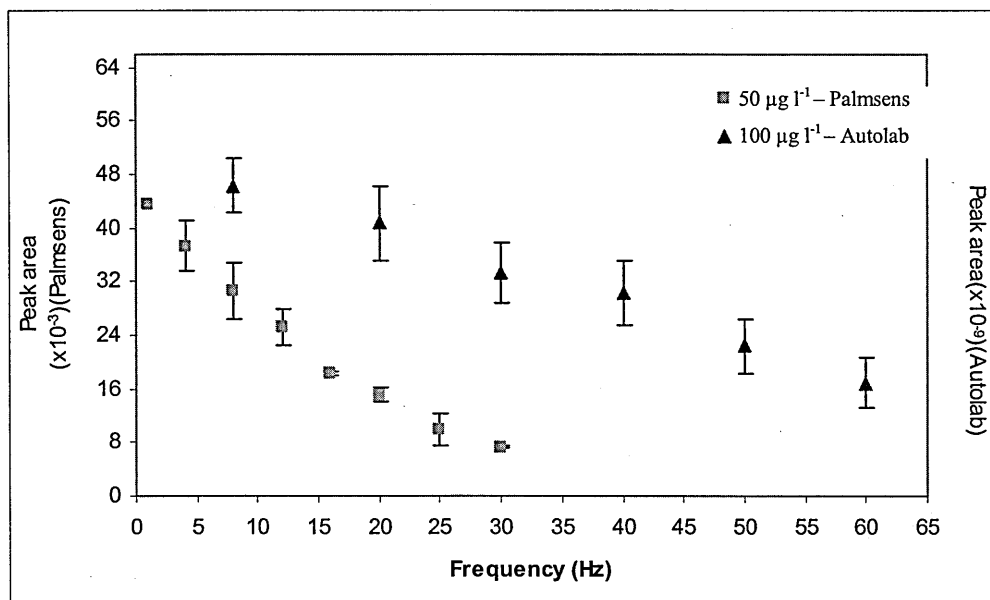


Figure 5.5 Optimisation of frequency parameters for square-wave analysis of 100 and 50 $\mu\text{g l}^{-1}$ lead using Autolab and Palmsens, respectively. Experiments carried out in 0.05 M HCl. Other conditions as in Figure 5.3.

In the previous studies, it was observed that when gold electrodes were employed for lead quantification, the presence of hydrochloric acid was necessary to obtain good response on gold electrode (Richter et al., 2003). The same electrolyte leads to the best signals for lead analysis. To choose the best concentration of hydrochloric acid, experiments involving lead deposition at different hydrochloric acid (HCl) concentrations, ranging from 0.01M to 0.4M were done. As shown in Figure 5.6, the current peak area for 100 $\mu\text{g l}^{-1}$ Pb (II) obtained between 0.01 M and 0.05 M was practically constant. There was some improvement on peak area between 0.05 M and 0.4 M HCl. The data shows that the use of higher HCl concentration could provide better lead (II) peak. However, Richter et al. (2003) reported a decrease in the signal at higher concentration of HCl due to the production of gas bubbles on the electrode surface and the gold electrode destroyed. In our study using SPGE, we chose 0.05M HCl for lead (II) quantification as compromised between peak area signal and the ability to reuse the SPCE by cleaning it electrochemically.

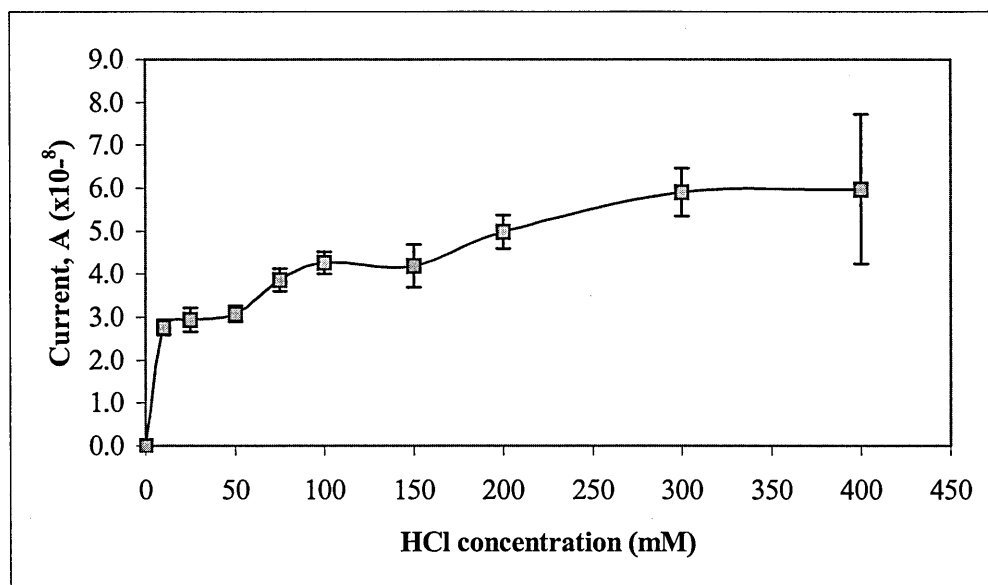


Figure 5.6 Optimisation of chloride parameters for square-wave analysis of $100 \mu\text{g l}^{-1}$ lead using Autolab. Experiments carried out in 0.05 M HCl. Other conditions as in Figure 5.3.

From the results obtained in these optimisation measurements, the following conditions were selected for the subsequent studies using both equipments: deposition potential = -0.7 V , deposition time = 120 s; frequency = 8 Hz; amplitude = 0.025 V and step amplitude = 0.05 V. At the end of each analysis, cleanup of the SPGE was done at 0.8V for 90s, to ensure that the deposited metal was completely stripped from the electrode.

The screen-printed gold electrodes were pretreated electrochemically using cyclic voltammetry in 0.5M H_2SO_4 /0.01M KCl, from 0 to 1.5V at 100 mV for ten cycles before use. This pretreatment method appeared to increase the surface functionalities and roughness or to remove surface contaminants and resulted in enhanced electrochemical activity (Morrin *et al.*, 2003). In between analysis, the electrode will be cleaned using the same media at 0.8 V for 90 s. The electrochemical cleaning procedure helped remove any deposited lead from the gold electrode, making it ready for subsequent analysis. A 90 s was found to be sufficient for this regeneration process. Figure 5.7 presents a series of 25 repetitive measurements in a solution of 0.1 M HCl containing $100 \mu\text{g l}^{-1}$ of lead. The repeatability obtained for this series

(RSD=3.17%) attests to the good performance of a single SPGE for lead analysis. The good performance of the SPGE present advantages, as low cost, ease of handling and absent of toxic waste, as in the cases of mercury drop electrodes and mercury film electrode. The use of only 100 μl volume of samples also contributes to waste decrease.

Under these conditions, the linear concentration range in which lead (II) could be detected on SPGE was evaluated. Figure 5.8 and 5.9 illustrated two series of square-wave stripping responses for lead over the range of 10 to 50 $\mu\text{g l}^{-1}$ (A) and 25 to 300 $\mu\text{g l}^{-1}$ (B). The first series (Figure 5.8) was performed with 240 s deposition time. The detection limit was estimated as 2 $\mu\text{g l}^{-1}$. The correlation coefficient calculated for this series was $r^2 = 0.9963$. Figure 5.9 shows the signal obtained with solutions containing lead in the range of 25 – 300 $\mu\text{g l}^{-1}$, using a deposition time of 120 s. The correlation coefficient calculated for this series was $R = 0.9991$. Detection limit was estimated as 5.8 $\mu\text{g l}^{-1}$. The linear ranges are wide enough to perform quantitative determinations of lead (II) after appropriate dilutions of samples. The detection limits were evaluated with the criterion $3xS_b/m$, where S_b is the standard deviation of the intercept and m is the slope of the linear plot.

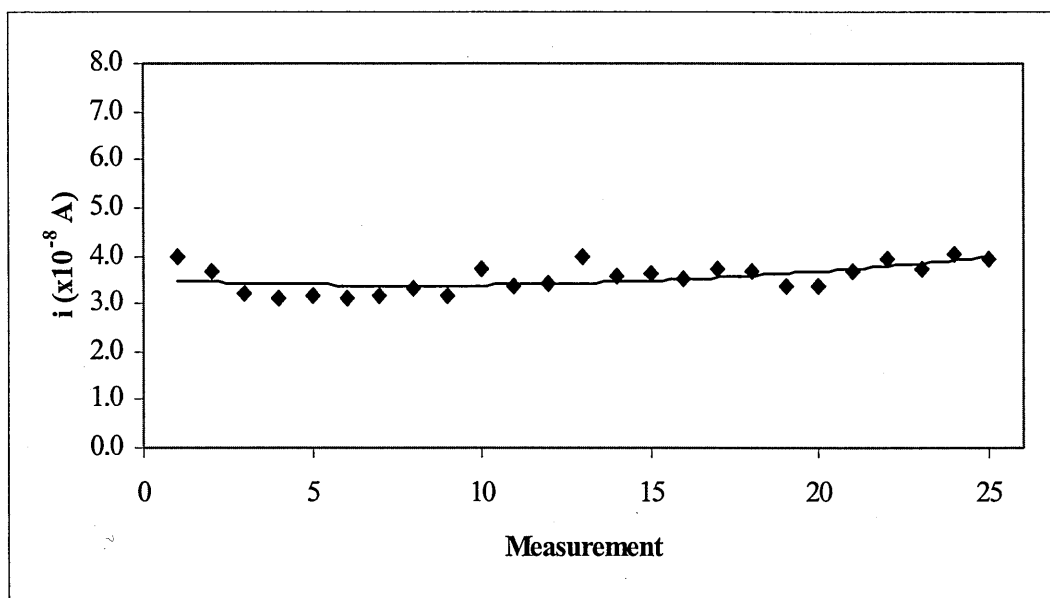


Figure 5.7 Repetitive square-wave stripping voltammograms for 100 $\mu\text{g l}^{-1}$ of lead in 0.1 M HCl. Deposition potential = -0.7 V, deposition time=120s, frequency = 8 Hz, amplitude = 0.02505 V. Cleanup process= 0.8 V (for 90 s).

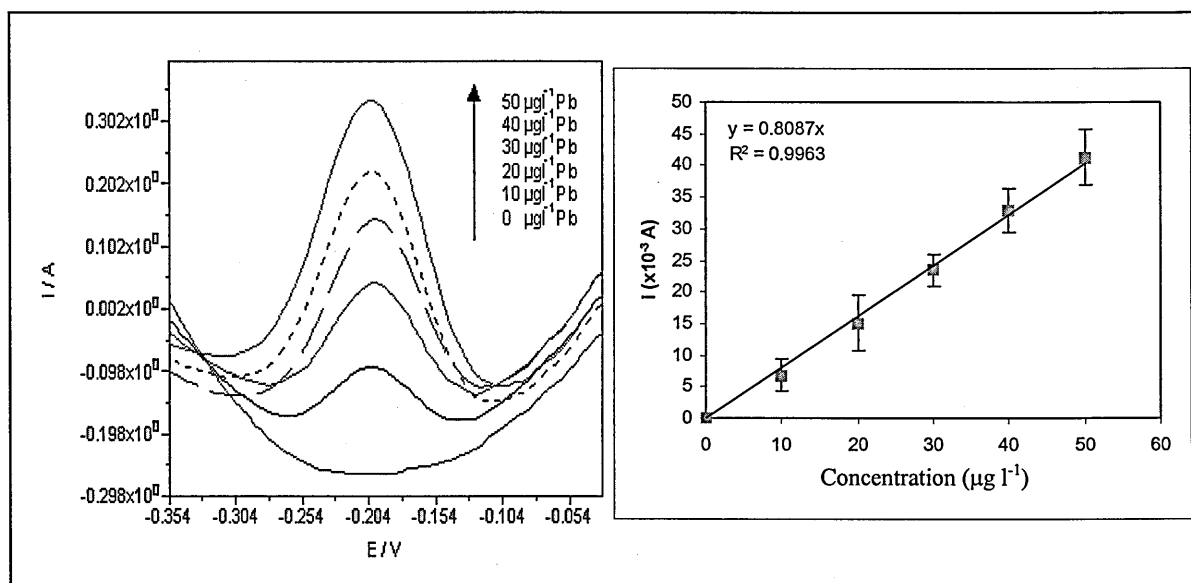


Figure 5.8: Square-wave stripping responses for distinct concentrations of lead from 10 to 50 $\mu\text{g l}^{-1}$ range. Deposition time: 240s. Other conditions as in Figure 5.3.

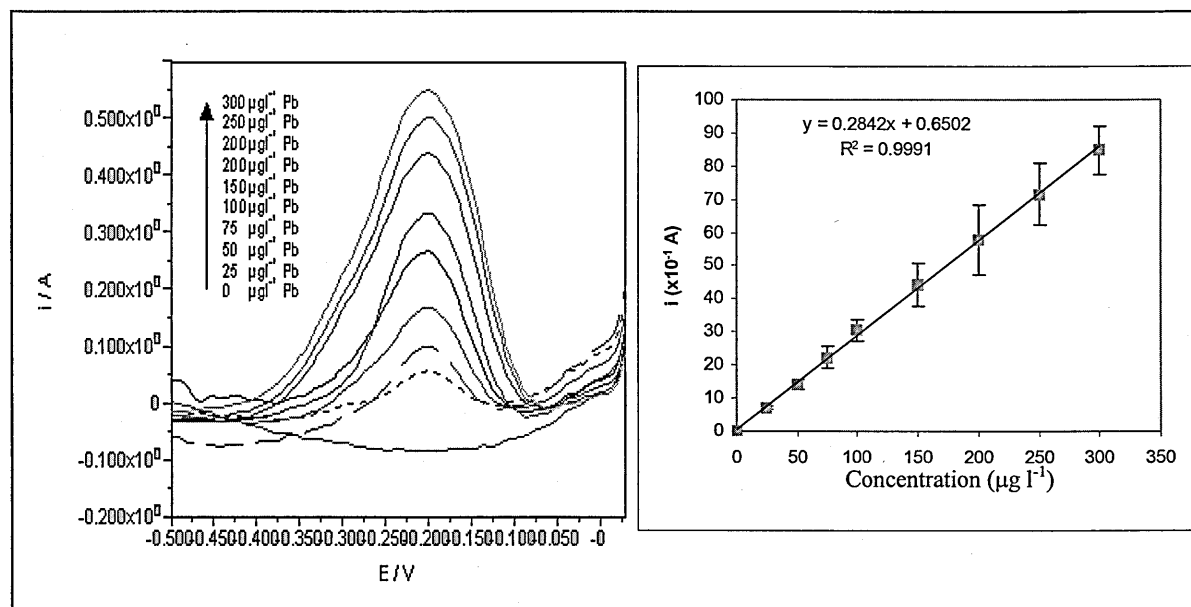
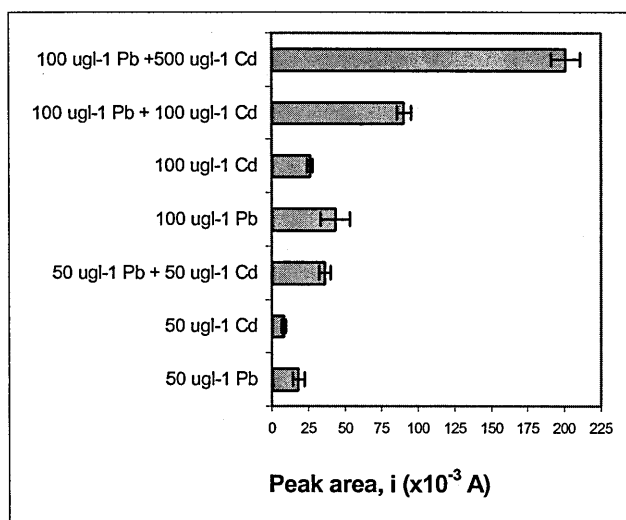


Figure 5.9: Square-wave stripping responses for distinct concentrations of lead from 25 to 300 $\mu\text{g l}^{-1}$ range. Deposition time: 120s. Other conditions as in Figure 5.3.

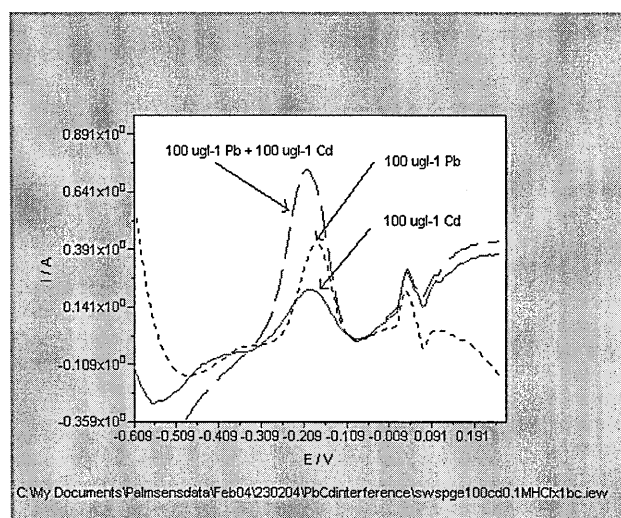
5.3.2.4 Interference Study

The selectivity of the proposed method was investigated by examining the effect of co-existing ions on the responses for Pb (II). The results indicated that cadmium has profound effect on the stripping responses of Pb (II) and the result is presented in Figure 5.10 (a) and 5.10 (b). Figure 5.10 (a) shows the peak area of Pb (II) when addition of 50, 100 and 500 $\mu\text{g l}^{-1}$ Cd (II) to 50 and 100 $\mu\text{g l}^{-1}$ Pb (II). Generally, sensitivity of 100 $\mu\text{g l}^{-1}$ Pb (II) (peak at around -0.18 V) is higher than the signal achieved for 100 $\mu\text{g l}^{-1}$ Cd (II) (peak at around -0.195) (Figure 5.10 (b)). There is significant increased in the stripping response of 100 $\mu\text{g l}^{-1}$ lead (II) in the presence of more than 50 $\mu\text{g l}^{-1}$ cadmium (II) (Figure 5.10(a)). This is due to cadmium (II) peak appearing very close to lead (II) peak as can be seen in Figure 5.10 (b) and both peaks overlap when the two metals are together in solution. Bonfil et al. (2002) reported that there is no separation between lead and cadmium peaks up to a concentration of 4 mM (828 $\mu\text{g l}^{-1}$) using conventional gold working electrode. A calibration graph for cadmium (II) in 0.05 M HCl using SPGE was therefore constructed in this study and the results indicated that a linear calibration graph for cadmium starts only from 100 $\mu\text{g l}^{-1}$ (Figure 5.19). Lower than this value, the Cd (II) peak is not reproducible. The overlapping of Pb (II) and Cd (II) peak could be a problem when analyzing Pb samples with high concentration of cadmium (II). However, the use of standard addition method should overcome these problems and it is suggested for all future testing using the gold screen-printed electrodes.

Possible interference by other ionic species in the stripping voltammetric detection was investigated by the addition of 50, 100 and 500 $\mu\text{g l}^{-1}$ chromium, mercury, arsenic, ferum, zinc, copper and calsium to a solution containing 50 $\mu\text{g l}^{-1}$ lead (II) using the optimized conditions. The results of this study are summarized in Figure 5.11. There is an increase in the stripping response of lead (II) in the presence of copper (II), while in the presence of 500 $\mu\text{g l}^{-1}$ As^{3+} ; there is more than 50 % suppression of the stripping response. Other metals do not show significant effect on stripping response of lead (II).



(a)



(b)

Figure 5.10: Effect of the addition of Cd (II) on the stripping response of lead (II) in 0.05 M HCl (a). Stripping voltammograms showing the effects of cadmium (II) on the stripping response of lead (II) in 0.05 M HCl (b).

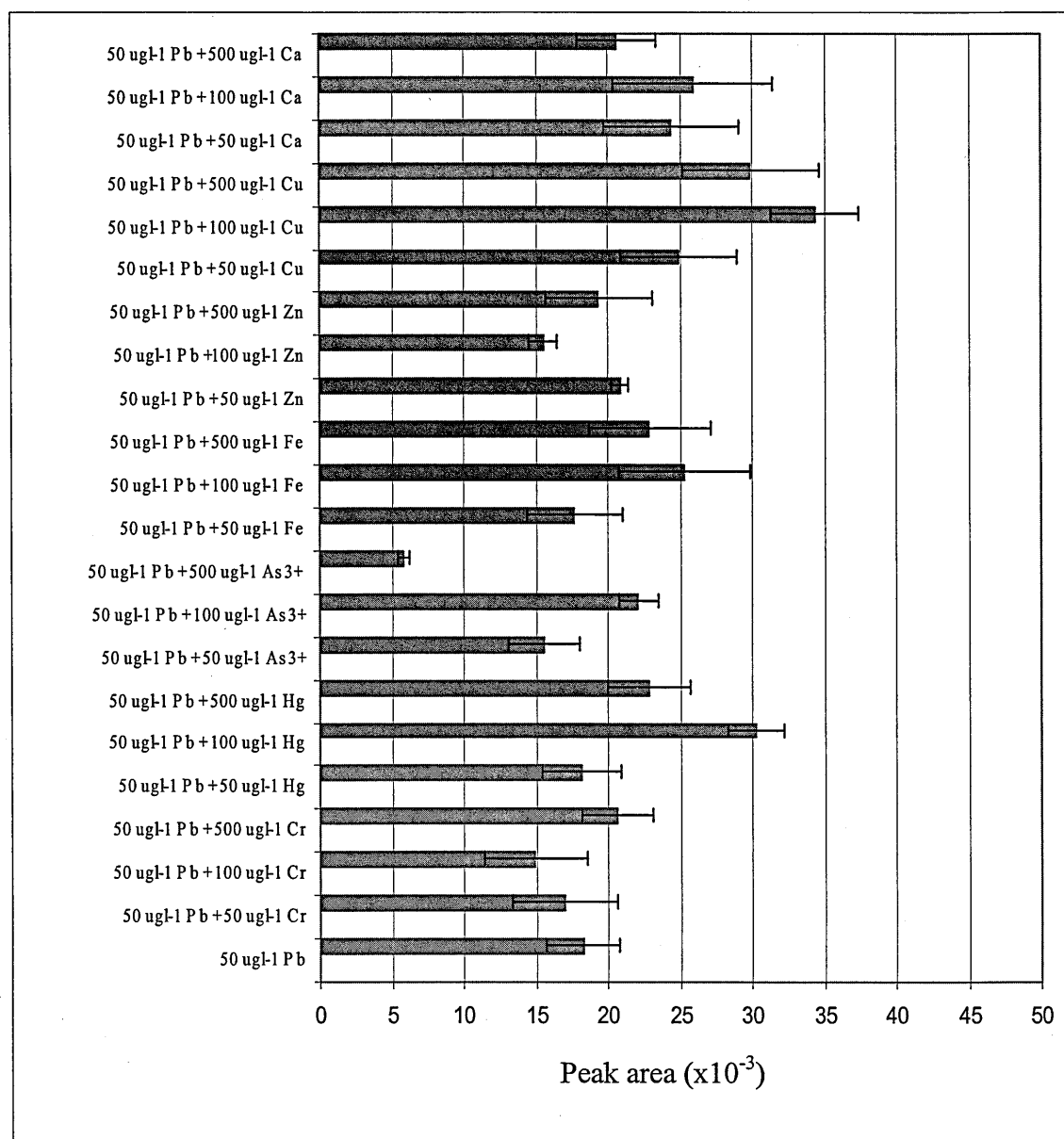


Figure 5.11 Intereference study on Pb (II) with other metals; chromium, mercury, arsenic(III), ferum, zinc, copper and calsium.

5.3.2.5 Application to wastewater and soil extract samples

The SPGE and SPMFE coupled to the portable battery-powered potentiostat were applied to the square wave voltammetry determination of Pb in wastewater samples, acetic acid and aqua regia-extracted soil samples. To perform this analysis, each sample was diluted from 10 - 62.5 times depending on Pb original contents in the samples. The results obtained were compared with those obtained by ICP-MS and SPMFE (Table 5.1 and 5.2). The concentrations of lead (II) in the samples were extrapolated from the standard addition plot (Fig. 5.12 and 5.13) constructed using the standard of addition method. Standard of addition method is a well-known method for circumventing the problems associated with matrix effects common to many analytical methods.

Table 5.1: Relation of metals presented in the wastewater samples and the results found for lead by square-wave analysis.

