



UNIVERSITY OF NAIROBI

**DEVELOPMENT OF POLYACRYLIC ACID BASED
ELECTROCHEMICAL SENSOR FOR DETECTION OF SELECTED
HEAVY METALS IN WATER**

BY

MOSES ONDABU OYAGI

BSc. MSc. (Nairobi)

I80/95787/2014

A thesis Submitted in Fulfillment of the Requirements for the Award of the Degree of Doctor of
Philosophy in Chemistry of the University of Nairobi.

2017

DECLARATION

I declare that ‘development of polyacrylic acid based electrochemical sensor for detection of selected heavy metals in water’ is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other people’s work or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi’s requirements.

----- Date: -----
MOSES ONDABU OYAGI
I80/95787/2014
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF NAIROBI

This thesis has been submitted for examination with our approval as the university supervisors

----- Date: -----
DR. PETERSON M. GUTO
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF NAIROBI

----- Date: -----
DR. JOHN O. ONYATTA
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF NAIROBI

----- Date: -----
PROF. GEOFFREY N. KAMAU
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF NAIROBI

DEDICATION

I dedicate this work to my beloved wife Fridah, my lovely son Adrian, my parents and siblings for making it possible to complete this study.

ACKNOWLEDGEMENTS

I thank God Almighty for giving me the strength and resilience to complete this work. Without His constant help and mercy, nothing would have been possible. All glory goes to Him.

I would like to extend my sincere gratitude to my supervisors and mentors; Prof. Geoffrey N. Kamau, Dr. Peterson O. Guto and Dr John O. Onyatta, all from the University of Nairobi for their valuable guidance and encouragements which has made my pursuance of this degree possible. I especially extend my gratitude to Dr. Peterson Guto, my principal supervisor, for his constant help, availability and enthusiasm right from the proposal stage to the end. His comments, suggestions and assistance served to help clarify many technical ideas.

I wish to pass my sincere gratitude to all members of the Chemistry Department, University of Nairobi, who have been helpful throughout my studies and in particular Dr. Immaculate Michira for her encouragements and advice.

My appreciation goes to my mum, siblings and the extended family members for their support and believing in me throughout my studies. Thank you for always caring for me and my family.

I pass my gratitude to my beloved wife, Fridah, for standing by me throughout this journey. I am very grateful for your encouragements and believing in my abilities and for your understanding during the long hours spent in the laboratory. I appreciate your support during the write up of this thesis.

I wish to pass my sincere gratitude to my friends Drs. Stephen Mailu and Peter Ndangili. Thank you for motivating me and just making me happy everytime we meet or speak. You have been helpful in different ways. Your encouragements and advice contributed a great deal to my completion of this work.

I would also like to thank Third World Academy of Sciences (TWAS), Italy and Deans' Research Grant (DRG), University of Nairobi for funding my studies. Thank you for your generous financial support that made it possible to complete this work.

ABSTRACT

A simple differential pulse anodic stripping voltammetric sensor based on Polyacrylic acid/Glassy carbon electrode has been developed for simultaneous analysis of lead(II), cadmium(II) and cobalt(II) ions. Electrochemical characterization of the Polyacrylic acid/Glassy carbon electrode (PAA/GCE) using potassium ferricyanide standard revealed that the diffusion coefficients (D_0) for the PAA/GC and GC plain electrodes were $0.0318 \times 10^{-7} \text{ cm}^2/\text{s}$ and $0.00122 \times 10^{-7} \text{ cm}^2/\text{s}$ respectively. The apparent electrochemical electron transfer rate constants (k^0) were $9.074 \times 10^{-3} \text{ cm/s}$ and $0.0883 \times 10^{-3} \text{ cm/s}$ for PAA/GC and GC plain electrodes respectively. The D_0 and k^0 of the modified and unmodified electrodes indicates that modification of the GC electrode with PAA led to easier and faster charge transfer at the electrode surface.

With differential pulse anodic stripping voltammetry, the following optimal conditions were determined: Deposition potential: -0.8 V, Accumulation time: 300 sec, amplitude: 0.06 V, pulse period: 0.02 sec, pulse width: 0.01 sec and sampling width: 0.0033 sec and pH 6.0.

Accuracy, precision, linearity, range, limits of detection and quantification validation parameters were determined for this sensor in accordance with the ISO/IEC 17025 guidelines. The recovery degree for accuracy was in the range of 101 to 110% for Pb(II), 100 to 105% for Cd(II) and 93 to 104% for Co(II). These lies between the imposed limits of 90 to 110%. The precision was found to be less than 10% for ten determinations in line with the required 10% at the limit of quantitation. Linear concentration range was also investigated and found to lie in the range of 125 – 7.8 μM Pb(II), 16 – 2 μM Cd(II) and 2 – 0.125 mM Co(II). Also, limits of detection also found to be 0.9 nM Pb(II), 1.9 mM Cd(II) and 11.0 μM Co(II) and while limits of quantitation were 3.0 nM Pb(II), 6.3 nM Cd(II) and 36.7 μM Co(II). The effects of foreign substances like Cu^{2+} , SO_4^{2-} , K^+ , Na^+ , Cl^- , NH_4^+ and O_2 were found to have no significant effect on the electrochemical

responses of the three heavy metals. These results confirms that this sensor provides accurate, reliable and consistent results for the determination of Pb(II), Cd(II) and Co(II) heavy metals in waters.