Waste-water Samples	Sample 1 ICP-MS ($\mu\text{g l}^{-1}$) ^a	Sample 1 SWSV ($\mu\text{g l}^{-1}$) ^b SPGE ^c	Sample 1 SWSV ($\mu\text{g l}^{-1}$) SPMFE ^d	Sample 2 ICP-MS ($\mu\text{g l}^{-1}$)	Sample 2 SWSV ($\mu\text{g l}^{-1}$) SPGE ^f	Sample 2 SWSV ($\mu\text{g l}^{-1}$) SPMFE ^g
Lead (Pb)	890 $\pm$ 0.02	983.4 $\pm$ 10.70 ^e	955.4 $\pm$ 0.70	740 $\pm$ 0.09	774 $\pm$ 5.74	862.6 $\pm$ 0.18
Arsenic (As)	< 0.05	-	-	< 0.05	-	-
Cadmium (Cd)	620 $\pm$ 0.04	-	739.8 $\pm$ 1.21	630 $\pm$ 0.009	-	517.1 $\pm$ 0.5
Chromium (Cr)	< 0.05	-	-	< 0.05	-	-
Copper (Cu)	180 $\pm$ 0.01	-	155.9 $\pm$ 0.92	180 $\pm$ 0.01	-	-
Nickel (Ni)	1350 $\pm$ 0.04	-	-	1300 $\pm$ 0.08	-	-
Zinc (Zn)	223000 $\pm$ 5.91	-	-	215000 $\pm$ 4.86	-	-
Mercury (Hg)	< 0.05	-	-	< 0.05	-	-

^aICP-MS – inductively coupled plasma-mass spectrometry

^bSWSV – square wave stripping voltammetry

^cSPGE - screen-printed gold electrode

^dSPMFE – screen-printed mercury film electrode

^evalue is the mean of triplicate determination $\pm$ standard deviation

^fSPGE +100 μl sample + Experimental condition: deposition potential= -0.7 V, deposition time = 120s, frequency = 8 Hz, step potential = 5 mV, amplitude = 25 mV.

^gSPCE + 100 μl sample + 80 mg l^{-1} Hg in 0.1M HCl + 0.5M NH_4Ac . Experimental condition: deposition potential= -1V, deposition time = 120s, frequency = 20 Hz, step potential = 5 mV, amplitude = 25 mV.

Table 5.2: Determination of lead (II) in acetic acid and aqua regia extracted soil samples.

Technique	ICP-MS ($\mu\text{g l}^{-1}$)								SWSV ($\mu\text{g l}^{-1}$)	SWSV ($\mu\text{g l}^{-1}$)
									SPGE	SPMFE
Metals	Pb	Cd	Cu	Zn	Ni	As	Cr	Hg	Pb	Pb
S3b	2853 $\pm$ 35.4	195 $\pm$ 2.10	1882 $\pm$ 37.8	27750 $\pm$ 159.7	85 $\pm$ 2.01	< 10	< 10	< 20	2894.2 $\pm$ 1.9	2220.36 $\pm$ 0.65
S4b	1537 $\pm$ 27.1	17 $\pm$ 2.08	262 $\pm$ 40.9	726 $\pm$ 16.3	83 $\pm$ 2.01	< 10	< 10	< 20	1219 $\pm$ 0.05	1171.3 $\pm$ 0.67
S5c	664	44	540	2052	189	nc	< 10	nc	741.4 $\pm$ 8.8 ^a	636.5 $\pm$ 0.5
S6c	5294	35	1197	5122	101	3450	< 10	< 20	5638 $\pm$ 3.7	5743.8 $\pm$ 0.64
S7c	2423 $\pm$ 20.8	12 $\pm$ 3.7	1853 $\pm$ 3.5	2918 $\pm$ 21.8	142 $\pm$ 2.1	655 $\pm$ 10.6	10 $\pm$ 3.6	32 $\pm$ 3.2	2272.8 $\pm$ 2.1	1981.6 $\pm$ 0.4
S8c	3046 $\pm$ 26.2	< 5	1087 $\pm$ 2.8	1006 $\pm$ 18	85 $\pm$ 2.2	654 $\pm$ 10.6	< 10	< 20	2580 $\pm$ 2.72	2284.7 $\pm$ 0.05
S9c	2799 $\pm$ 44	50 $\pm$ 34.60	1298 $\pm$ 42.2	8843 $\pm$ 92.3	61 $\pm$ 28.10	1756 $\pm$ 49.9	< 10	< 20	2340.5 $\pm$ 1.57	2765.2 $\pm$ 0.73

b – acetic acid extract

c – aqua regia extract

S - sample

nc – not certified

^aValue is the mean of triplicate determination $\pm$ standard deviation

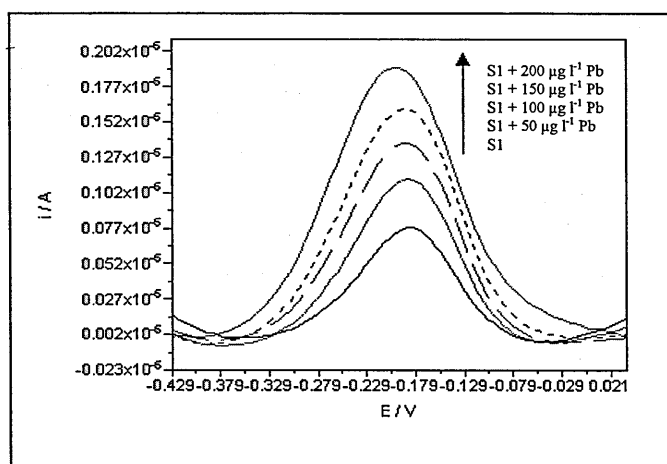


Figure 5.12 Typical square-wave voltammograms and for the determination of lead (II) in waste-water sample on SPGE. Standard addition solutions of lead (II) were added in 50, 100, 150 and 200 $\mu\text{g l}^{-1}$ increments to the sample. Other conditions as in Figure 5.3. The error bars represent standard deviation of 3 independent measurements.

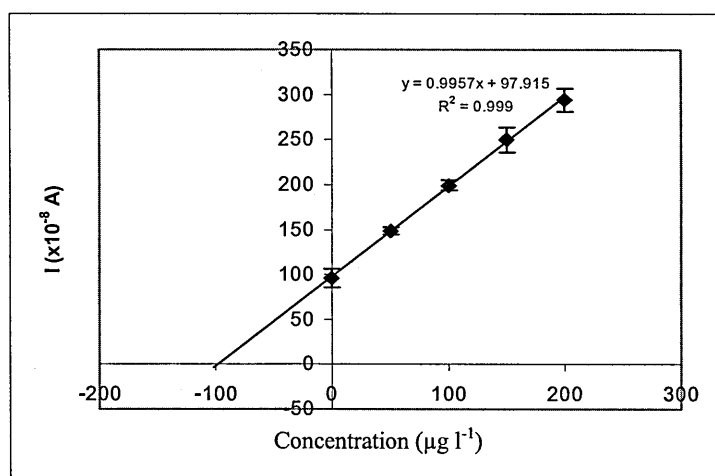


Figure 5.13 Standard addition plot for the determination of lead (II) in waste-water sample on SPGE. Standard addition solutions of lead (II) were added in 50, 100, 150 and 200 $\mu\text{g l}^{-1}$ increments to the sample. Other conditions as in Figure 5.3. The error bars represent standard deviation of 3 independent measurements.

As can be seen from Table 5.1 and 5.2, there is fairly close agreement between the results obtained with ICP-MS, stripping voltammetry determinations at SPGE and SPMFE. The good agreement of these results proves the performance of the method. Lead (II) concentrations determined with SWASV coupled to SPGE and SPMFE for

wastewater samples were much higher than the concentrations obtained with ICP-MS (Table 5.1). Typical stripping voltammograms for the wastewater sample is presented in Figure 5.12. The stripping voltammetric measurements yielded well defined stripping peaks for the waste-water samples and for the standard additions of various concentrations of lead (II) solutions. Apart from the element of interest, the wastewaters analyzed contain many other elements, which are listed in Table 5.1 and 5.2. Although Cd (II) concentration is almost at the same to Pb (II) in wastewater samples, it doesn't effects the level of measured Pb (II). This is because with addition of increase concentration of Pb (II) into the samples solution will increase the stripping peak for lead while the concentration of Cd (II) remains the same. The only drawback is that the simultaneous analysis of Cd (II) and Pb (II) could not be performed in one single analysis. The high level of Zn and Cu in the wastewater have not significantly effect the level of Pb (II) and these results agree with the results achieved previously with syntetic lead (II) samples using syntetic solutions (Figure 5.11).

The results for lead (II) in acetic acid and aqua regia extracts of soils are shown in Table 5.2. A complete lead (II) peak for quantification is obtained and the peak doesn't have any problems due to peak shape and its occurrence on a shoulder as reported by Beni et al. (2004) using linear sweep voltammetry. There is a good agreement between data obtained by ICP-MS and SPGE for both extracts (Figure 5.14). In this two extraction media, detection of lead (II) seems to be feasible. The same table also shows the influence of the presence of additional metal species on the response of the SWSV method to the lead (II) ion. Due to low level of cadmium (II) in all soil samples analysed, we expect the effect may be due arsenic and copper (II). The SWSV results were compared to those obtained by ICPMS methods by means of a correlation plot and is shown in Figure 5.7(c). A relatively high correlation coefficient value for results obtained between SWV and ICPMS ($r^2=0.9681$, $n=9$) shows good agreement between the methods, over a wide range of lead concentration levels. The equation of the line best fit between the two methods is $y= 0.9494x + 198.45$. Also, the slope obtained is close to 1 and would correspond to a perfect agreement between the two methods. The method was tested for field analysis at SENSPOL Technical Meeting on Problems related to Diffuse Pollution Sources: Characterization of Sediment, Dredged Material and Groundwater, Koblenz,

Germany, 28 - 31 October 2003. The results obtained for some spiked surface and ground water samples are presented in Table 5.3. The correlation result was presented in Figure 5.15. There was good correlation ($r^2=0.9933$) between two method tested for lead (II) analysis for results no. 24 - Cranfield University, i.e. screen-printed gold electrode and GFASS as compared to results presented by other agency (no.22). The correlation results prove that the method suitable to be used for lead (II) analysis in surface and ground water samples.

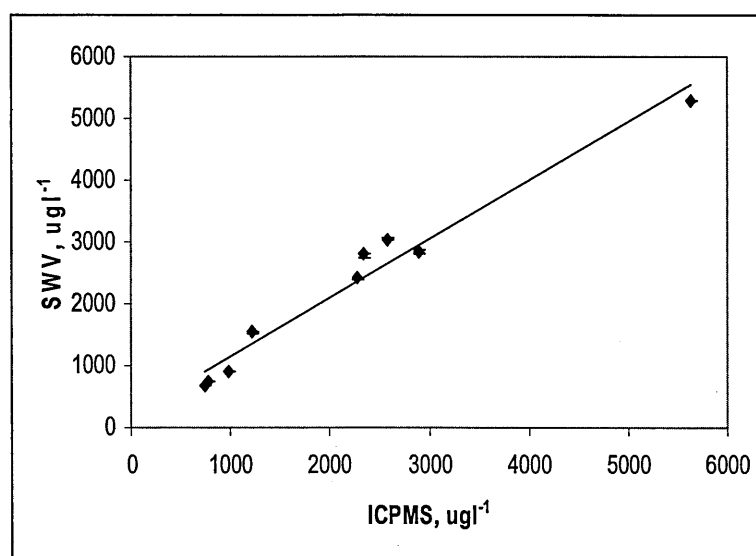


Figure 5.14: Correlation between SWV and ICPMS for the determination of lead (II) in wastewater and soil samples.

Table 5.3 : Results for Lead (II) in 0.05 M HCl analysis using square wave voltammetry technique. The analysis was done at SENSPOL Technical Meeting on Problems related to Diffuse Pollution Sources: Characterization of Sediment, Dredged Material and Groundwater Koblenz, Germany, 28 - 31 October 2003.

Sample	Results Pb ($\mu\text{g l}^{-1}$)
Iffezheim, Surface Water	<10
Iffezheim, Surface Water, P2	29.3
Groundwater, Urmitz, U P1	16.5
Groundwater, Niederwerth, NWP3	43.16

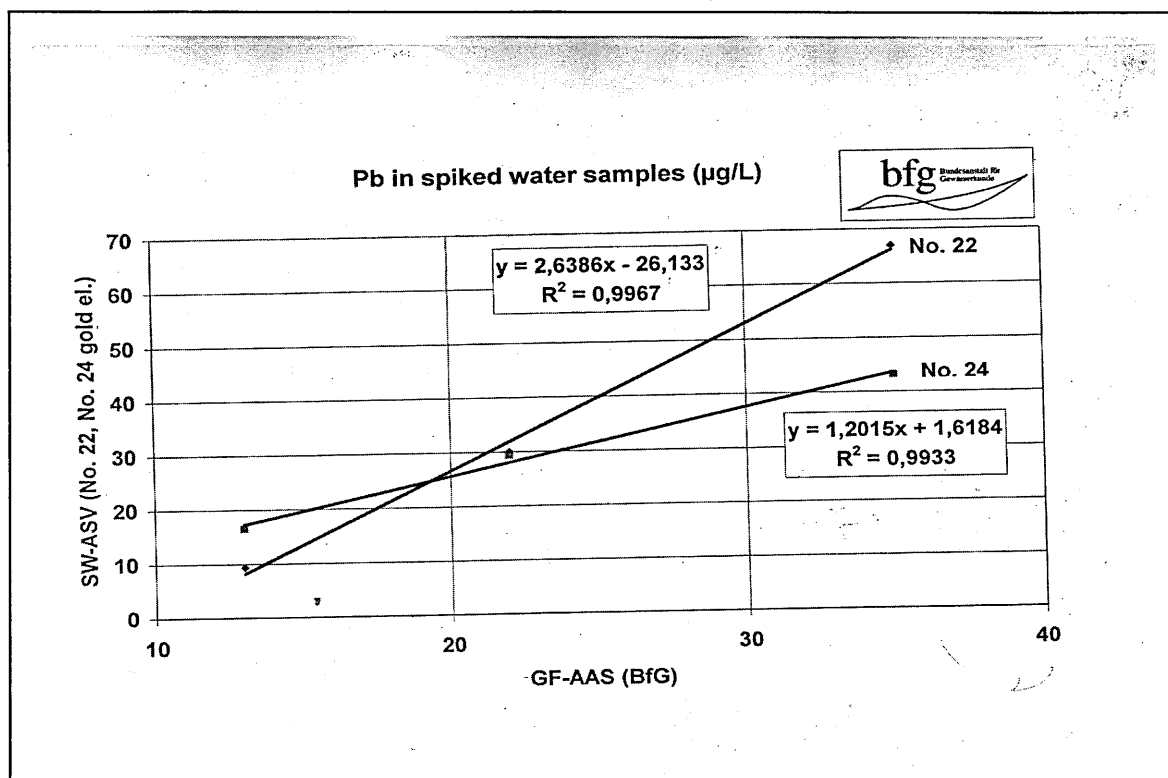


Figure 5.15: Results on correlation between two methods tested for lead (II) analysis (GF-AAS vs Screen-printed gold electrode (No.24) at SENSPOL Technical Meeting on Problems related to Diffuse Pollution Sources: Characterization of Sediment, Dredged Material and Groundwater, Koblenz, Germany, 28 - 31 October 2003. No. 22 results produced by other group which based on mercury coated screen-printed electrode.

5.4 DETERMINATION OF CADMIUM (II)

Publications on Cd (II) analysis using gold electrode are scarce. Bonfil et al. (2002), Bonfil & Kirowa-Eisner (2002) reported the use of gold and silver electrodes for Cd (II) using subtractive anodic stripping voltammetry and square-wave anodic stripping voltammetry. However, at gold electrodes, the lead and cadmium peaks overlap at concentrations of cadmium higher than 250 nM at deposition times greater than 30 s. The characteristics of the ASV were determined by the UPD phenomenon. Self-assembled monolayer of ω -mercaptocarboxylic acid monolayer on gold electrodes was used for the very sensitive and selective analysis of ultralow levels of cadmium (II) with detection limit of 6.18 part pertrillion (ppt) with Osteryoung square wave stripping voltammetry (Turyan & Mandler, 1994). Cadmium (II) could also be detected using screen-printed gold electrode with square-wave voltammetry. As reported in section 5.3.2.4 on the interference study of lead, Cd (II) is detected and overlap with Pb (II) in 0.05 M HCl at Cd (II) concentration more than 50 $\mu\text{g l}^{-1}$. This section will explain about the calibration graph and detection limit of Cd (II) in 0.05 M HCl.

5.4.1 Results of cadmium (II) analysis

Figure 5.16 shows the peak for 0, 50, 100, 150, 200, 250, 300, 400, 500 and 750 $\mu\text{g l}^{-1}$ Cd (II) in 0.05 M HCl. These peaks appear at between -0.2 to -0.3 V when square-wave stripping voltammetry was used. There is no peak observed at 10 and 25 $\mu\text{g l}^{-1}$ Cd (II). This is due to insufficient amount of Cd (II) deposited on the surface of the gold electrode.

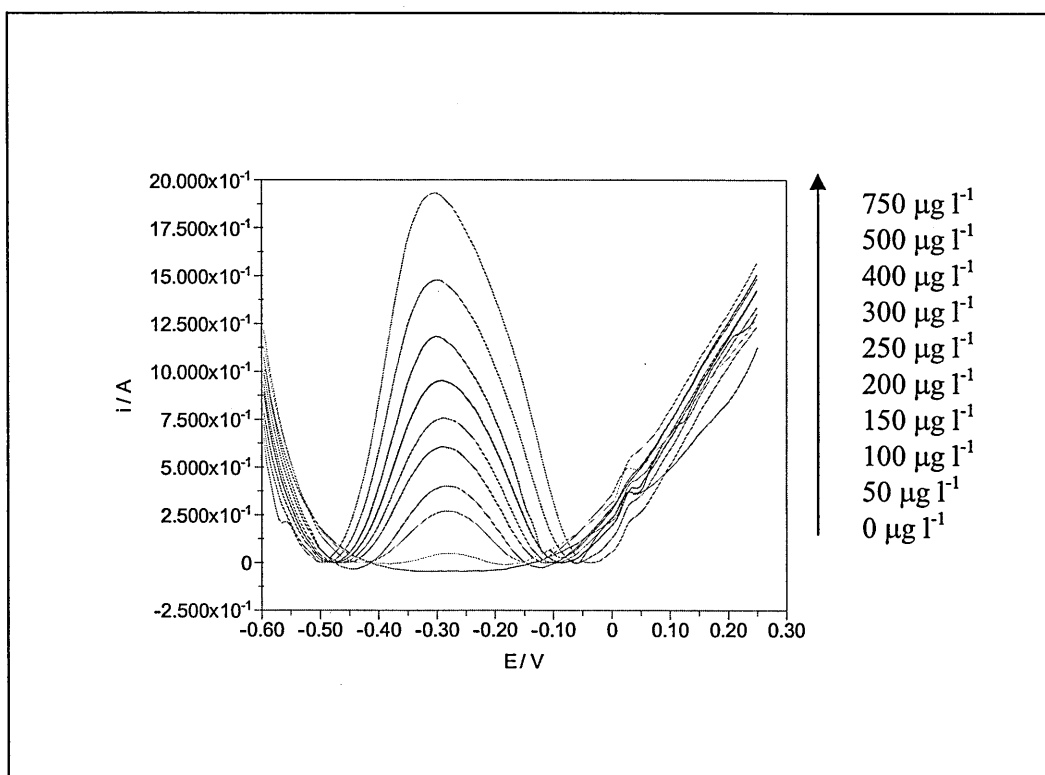


Figure 5.16: Stripping voltammograms for 0, 50 and 100 $\mu\text{g l}^{-1}$ Cd (II) in 0.05 M HCl. Deposition potential = -0.7 V, deposition time = 120s, Frequency = 8 Hz, step potential: 0.05 V; amplitude: 0.025 V.

The effect of different concentration of chloride on stripping voltammograms of 100 $\mu\text{g l}^{-1}$ Cd (II) is shown in Figure 5.17. The use of higher concentration of HCl seems to decrease the peak area of Cd (II) due to high background interferences from other peaks.

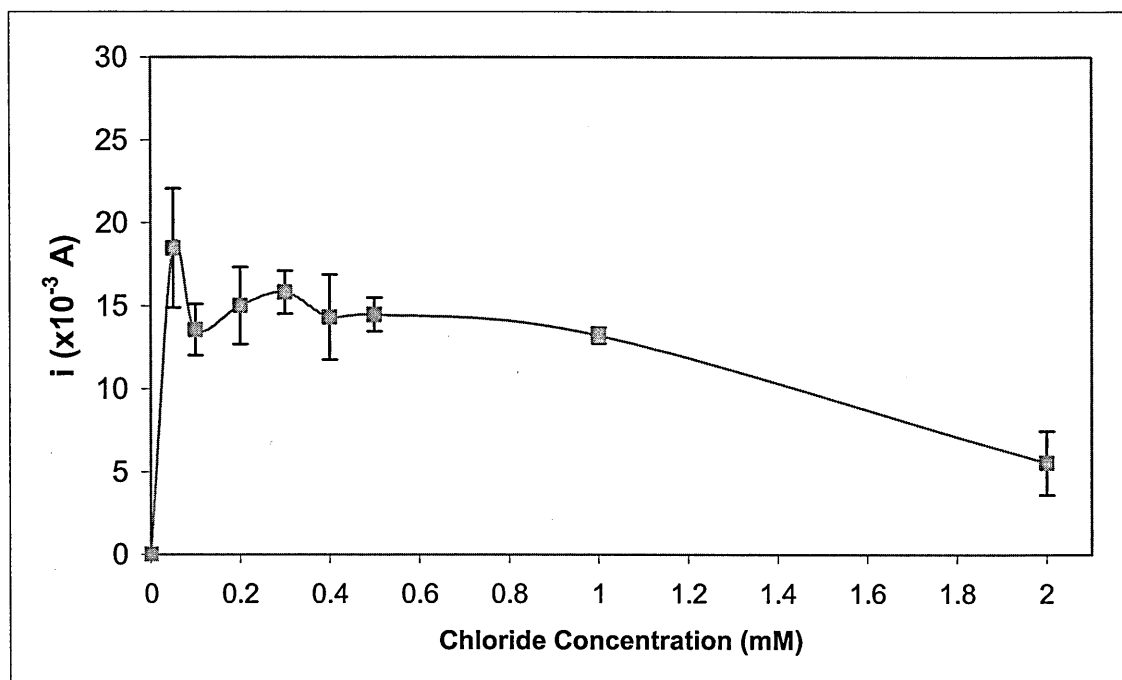


Figure 5.17: Graph on the effect of various concentration of HCl on stripping peak of $100 \mu\text{g l}^{-1}$ Cd (II). Deposition potential = -0.7 V , deposition time = 120s, Frequency = 8 Hz, step potential: 0.05 V; amplitude: 0.025 V.

Figure 5.18 shows 25 repetitive measurements of $50 \mu\text{g l}^{-1}$ Cd (II) in 0.05 M HCl on a same electrode. New electrode was cleaned using 0.5 M H_2SO_4 /0.01M KCl as previously mentioned in section 5.3.2.3 on Pb (II) analysis. In between analysis the electrode was also cleaned with same solution of 0.5 M H_2SO_4 /0.01M KCl for at 0.8V for 120 s. The Cd (II) peak area was low at first few determinations and starts to increase as measurements number increase.

The calibration graph for Cd (II) in 0.05 M HCl is shown in Figure 5.19. The range for Cd (II) is from 50 to $750 \mu\text{g l}^{-1}$. The calibration graph starts only at $50 \mu\text{g l}^{-1}$ due to lack of sensitivity of the SPGE at lower concentration. Detection limit calculated as three times standard deviation of 25 repetitive of $50 \mu\text{g l}^{-1}$ is $9.6 \mu\text{g l}^{-1}$.

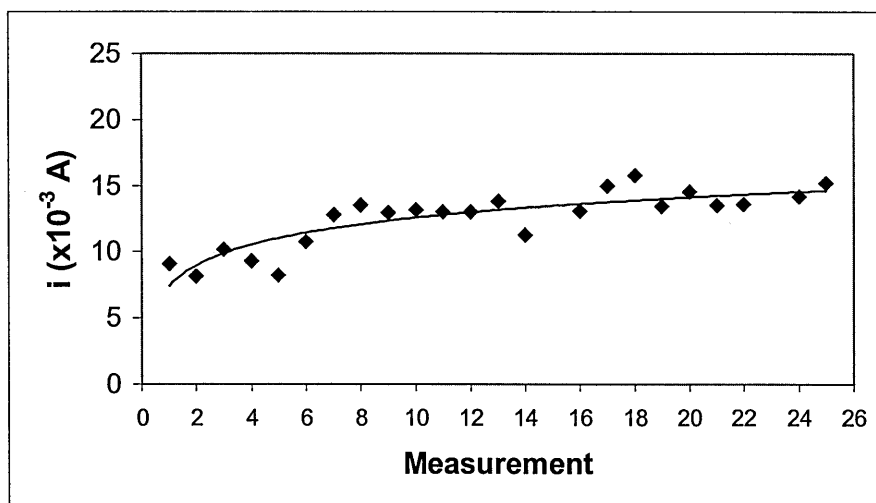


Figure 5.18: Repetitive square-wave stripping voltammograms for $50 \mu\text{g l}^{-1}$ of Cd (II) in 0.05 M HCl. Deposition potential = -0.7 V, deposition time = 120 s, frequency = 8 Hz, dep amplitude = 0.02505 V. Cleanup process = 0.8 V (for 90s).

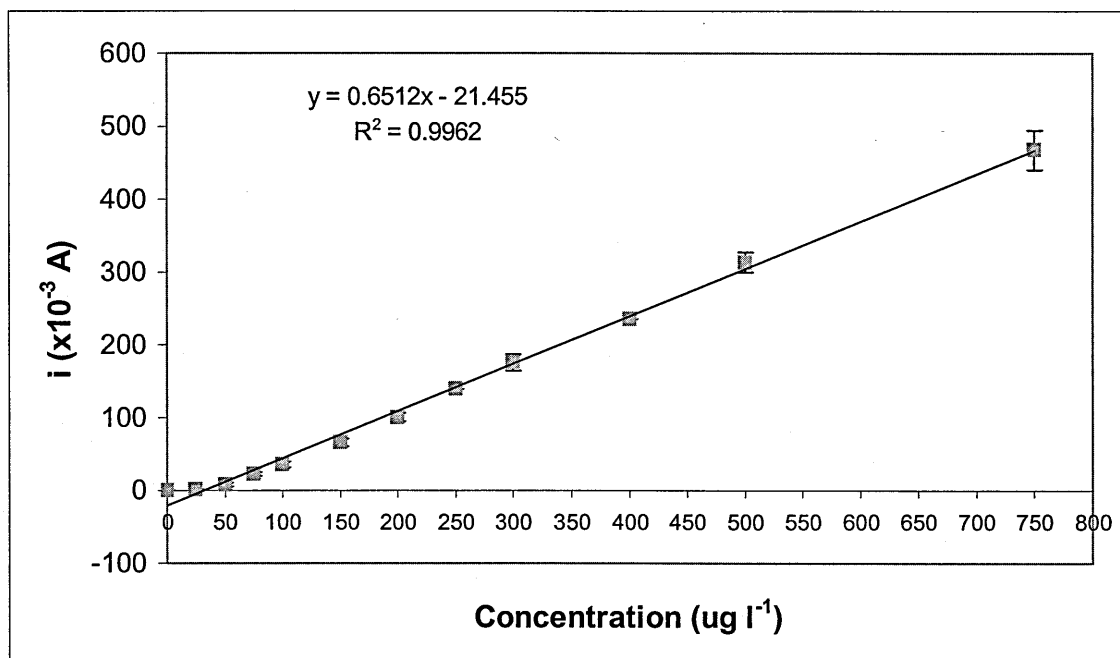


Figure 5.19: Calibration Graph for Cd (II) in 0.05 M HCl. Others condition is same in Figure 5.16.

5.5 CONCLUSION

In this study, we have demonstrated that SPGE can be successfully used for lead (II) quantification, even in samples with complex matrix such as wastewater and soil samples. The disposable three electrode configuration screen-printed gold electrodes coupled to the portable potentiostat used in this work offer some attractive features for field applications, including the use of microlitre ($\leq 100 \mu\text{l}$) sample solution, without de-aeration or stirring steps, low cost, no toxicity from mercury used and role as a complementary screening or early tool indicating the presence of lead contamination. Although the electrode is meant for disposable use, we prove that cleaning with H_2SO_4 could remove the deposited lead (II) and the electrode can be reused for up to 20 subsequent analysis.

The coupling of the screen-printed gold electrode and the portable electrochemical analyzer using the square-wave technique proved to be an excellent tool in the determination of the available lead in soils as well as wastewater samples. Though the performance of the SPGE used for square-wave measurements measured comparable to those at SPMFE; the only downside is the undesirable effect of cadmium if present at high concentration in the samples. A more systematic study involving the use of different electrolytes will be tested for separating the lead (II) and cadmium (II) peak.

There is possibility of detecting Cd (II) in 0.05 M HCl using screen-printed gold electrodes. However, due to Cd (II) peak proximity to lead (II) in 0.05 M HCl solution, the Cd (II) peak will overlap with lead (II) if lead (II) ions are present in the solution. Generally, Pb (II) is more sensitive than Cd (II) on screen-printed gold electrode.

CHAPTER 6

6.0 DETECTION OF ARSENIC (III) USING SCREEN-PRINTED GOLD ELECTRODES

6.1 OUTLINE

In this chapter, reviews and analysis of arsenic (III) with screen-printed gold electrodes using linear sweep voltammetry are described.

6.2 INTRODUCTION

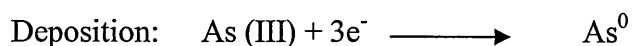
Arsenic contamination is a worldwide problem and has become a challenge for world scientists. There are many detection methods have been develop for determination of trace levels of arsenic (Burguera & Burguera, 1997; Karthikeyan & Hirata, 2003; Cavicchioli et al., 2004; Francesconi & Kuehnelt, 2004). The methods include hydride generation atomic absorption spectrometry and hydride generation atomic fluorescence spectrometry (Amankwah & Fasching, 1985), graphite furnace atomic absorption spectrometry (Lima et al., 2003; Shemirani et al., 2004), inductively coupled plasma (ICP) techniques, with atomic fluorescence spectrometry (AFS) (Gomez-Ariza et al., 2000; Cava-Montesinos et al., 2003) or mass-spectrometry (MS) (Brisbin et al., 2002; Wang et al., 2001), high-performance liquid chromatography with ICP-MS (Nakazato et al., 2002; Garcia-Manyes, 2002) and neutron activation analysis (Sun & Yang, 1999). Most of these methods are sensitive and provide good and reliable results. However, methods based on these techniques are expensive, time consuming due to complicated procedures, require special sample pre-treatment and cannot be used for field monitoring of a large number of samples. Majority of commercially available field sensors are still based on colorimetric techniques, which offer rapid but not necessarily correct results in on-site conditions (Hung et al., 2004). Therefore, to solve these problems, a rapid and sensitive sensor needs to be developed. Electroanalytical techniques have excellent sensitivity and the availability of low-cost portable equipment are ideal for field analysis and therefore provide a possible mean of fulfilling this requirement.

Stripping voltammetry and potentiometry techniques have been used widely for the determination of As (III), these involve cathodic stripping voltammetry (CSV), anodic

stripping voltammetry (ASV) or potentiometry with chemical or constant current. These techniques are preferred because the substance of interest is pre-concentrated on the working electrode and frequently used to determine traces of As. As (III) analysis for CSV is normally carried out using a hanging mercury drop electrode (Holak, 1980; Sadana, 1983; Zima & van den Berg, 1994; Greulach & Henze, 1995; Li & Smart, 1996; Henze et al., 1997; Barra & Santos, 2001; Ferreira & Barros, 2002). Arsenic will be preconcentrated in highly acidic media with further scanning in the cathodic direction to obtain peak due to the formation of arsine (Holak, 1980). In order to increase sensitivity, the accumulation step involves the use of an adsorption or deposition inducer to immobilize the analyte on the working electrode. As a rule, copper or selenium ions are added in excess to the solution containing arsenic ions (Cavicchioli et al., 2004).

There are a large number of reported research papers details method of arsenic detection (Sun et al., 1997; Kopanica & Novotný, 1998; Huang & Dasgupta, 1999; Rasul et al., 2002; Švancara et al., 2002; Feeney & Kounaves, 2000, 2002; Prakash et al., 2003; Dai et al., 2004). Gold is the most suitable working electrode for the determination of arsenic using stripping voltammetry or potentiometry. Au provides a more sensitive response toward As oxidation than the other electrode materials studied and has a higher hydrogen over voltage than platinum (Dai et al., 2004), which reduces the problem of simultaneous evolution of hydrogen (Forsberg et al., 1975) while depositing arsenic. Gold also displays better reversibility of the electrode reactions for arsenic in both the plating and stripping step (Forsberg et al., 1975), which results in higher and sharper oxidation peaks (Sun et al., 1977; Forsberg et al., 1975). Table 6.1 shows a summary for arsenic detection on gold and gold film electrode using stripping voltammetry and potentiometry.

In anodic stripping voltammetry, As (III) and As (V) are initially reduced to As^0 at a sufficiently negative potential and a film is formed on the surface of the gold working electrode (Li and Smart, 1996). However, As (V) requires the application of a more negative potential due to poor electroactivity.





In the stripping process (subsequent reoxidation of arsenic), which leading to the anodic current recorded in the voltammogram, it is generally assumed that the reaction stops at the trivalent stage:

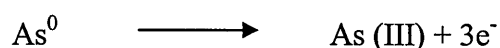


Table 6.1: Summary for arsenic detection on gold or gold film electrode using stripping voltammetry and potentiometry.

Authors	Electrode material	technique	Electrolytes	Deposition time /s	Detection limit / $\mu\text{g l}^{-1}$
Dai et al, 2004.	Gold nanoparticle-modified glassy carbon	LSV and SWV	1 M HCl	180s	0.0096 and 0.014
Feeney and Kounaves, 2000, 2002.	Gold ultramicroelectrode array	SWV	2 M HCl	300	0.013
Kopanica & Novotný, 1998.	Gold disk	DPASV	1 M H_2SO_4 + 0.1 M HCl	300	0.15
Sun et al.	Rotating gold disk	ASV	2 M HCl	240	0.19
Prakasah et al., 2003	Rotating gold disk	DPASV	0.25 N HCl	60	2
Huang & Gasgupta, 1999.	Gold film on Pt wire	ASV	4.5 M HCl	80	0.5
Švancara et al., 2002.	Gold film plated carbon paste	CCSA	1 M HClO_4 + 0.2 M HCl	-	3 (As(III)) 0.5 (As(V))
Rasul et al., 2002	Gold film-glassy carbon	ASV	4.5 M HCl	120	1.2
Viltchinskia et al., 1997.	Gold film graphite	ASV	0.1 M HCl	30-100	<0.4
Davis et al., 1978	Gold film on pyrolytic graphite	ASV	7.3 M HCl	120	-
Munoz & Palmero, 2004a,b	Co-deposition Au (III) and As (III) at Glassy-carbon electrode.	PSA	1.2 M HCl	120 60	0.55 (As(III)) 0.79 (As(V))

LSV-Linear sweep voltammetry, SWV-Square-wave voltammetry, DPASV-Differential pulse anodic stripping voltammetry, CCSA-Constant current stripping analysis, ASV-Anodic stripping voltammetry, PSA-Potentiometric Stripping Analysis

However, most of the stripping voltammetry methods available use conventional electrodes in an electrochemical cell. The modifications made were only on the working electrode. This section reports the possibility of using screen-printed gold-electrode for arsenic (III) analysis in synthetic solution. The method was optimized for several parameters using linear sweep voltammetry. Possibilities of interferences from other metals were also tested.

6.3 EXPERIMENTAL

6.3.1 Chemicals

The electrolyte used in the experiments was 1 M HCl for As (III). The other chemicals used were described in previous section for lead (II) and cadmium (II) analysis as described in chapter 5.

6.3.2 Electrode Fabrication

The screen-printed gold electrodes were used for the arsenic (III) analysis and were fabricated as described in chapter 2.

6.3.3 Procedure

Autolab Electrochemical Analyser and PalmSens were used for electrochemical measurements. Tests were carried out by placing a 100 μ l sample on a three-electrode strip for each experiment in unstirred and non-deaerated solution. Linear sweep voltammetry technique was used by deposition of the target arsenic (III) at a potential of -0.3 V (vs. screen-printed Ag/AgCl) for 60 s. New electrode was used in every analysis. The SPGEs were used without prior cleaning with 0.5 M H_2SO_4 /0.01M HCl.

6.4 RESULTS

Several techniques were tested for As (III) analysis, namely constant current chronopotentiometry (CCSCP), square-wave voltammetry (SWV) and linear sweep voltammetry (LSV). The gold electrodes were used without prior cleaning with 0.5 M H_2SO_4 /0.01M KCl. This is due to the use of high concentration of HCl in the analysis (1 Molar) could make the gold film on the electrode dissolve (the active gold surface for the deposition was reduced) because of the tendency for complex formation

between the gold and chloride ion (Sun et al., 1997), the reused of the electrodes even after cleaning would effect the reproducibility of the stripping peaks.

Stripping potentiometry has been used during the past 23 years for the determination and speciation of arsenic in several samples (Munoz & Palmero, 2004). Figure 6.1 shows the chronopotentiometry stripping analysis (CCSCP) of $0 \mu\text{g l}^{-1}$ and $50 \mu\text{g l}^{-1}$ As (III) in 1 M HCl. Arsenic (III) peak appears at 0.14 V as compared to no peak observe in $0 \mu\text{g l}^{-1}$ As (III). However, there is an interfering peaks appear at more negative potential around 0.058 V. This interfering peak is due to the present of chloride in the electrolyte.

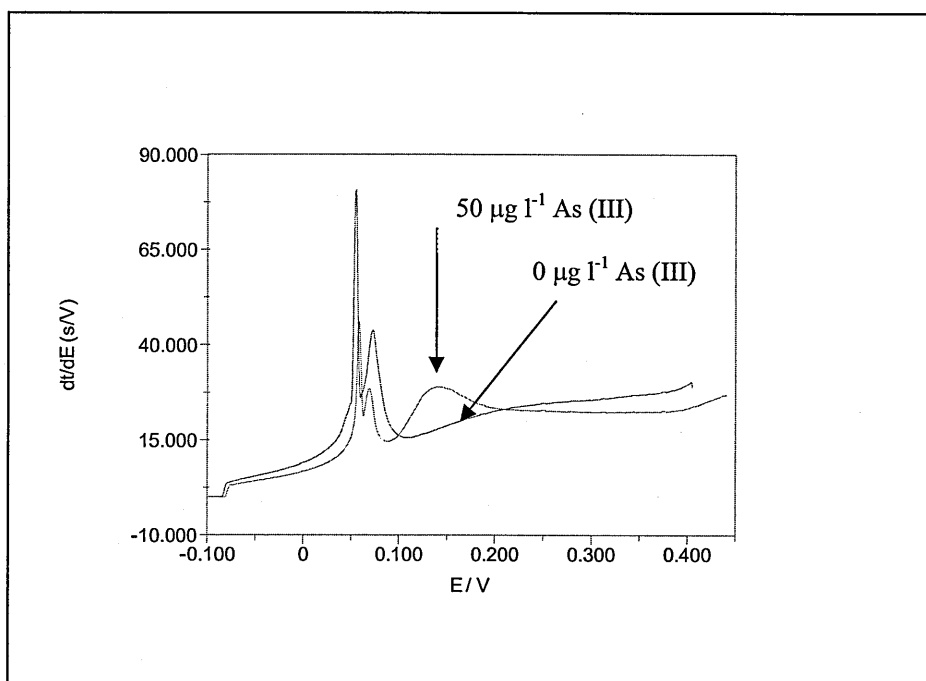


Figure 6.1 : Chronopotentiometry Stripping Analysis of 0 and $50 \mu\text{g l}^{-1}$ As (III) in 1 M HCl. Deposition potential = -0.1 V, deposition potential = 120 s and stripping current= $1 \mu\text{A}$.

Figure 6.2 shows $0 \mu\text{g l}^{-1}$ and $50 \mu\text{g l}^{-1}$ As (III) in 1 M HCl when square-wave anodic stripping voltammetry (SWASV) is used. The As(III) peak appears at 0.262 V. There is still a present of interfering peak before the As (III) peak. For the rest of the analysis, linear-sweep voltammetry has been the technique of choice because good reproducibility obtained as compared to CCSCP and SWASV. Figure 6.3 shows a

typical peaks for 0 and 50 $\mu\text{g l}^{-1}$ As(III) in 1 M HCl when LSV is used. The peak appears at potential of 0.29 V after interfering peak at 0.08 V.

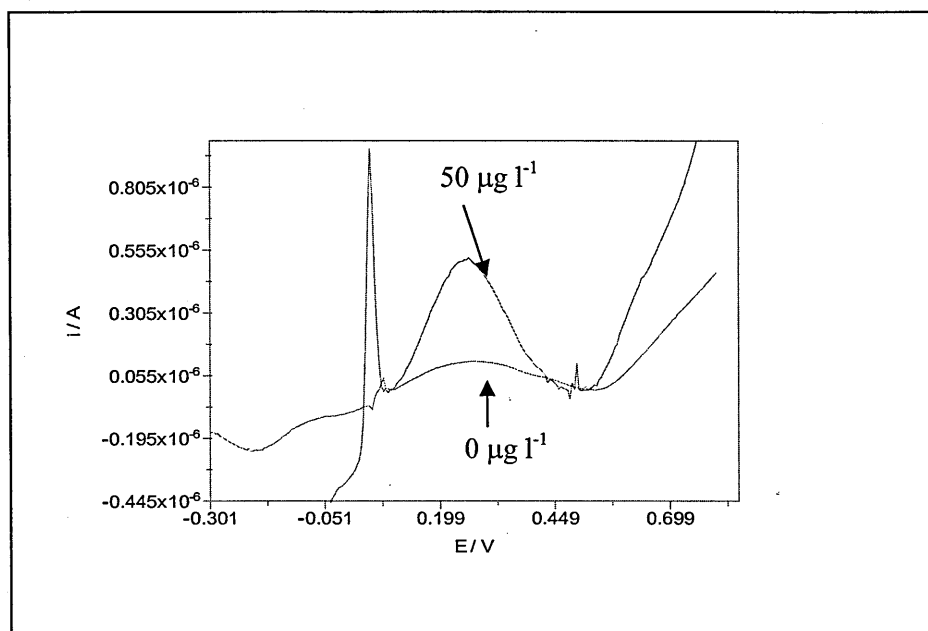


Figure 6.2: Square-Wave Stripping Voltammetry analysis of 0 and 50 $\mu\text{g l}^{-1}$ As (III) in 1 M HCl. Deposition potential = -0.3 V, deposition time = 60s, frequency = 25 Hz, step potential = 0.0495 and amplitude = 0.02505V.

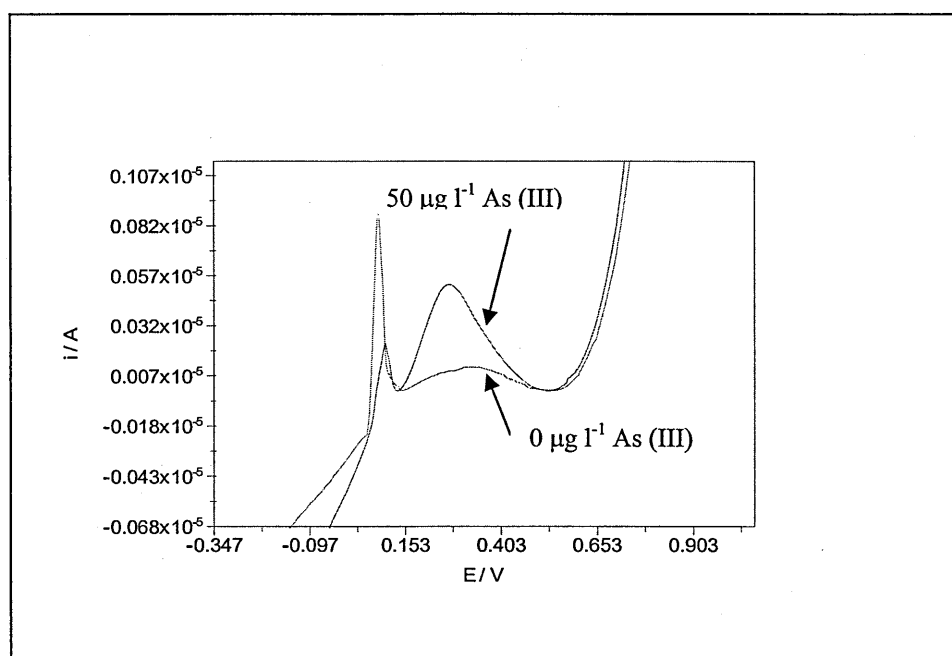


Figure 6.3: Linear sweep voltammetry of 0 $\mu\text{g l}^{-1}$ and 50 $\mu\text{g l}^{-1}$ As (III) in 1 M HCl. Begin potential = -0.3 V, end potential = 0.8 V, step potential = 0.00244, scan rate = 200 mV, equilibrium time = 60 s.

All of the studies summarized in Table 6.1 show that HCl is the most suitable supporting electrolyte for the electrochemical stripping determination of arsenic. This is because the stripping medium must be acidic in order to avoid the formation of hydrolysed species during the stripping step (Sun et al., 1997; Hamilton et al., 1980; Sadana, 1983; Davis et al., 1978). This electrolyte also aided in achieving good sensitivity and the stripping peak was also narrow which indicated that the charge-transfer reaction was fast and reversible. The importance of chloride ions for arsenic analysis has been highlighted in other studies. With increasing HCl concentration, more chloride ions tightly bind in double layer around the electrode and acts as an ionic bridge between the working electrode and arsenic ion. This makes the redox reaction more reversible (Arnold, 1967).

Figure 6.4 shows the effect of chloride ion on linear sweep voltammetry of As (III). When the assay is carried out without HCl, no As (III) or interfering peak can be seen. The As (III) starts to appear even with lower concentration of HCl (0.1 M) and the As (III) peak area increase accordingly with increasing HCl concentration. However, to choose the best concentration of HCl for the rest of the analysis, the effect of interference peak has to be taken into consideration. The higher concentration of HCl used will increase the interfering peak and decrease the As (III) peak area (Figure 6.4). It was also noticed that reproducibility decreased that may be due to adsorbed chloride on the gold substrate (Feeney & Kounaves, 2000). On the other hand, it is necessary to minimise the amount of acidic waste generated. For the rest of the analysis, 1 M HCl was used due to compromise between peak area and interfering peak.

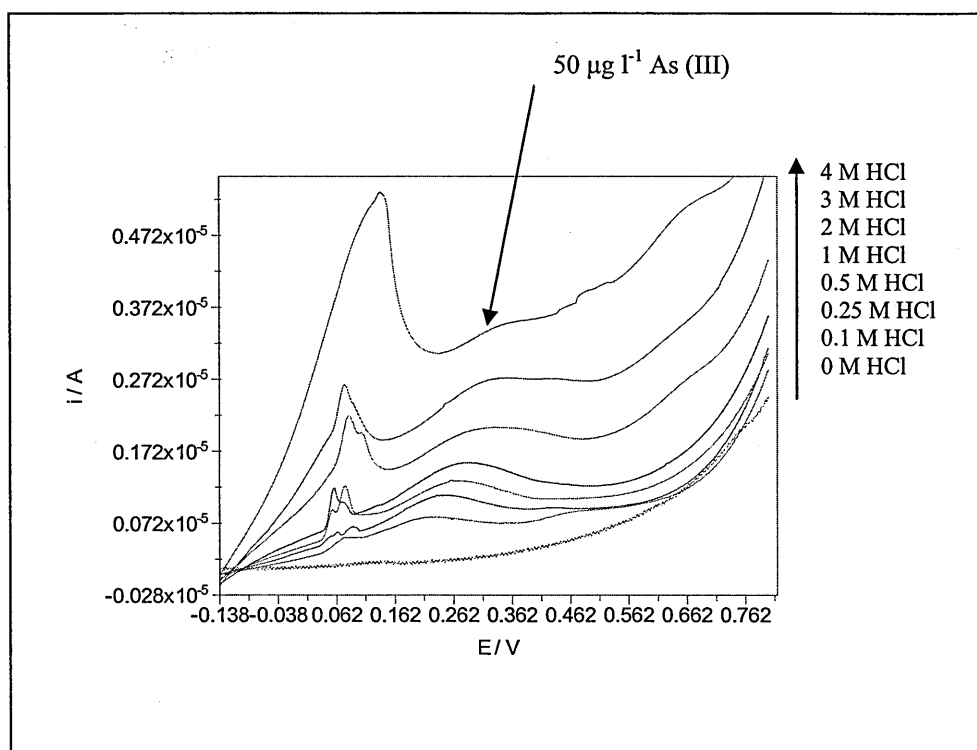


Figure 6.4: Stripping voltammograms on the effect of chloride ion on linear sweep voltammetry of $50 \mu\text{g l}^{-1}$ As (III) in . Begin potential = -0.3 V , end potential = 0.8 V , step potential = 0.00244 , scan rate = 200 mV , equilibrium time = 60 s .

Figure 6.5 shows the effect of deposition time for As (III). The stripping peak area increases when the stripping time increasing until about 120 s . After this time, the gold electrode becomes saturated and the increase in deposition time doesn't improve the peak area. A deposition of 60 seconds was used in this study to prevent possible error resulted from the electrode surface saturation.

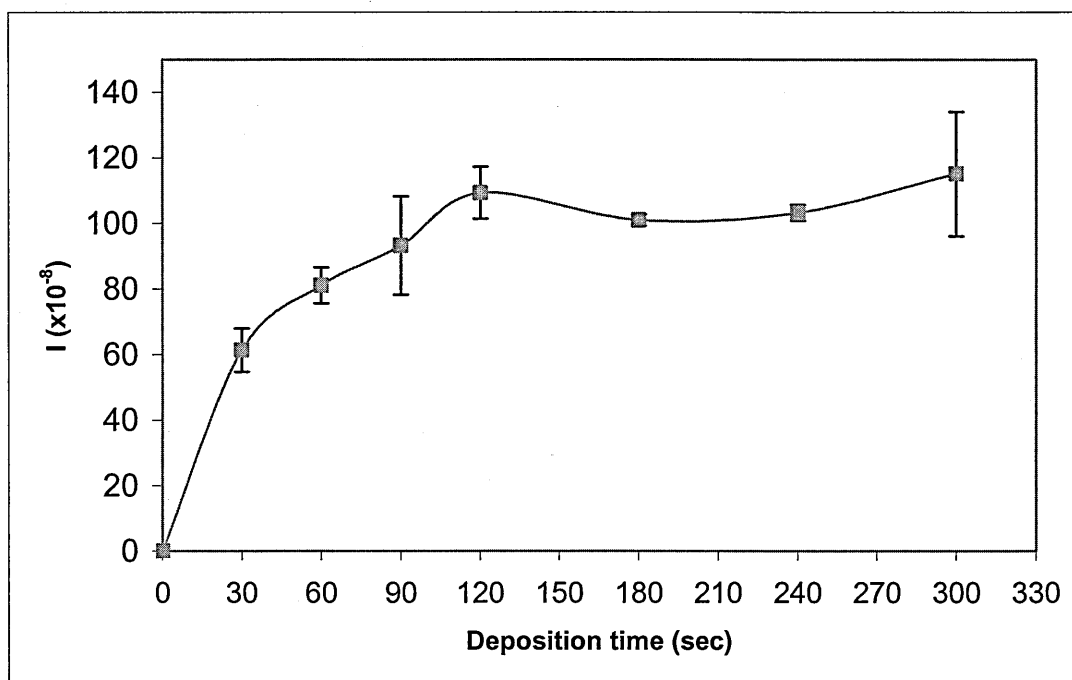


Figure 6.5: Effect of deposition time on LSV of $50 \mu\text{g l}^{-1}$ As (III) in 1 M HCl. Begin potential = - 0.3 V, end potential = 0.8 V, step potential=0.00244, scan rate=200 mV.

Figure 6.6 shows the effect of deposition potential for As (III). There is very small peak appears at -0.1 V. At this potential there is very little amount of As (III) deposited on the gold electrode. The peak starts to increase at -0.2 V until -0.6 V. The deposition potential -0.3 V was chosen due to not much difference in peak current occurs in the range of -0.2 to -0.6 V. However, at -0.7 V, the As (III) peak starts to deteriorate due to appearance of interference from other peak at very negative potential.

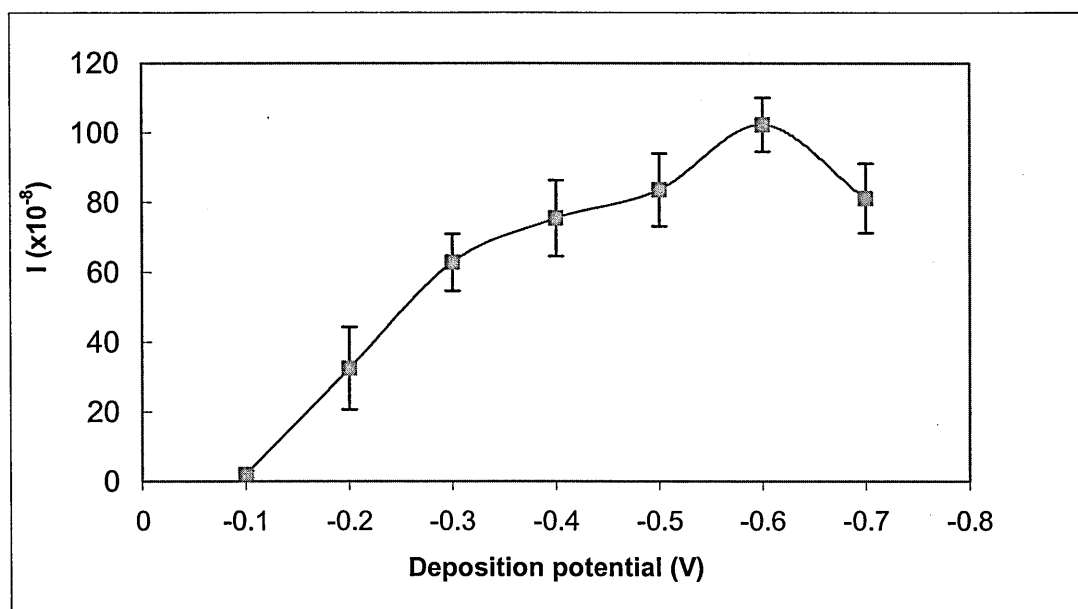


Figure 6.6: The effect of deposition potential for $50 \mu\text{g l}^{-1}$ As (III) in 1 M HCl. Other parameters are same as in Figure 6.4.

The effect of scan rate is shown in Figure 6.7. Although there is some improvement when higher scan rate is used, however the improvement is insignificant when we almost the same calibration graph for 100 mV s^{-1} and 200 mV s^{-1} was obtained. For the rest of the analysis, 200 mV s^{-1} was chosen to provide the necessary sensitivity and a well defined peak.

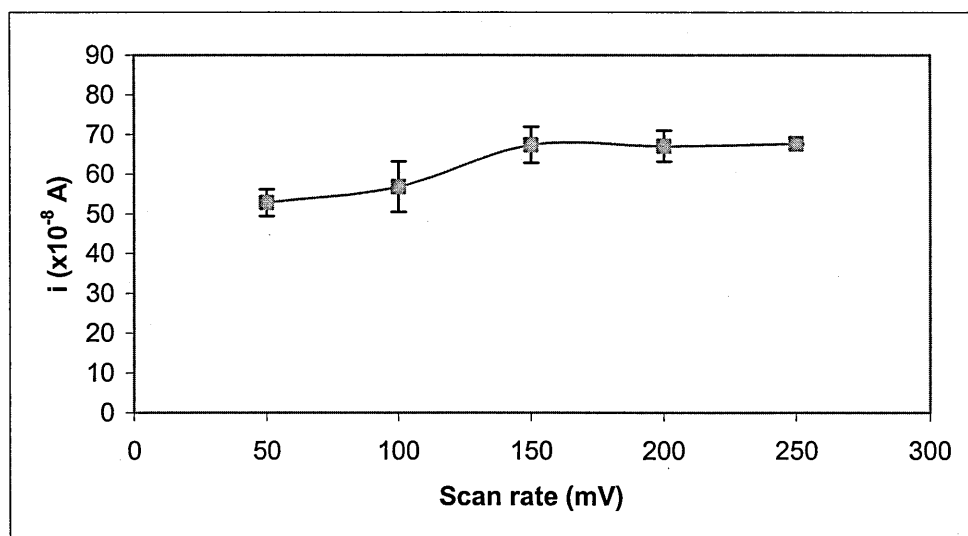


Figure 6.7: Effect of scan rate on $50 \mu\text{g l}^{-1}$ As (III) in 1 M HCl. Other parameters are same as in Figure 6.4.

Figure 6.8 shows the effect of other metals on stripping voltammetry of As (III) in 1 M HCl. Three different concentration of calcium, cadmium, chromium, ferum, lead and zinc from 100, 200 and 1000 $\mu\text{g l}^{-1}$ were added to 100 $\mu\text{g l}^{-1}$ of As (III) in 1 M HCl. There are no significant interferences from calcium, cadmium, chromium, ferum and zinc on As (III) peak. Lead, copper and mercury have been found to present the most serious effects on stripping voltammetry of As (III) because the stripping peaks of Pb (II), Cu (II) and Hg (II) are close to the stripping peak of As and intermetallic compounds of Cu-As and Hg-As can be formed at the working electrode (Kopanica & Novotny, 1998; Huang & Dasgupta, 1999). The addition of lead in the As (III) solution seems to decrease the peak area of As(III) and there is an appearance of lead (II) peak at more negative potential when 1000 $\mu\text{g l}^{-1}$ Pb added (II) (Figure 6.9(d)).

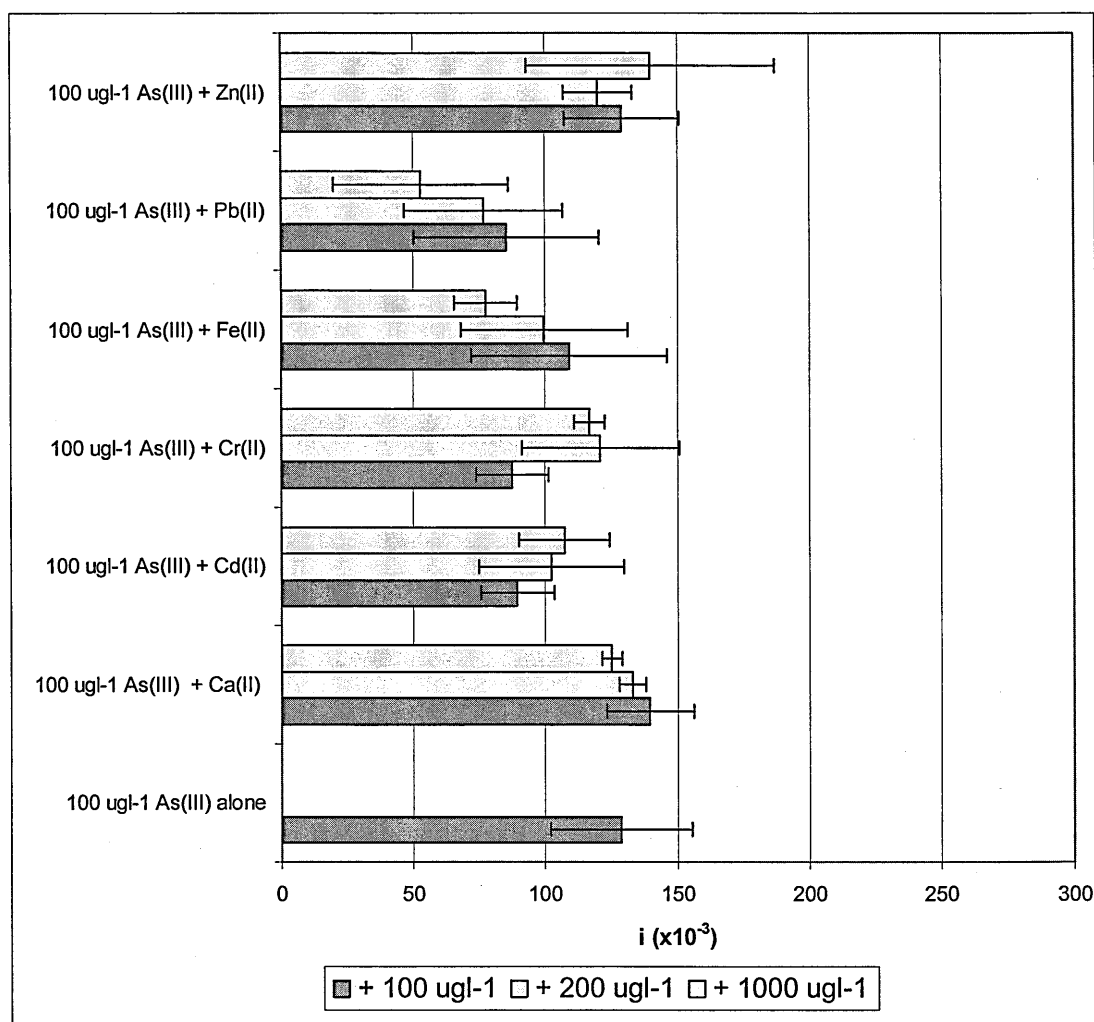


Figure 6.8: Effect of other metals on the stripping voltammetry of As (III) in 1 M HCl.

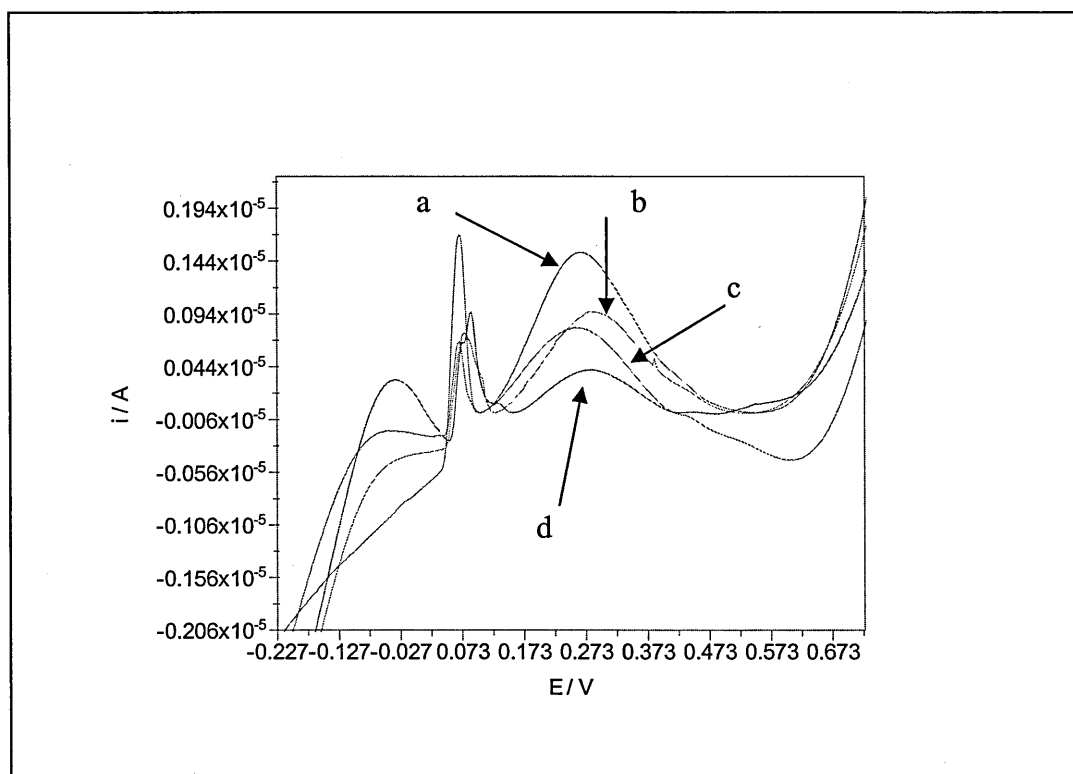


Figure 6.9: Voltammograms of $100 \mu\text{g l}^{-1}$ As (III) (a), and addition of $100 \mu\text{g l}^{-1}$ Pb (II) (b), $200 \mu\text{g l}^{-1}$ Pb (II) (c) and $1000 \mu\text{g l}^{-1}$ Pb (II) (d) in 1 M HCl.

As in the case of copper, addition of 100 and $200 \mu\text{g l}^{-1}$ Cu will reduce the As (III) peak and Cu (II) peak appears at positive potential (Figure 6.10). The addition of $1000 \mu\text{g l}^{-1}$ Cu in the As (III) solution significantly increased the peak area of Cu (II) with small As (III) shoulder peak. This is due to having nearby oxidation peaks, Cu and As (III) have been shown to combine strongly (Forsberg et al., 1975). The use of other method such as potentiometric also gives the same results as above reported and requires that the samples contain low Cu (II) content. In samples where Cu (II) concentration is high, it should be necessary to modify the method with an additional step such as ion-exchange separation in order to eliminate Cu (II) effectively from the sample prior to analysis (Svancara et al., 2002). The effect of Hg (II) on stripping of $100 \mu\text{g l}^{-1}$ As(III) is presented in Figure 6.11. There is an appearance of small peak at positive potential towards Arsenic (III) peak when $200 \mu\text{g l}^{-1}$ As (III) is added. New peak appears at 0.46 V when $1000 \mu\text{g l}^{-1}$ Hg (II) is added in the solution although there is still a small peak previously from As (III) which doesn't fully disappear.

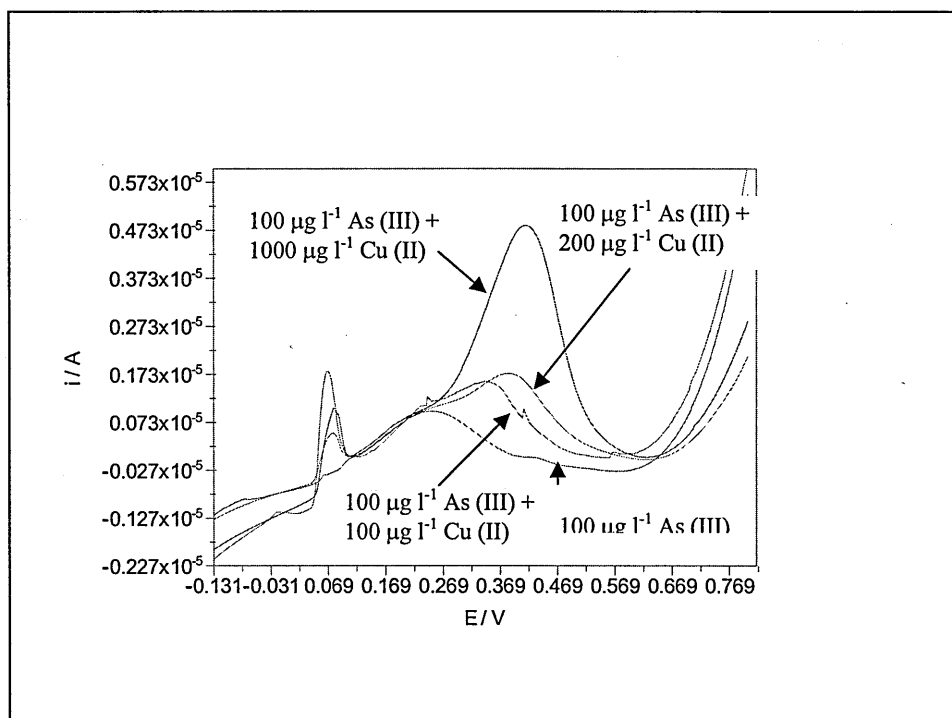


Figure 6.10: Voltammograms of 100 µg l⁻¹ As (III), and addition of 100 µg l⁻¹ Cu (II), 200 µg l⁻¹ Cu (II), 1000 µg l⁻¹ Cu (II) in 1 M HCl.

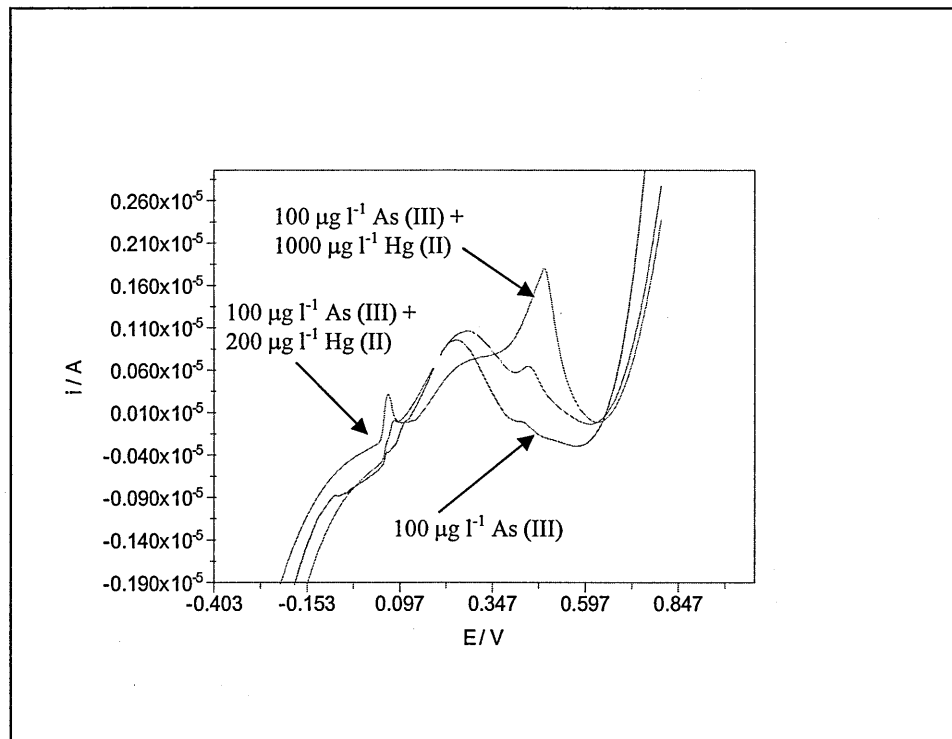


Figure 6.11: Voltammograms of 100 µg l⁻¹ As (III), and addition 200 µg l⁻¹ Hg (II), 1000 µg l⁻¹ Hg (II) in 1 M HCl.

Calibration voltammograms for As (III) from $0 \mu\text{g l}^{-1}$ to $150 \mu\text{g l}^{-1}$ is shown in Figure 6.12. Figure 6.13 shows the calibration graph for As (III). The calibration graph reach plateau at $200 \mu\text{g l}^{-1}$ due to the saturation of the gold electrode surface. The change of scan rate to 200 mV s^{-1} doesn't significantly improve the peak signal (area). Detection limit calculated as $3 \times$ standard deviation of blanks was calculated as $0.7 \mu\text{g l}^{-1}$ for 60 s deposition time. The detection limit is lower than the World Health Organization's arsenic guideline value of $10 \mu\text{g l}^{-1}$ in drinking water.

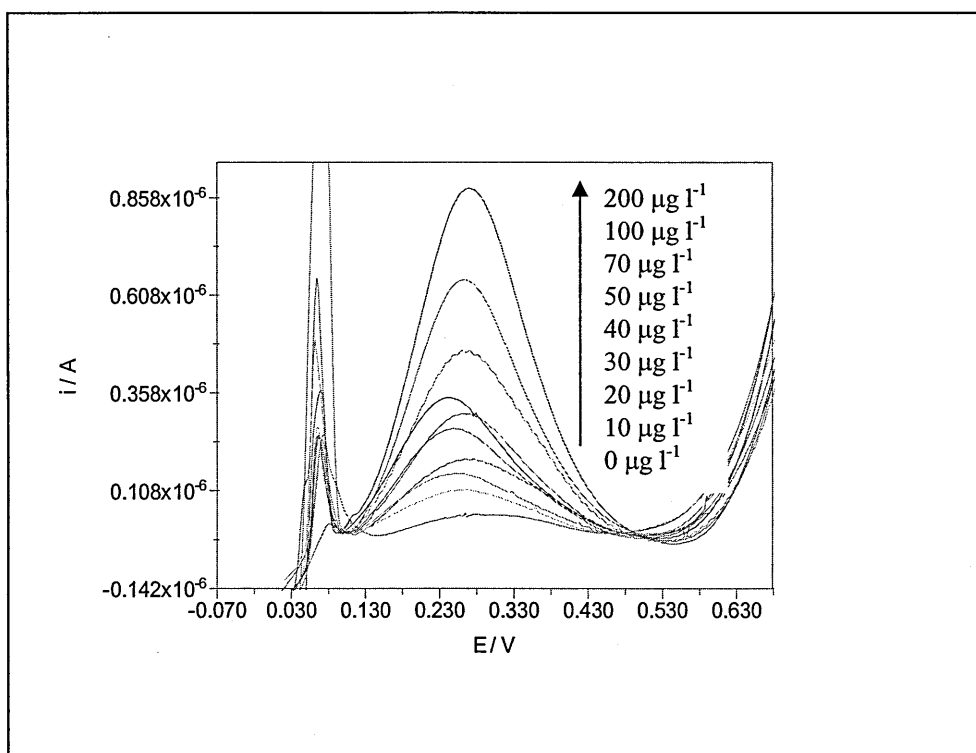


Figure 6.12: Voltammograms for As (III) from 0, 10, 20, 30, 40, 50, 70, 100 and $200 \mu\text{g l}^{-1}$ As (III) in 1 M HCl. Begin potential = -0.3 V , end potential = 0.8 V , step potential = 0.00244 , scan rate = 100 mV , equilibrium time = 60 s .

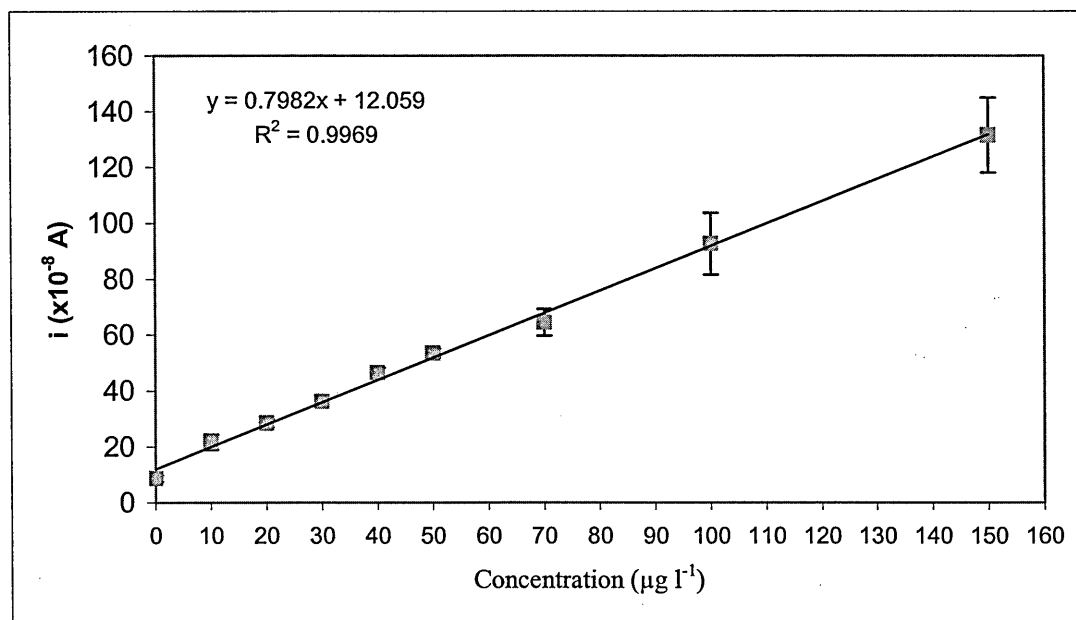


Figure 6.13: Calibration graph for As (III) from 0 to 200 $\mu\text{g l}^{-1}$ As (III) in 1 M HCl. Begin potential = - 0.3 V, end potential = 0.8 V, step potential = 0.00244, scan rate = 100 mV s^{-1} , equilibrium time = 60 s.

Tap water, soils samples extracted with acetic acid and aqua regia and urine samples were tested for As (III) using the proposed method. However, there is difficulty to obtained reproducible peaks for As (III) and these might be due to the presence of other chemicals in the extracted soil and urine samples which interferes with the results. Attempts to use sodium sulphide and heat the whole solution to reduce As (V) to As (III) do not produce promising results. Further studies require focusing on the samples analysis for As (III) in various environmental samples.

6.5 CONCLUSION

Electrochemical techniques to date have proven to be able to give reliable results in laboratory conditions and as such have potential for a further development into a mobile, low cost analytical device capable of fulfilling the requirements of a rapid and accurate sensor. The novelty of this work on As (III) analysis demonstrates the possibility to analyse As (III) using screen-printed gold electrodes. The determination limit was 0.7 $\mu\text{g l}^{-1}$ of As (III) for a deposition time of 1 min and the precision, and accuracy of the method were good. The method developed is simple and requires relatively short analysis time. The most advantageous seems that the method employs

an inexpensive and disposable working electrode. This minimizes the problems connected with the use of solid gold electrodes.

CHAPTER 7

7.0 DISCUSSION

7.1 INTRODUCTION

Monitoring of heavy metals in the environment is of great importance for ecological assessments as well as for understanding the dissemination of pollutants. Contamination by these metals is indeed widespread all over the world. The aim of the work reported in this thesis was to fabricate single-use metal ion sensor incorporating a three-electrode configuration for on-site analysis. The developed sensor was coupling to square-wave, chronopotentiometric stripping technique and linear sweep voltammetry and optimized for heavy metal detection. These tasks were successfully tested by applying this device for detection of heavy metal ions in aqueous synthetic solutions and also contaminated the waste-water and extracted soil samples. The purpose of producing this type of sensor due to its practicality and economic interests such as cost, speed, simplicity, sensitivity, reproducibility, robustness and applicability in the field.

The heavy metal ions sensor develops in this work were based on the use of screen-printed electrodes. This process was carried out in stages include method optimization and finally device testing in real sample. The characterization and application of the developed screen-printed electrochemical sensor is discussed in the following section.

7.2 FABRICATION, ELECTROCHEMICAL AND SURFACE CHARACTERISATION OF SCREEN-PRINTED ELECTRODES

The electrochemical characteristics of the in-house fabricated thick-film carbon and gold electrodes were studied so that valuable insights into electroanalytical behaviour can be obtained. There are many reported number of publications on the electrochemical characteristics of thick film-carbon electrodes (Osborne *et al.*, 1996; Wang *et al.*, 1996b, 1998b; Cui *et al.*, 2001; Grennan *et al.*, 2001; Morrin *et al.*, 2003). However, only little information regarding the surface of the gold screen-printed electrode are available (Susmel *et al.*, 2003).

7.2.1 Voltammetric characterization of the working electrode

Carbon electrodes are particularly attractive for sensing applications. Of the different forms of carbon, that the most used is glassy carbon electrode (GCE) due to its low permeability to gases, low porosity, hardness, good electrical conductivity, inertness and good positive potential range. The use of screen-printed carbon and gold electrodes in this study was to examine them for electrochemical sensing. Due to the interest in these electrodes, tests have to be done to examine the potential window of the proposed electrodes and to understand the electron-transfer processes at the SPCE and SPGE surfaces. The electron-transfer process involves evaluating the heterogeneous rate constant, k^0 . Using the increase in peak separation (ΔE_p) obtained from the current-potential curves, serves as an index of the ease of electron transfer at the SPCE surface. Estimation of the SPCE's active surface area can also be carried out.

The potential window for SPCE-Acheson, SPCE-Ercon and SPGE were examined in different supporting electrolytes because the working range available for the detection of electroactive species can be affected by several factors including supporting electrolytes decomposition and electrode dissolution. Comparison was made using GCE and GDE. Figure 2.4 shows the effect of different electrolytes used, using LSV. Different supporting electrolytes used will give different negative potential limits and these can be explained from the influence of pH on the potential of the hydrogen evolution reaction. Under less acidic conditions (0.1 M HCl + 0.5 M ammonium acetate, pH 4.6), the SPCE-Ercon operable in the potential range -1.1 V to ~ 1.1 V, (Figure 2.4 (a)). The influence of positive pH obviously seen in 0.025 M Tris-HCl/0.1 M KCl with wider potential window (Figure 2.4 (b)). SPGE and GDE show almost the same negative potential limit but GDE has more positive potential window. The most negative potential signals the commencement of hydrogen evolution on the electrode and the most positive potential is for the oxidation of the hydroxide ions on the electrode.

The electrochemical characterization of the SPCE and SPGE were carried out with potassium ferrocyanide, $K_4Fe(CN)_6$ using CV and chronoamperometry. A comparison of cyclic voltammograms for the ferrocyanide redox couple using glassy carbon, gold disc and screen-printed electrodes were made. Peak separations increased from 83-

116 mV for glassy carbon electrodes and 245-482 mV for SPCE-Acheson, 297-609 for SPCE-Ercon screen-printed carbon electrodes, using scan rates ranging from 10 to 100 mV s^{-1} , respectively. For gold disc electrode (GDE), peak separations increased from 71-90 mV s^{-1} using scan rates ranging from 50 - 200 mV s^{-1} , whilst screen-printed gold electrode shows peak separations from 184 - 446 mV s^{-1} from scan rate 10 - 75 mV s^{-1} . Such a higher peak separation for screen-printed carbon or gold electrodes as compared to glassy carbon and gold disc electrode suggests that the reaction at the surface of the screen-printed electrode is quasi-reversible. It was also observed for SPCEs and SPGE that the higher the scan rate, the higher the peak current, and the oxidation peak potential also shifted slightly to more positive values. The peak definition also worsened for higher scan rates. The electron transfer reaction was quasi-reversible because the rate of electron transfer was too slow to keep the $\text{Fe(CN)}_6^{4-}/\text{Fe(CN)}_6^{3-}$ couple in equilibrium as the potential changed.

The effect on the electron transfer activity and overall analytical performance of the SPCE and SPGE was due to the presence of inhibitory organic layer in the carbon ink used for screen-printing. The SPCE and SPGE did not display the ideal reversible behaviour of a fully conductive electrode (GCE and GDE) that might be expected for potassium ferrocyanide. This unattractive behaviour suggested a lower graphite-carbon/gold loading compared to GCE/GDE. The hydrophobic character of the SPCE and SPGE surface resulted in the quasi-reversible behaviour of the redox couple at the SPCE whereas the same substance measured at the GCE and GDE exhibited a fair reversibility.

The heterogenous electron transfer rates (k^0) at the glassy carbon, screen-printed carbon/gold electrodes, respectively, were determined to be $6.3 \times 10^{-2} \text{ cm s}^{-1}$ (glassy carbon), $9.4 \times 10^{-5} \text{ cm s}^{-1}$ (SPCE-Acheson), $3.7 \times 10^{-5} \text{ cm s}^{-1}$ (SPCE-Ercon) and $1.41 \times 10^{-4} \text{ cm s}^{-1}$ (SPGE). The k^0 value obtained for SPCE is in the range expected for quasi-reversible reactions (Erlenkotter *et al.*, 2000). Palchetti (1998) and Kadara (2003) obtained a value of 7.4×10^{-4} and $1.36 \times 10^{-4} \text{ cm s}^{-1}$, respectively for SPCEs used for detecting heavy metal ions while Grennan *et al.* (2001) reported a value of $2.2 \times 10^{-3} \text{ cm s}^{-1}$ for SPCEs used in an amperometric biosensor system. For the GCE used in this work, a k^0 value of 6.3×10^{-2} was obtained. This value is higher than the values reported by Grennan *et al.* (2001) ($1.2 \times 10^{-2} \text{ cm s}^{-1}$) and Morrin *et al.* (2003)

($5.9 \times 10^{-2} \text{ cm s}^{-1}$). This decrease in the electron transfer rate for screen-printed carbon and gold electrodes might be attributed to the presence of the polymeric binder in the carbon and gold ink, which serves to inhibit electron transfer rates. The same poorly reversible redox reaction of the ferri/ferrocyanide redox couple was also reported by Wang *et al.* (1996), who determined heterogenous rate constant of $8.2 \pm 0.8 \times 10^{-3} \text{ cm s}^{-1}$ and anodic peak separations of 80-130 mV for preactivated electrodes prepared using the same ink. Wang *et al.* (1996) reported that increases in the activity of electrodes made from the same ink with increases in curing temperatures (225°C) in terms of effects on the heterogenous electron transfer rates of the ferrocyanide redox couple. This improvement of electroactivity has also been reported for electrodes prepared from other inks (Wang *et al.*, 1996; Erlenkotter *et al.*, 2000).

7.2.2 Surface characterization of the working electrode

SEM examination of the screen-printed carbon and gold electrode working surface was carried out to understand the morphological features imposed by screen-printing. As observed from images of SPCE (Figure 4.12 (a)), the surface of SPCE appears to be relatively rough. This is due to graphite particles randomly distributed and oriented on the polyester sheet. The roughness and presence of clusters on the electrode surface have been reported to result in an increase in its active surface area (Moreno-Baron *et al.*, 2003). No visual difference was observed between the SEM presented and a SEM of an electrochemically cleaned screen-printed carbon electrode with 0.1 M HCl.

The surface structure of screen-printed gold working electrode is shown in Figure 5.1. The surface structure shows a group of micro granule combined with each other with polymeric binder and each granule has diameter $< 1 \text{ }\mu\text{m}$. The micro granule groups are not uniformly distributed with pin holes shown on the electrode surface. There is no significantly change on the SPGE working surface morphology when cleaning performs with 0.5 M H_2SO_4 by means of cycling 10 times from 0 to 1.5 V using cyclic voltammetry. The small granule shape could significantly contribute to an increase in active surface area of the gold electrode. The analysis of the SPGE shows that the main contents of the surface are gold (Au), with a small amount of carbon and oxygen (Figure 5.1).

The inks used for screen-printing are composed of three principal components: (1) organic polymer binder, (2) solvent; (3) graphite/gold particles. During the curing process the solvent evaporated, leaving a printed binder and carbon/gold mix. It is the combination of these two components which lend the electrodes their specific electrochemical properties (Osborne *et al.*, 1996). In the case of screen-printed gold electrode, the features of ink component (1) and (3) are clearly shown in the electron micrographs (Figure 5.1). According to Osborne *et al.* (1996), the surface which exposed polymer binder, which exhibits poor surface conductivity, give rise to film charging effects.

7.3 HEAVY METAL IONS DETECTION ON SCREEN-PRINTED ELECTRODES

There is a huge potential for the development of voltammetric environmental trace-metal sensor analysis, particularly in the field of on site measurements where other techniques are much more limited for various reasons. High sensitivity and reliability are required for *in situ*, long term, unattended, voltammetric, trace-metal monitoring. Only Hg electrodes have fulfilled these conditions, despite the efforts made over the last 15 years to develop Hg-free electrodes. However, conventional electrochemical cells and classical bulky electrodes represent a problem for decentralized measurements which require large volume of sample solutions. Improvement can be achieved by their replacement with miniaturized and planar electrodes that can be used as drop on sensor (Palchetti *et al.*, 2001).

The applications of electrochemical techniques with disposable screen-printed electrodes for heavy metals analysis have been reported in many journals (Wang and Tian, 1992; 1993a; 1993b; Wang *et al.*, 1993; Neuhold *et al.*, 1995; Desmond *et al.*, 1996; 1998; Palchetti *et al.*, 1999; Honeychurch *et al.*, 2000; Choi *et al.*, 2001; Zen *et al.*, 2002; Masawat *et al.*, 2003). However, most of the cases used two-electrode screen-printed system (Wang and Tian, 1992; 1993a; 1993b; Wang *et al.*, 1993; Palchetti *et al.*, 1999) or where the screen-printed working electrode is placed alongside conventional silver/silver chloride reference electrode and platinum counter electrode in a conventional electrochemical cell (Neuhold *et al.*, 1995; Palchetti *et al.*, 1999; Honeychurch *et al.*, 2000). Not many have involved the use of integrated three-

electrode system (screen-printed working, counter and reference electrodes), printed onto a planar substrate.

In this research, the use of integrated screen-printed carbon/gold working electrode, Ag/AgCl reference electrode and carbon counter electrode for detections of heavy metal ions were carried out. To greatly enhance the analytical power and scope of the bare SPCE, the formation of another metal layer onto the electrode surface, favourable to the deposition of metal to be analysed was applied. Mercury ions were used *in situ* for the formation of metal layer (metallic film) onto the bare SPCE surface. The heavy metals detection on mercury film SPCE could further enhanced by using peptides which can chelate with more metals in the solution. To find alternative for mercury electrode as it is known as a toxic substance, gold ink was printed on the graphite base working electrode to provide a screen-printed gold electrode. The SPGE proves to be useful for analysis of lead (II), cadmium (II) and arsenic (III).

7.3.1 Mercury film screen-printed carbon electrode-stripping voltammetric detection cadmium (II), lead (II) and copper (II)

Voltammetric techniques are based on the recording of the current, I , which flows between the working electrode (WE) and an auxiliary electrode (AE), due to the reduction or oxidation of the test element, as function of the potential, E , imposed on the WE and expressed with respect to that of a reference electrode (RE). In this work, the applicability and suitability of mercury film screen-printed carbon electrode (MFSPCE) to voltammetric stripping analysis of heavy metal ions was tested using inks (graphite and Ag/AgCl) obtained from Ercon (USA). The aim was to make comparison between the results obtained for lead (II) using MFSPCE and screen-printed gold electrode (SPGE) for waste-water and extracted soil samples analysis. The MFSPCE was produced by *in situ* deposition of mercury (II) with the target analytes onto the bare SPCE. The mercury film formed on the SPCE strips behaved satisfactorily and allowed the detection of cadmium (II), lead (II) and copper (II) with very close characteristics to what can be expected on mercury drop electrodes.

A 0.1 M HCl + 0.5 M NH₄Ac (pH 4.5) was chosen as the supporting electrolyte to use for separate cadmium (II), lead (II) and copper (II) analysis on the basis of sensitivity

as well as the need for chloride ions in the test solution for stabilizing the potential of the on-board screen-printed Ag/AgCl reference electrode (Desmond *et al.*, 1997; 1998; Choi *et al.*, 2001). This pH was used for supporting medium due to the problem of hydrogen evolution at low pH and thus reduces the sensitivity of cadmium (II). Hydrochloric acid (HCl) was used instead of potassium chloride (KCl) because HCl is normally free from metal ion impurities compared to KCl (Kadara *et al.*, 2003) and ammonium acetate is added to increase the pH around 4.5.

In situ plating of the bare SPCE was the chosen mode of forming mercury film on the electrode because it simplified the experimental procedure. Increasing mercury concentrations produced larger peak areas for all three metals. There is decreased in peak area was observed for Hg (II) concentrations up to 200 mg l⁻¹ for cadmium (II), lead (II) and copper (II). This might be due to the electrode saturation with metal-amalgam. Using SWASV, an optimized mercury (II) ion concentration of 80 mg l⁻¹ was used for co-deposition with cadmium (II), lead (II) and copper (II) onto the bare SPCE.

Longer deposition times also showed larger peak areas for all three metals. However, saturation was reached for deposition time longer than 250 s for lead (II) and 180 s for copper (II), respectively, again may be because of electrode saturation with metal-amalgam. Deposition longer than required can also deplete the metal ions in the bulk solution or lead to the formation of intermetallic complexes, all of which will affect the definition and size of the stripping peaks (Wilson and Dewald, 2001). The presence of break-point in the peak area vs. accumulation time plot may be due to the changes in the mechanism of deposition after longer periods (Honeychurch *et al.*, 2002). Such changes in the dynamics of deposition might be a result of changes in the electrode surface during the accumulation period.

The deposition potential was also found to affect the stripping peaks. The more negative the deposition potential, the larger the peak area, although above -1.3 V (for cadmium (II) and lead (II)) and -1.1 V (for copper (II)) produced voltammograms with higher background readings, probably due to the electrohydrolytic evolution of hydrogen (Choi *et al.*, 2001). Since the stripping peak for cadmium (II) is only observed at -0.9 V, deposition potentials above -0.9 V are required for the analysis of

this metal. Optimal readings were obtained for all the three metals after the use of -1 V deposition potential, and therefore this value was chosen for further experiments.

The performance characteristics of the developed MFSPCE for 120s deposition time show that the linear range for cadmium (II), lead (II) and copper (II) were 0 to 200 $\mu\text{g l}^{-1}$. The detection limit recorded for cadmium (II), lead (II) and copper (II) were 2, 1 and 5 $\mu\text{g l}^{-1}$ with relative standard deviation (RSD) of 6.5 %, 6.9 % and 7.5 %, respectively. The performance did not suffer in comparison to other results reported for cadmium (II), lead (II) and copper (II) detection at mercury film plated SPCEs considering the conditions applied to this work. For instance, Wang and Tian (1992) obtained limits of detection of 0.05 and 0.03 $\mu\text{g l}^{-1}$ for cadmium (II) and lead (II) using SWASV, respectively. Wang *et al.* (1993) reported detection limits of 0.1 $\mu\text{g l}^{-1}$ for SWASV for measurements of lead (II) using deposition time of 240s. Employing DPASV with no stirring of solutions involved during the deposition step, Desmond *et al.* (1998) reported high detection limits of 71 and 64 $\mu\text{g l}^{-1}$ for cadmium (II) and lead (II), respectively using a longer deposition time of 300s. Palchetti *et al.* (1999), using a stirring step as part of the measurement protocol, quoted detection limits of 1 and 0.4 $\mu\text{g l}^{-1}$ using SWASV for cadmium (II) and lead (II). A longer deposition time of 180s was employed with the SWASV measurements. Recently, Palchetti *et al.* (2005), reported detection limit of 0.3, 1 and 0.5 $\mu\text{g l}^{-1}$ for lead (II), cadmium (II) and copper (II) using carbon working electrode modified sensor coupled with a portable commercial electroanalytical instrumentation for on-site monitoring. The higher limit of detection obtained for the analysis of copper (II) may be due to its poor solubility in mercury when compared to cadmium (II) and lead (II) (Honeychurch *et al.*, 2002), which would therefore result in the formation of less amount of amalgam. The use of screen-printed carbon electrodes without chemical modification has been reported to yield detection limits of 8.2 for Cu (II) after DPASV analysis of copper (II) (Honeychurch *et al.*, 2002).

7.4 DETECTION WITH CYSTEINE MODIFIED MERCURY FILM SCREEN-PRINTED CARBON ELECTRODE

The use of amino acids and peptides has been widely exploited for the stripping analysis of Cu (II) at trace levels (Yang *et al.*, 2003; Bai *et al.*, 1998; Tanaka &

Yoshida, 1982). Constant current stripping chronopotentiometry (CCSCP) was investigated for the determination of cadmium (II), lead (II) and copper (II) since it can provide a much wider linear range for the determination of metals, which therefore results in the possibility of samples analysis over a wide concentration range (Kadara *et al.*, 2003; Kadara and Tothill, 2004). This technique has been reported to be very insensitive to oxygen and organic matter (Economou and Fielden, 1997), factors that make it an ideal candidate for on-field determination of metal ions. This technique has been tested for determinations of cadmium (II), lead (II) and copper (II) together with peptides to enhance the sensitivity of the technique.

The stripping peak of cadmium (II), lead (II) and copper (II) were improved $> 60\%$, $> 80\%$ and $> 100\%$, respectively by the addition of cysteine when chronopotentiometric stripping analysis was performed. A greater signal enhancement for $\text{Cu} > \text{Pb} > \text{Cd}$ shows that copper has the highest affinity for the peptide than lead and cadmium. The peak enhancement observed for three metals tested in the presence of cysteine suggests the suitability of this compound for use in sensor modification. The enhancement in sensitivity has also been reported for Cu (II) peak at 10-35 mM cysteine when anodic stripping voltammetry and potentiometric stripping analysis were used (Bai *et al.*, 1998; Tanaka and Yoshida, 1982).

The strategy in developing this sensor is to use lower concentration of mercury (10 mg l^{-1}) by adding 40 mM cysteine (for cadmium (II)), 20 mM cysteine (for lead (II) and copper (II)) in the system for the formation of mercuric cysteinate film on the electrode at deposition potential -1 V . The use of 80 mg l^{-1} mercury by other researchers for preplating mercury (Wang and Tian, 1992; Choi *et al.*, 2001) could be reduced and minimized the mercury waste.

The determination of copper on cysteine modified mercury film electrode SPCE was more complicated by the large background signal obtained at its stripping potential. The use of more concentrated chloride solutions (2 M HCl) can significantly improve the copper analysis (Kadara *et al.*, 2003). The use of higher amounts of peptide is not recommended since it can block the electrode surface after excess formation of complexes with the metals. It can also yielded high background readings and solubility problems, and therefore a compromising 40 mM cysteine was chosen for

cadmium (II) and 20 mM for lead (II) and copper (II) for further tests. A shift to more negative potential was observed for cadmium (II) peak as the concentration increase. This phenomenon could be a substitution of the 1:2 complex by a 1: 1 complex occur due to a decrease in the relative amount of thiol, thus shifting the stripping potential to more negative values (Diaz-Cruz *et al.*, 1997; Mandieta *et al.*, 1996).

Calibration plots were obtained for cadmium (II) and lead (II) in the presence of cysteine for metals concentrations over the 0 – 800 $\mu\text{g l}^{-1}$ range. The potentiograms display well defined peaks which increased proportionally to the concentration of cadmium (II) and lead (II). Whilst, for copper (II), the calibration graph was 0 – 400 $\mu\text{g l}^{-1}$. The lowest detection limit calculated for cadmium (II) and lead (II) were 0.4 and 0.7 $\mu\text{g l}^{-1}$ in 0.025 M Tris-HCl/0.1 M KCl. Whilst for copper (II), 3 $\mu\text{g l}^{-1}$ was the lowest concentration detected in 2 M HCl. The low detection limit allows the determination of drinking water samples.

The analytical performance of the developed sensors was examined by analyzing the content of cadmium (II), lead (II) and copper (II) in waste water and soil extracts. In order to correct interferences from the presence of other metals or compounds in the matrix of the sample, standard additions were used for the determination of the metals content. Preliminary studies on the effect of acidic matrices could have on the determination of both cadmium (II) and lead (II) were performed. The effect of the presence of high concentrations of copper (II) was also investigated.

Analysis of different concentrations of cadmium (II) and lead (II) after additions of acetic acid or aqua regia according to possible samples dilutions produced a decrease in the signal for some of the concentrations tested. Such a decrease yielded peak areas close to those recorded for the analysis of cadmium (II) and lead (II) with unmodified electrodes. The addition of larger volume of either 0.11 M acetic acid or aqua regia (which was consistent with the volume of sample to employ) may have decreased the pH of the solution to more acidic value, making the protonated form of the peptide to predominant. Hence, the competition between protons and the metal for the active groups of the ligand result in lower binding of the metal, thus ‘unmodifying the sensor’. This could explain why the signals were close to those given by unmodified

screen-printed electrodes and also the lower slopes obtained for each plot. A moderate shift of the stripping potential was also observed (to more positive values), suggesting the change in pH. A more concentrated buffer was therefore required for the analysis of real samples. The standard addition method was also employed to correct any other matrix effect.

Analysis of environmental samples after correction of matrix effects yielded values for cadmium (II), lead (II) and copper (II) contents that showed good correlation with to those given by ICP-MS with cysteine modified sensor. Nevertheless, the accuracy of the samples analysis was demonstrated, since correlations close to 1 were obtained for plots constructed for the metal content given by the modified sensor vs. the metal content given by ICP-MS.

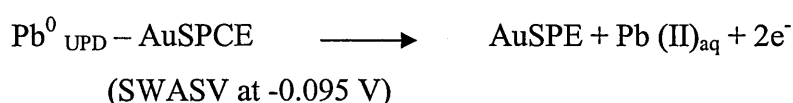
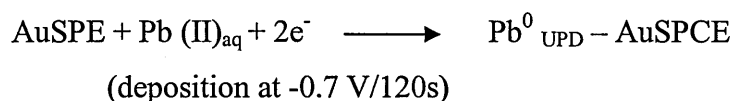
7.5 METAL IONS ANALYSIS DETECTION USING SCREEN-PRINTED GOLD ELECTRODE

Square-wave voltammetry was used to follow the lead stripping process at the SPGE because it offers certain advantages over the linear sweep and differential pulse voltammetry for rapid analysis. The analysis time is drastically reduced; a complete voltammogram can be recorded within a few seconds, compared with about 2-3 min in differential pulse voltammetry. The use of screen-printed gold electrodes for heavy metals analysis was to replace the mercury electrode systems, which are now becoming more and more out of favour for anything other than highly-specialised operations. Furthermore, there might be future regulations and occupational health considerations may severely restrict the use of mercury as an electrode material. Mercury-free analysis is a highly desirable alternative to existing techniques. Screen-printed gold coated electrodes usage was first reported by Wang (1993a, 1993b) on the analysis of lead and mercury in urine and water samples. In this study, gold ink was printed on the graphite support and use together with onboard silver/silver chloride and graphite carbon electrode.

7.5.1 Lead (II) and Cadmium (II) Analysis

It has been shown that silver working electrodes are more suitable for the detection of lead (II) than gold working electrodes (Brand *et al.*, 1997; Kirowa-Eisner *et al.*, 1999). However, in this work, screen-printed gold electrode comprising a three

electrode configuration system was successfully developed for lead (II) analysis using square-wave stripping voltammetry. The lead (II) peak is well defined and this might be due to the use of different substrate (gold ink) for the electrode substrate. At this electrode, the electrodeposition of metals proceeds via the formation of a monolayer of the target metal (underpotential deposition, UPD) followed by the deposition of the bulk-phase metal. Following is the possible mechanism:



Investigations on mixtures of lead and cadmium demonstrate that underpotentially deposited Pb atoms are stripped after cadmium atoms in 0.05 M HCl. This as contrast as reported by Bonfil *et al.* (2002) that Pb atoms are stripped before the cadmium atoms in HNO₃ (10 mM) + KCl (10 mM). The use of different gold substrate and electrolyte in the system analysis might influence the appearance of the peak and the study on effect of other electrolytes or mixtures of electrolyte should be studied for UPD lead and cadmium. In this study, HCl has been used as electrolytes because the presence of Cl⁻ anions in the solution enhanced the Pb²⁺ UPD signal and if the absence of acid, no Pb UPD peak was observed (Bonfil *et al.*, 2002). The use of HCl was also reported by other researcher (Richter *et al.*, 2003). However, a decreased in the signal at higher concentration of HCl was reported due to the production of gas bubbles on the electrode surface, which destroys the gold electrode (Richter *et al.*, 2003). It has been postulated that the gold film was oxidised in the presence of high chlorine concentration to the AuCl₄⁻ complex. This would decrease the active gold surface area and result in poor reproducibility (Sun *et al.*, 1997). And on the other hand, it is necessary to minimize the amount of acidic waste generated. Therefore, a concentration of 0.05 M HCl was selected for lead (II) quantification using screen-printed gold electrodes. This compromised HCl concentration allowed the reuse of the gold electrode after electrochemical cleaning.

The optimization method for lead (II) in 0.05 M HCl was done on two different equipments, Autolab and PalmSens. PalmSens was used due to its portability and could provide the opportunity to analyse lead (II) for on-site analysis. For this analysis, deposition potential of -0.7 V was employed for lead measurements. The use of more negative potential is not advisable since there was interference from H₂ generation. The gas generated could cause some irregularity to the signals and the baseline was less reproducible. The SPGE produced in-house was meant for disposable use sensor. However, pretreatment electrochemically for ten cycles appeared to increase surface functionalities and remove surface contaminants which results in enhanced electrochemical activity. This is an advantage for UPD-SV because only low concentrations of metals being deposited, so that the structure of the electrode surface therefore remains mainly unchanged, resulting in a good repeatability of the analytical response. The response from the SPGE system is reproducible for 20 runs on each electrode strip. This was evaluated from a series of 25 repetitive measurements on the same electrode strip for 100 µg l⁻¹ following 120 deposition times. The relative standard deviation was 3.17%.

The method was evaluated for increasing concentration of lead (II) in synthetic solution of 0.05 M HCl. Two different deposition times could be applied. For high levels of lead analysis, 120 s deposition times should be enough to provide linear range 25 – 300 µg l⁻¹, with detection limit of 5.8 µg l⁻¹ achieved. The SPGE could also be used for higher deposition time 240 s if low concentration of lead (II) is of interest. Linear range from 10 to 50 µg l⁻¹ can be constructed with lower detection limit of 2 µg l⁻¹ obtained. This detection limit is sufficient enough for analysis of lead (II) in drinking water and lower than EU, US EPA and WHO requirement for drinking water quality of 50, 15 and 10 µg l⁻¹, respectively. The analytical outputs generated with Autolab and PalmSens were compared and a relatively high correlation coefficient value ($r^2=0.9987$) was obtained for lead (II) calibration in the range 25-300 µg l⁻¹ with a slope value of 0.97743, that is close to unity, indicates that there is good agreement in the output obtained for both instruments. Kadara & Tothill, (2004) was also reported high correlation coefficient with slope value close to unity. Thus, the portable battery-powered potentiostat offers tremendous potential for obtaining

analytical information of heavy metal pollutants at contaminated sites in much simpler and cheaper manner.

Certain metal cations can interfere with the determination of Pb^{2+} by UPD voltammetry by the formation of lead-cation solid solutions (Honeychurch *et al.*, 2001). The possible interferences on SWASV measurements of lead (II) at SPGE when different concentration of other metals such as chromium, mercury, arsenic, ferum, zinc, copper and calcium added were evaluated. Generally, lead (II) is more sensitive on SPGE as compared to cadmium (II). However, due to proximity of lead (II) and cadmium (II) peak potential on the SPGE, addition of at least $50 \mu\text{g l}^{-1}$ cadmium (II) could increase the peak area of $50 \mu\text{g l}^{-1}$ lead (II). In real waste-water analysis performed on SPGE, although the level of lead (II) and cadmium (II) were almost close to each other, method of standard addition seems to provide reliable results due to addition of only one metals in the sample solution. There are effects of copper (II) and arsenic (III) on lead (II) analysis. As^{3+} reduced the lead response significantly. The effect of other metals did not significantly influence the levels of lead (II) being analysed in the synthetic samples. These cation interferences appear at concentrations which are higher than those normally obtained in drinking water and tap water samples and probably will not interfere in the determination of lead in typical samples of interest.

In order to evaluate the performance of the SPGE, three replicate determinations of lead (II) in waste-water and extracted soil samples were carried out using the standard addition method. As can be seen, there is fairly close agreement between some of the results obtained with ICP-MS. A correlation plot between the results obtained for different soil samples using SWASV coupled to the integrated screen-printed three-electrode device and ICP-MS was evaluated. A relative high correlation coefficient value for results obtained between SWASV and ICP-MS shows good agreement between the methods. Also the slope of the correlation curve is close to 1 and this confirms the validity of the proposed method (SWASV incorporating *in situ* SPGE) for lead (II) detection in environmental samples using 0.05 M HCl medium. These data demonstrate that the proposed SPGE has promise for the determination of lead (II) in waste-water and extracted soil samples.

Cadmium was also tested due to the test of interference on lead (II). Calibration graph for cadmium (II) in 0.05 M HCl could only start at $50 \mu\text{g l}^{-1}$ since lower than that value no cadmium (II) peak observed. Cadmium (II) could only be detected on SPGE at higher levels and this is not possible for analyzing cadmium (II) in drinking water. Permitted levels for cadmium (II) were $5 \mu\text{g l}^{-1}$ (for EU and US EPA) and $3 \mu\text{g l}^{-1}$ (WHO).

7.5.2 Arsenic (III) analysis

In general, electrochemical methods offer possibilities to determine arsenic and arsenic compounds at low concentrations. These techniques offer the different advantages: simple instrumentation and operation, low cost, high sensitivity and excellent selectivity which allows diversifying the oxidation states of arsenic featuring different level of toxicity. Gold was found to be superior to platinum as a working electrode material for As (III) analysis due to a higher hydrogen overvoltage. This solved the problem of simultaneous evolution of hydrogen while depositing arsenic (Forsberg *et al.*, 1975). Besides, gold displays better reversibility of the electrode reactions for arsenic in both the plating and stripping steps (Forsberg *et al.*, 1975), which results in higher and sharper oxidation peaks (Sun *et al.*, 1997; Forsberg *et al.*, 1975). However, the response of gold electrode is very strongly dependent on the past history, pretreatment and the oxide films formations (Sun *et al.*, 1997). The solid gold electrodes have high costs and require permanent control and care of the quality of their surface. From time to time, special treatment procedures such as polishing, activating or regeneration of the electrode surface are necessary to avoid possible oxidation of the electrode surface and its memory effects (Sadana, 1983). Besides, elemental arsenic is not electrically conductive, and once deposited at an active electrode site, it passivates the location to further deposition of electroactive species (Davis *et al.*, 1978; Forsberg *et al.*, 1975), including more arsenic. Such troubles can be overcome by using disposable screen-printed gold electrodes.

In this work, arsenic (II) was tested using three different techniques, chronopotentiometry (CCSCP), square-wave anodic voltammetry (SWASV) and linear sweep voltammetry (LSV) on screen-printed gold electrodes. The most

important difference between potentiometric and voltammetric stripping is that in potentiometric no current passes through the working electrode during stripping. This makes the technique insensitive to interferences from electro-active substances present in the sample. In voltammetric stripping, such substances give rise to background currents that overlap the current stripping peaks. Good sensitivity could be achieved using the three techniques. However, in term of reproducibility, LSV shows the best in term of stability of the peak obtained on several different electrodes. It had been stated that ASV of As (III) with gold suffers from lack of reproducibility and several attempts were performed in order to overcome the problems. The electrode was re-plated and washed with the samples before each measurement (Greulach & Henze, 1995). Another way was to reactivate the electrode surface before each measurement was found to improve reproducibility.

In SWASV determinations of arsenic, the stripping medium must be acidic in order to avoid the formation of hydrolysed species during stripping step (Hamilton *et al.*, 1980; Jagner *et al.*, 1981; Sadana, 1983). In this work, HCl has been used as supporting electrolyte. This is because HCl is the most widely used supporting electrolyte for the electrochemical determination of arsenic (Jagner *et al.*, 1981; Sun *et al.*, 1997). The acid provide good sensitivity and the stripping peaks were also narrow indicated that the charge-transfer reaction was fast and reversible (Wang and Greene, 1983; Sun *et al.*, 1997). Various authors have concluded that the use of higher concentrations of HCl increases the arsenic stripping signals (Huang and Dasgupta, 1999; Sun *et al.*, 1997). However, in this work, the use of higher HCl have been avoided because it offered no significant increase in arsenic signal but increase the interfering peak which appears before the arsenic (III) peak and there was also more irreproducibility that may be due to adsorbed chloride on the gold substrate (Feeney and Kounaves, 2000). Hence, 1 M HCl has been used as compromised between the response and the interference peak. Therefore, the presence of chloride in the samples can be interpreted as being necessary to provide well-defined stripping reactions for arsenic (Kopanica and Novotny, 1998), because of the chlorine ions strongly complex arsenic to AsCl_3 (Sun *et al.*, 1997; Feeney and Kounaves, 2000). Arnold and Jonson (1967) described that the As (III) species in aqueous solutions contained HCl at increasing concentration are found to be consistant with the existence of the following species: H_3AsO_3 , $\text{As}(\text{OH})_2^+$, $\text{As}(\text{OH})_2\text{Cl}$, $\text{As}(\text{OH})\text{Cl}_2$, and AsCl_3 . Since $\text{As}(\text{OH})_2\text{Cl}$ and

As(OH)Cl_2 are thought to be the actual species take part in the electron-transfer reaction on the electrode, the deposition efficiency of As(III) become higher as the amount of $\text{As(OH)}_2\text{Cl}$ and As(OH)Cl_2 increases with increasing HCl concentration. Moreover, more chloride ions tightly bound in double layer around the electrode act as a bridge between the arsenic ion and the electrode, which makes the redox reaction more reversible (Stojanovic *et al.*, 1990). However, the drawback of HCl was reported when arsenic was determined, chlorine was generated at the auxiliary electrode concurrent with arsenic deposition at the working electrode. The chlorine would then diffuse to the working electrode where it would readily oxidize the gold electrode surface, thereby making it inactive (Aldstadt and Martin, 1996).

In the optimization method of arsenic (III), the sensitivity of the arsenic response increased with increasing electrolysis time (Figure 6.5). However, the deposition time range was limited by the saturation of electrode surface at longer electrolysis time until up to 120 s. Relatively, at longer electrolysis time, higher mean deviation observed which may be associated with increased hydrogen evolution. Hence, to avoid problems that may arise from excessive hydrogen evolution, it is recommended to use short preconcentration times (Munoz and Palmero, 2004). Another important parameter was deposition potential. Increase deposition potential from -0.1 V until up to -0.6 V would increase the response of As (III) (Figure 6.6). Hence, deposition potential of -0.3 V was used throughout the analysis due to compromise between response and interference from the electrode. The use of higher potential create bigger interference peak, which could reduce the arsenic response. Frequency was another parameter tested for arsenic (III). There was no significant improvement on stripping response when changing the frequency from 50 to 250 Hz. Therefore, 200 Hz was chosen for future analysis in the measurement of As (III).

Possible interferences in the arsenic determination by stripping voltammetry are the ion metals, which can be reduced at the gold electrode or at the same electro-deposition potential than arsenic: Pb (II), Hg (II) and Cu (II). The stripping peak potentials for other gold-soluble elements, which are oxidised at potentials close to that of arsenic, may be separated by varying the chloride concentration in the stripping medium with calcium chloride and hydrochloric acid. Special attention was made to Cu (II) and Hg (II) ions whose interference due to the capability to form

binary intermetallic compounds with arsenic such as Cu_3As_2 and represent the most problematic point in the SWASV. In samples with an excess of Cu (II) versus arsenic, it should be necessary to modify the method with an additional step such as ion-exchange separation in order to eliminate Cu (II) effectively from the sample prior to analysis (Svancara *et al.*, 2002). Other authors avoided this interference by a completely re-oxidation of copper, prior to stripping of arsenic, at a potential different than potential of arsenic (Hua *et al.*, 1987).

CHAPTER 8

8.0 CONCLUSIONS & FUTURE WORK

8.1 OUTLINE

This chapter presents an overview of the major results reported in this thesis including some suggestions for future work.

8.2 CONCLUSION

The work described in this thesis has detailed the development process undertaken for the production of disposable electrochemical screen-printed sensors for heavy metals detection in environmental matrices. The process included the fabrication of the electrodes and characterisation, which enabled various sensing devices to be established and used for metal ion detection and determination. This sensor must meet the requirements of decentralised tests such as reliable and sensitive, portable and easy to use with low cost and amenable to masss-production. Below is a summary of the findings:

- The screen-printing technique was used for the production of disposable carbon/gold electrodes. The carbon and gold electrodes provided a convenient base for electrochemical metal ion sensors as well as being cheap to produce.
- The electrochemical behaviour of carbon/gold working electrodes was investigated. The characterisation of the screen-printed electrode (SPE) showed that its surface structure and electrochemical activity is dependent on the electrode material. The screen-printed carbon electrode (SPCE) and screen-printed gold electrode (SPGE) did not display the ideal reversible behaviour that might be expected for potassium ferrocyanide. This unattractive behaviour suggests a low graphite-carbon and gold loading.

- SPCE-Acheson shows that in alkaline solution (0.025 M Tris-HCl/0.1 M KCl), potential window in the range -1.2 V to 0.7 V achieved, while slightly acidic condition in 0.1 M HCl + 0.5 M ammonium acetate (pH 4.6), the SPCE-Ercon is operable in the potential range - 1.1 V to ~ + 1.1 V. SPGE has shorter potential window in acidic solution (0.05 M HCl) from -0.5 V to + 0.7 V.
- The surface of SPCE-Acheson and SPCE-Ercon appear to be relatively rough due to graphite particles randomly distributed and oriented on the polyester sheet. For SPGE, the surface structure shows a group of micro-granule combined with each other with polymeric binder and each granule has diameter < 1 μm . The micro-granule groups are not uniformly distributed with pin holes shown on the electrode surface.
- The fabricated three-electrode configuration system coupled with SWASV CCSCP and LSV provided a means of a relatively inexpensive on-site detector for trace levels of lead (II), copper (II), cadmium (II) and arsenic (III). Detection of these metals was carried out on mercury film screen-printed carbon (MFSPCE), the modified SPCEs using cysteine, bare screen-printed gold electrode and the optimised procedures developed for measurements.
- The preconcentration of lead (II), cadmium (II) and copper (II) metallic phase from aqueous solutions was favoured when co-depositing with mercury onto the bare carbon electrode surface. The bare SPCE acted as supports for the *in situ* plating of mercury films or modified with cysteine. With the optimised working conditions, the results obtained indicate that the screen-printed electrochemical sensors are sensitive and reproducible enough for the SWASV and CCSCP determination of cadmium (II), lead (II) and copper (II) in the parts per billion range.

- Limits of detection below $6 \mu\text{g l}^{-1}$ were obtained for cadmium (II), lead (II) and copper (II) detection at MFSPCE coupled to SWASV, whilst $< 3 \mu\text{g l}^{-1}$ detection limit was detected for MFSPCE modified with cysteine coupled to CCSCP. The reproducibility of the measurements (RSD, %), which also contributed to interest in the electrochemical system, was below 15 % for both film electrodes.
- Due to toxicity of mercury and interest in a search for new electrode material, screen-printed gold electrode has been selected for heavy metals analysis. Lead (II) in 0.05 M HCl is detected on SPGE using underpotential deposition-square-wave stripping voltammetry (UPD-SWAV).
- A linear concentration range of 0 - $50 \mu\text{g l}^{-1}$ and 0 - $300 \mu\text{g l}^{-1}$ with detection limit of 2 and 5.8 were obtained for lead (II), with measurement times of four and two minutes, respectively.
- The SPGE is meant for single-use drop on sensor for on-site detection of lead (II). However, the electrodes can be reused for up to 20 times after cleaning with 0.5 M sulphuric acid.
- Although the performance of the SPGE used for SWASV measurements quite good for lead (II) analysis, the only downside is the undesirable effect of cadmium (II) at concentration $> 50 \mu\text{g l}^{-1}$. To eliminate the effect of cadmium (II), method of standard addition should be used for the analytical work on real samples.
- Cadmium (II) was also tested with SPGE in 0.05 M HCl. Cadmium (II) was not sensitive at $< 50 \mu\text{g l}^{-1}$. A linear calibration graph for cadmium (II) obtained from 50 to $750 \mu\text{g l}^{-1}$ and detection limit of $9.6 \mu\text{g l}^{-1}$ achieved for 120 s preconcentration times.

- Arsenic (III) was detected using SPGE in 1 M HCl. Low detection limit ($0.7 \mu\text{g l}^{-1}$) and a linear calibration graph range 0.7 to $150 \mu\text{g l}^{-1}$ show the possibility of detecting As (III) in environmental samples.
- Other metals such as lead (II), copper (II) and mercury (II) interfere with arsenic (III) measurements in 1 M HCl.
- For environmental samples, the usefulness of SWASV and CCSCP coupled to screen-printed carbon/gold electrodes (SPEs) for the determination of cadmium (II), lead (II) and copper (II) in waste-water samples and in extracts obtained by solid-liquid phase extraction of soil (or sediments) samples was evaluated. Overall, the results show that the developed screen-printed electrochemical sensors are capable of measuring copper, lead and cadmium in water waste-samples and in soil (or sediments) samples.
- The results of the sample analysis carried out in this work could help in speeding up the development and optimisation of cheap and time-saving electrochemical sensor devices for getting useful information and benefit of workers in integrated water-soil in solving environmental problems from heavy metal contamination.

8.3 FUTURE WORK

- The suitability and application of the integrated planar screen-printed three electrodes system for electrochemical sensing has been highlighted in this work. However, improving the characteristics of the graphite-carbon based ink and gold used in printing the carbon- and gold-working electrode must be achieved through developing custom-made inks with the appropriate electrochemical characteristics that should eliminate problems of organic and inorganic impurity for the detection of heavy metals.

- Other strategies could be done on the design of the electrode from single electrode format into a microdisk/microfabricated array format. Combining the attractive features of the microelectrode array and screen-printing technology could produce well defined, highly reproducible microdisk arrays geometry with enhanced deposition efficiency, large “collective” response and low cost.
- The use of other more novel peptides/ligands/modifying agents should be explored that could increase the sensitivity and reduce the detection limits of analysis need to be examined. This could involve the direct bulk modification of the graphite-carbon ink with chemical reagents (printable ligands with metal complexing properties).
- Other electrolyte compositions should be tested to find the most suitable one for lead (II) and cadmium (II) to separate these peaks on SPGE. In case of arsenic analysis, the reduction of arsenic (V) before the measurements is necessary. Reagents use for reduction of arsenic (V) to arsenic (III) should be tested to reduce interferences and enhance the sensitivity of the technique.
- Application of the SPCE/SPGE to evaluating concentrations of heavy metal ions in sites conditions.

REFERENCES

- Albareda-Sirvent, M., Merkoci, A. & Alegret, S.** (2000). Configurations used in the design of screen-printed enzymatic biosensors. A review. *Sensors and Actuators B* **69**, 153-163.
- Aldstadt, J.H., and Martin, A.F.** (1996). Flow injection method for the determination of arsenic (III) at trace levels in alkaline media. *The Analyst* **121**(10), 1387-1391.
- Amankwah, S.A. and Fasching, J.L.** (1985). Separation and determination of arsenic(V) and arsenic(III) in sea-water by solvent extraction and atomic-absorption spectrophotometry by the hydride-generation technique. *Talanta* **32**, 111-114.
- Antonelli, M.L., Carunchio, V., Luciani, M., and Vici, G.** (1987). Determination of the urease activity and the relative inhibition in the presence of some metal ions: a microcalorimetric study. *Thermochim. Acta* **122**, 95-103.
- Apostoli, P.** (2002). Elements in environmental and occupational medicine. *Journal of Chromatography B* **778**, 63-97.
- Arnold, J.P., and Johnson, R.M.** (1969). Polarography of arsenic. *Talanta* **16**, 1191-1207.
- Arrigan, D.W.M. & Bihan L.L.** (1999). A study of L-cysteine adsorption on gold via electrochemical desorption and copper(II) ion complexation. *Analyst* **124**, 1645-1649.
- Arrigan, D.W.M., Bihan, L.L. & Pickup M.J.** (1999). Apparent enhanced underpotential voltammetry of lead (II) at a spontaneously adsorbed monolayer-coated gold electrode. *Analyst* **124**, 1797-1802.
- ATSDR** (2004). Public health statement for copper.
<http://www.atsdr.cdc.gov/toxprofiles/phs132.html>. (Access on 12.08.04).
- Aw, T.C. and Vale, J.A.** (1996). Poisoning from metals. In *Oxford textbook of medicine*, pp. 1105-1115. Edited by Weatherall, Ledingham, J.G.G. & Warrel, D.A. 3rd. Ed. Vol. 1 Oxford: Oxford University Press.
- Bachmann, T.T.** (1999). Development of disposable sensors for rapid multianalyte detection: Acetylcholinesterase and microbial biosensors. *PhD thesis*, Institute of Technical Biochemistry, University of Stuttgart, Germany.
- Bai, Y., Ruan, X., Mo., J. & Xie, Y.** (1998). Potentiometric stripping analysis of copper using cysteine modified mercury film electrode. *Analytica Chimica Acta* **373**, 39-46.
- Baird, C.** (1999). *Environmental Chemistry*, 2nd Ed. W.H Freeman and Company.

- Barra, C.M., Correla dos Santos, M.M.** (2001). Speciation of inorganic arsenic in natural waters by square-wave cathodic stripping voltammetry. *Electroanalysis* **13**(13), 1098-1104.
- Beard, S.J., Hughes, M.N., and Poole, R.K.** (1995). Inhibition of the cytochrome bd-terminated NADH oxidase system in *Escherichia coli* K-12 by divalent metal cations. *FEMS Microbiology Letters* **131**, 205-210.
- Beinrohr, E., Csemi, P., Manova, A. and Dzurov, J.** (1994). Absolute analysis of trace metals through galvanostatic stripping chronopotentiometry with signal accumulation. *Fresenius J. Anal. Chem.* **349**, 625-632.
- Beinrohr, E., Čaktr, M., Dzurov, J., Kottaš, P. and Kozakova, E.** (1996). Calibrationless determination of mercury by flow-through stripping coulometry. *Fresenius J. Anal. Chem.* **356**, 253-258.
- Beinrohr, E., Čakrt, M., Dzurov, J., Jurica, L. and Broekaert, J.A.C.** (1999). Simultaneous calibrationless determination of zinc, cadmium, lead, and copper by flow-through stripping chronopotentiometry. *Electroanalysis* **11**(15), 1137-1144.
- Bello Rodriguez, B.** (2004). Development of biosensors and chemical sensors for the determination of metals in water and soil extracts. *PhD thesis*, Biotechnology Centre, Cranfield University, U.K.
- Bello Rodriguez, B., Bolbot, J.A. and Tothill, I.E.** (2004a). Amperometric Analysis of the Effect of Heavy Metals on the Activity of Isocitric Dehydrogenase. *Analytical Letters*, **37**(3), 415-433.
- Bello Rodriguez, B., Bolbot, J.A. and Tothill, I.E.** (2004b). Development of urease and glutamic dehydrogenase amperometric assay for heavy metals screening in polluted samples. *Biosensors and Bioelectronics*, **19**(10), 1157-1167.
- Beni, V., Newton, H.V., Arrigan, D.W.M., Hill, M., Lane, W.A. & Mathewson, A.** (2004). Voltammetric behaviour at gold electrodes immersed in the BCR sequential extraction scheme media. Application of underpotential deposition-stripping voltammetry to determination of copper in soil extracts. *Analytica Chimica Acta* **502**, 195-206.
- Bersier, P.M., Howell, J and Bruntlett, C.** (1994). Advanced Electroanalytical Techniques Versus Atomic Absorption Spectrometry, Inductively Coupled Plasma Atomic Emission Spectrometry and Inductively Coupled Plasma Mass Spectrometry in Environmental Analysis. *Analyst* **119**, 219 – 232.

- Bertocchi, P.**, Ciranni, E., Compagnone, D., Mageauru, V., Palleschi, G., Pirvutoiu, S. and Valvo, L. (1999). Flow injection analysis of mercury (II) in pharmaceuticals based on enzyme inhibition and biosensor detection. *J. Pharm. Biom. Anal.* **20**, 263-269.
- Blake, D.A.**, Chakrabarti, P., Khosraviani, M., Hatcher, F.M., Westhoff, C.M., Goebel, P., Wylie, D.E., and Blake II, R.C. (1996). Metal binding properties of a monoclonal antibody directed toward metal-chelate complexes. *J. Biol. Chem.* **271** (44), 27677-27685.
- Blake, D.A.**, Blake II, R.C., Khosraviani, M. and Pavlov, A.R. (1998). Immunoassays for metal ions. *Analytica Chimica Acta* **376**, 13-19.
- Blake, D.A.**, Jones, R.M., Blake II, R.C., Pavlov, A.R., Darwish, I.A. and Yu, H. (2001). Antibody-based sensors for heavy metal ions. *Biosensors and Bioelectronics* **16**, 799-809.
- Bond, A.M.**, Mahon, P.J., Schiewe, J. and Vicente-Beckett, V. (1997). An inexpensive and renewable pencil electrode for use in field-based stripping voltammetry. *Analytica Chimica Acta* **345**, 67 – 74.
- Bonfil, Y.**, Brand, M & Kirowa-Eisner, E. (1999). Determination of sub- $\mu\text{g l}^{-1}$ concentrations of copper by anodic stripping voltammetry at the gold electrode. *Analytica Chimica Acta* **387**, 85-95.
- Bonfil, Y.**, Brand, M & Kirowa-Eisner, E. (2000). Trace determination of mercury by anodic stripping voltammetry at the rotating gold electrode. *Analytica Chimica Acta* **424**, 65-76.
- Bonfil, Y.** and Kirowa-Eisner, E. (2002). Determination of nanomolar concentrations of lead and cadmium by anodic-stripping voltammetry at the silver electrode. *Analytica Chimica Acta* **457**, 285-296.
- Bonfil, Y.**, Brand, M & Kirowa-Eisner, E. (2002). Characteristics of subtractive anodic stripping voltammetry of Pb and Cd at silver and gold electrodes. *Analytica Chimica Acta* **464**, 99-114.
- Bontidean, I.**, Berggren, C., Johansson, G., Csoregi, E., Mattiasson, B., Lloyd, J.R., Jakeman, K.J. & Brown, N.L. (1998). Detection of heavy metal ions at femtomolar levels using protein-based biosensors. *Analytical Chemistry* **70**, 4162-4169.

- Brack, W.,** Paschke, A., Segner, H., Wennrich, R., and Schüürmann, G. (2000). Urease inhibition: a tool for toxicity identification in sediment elutriates. *Chemosphere* **40**, 829-834.
- Brand, M.,** Eshkenazi, I., and Kirowa-Eisner, E. (1997). The silver electrode in square-wave anodic stripping voltammetry. Determination of Pb^{2+} without removal of oxygen. *Analytical Chemistry* **69**, 4660-4664.
- Brisbin, J.A.,** B'Hymer, C. & Caruso, J.A. (2002). A gradient anion exchange chromatographic method for the speciation of arsenic in lobster tissue extracts. *Talanta* **58**, 133-145.
- Brown, R.J.C** and Milton, M.J.T. (2005). Analytical techniques for trace element analysis: an overview. *Trends in Analytical Chemistry* **24**(3), 266-274.
- Burguera, M.** and Burguera, J.L. (1997). Analytical methodology for speciation of arsenic in environmental and biological samples. *Talanta* **44**, 1581-1604.
- Cakir, S.,** Biçer, E. & Cakir, O. (1999). Electrochemical study of cystine in the presence of cadmium and folic acid. *Journal of Inorganic Biochemistry* **77**, 249-255.
- Cammann, K.,** Buscher, W., Breer, C.B., Riepe, H.G., Rosenkranz, B. & Twiehaus, T. (2000). Inorganic compounds: heavy metal. In *Biosensors for environmental monitoring*, pp. 368. Edited by Bilitewski, U., Turner, A.P.F. Amsterdam: Academic Publishers.
- Cassidy, J. F.,** Doherty, A.P. & Vos, J.G. (1998). Amperometric methods of detection. In *Principles of chemical and biological sensors*, pp.91-92 . edited by Diamond, D. New York: John Wiley & Sons, Inc.
- Cava-Montesinos, P.,** Cervera, M.L., Pastor, A. and Guarda, M.d.l. (2003). Determination of arsenic and antimony in milk by hydride generation atomic fluorescence spectrometry. *Talanta* **60**, 787-799.
- Cavicchioli, A.,** La-Scalea, M.A. and Gutz, I.G.R. (2004). Analysis and speciation of traces of arsenic in environmental, food and industrial samples by voltammetry: a Review. *Electroanalysis* **16**(9), 697 -711.
- Chakrabarti, P.,** Hatcher, F.M., Blake II, R.C., Ladd, P.A., and Blake, D.A (1994). Enzyme immunoassay to determine heavy metals using antibodies to specific metal-EDTA complexes: optimization and validation of an immunoassay for soluble indium. *Anal. Biochem.* **217**, 70-75.

- Choi, J-Y.,** Seo, K., Cho, S-R., Oh, J-R., Kahng, S-H. and Park, J. (2001). Screen-printed anodic stripping voltammetric sensor containing HgO for heavy metal analysis. *Analytica Chimica Acta* **443**, 241-247.
- Christian, G.D.** (1994). *Analytical Chemistry*, 5th edn. New York: John Wiley & Sons.
- Compagnone, D.,** Palleschi, G., Varallo, G., and Imperiali, P. (1995). Amperometric biosensor for the determination of heavy metals. *SPIE Proceedings* **2504**, 141-152.
- Compagnone, D.,** Lupu, A.S., Ciucu, A., Magearu, V., Cremisini, C., and Palleschi, G. (2001). Fast amperometric FIA procedure for heavy metal detection using enzyme inhibition. *Analytical Letters* **34**(1), 17-27.
- Connor, M.,** Dempsey, E., Smyth, M.R., and Richardson, D.H.S. (1991). Determination of some metal ions using lichen-modified carbon paste electrodes. *Electroanalysis* **3**, 331-336.
- Corbisier, P.,** Lelie, D.v.d., Borremans, B., Provoost, A., Lorenzo, V.,d., Brown, N.L., Lloyd, J.R., Hobman, J.L., Csoregi, E., Johansson, G. & Mattiasson, B. (1999). Whole cell- and protein-based biosensors for the detection of bioavailable heavy metals in environmental samples. *Analytica Chimica Acta* **387**, 235-244.
- Cserey, A.** (2004). Determination of lead (II) with rotating sample system. *Electroanalysis* **16**(16), 1299-1304.
- Currel, G.** (2000). *Analytical Instrumentation: performance characteristics and quality*. Chichester: John Wiley & Sons.
- Dai, X.,** Nekrassova, O., Hyde, M.E. & Compton, R.G. (2004). Anodic stripping voltammetry of arsenic (III) using gold nanoparticle-modified electrodes. *Analytical Chemistry* **76**, 5924-5929.
- Danzer, T.,** and Schwedt, G. (1996). Chemometric methods for the development of a biosensor system and the evaluation of inhibition studies with solutions and mixtures of pesticides and heavy metals. Part I. Development of an enzyme electrodes system for pesticide and heavy metal screening using selected chemometric methods. *Analytica Chimica Acta* **318**, 275-286.
- Darwish, I.A.,** and Blake, D.A. (2002). Development and validation of a one-step immunoassay for determination of cadmium in human serum. *Anal. Chem.* **74**, 52-58.
- Davis, P.H.,** Dulude, G.R., Griffin, R.M., Matson, W.R. & Zink, E.W. (1978). Determination of total arsenic at the nanogram level by high-speed anodic stripping voltammetry. *Analytical Chemistry* **50** (1), 137-143.

- Desmond, D.**, Lane, B., Alderman, J., Hall, G., Alvarez-Icaza, M., Garde, A., Ryan, J., Barry, L., Svehla, G., Arrigan, D.W.M. and Schniffner, L. (1996). An ASIC-Based System for Stripping Voltammetric Determination of Trace Metals. *Sensors and Actuators B* **34**, 466 – 470.
- Desmond, D.**, Lane, B., Alderman, J., Glennon, J.D., Diamond, D. and Arrigan, D.W.M. (1997). Evaluation of miniaturised solid state reference electrodes on a silicon based component. *Sensors and Actuators B* **44**, 389-396.
- Desmond, D.**, Lane, B., Alderman, Arrigan, D.W.M. and Glennon, J.D. (1998). An Environmental Monitoring System for Trace Metals Using Stripping Voltammetry. *Sensors and Actuators B* **48**, 409 – 414.
- Diaz-Cruz, M.S.**, Mendieta, J., Tauler, R., and Esteban, M. (1997). Cadmium-binding properties of glutathione: a chemometrical analysis of voltammetric data. *J. Inorg. Biochem.* **66**, 29-36.
- Dolphin, D.**, Avramovic, O., and Poulson, R. (1989). *Glutathione: chemical, biochemical and medical aspects. Part A*. John Wiley and Sons. Wiley-interscience publication.
- Donlan , A.M.**, Moody, G.J., and Thomas, J.D.R. (1989). A prospect for the analysis of species acting as enzyme inhibitors as illustrated by heavy metal inhibition. *Analytical Letters* **22**(8), 1873-1896.
- Doyle, M.J.**, Halsall, H.B., and Heineman, W.R. (1982). Heterogenous immunoassay for serum proteins by differential pulse anodic stripping voltammetry. *Anal. Chem.* **54**, 2318-2322.
- Economou, A.**, and Fielden, P.R. (1997). Applications, potentialities and limitations of adsorptive stripping analysis on mercury film electrodes. *Trends. Anal. Chem.* **16**(5), 286-292.
- Erlenkotter. A.**, Kottbus, M. and Chemnitius, G.C. (2000). Flexible Amperometric Transducers for Biosensors Based on a Screen-Printed Three-Electrode System. *Journal of Electroanalytical Chemistry* **481**, 82 – 94.
- Esteban, M.** and Casassas, E. (1994). Stripping Electroanalytical Techniques in Environmental Analysis. *Trends in Analytical Chemistry* **13**(3), 110 – 117.
- EU** (2004). Water policy in the European Union.
<http://europa.eu.int/comm/environment/water.index.html>. (Access on 20/12/2004).

- Evtugyn, G.A.,** Budnikov, H.C., and Nikolskava, E.B. (1998). Sensitivity and selectivity of electrochemical enzyme sensors for inhibitor determination. *Talanta* **46**, 465-484.
- Ewing, G.W.** (1994). *Analytical Instrumentation Handbook*, 2nd Edition, Revised & Expanded.
- Feeney, R.** and Kounaves, S.P. (2000). On-site analysis of arsenic in groundwater using a microfabricated gold ultramicroelectrode array. *Analytical Chemistry* **72**, 2222-2228.
- Feeney, R.** and Kounaves, S.P. (2002). Voltammetric measurement of arsenic in natural waters. *Talanta* **58**, 23-31.
- Fennouh, S.,** Casimiri, V., Geloso-Meyer, A., and Burstein, C. (1998). Kinetic study of heavy metal salt effects on the activity of L-lactate dehydrogenase in solution or immobilized on an oxygen electrode. *Biosensors and Bioelectronic* **13**, 903-909.
- Ferreira, M.A.** and Barros, A.A. (2002). Determination of As (III) and arsenic (V) in natural waters by cathodic stripping voltammetry at a hanging mercury drop electrode. *Analytica Chimica Acta* **459**, 151-159.
- Fisher, E.** and van den Berg, C.M.E. (1999) Anodic stripping voltammetry of lead and cadmium using a mercury film electrode and thiocyanate. *Analytica Chimica Acta* **385**, 273-280.
- Fleit, E. & Lakatos, G.** (2003). Accumulative heavy metal patterns in the sediment and biotic compartments of the Tisza watershed. *Toxicol. Lett.* **140-141**, 323-332.
- Fogg, A.G & Wang, J.** (1999). Terminology and convention for electrochemical stripping analysis. *Pure Appl. Chem.* **71(5)**, 891-897.
- Forsberg, G.,** O’Laughlin, J.W. and Mergargle, R.G. (1975). Determination of arsenic by anodic stripping voltammetry and differential pulse anodic stripping voltammetry. *Analytical Chemistry* **47(9)**, 1586-1592.
- Francesconi, K.A.** and Kuehnelt, D. (2004). Determination of arsenic species: A critical review of methods and application, 2000-2003. *Analyst* **129**, 373-395.
- Galan-Vidal, C.A.,** Munoz, J., Dominguez, C. and Alegret, S. (1995). Chemical Sensors, Biosensors and Thick-Film Technology. *Trends in Analytical Chemistry* **14(5)**, 225 – 231.
- Garcia-Manyes, S.,** Jiménez, G., Padró, A., Rubio, R. & Rauret, G. (2002). Arsenic speciation in contaminated soils. *Talanta* **58**, 97-109.

- Gardea-Torresday, J., Darnall, D., and Wang, J.** (1988). Bioaccumulation and measurement of copper at an alga-modified carbon paste electrode. *Anal. Chem.* **60**, 72-76.
- Gayet, J-C., Haouz, A., Geloso-Meyer, A., and Burstein, C.** (1993). Detection of heavy metal salts with biosensors built with an oxygen electrode coupled to various immobilized oxidases and dehydrogenases. *Biosensors and Bioelectronic* **8**, 177-183.
- Gil, E.D. and Ostapczuk, P.** (1994). Potentiometric stripping determination of mercury (II), selenium (IV), copper (II) and lead (II) at a gold film electrode in water samples. *Analytica Chimica Acta* **293**, 55-65.
- Gil, M.H., and Piedade, A.P.** (1992). Covalent binding of urease on ammonium-selective potentiometric membranes. *Biosens. Bioelectron.* **7**, 645-652.
- Gooding, J.J., Hibbert, D.B. and Yang, W.** (2001). Electrochemical metal ion sensors. Exploiting amino acids and peptides as recognition elements. *Sensors* **1**, 75-90.
- Gomez-Ariza, J.L., Sanchez-Rodas, D., Giraldez, I. and Morales, E.** (2000). Comparison between ICP-MS and AFS detection for arsenic speciation in environmental samples. *Talanta* **51**, 257-268.
- Gopel, W., Hesse, J. and Zemel, J.N.** (1993). *Sensors: A Comprehensive Survey* Vol. 8, VCH Publishers.
- Grabarczyk, M. and Korolczuk, M.** (2003). Determination of labile chromium in water samples by catalytic adsorptive stripping voltammetry in on-line system. *Electroanalysis* **15**(5-6), 524-528.
- Grennan, K., Killard, A.J. and Smyth, M.R.** (2001). Physical Characterisation of a screen-printed electrode for use in an amperometric biosensor system. *Electroanalysis* **13**(8-9), 745-750.
- Grimalt, J.O., Ferrer, M. and Macpherson, E.** (1999). The mine tailing accident in Aznalcollar, *Sci. Total Environ.* **242**, 3-11.
- Greulach, U. and Henze, G.** (1995). Analysis of arsenic (V) by cathodic stripping voltammetry. *Analytica Chimica Acta* **306**, 217-223.
- Gunzler, H. and Williams, A.** (2001). *Handbook of Analytical Techniques*. Vol. 2, Wiley – VCH Verlag GmbH, D-69469 Weinheim (FRG).
- Hamilton, T.W. and Ellis, J.** (1980). Determination of arsenic and antimony in electrolytic copper by anodic stripping voltammetry at a gold film electrode. *Analytica Chimica Acta* **119**, 225-233.

- Han, S.,** Zhu, M., Yuan, Z., and Li, X. (2001). A methylene blue-mediated enzyme electrode for the determination of trace mercury (II), mercury (I), methylmercury, and mercury-glutathione complex. *Biosensors and Bioelectronic* **16**, 9-16.
- Henze, G.,** Wagner, W. and Sander, S. (1997). Speciation of arsenic(V) and arsenic (III) by cathodic stripping voltammetry in fresh water samples. *Fresenius J Anal Chem* **358**, 741-744.
- Herzog, G.** and Arrigan, D.W.M. (2003a). Application of disorganized monolayer films on gold electrodes to the prevention of surfactant inhibition of the voltammetric detection of trace metals via anodic stripping of underpotential deposits: detection of copper. *Analytical Chemistry* **75**(2), 319-323.
- Herzog, G.** and Arrigan, D.W.M. (2003b). Comparison of 2-mercaptoethane sulfonate and mercaptoacetic acid disorganized monolayer-coated electrodes for the detection of copper via underpotential deposition-stripping voltammetry. *Electroanalysis* **15**(15-16), 1302-1306.
- Herzog, G.,** Beni, V., Dillon, P.H., Barry, T. & Arrigan, D.W.M. (2004). Effect of humic acid on the underpotential deposition-stripping voltammetry of copper in acetic acid soil extract solutions at mercaptoacetic acid-modified gold electrodes. *Analytica Chimica Acta* **511**(1), 137-143.
- Herzog, G.** and Arrigan, D.W.M. (2005). Determination of trace metals by underpotential deposition-stripping voltammetry at solid electrodes. *Trends in Analytical Chemistry* **24** (3), 208-217.
- Heyrovsky, M.,** Mader, P., Vesela, V. & Fedurco, M. (1994). The reaction of cystine at mercury electrodes. *J. of Electroanalytical Chemistry* **369**, 53-70.
- Heyrovsky, M.,** Mader, P., Vavricka, S., Vesela, V. & Fedurco, M. (1997). The anodic reactions at mercury electrodes due to cysteines. *Journal of Electroanalytical Chemistry* **430**, 103-117.
- Higson, S.P.J.** (2003). Electroanalytical Techniques, in *Analytical Chemistry*. Oxford: Oxford University Press.
- Holak, W.** (1980). Determination of arsenic by cathodic stripping voltammetry with a hanging mercury drop electrode. *Analytical Chemistry* **52**, 2189-2192.
- Honeychurch, K.C.,** Hart, J.P. and Cowell, D.C. (2000). Voltammetric Behaviour and Trace Determination of Lead at a mercury-free screen-printed carbon electrode. *Electroanalysis* **12**(3), 171 – 177.

- Honeychurch, K.C.,** Hart, J.P. and Cowell, D.C. (2001). Voltammetric studies of lead at a 1-(2-Pyridylazo)-2-Naphthol modified screen-printed carbon electrode and its trace determination in water by stripping voltammetry. *Analytica Chimica Acta* **431**, 89 – 99.
- Honeychurch, K.C.,** Hawkins, D.M., Hart, J.P. and Cowell, D.C. (2002). Voltammetric behaviour and trace determination of copper at a mercury-free screen-printed carbon electrode. *Talanta* **57**, 565-574.
- Honeychurch, K.C.,** Hart, J.P. (2003). Screen-printed electrochemical sensors for monitoring metal pollutants. *Trends in Analytical Chemistry* **22** (7+8), 456-469.
- Hua, C.,** Jagner, D., and Renman, L. (1987). Automated determination of total arsenic in sea water by flow constant-current stripping analysis with gold fibres electrodes. *Analytica Chimica Acta* **201**, 263-268.
- Huang, H.** and Dasgupta, P.K. (1999). A field-deployable instrument for the measurement and speciation of arsenic in potable water. *Analytica Chimica Acta* **380**, 27-37.
- Hudson-Edwards, K.,** Macklin, M.G., Jamieson, H.E., Brewer, P.A., Coulthard, T.J., Howard, A.J., and Turner, J.N. (2003). The impact of tailings dam spills and clean-up operations on sediment and water quality in river systems: the Rios Agrio-Guadiamar, Aznalcollar, Spain. *Applied Geochemistry* **18**, 221-239.
- Hung, D.Q.,** Nekrassova, O. and Compton, R.G. (2004). Analytical methods for inorganic arsenic in water: a review. *Talanta* **64**, 269-277.
- Jagner, D.,** Josefson, M., and Westerlund, S. (1981). Determination of arsenic (III) by computerized potentiometric stripping analysis. *Analytical Chemistry* **53**, 2144-2146.
- Ju, H.** and Leech, D. (2000). Electrochemical study of a metallothionein modified gold disk electrode and its action on Hg^{2+} cations. *Journal of Electroanalytical Chemistry* **484**, 150-156.
- Jung, K.,** Bitton, G., Koopman, B. (1995). Assessment of urease inhibition assays for measuring toxicity of environmental samples. *Wat. Res.* **29**(8), 1929-1933.
- Junter, G.A.** (1988). *Electrochemical detection techniques in the applied biosciences*. Ellis Horwood Ltd. Series in Biochemistry and Biotechnology.
- Kadara, R.O.** (2003). Development of electrochemical sensors for heavy metal ions detection in environmental samples. *PhD thesis*, Biotechnology Centre, Cranfield University, U.K.

- Kadara, R.O.**, Newman, J.D. and Tothill, I.E. (2003). Stripping chronopotentiometric detection of copper using screen-printed three-electrode system – application to acetic acid bioavailable fraction from soil samples. *Analytica Chimica Acta* **493**, 95-104.
- Kadara, R.O.** and Tothill, I.E. (2004). Stripping chronopotentiometric measurements of lead (II) and cadmium (II) in soils extracts and wastewaters using a bismuth film screen-printed electrode assembly. *Anal. Bioanal. Chem.* **378**, 770-775.
- Kadara, R.O.** and Tothill, I.E. (2005). Resolving the copper interference effect on the stripping chronopotentiometric response of lead (II) obtained at bismuth film screen-printed electrode. *Talanta*, (in press).
- Kamtekar, S.D.**, Pande, R., Ayyagari, M.S., Marx, K.A., Kaplan, D.L., Kumar, J., and Tripathy, S.K. (1995). A chemiluminescence-based biosensor for metal ion detection. *Mat. Sci. Engineering C* **3**, 79-83.
- Karthikeyan, S.** and Hirata, S. (2003). Arsenic speciation in environmental samples. *Analytical letters* **36**(11), 2355-2366.
- Keyhani, J.**, Keyhani, E., Einollahi, N., Minai-Tehrani, D., and Zarchipour, S. (2003). Heterogenous inhibition of horseradish peroxidase activity by cadmium. *Biochim, Biophys. Acta* **1621**, 140-148.
- Khosraviani, M.**, Pavlov, A.R., Flowers, G.C. and Blake, D.A. (1998). Detection of Heavy Metals By Immunoassay: Optimisation and Validation of A Rapid, Portable Assay For Ionic Cadmium. *Environmental Science Technology* **32**, 137 –142.
- Khosraviani, M.**, Blake II, R.C., Pavlov, A.R., Lorbach, S.C., Yu, H., Delehanty, J.B., Brechbiel, M.W., and Blake, D.A. (2000). Binding properties of a monoclonal antibody directed toward lead-chelate complexes. *Bioconj. Chem.* **11**, 267-277.
- Kirowa-Eisner, E.** and Tzur, M.B.D. (1999). Determination of sub-nanomolar concentrations of lead by anodic-stripping voltammetry at the silver electrode. *Analytica Chimica Acta* **385**, 325-335.
- Kissinger, P.T.** (2000). Electrochemistry for the non-electrochemist. *Current Separations* **20**:2, 51-53.
- Khoo, S.O.** and Guo, S.X. (2002). Rapidly renewable and reproducible mercury film coated carbon paste electrode for anodic stripping voltammetry. *Electroanalysis* **14**(12), 813-822.
- Komaba, S.**, Fujino, Y., Matsuda, T., Osaka, T., and Satoh, I. (1998). Biological determination of Ag(I) and argininr by using the composite film of electroinactive polypyrrole and polyion complex. *Sensors and Actuator B* **52**, 78-83.

- Kopanica, M.** and Novotný, L. (1998). Determination of traces of arsenic (III) by anodic stripping voltammetry in solutions, natural waters and biological material. *Analytica Chimica Acta* **368**, 211-218.
- Kormos, F.,** Lengauer, A.N. (1999). Studies concerning the biomonitoring of the degree of environmental pollution using a urea-sensitive enzymatic sensor. *LRA*. **12**, 27-30.
- Kozlowski, H.,** Bal Wojciech., Dyba, M. & Kowalik-Jankowska, T. (1999). Specific structure-stability relations in metallopeptides. *Coordination Chemistry Reviews*. **184**, 319-346.
- Krawczyk, T.K.** (1998). Analytical applications of inhibition of enzymatic reactions. *Chem. Anal.* **43**, 135-158.
- Krawczyk, T.K.,** Moszczyńska, M., Trojanowicz, M. (2000). Inhibitive determination of mercury and other metal ions by potentiometric urea biosensor. *Biosens. Bioelectron* **15**, 681-691.
- Kröger, S.,** and Turner, A.P.F. (1997). Solvent-resistant carbon electrodes screen-printed onto plastic for use in biosensors. *Anal. Chim. Acta*. **347**, 9-18.
- Landis, W.G.** and Yu, M-H. (1998). *Introduction to environmental toxicology: impacts of chemicals upon ecological systems*, 2nd Ed.. Boca Raton: Lewis Publishers.
- Lanphear, B.P.** (1998). The paradox of lead poisoning and prevention. *Science* **281**, 1617-1618.
- Li, H.** and Smart, R.B. (1996). Determination of sub-nanomolar concentration of arsenic (III) in natural waters by square wave cathodic stripping voltammetry. *Analytica Chimica Acta* **325**, 25-32.
- Lima, E.C.,** Brasil, J.L. and Vaghetti, J.C.P. (2003). Evaluation of different permanent modifiers for the determination of arsenic in environmental samples by electrothermal atomic absorption spectrometry *Talanta* **60**, 103-113.
- Liu, A-C.,** Chen, D-c, Lin, C-C, Chou, H-H. and Chen, C-h. (1999). Application of cysteine monolayers for electrochemical determination of sub-ppb copper (II). *Anal. Chem.* **71**, 1549-1552.
- Lloyd, J.R.** and Lovley, D.R. (2001). Microbial detoxification of metals and radionuclides. *Current Opinion in Biotechnology* **12**, 248-253.

- Locatelli, C.** and Torsi, G. (1999). Cathodic and anodic stripping voltammetry: simultaneous determination of As-Se and Cu-Pb-Cd-Zn in the case of very high concentration ratios. *Talanta* **50**, 1079 – 1088.
- Maccà C,** Soldà L, Voltan R, Calliari I (2004). Stripping Chronopotentiometry with Dry-Preservable Supporting Electrolyte. *Electroanalysis* **16**(16), 1311-1317.
- Manahan, , S.E.** (2000). *Fundamentals of environmental chemistry*, 2nd Ed. Boca Raton: Lewis Publishers.
- Mandieta, J.,** Diaz-Cruz, M.S., Tauler, R., and Esteban, M. (1996). Application of multivariate curve resolution to voltammetric data. II. Study of metal-binding properties of the peptides. *Anal. Biochem.* **240**, 134-141.
- Marx, A.,** and Hock, B. (1998). Characterisation of a new monoclonal antibody against mercury (II). *Anal. Letters* **31** (10), 1633-1650.
- Marx, A.,** Krötz, E. and Hock, B. (1998). Improvement of an enzyme immunoassay for the determination of mercury (II). *Anal. Letters* **31** (10), 1651-1661.
- Masawat, P.,** Liawruangrath S. and Slater, J.M. (2003). Flow injection measurement of lead using mercury-free disposable gold-sputtered screen-printed carbon electrodes (SPCE). *Sensors and Actuators B* **91**, 52-59.
- McCarron, T.** (1999). Electrochemistry ions in solutions. <http://www.tannerm.com> (accessed on 25/12/2004).
- Md Noh, M.F.N.,** Kadara, R.O., Tothill, I.E. (2004). Chronopotentiometric Analysis of Cadmium (II) and Lead (II) using Cysteine modified screen-printed electrode. In: Valiente M, Perez G, Gaona X, Ree DV, Alcock S (Eds) (2004). Senspol Technical Meeting, Sevilla Proceedings, Cranfield University, Cranfield.
- Mejare, M.** and Bulow, L. (2001). Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals. *Trends in Biotechnology* **19**(2), 67-73.
- Mielke, H.W.** (1999). Lead in the inner cities. *Am. Sci.* **87**, 62-73.
- Moreno-Baron L,** Merkoci A, Alegret S (2003). Graphite-epoxy composite as an alternative material to design mercury free working electrodes for stripping voltammetry *Electrochimica Acta* **48**, 2599-2605.
- Morrin, A.,** Killard, A.J. & Smyth, M.R. (2003). Electrochemical characterization of commercial and home-made screen-printed carbon electrodes. *Analytical letters* **36**(9), 2021-2039.

- Muñoz, E.** and Palmero, S. (2004). Analysis and speciation of arsenic by stripping potentiometry: a review. *Talanta*, 000, 000-000.
- Muñoz, E.** and Palmero, S. (2004a). Potentiometric stripping of arsenic (III) using a wall-jet flow cell and gold (III) solution as chemical reoxidant. *Electroanalysis* 16(18), 1528-1535.
- Muñoz, E.** and Palmero, S. (2004b). Speciation of arsenic by potentiometric stripping analysis using gold (III) solution as chemical reoxidant and a wall-jet flow cell. *Electroanalysis* 16(23), 1956-1963.
- Newman, J.D.,** White, S.F., Tothill, I.E., and Turner, A.P.F. (1995). Catalytic materials, membranes, and fabrication technologies suitable for the construction of amperometric biosensors. *Anal. Chem.* 67, 4594-4599.
- Nakazato, T.,** Tao, H., Taniguchi, T. & Isshiki, K. (2002). Determination of arsenite, arsenate, and monomethylarsonic acid in seawater by ion-exclusion chromatography combined with inductively coupled plasma mass spectrometry using reaction cell and hydride generation techniques. *Talanta* 58, 121-132.
- Narinesingh, D.,** Mungal, R., and Ngo, T.T. (1994). A screening method for trace mercury analysis using flow injection with urease inhibition and fluorescence detection. *Analytica Chimica Acta* 292, 185-190.
- New Mexico State University** (2003). Physical chemistry laboratory websites. <http://www.chemistry.nmsu.edu/studntres/chem435/Lab13>. (accessed on 14/2/2003).
- Nordberg, M.** (1998). Metallothioneins: historical review and state of knowledge. *Talanta* 46, 243-254.
- Oehlschläger, K.,** Hüttl, R., and Wolf, G. (1998). Calorimetric investigations of urease inhibition by heavy metal ions. *Thermochim. Acta* 310, 185-189.
- Olsen, K.B.,** Wang, J., Setiadji, R. and Lu, J. (1994). Field Screening of chromium, cadmium, zinc, copper, and lead in sediments by stripping analysis. *Environ. Sci. Technol.* 28, 2074-2079.
- Osborne, M.D.,** Seddon, B.J., Dryfe, R.A.W., Lager, G., Loyall, U., Schafer, H. and Girault, H.H. (1996). Excimer laser-induced electrochemical activity in carbon ink films. *J. Electroanal. Chem.* 417, 5-15.
- Palchetti, I.** (1998). Screen-Printed Sensors for Heavy Metal Detection. *PhD thesis*, Biotechnology Centre, Cranfield University, U.K.

- Palchetti, I.,** Cagnini, A., Mascini, M. and Turner, A.P.F. (1999). Characterisation of Screen-Printed Electrodes for Detection of Heavy Metals. *Mikrochimica Acta* **131**, 65–73.
- Palchetti, I.,** Laschi, S. and Mascini, M. (2005). Miniaturised stripping-based carbon modified sensor for in field analysis of heavy metals. *Analytica Chimica Acta* **530**(1), 61–67.
- Pauliukaite, R.,** Metelka, R., Svancara, I., Krolicka, A., Bobrowski, A., Vytras, K., Norkus, E. and Kalcher, K. (2002). Carbon paste electrodes modified with Bi₂O₃ as sensors for the determination of Cd and Pb. *Anal. Bioanal. Chem.* **374**, 1155–1158.
- Popek, E.P.** (2003). Sampling and analysis of environmental chemical pollutants: A complete guide. 1st Ed. Academic Press. Elsevier, San Diego.
- Prakash, R.,** Srivastava, R.C. & Seth, P.K. (2003). Direct estimation of total arsenic using a novel metal side disk rotating electrode. *Electroanalysis* **15**(17), 1410–1414.
- Preininger, C.** and Wolfbeis, O.S. (1996). Disposable cuvette test with integrated sensor layer for enzymatic determination of heavy metals. *Biosens. Bioelectron.* **11**, 981–990.
- Prellswaid, A.** and Schwedt, G. (1994). A screening procedure for heavy metals in soil extracts by alcohol dehydrogenase and urease inhibition. *Acta Hydrochim. Hydrobiol.* **22**, 70–75.
- Prichard, E.,** MacKay, G.M. and Points, J. (1996). *Trace Analysis: A Structured Approach to Obtaining Reliable Result*, Crown Copyright.
- Rasul, S.B.,** Munir, A.K.M., Hossain, Z.A., Khan, A.H., Alauddin, M. & Hussam, A. (2002). Electrochemical measurement and speciation of inorganic arsenic in groundwater of Bangladesh. *Talanta* **58**, 33–43.
- Reeder, G.S.** and Heineman, W.R. (1998). Electrochemical Characterisation of a Microfabricated Thick-film Carbon Sensor for Trace Determination of Lead. *Sensors and Actuators B* **52**, 58–64.
- Reeve, R.N.** (1994). *ACOL: Environmental Analysis*, Chichester: John Wiley and Sons Ltd.
- Reeve, R.** (2002). Introduction to environmental analysis. Chichester: John Wiley and Sons Ltd.
- Richter, E.M.,** Pedrotti, J.J. & Angnes, L. (2003). Square-wave quantification of lead in rainwater with disposable gold electrode without removal of dissolved oxygen. *Electroanalysis* **15** No 23–24, 1871–1877.

- Rogers, K.R.** and Mascini, M. (1998). Biosensors for field analytical monitoring. *Field Anal. Chem. Technol.* **2**(6), 317-331.
- Rundle, C.C.** (2004). A beginners guide to ion-selective electrode measurements. <http://www.nico2000.net/Book/Guide1.html>. (Access on 20/12/04).
- Sadana, R.S.** (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.
- Senillou, A.,** Jaffrezic-Renault, N., Martelet, C., Cosnier, S. (1999). A miniaturized urea sensor based on the integration of both ammonium based urea enzyme field effect transistor and a reference field effect transistor in a single chip. *Talanta* **50**, 219-226.
- Shekhovtsova, T.N.,** Chernetskaya, S.V., Belkova, N.V., Dolmanova, I.F. (1994). The use of immobilized enzymes for the determination of metal ions. *J. Anal. Chem.* **49**, 709-714.
- Shemirani, F.,** Baghdadi, M. and Ramezani, M. (2004). Preconcentration and determination of ultra trace amounts of arsenic (III) and arsenic (V) in tap water and total arsenic in biological samples by cloud point extraction and electrothermal atomic absorption spectrometry. *Talanta*, **65** (4), 882-887.
- Shi, G.,** and Jiang, G. (2002). A dip-and-read test strip for the determination of mercury (II) ion in aqueous samples based on urease activity inhibition. *Analytical Science* **18**, 1215-1219.
- Shi, R.,** Stein, K., and Schwedt, G. (1997). Determination of mercury (II) traces in drinking water by inhibition of an urease reactor in a flow injection analysis (FIA) system. *Fresenius J. Anal. Chem.* **357**, 752-755.
- Shih, Y.,** Zen, J-M, Kumar, A.S., Lee, Y-C., and Huang, H-R. (2004). Determination of toxic lead level in cosmetic-hair dye formulations using a screen-printed silver electrode. *Bull. Chem. Soc. Jpn.* **77**, 311-312.
- Soldatkin, A.P.,** Volotovskiy, V., El'skaya, A.V., Jaffrezic-Renault, N., Martelet, C. (2000). Improvement of urease based biosensor characteristics using additional layers of charged polymers. *Anal. Chim. Acta* **403**, 25-29.
- Srinivasan, S.,** Chizmadzhev, Y.A., Bockris, J., Conway, B.E. and Yeager, E. (1985). *Comprehensive treatise of electrochemistry. Vol 10. Bioelectrochemistry.* Plenum Press, NY.

- Stankovich, M.T.**, and Bard, A.J. (1977). The electrochemistry of proteins and related substances I. Cystine and cysteine at the mercury electrode. *J. Electroanal. Chem.* **75**, 487-505.
- Stojanovic, R.S.**, Bond, A.M., and Butler, E.C.V. (1990). Liquid chromatography-electrochemical detection of inorganic arsenic using a wall jet cell with conventional and micro-sized platinum disk electrodes. *Analytical Chemistry* **62**(24), 2692-2697.
- Sun, Y-C**, Mierzwa, J. & Yang, M-H. (1997). New method of gold-film electrode preparation for anodic stripping voltammetric determination of arsenic (III and V) in seawater. *Talanta* **44**, 1379-1387.
- Sun, Y.C.** and Yang, J.Y. (1999). Simultaneous determination of arsenic (III,V), selenium(IV,VI), and antimony(III,V) in natural water by coprecipitation and neutron activation analysis. *Analytica Chimica Acta* **395**, 293-300.
- Susmel, S.**, Guilbault, G.G. and O'Sullivan, C.K. (2003). Demonstration of labelless detection of food pathogens using electrochemical redox probe and screen-printed gold electrodes. *Biosensors and Bioelectronics* **18**, 881-889.
- Švancara, I.**, Vytrás, K., Bobrowski, A. & Kalcher, K. (2002). Determination of arsenic at a gold-plated carbon paste electrode using constant current stripping analysis. *Talanta* **58**, 45-55.
- Tanaka, S.** and Yoshida, H. (1982). Enhancement effect of cysteine on anodic stripping voltammetry of copper. *J. Electroanal. Chem.* **137**, 261-270.
- Tothill, I.E.**, Turner, A.P.F. (1998). Biosensors: new developments and opportunities in the diagnosis of livestock diseases, International Atomic Energy Agency, 79-94.
- Thevenot, D.R.**, Toth, K., Durst, R.A. and Wilson, G.S. (2001). Electrochemical biosensors: recommended definitions and classification. *Biosensors & Bioelectronics* **16**, 121-131.
- Thompson, R.B.**, and Jones, E.R. (1993). Enzyme-based fiber optic zinc biosensor. *Anal. Chem.* **65**, 730-734.
- Town, R.M.** (1999). Effect of complexants and surfactants on the deposition and stripping steps in chronopotentiometric stripping analysis and anodic stripping voltammetry: implications for operationally defined speciation measurements. *Fresenius J Anal Chem* **363**, 474-476.
- Tu, Y-F.**, Fu, Z-Q., and Chen, H-Y. (2001). The fabrication and optimization of the disposable amperometric biosensor. *Sens. Actuators B* **80**, 101-105.

- Turyan, I.** and Mandler, D. (1997). Self-assembled monolayers in electrochemical chemistry: Application of ω -Mercaptocarboxylic acid monolayers for electrochemical determination of ultralow levels of cadmium (II). *Anal. Chem.* **66**, 58-63.
- University of Bath (2004).** Electrochemistry Refresher. <http://www.bath.ac.uk/e-learning/electures/lectures/voltammetry/var01.htm> (accessed on 11/8/2004).
- University of Saskatchewan (2004).** Lecture note. <http://electrochem.usask.ca>. (access on 20/8/2004).
- US EPA (2004).** Drinking water standards. <http://www.epa.gov/safewater/standards.html>. (accessed on 12/8/2004).
- Van Den Berg, C.M.G.,** Househam, B.C. & Riley, J.P. (1988). Determination of cystine and cysteine in seawater using cathodic stripping voltammetry in the presence of Cu(II). *Journal of Electroanalytical Chemistry* **239**, 137-148.
- Viltchinskaia, E.A.,** Zeigman, L.L., Garcia, D.M. & Santos, P.F. (1997). Simultaneous determination of mercury and arsenic by anodic stripping voltammetry. *Electroanalysis* **9**(8), 633-640.
- Virginia Tech (2004).** The chemistry hypermedia project: online resources for students, educators, and scientists. <http://www.chem.vt.edu/chem-ed/index.html>. (accessed on 11/8/2004).
- Volotovskiy, V.,** Nam, Y.J. and Kim, N. (1997). Urease-based biosensor for mercuric ions determination. *Sensor and Actuator B*, **42**, 233-237.
- Wang, J.,** and Green, B. (1983). Characteristics of a flow cell for the determination of arsenic (III) by stripping voltammetry. *J. Electroanal. Chem.* **154**, 261-268.
- Wang, J.** and Tian, B. (1992). Screen-printed stripping voltammetric and potentiometric electrodes for decentralised testing of trace lead. *Analytical Chemistry* **64**(15), 1706-1709.
- Wang, J.** and Tian, B. (1993a). Mercury-free disposable lead sensors based on potentiometry stripping analysis at gold-coated screen-printed electrodes. *Analytical Chemistry* **65**(11), 1529-1532.
- Wang, J.** and Tian, B. (1993b). Screen-printed electrodes for stripping measurements of trace mercury. *Analytica Chimica Acta* **274**, 1-6.
- Wang, J.,** Lu, J.M. and Yarnitzky, C. (1993). Screen-printed ultramicroelectrode arrays for on-site stripping measurements of trace metals. *Journal of Electroanalytical Chemistry* **361**(1-2), 77 - 83.

- Wang, J.** (1994). Decentralised electrochemical monitoring of trace metals: From disposable strips to remote electrodes- Plenary Lecture. *Analyst* **119**, 763 – 766.
- Wang, J.,** Pedrero, M., Sakslund, H., Hammerich, O. and Pingarron, J. (1996). Electrochemical activation of screen-printed carbon strips. *Analyst* **121**, 345-350.
- Wang, J.,** Tian, B., Nascimento, V.B. and Angnes, L. (1998). Performance of screen-printed carbon electrodes fabricated from different carbon inks. *Electrochimica Acta* **43(23)**, 3459-3465.
- Wang, J.,** Tian, B., Wang, J., Lu, J., Olsen, C., Yarnitzky, C., Olsen, K., Hammerstrom, D. and Bennett, W. (1999). Stripping Analysis into the 21 st Century: Faster, Smaller, Cheaper, Simpler and Better. *Analytica Chimica Acta* **385**, 429 – 435.
- Wang, J.** (2000). *Analytical Electrochemistry*. 2nd Edition, John Wiley and Sons, Inc., Publication, New York.
- Wang, J.,** Hansen, E.H. & Gammelgaard, B. (2001a). Flow injection on-line dilution for multi-element determination in human urine with detection by inductively coupled plasma mass spectrometry. *Talanta* **55**, 117-126.
- Wang, J.,** Lu, J., Hocevar, S.B. and Ogorec, B. (2001b). Bismuth-coated screen-printed electrodes for stripping voltammetric measurements of trace lead. *Electroanalysis* **13(1)**, 13–16.
- Webb, J.L.** (1996). Enzyme and metabolic inhibitors, Vol. II. Academic Press, New York.
- Williams, G.,** and D'Silva, C. (1994). Hand-held instrumentation for environmental monitoring. *Analyst* **119**, 187-190.
- WHO** (1998). *Guidelines for drinking-water quality*. Second Edition. World Health Organisation, Geneva. <http://www.who.int>. (Access on 12/12/2004).
- Wild, A.** (1993). *Heavy metals and Radionuclides*. In: Soils and the Environment. Cambridge University Press.
- Williams, R.E.,** Holt P-J., Bruce, N.C. and Lowe, C.R. (2000). Heavy metals. In *Biosensors for environmental monitoring*, pp. 213-225. Edited by Bilitewski, U., Turner, A.P.F. Amsterdam: Harwood Academic Publishers.
- Wilson, M.M.** and Dewald, H.D. (2001). Stripping potentiometry of indium in aqueous chloride solutions. *Microchem, J.* **69**, 13-19.

- Wittekindt, E.,** Werner, M., Reinicke, A., Herbert, A., and Hansen, P. (1996). A microtiter-plate urease inhibition assay sensitive, rapid and cost-effective screening for heavy metals in water. *Environm. Technol.* **17**, 597-603.
- Yang, W.,** Jaramillo, D., Gooding, J.J., Hibbert, D.B., Zhang, R., Willet, G.D. & Fisher, K.J. (2001). Sub-ppt detection limits for copper ions with gly-gly-his modified electrodes. *Chem. Commun.* 1982-1983.
- Yarnitzky, C.,** Wang, J. and Tian, B. (2000). Hand-held lead analyser. *Talanta* **51**, 333 – 338.
- Yoneyama, K.,** Fujino, Y., Osaka, T., and Satoh, I. (2001). Amperometric sensing system for the detection of urea by a combination of the pH-stat method and flow injection analysis. *Sensors and Actuators B* **76**, 152-157.
- Yu, M-H.** (2001). Environmental toxicology: impacts of environmental toxicants on living systems. Boca Raton: Lewis Publishers.
- Zaborska, W.,** Krajewska, B., Leszko, M., and Olech, Z. (2001). Inhibition of urease by Ni^{2+} ions. Analysis of reaction progress curves. *J. Mol. Catalysis B: Enzymatic* **13**, 103-108.
- Zen, J-M.,** Chung, H-H. and Kumar, A.S. (2000). Determination of lead (II) on a copper/mercury-plated screen-printed electrode. *Analytica Chimica Acta* **421**, 189-197.
- Zen, J-M.,** Yang, C-C. and Kumar, A.S. (2002). Voltammetric behaviour and trace determination of Pb^{2+} at a mercury-free screen-printed silver electrode. *Analytica Chimica Acta* **464**, 229-235.
- Zima, J.** and van den Berg, C.M.G. (1994). Determination of arsenic in sea water by cathodic stripping voltammetry in the presence of pyrrolidine dithiocarbamate. *Analytica Chimica Acta* **289**, 291-298.

PUBLICATIONS

Results from this thesis have been published and presented as follows:

Conference Posters

Md Noh, M.F., Kadara, R.O. and Tothill, I.E.. (2002). Chronopotentiometric analysis of cadmium (II) and lead (II) in soil using cysteine modified screen-printed electrode. 3rd *Senspol Technical Meeting, Krakow, Poland, 3-6 June 2003.*

Oral Presentations

Md Noh, M.F., Kadara, R.O. and Tothill, I.E.. (2002). Chronopotentiometric analysis of cadmium (II) and lead (II) using cysteine modified screen-printed electrode. *Senspol Technical Meeting, Seville, Spain, 6-9, November 2002.*

Md Noh, M.F. and Tothill, I.E.. (2003). Application of cysteine modified screen-printed electrode to chronopotentiometric stripping analysis of cadmium (II) in soil samples extracted with acetic acid. 4th Postgraduate Research Conference, Silsoe, 19 June 2003.

Md Noh, M.F. and Tothill, I.E.. (2003). Electrochemical determination of copper (II) and lead (II) by cysteine modified mercury and gold screen-printed electrode. *Senspol Technical Meeting, Koblenz, 28-31, October 2003.*

Proceedings

Md Noh, M.F.N., Kadara, R.O., Tothill, I.E. (2004). Chronopotentiometric Analysis of Cadmium (II) and Lead (II) using Cysteine modified screen-printed electrode. In: Valiente M, Perez G, Gaona X, Ree DV, Alcock S (Eds) (2004). *Senspol Technical Meeting, Sevilla Proceedings, Cranfield University, Cranfield.*

Research papers

Md Noh., Mohd F., Kadara, R.O. and Tohill, I.E*. Development of cysteine modified screen-printed electrode for the chronopotentiometric stripping analysis of cadmium (II) in waste-water and soil extract. *Analytical and Bioanalytical Chemistry* (Accepted).

Md Noh., Mohd F. and Tohill, I.E*. Development of disposable screen-printed gold electrode and its implementation for lead (II) analysis in waste-water and soil samples. (Submitted).