PATENTS

Differential pulse anodic stripping voltammetric sensor using Polyacrylic acid/glassy carbon electrode for simultaneous analysis of Lead(II), Cadmium(II) and Cobalt(II) ions. Filing date: 04/05/2016, Filing no: KE/P/2016/002467 at Kenya Industrial Property Institute (KIPI)

LIST OF PUBLICATIONS

1. Moses O. Oyagi, John O. Onyatta, Geoffrey N. Kamau, Peterson M. Guto. Simultaneous Determination of Trace Lead (II), Cadmium (II) and Cobalt (II) by Differential Pulse Anodic Stripping Voltammetry Using Polyacrylic acid/Glassy Carbon Electrode. International Journal of Sciences: Basic and Applied Research (IJSBAR), Vol. 23, No. 2, pp 324 - 334, July 2015.
2. Moses O. Oyagi, John O. Onyatta, Geoffrey N. Kamau, Peterson M. Guto. Validation of the Polyacrylic acid/Glassy Carbon Differential Pulse Anodic Stripping Voltammetric Sensor for Simultaneous Analysis of Lead (II), Cadmium (II) and Cobalt (II) ions. International Journal of Electrochemical Science. Vol. 11, pp 3852-3861, May 2016.
3. Moses O. Oyagi, John O. Onyatta, Geoffrey N. Kamau, Peterson M. Guto. Electrokinetic Properties of Polyacrylic Acid Film on Gold, Platinum and Glassy Carbon Electrodes in Aqueous Solutions. *Submitted to the International Journal of Bichemiphysics.*

TABLE OF CONTENTS

| | |
|--|--------------|
| DECLARATION | ii |
| DEDICATION | iii |
| ACKNOWLEDGEMENTS | iv |
| ABSTRACT | vi |
| PATENTS | viii |
| LIST OF PUBLICATIONS | viii |
| LIST OF FIGURES | xiii |
| LIST OF TABLES | xvi |
| LIST OF SCHEMES | xvii |
| LIST OF ABBREVIATIONS AND SYMBOLS | xviii |
| CHAPTER ONE | 1 |
| INTRODUCTION | 1 |
| 1.1 Background | 1 |
| 1.2 Problem statement | 5 |
| 1.3 Objectives..... | 5 |
| 1.3.1Major objective..... | 6 |
| 1.4 Justification and significance of the work | 6 |
| CHAPTER TWO | 8 |
| LITERATURE REVIEW | 8 |
| 2.1 Electrochemistry..... | 8 |
| 2.2 Electrochemical techniques..... | 9 |
| 2.2.1 Voltammetry | 13 |
| 2.2.2 Forms of Voltammetry | 15 |
| 2.2.2.1Potential Sweep Voltammetry | 16 |
| 2.2.2.2 Differential-Pulse Voltammetry | 17 |
| 2.2.2.3 Stripping Voltammetry | 17 |
| 2.2.2.3.1Anodic-Stripping Analysis | 18 |

| | |
|---|-----------|
| 2.2.2.3.2 Cathodic-Stripping Analysis..... | 20 |
| 2.3 Electrochemical sensors | 21 |
| 2.3.1 History of electrochemical sensors | 22 |
| 2.3.1.1 Technological advancements in electrochemical sensors | 22 |
| 2.3.2 Main types of electrochemical sensors methods | 24 |
| 2.3.2.1 Amperometry-based sensors..... | 25 |
| 2.3.2.2 Potentiometry-based sensors | 26 |
| 2.3.2.3 Conductometric sensors..... | 26 |
| 2.3.2.4 Biosensors..... | 27 |
| 2.3.3 Polymers | 28 |
| 2.4 Heavy metal pollutants..... | 31 |
| 2.4.1 Sources of heavy metal pollutants | 31 |
| 2.4.2 Implication of heavy metals pollutants on human health | 33 |
| 2.4.2.1 Lead | 34 |
| 2.4.2.3 Cobalt..... | 37 |
| CHAPTER THREE | 39 |
| MATERIALS AND METHODS | 39 |
| 3.1 Chemicals and solutions..... | 39 |
| 3.2 Apparatus | 39 |
| 3.3 Cleaning of the glassware | 39 |
| 3.4 PAA film preparation and optimization | 40 |
| 3.4.1 Procedure | 40 |
| 3.4.2 Electrochemical characterization of the PAA/GCE | 40 |
| 3.5 Optimization of the sensor | 41 |

| | |
|--|-----------|
| 3.5.1 Buffer pHs (acetate buffer)..... | 41 |
| 3.5.2 Pulse amplitude..... | 41 |
| 3.5.3 Deposition time..... | 42 |
| 3.5.4 Pulse width | 42 |
| 3.5.5 Sampling widths | 42 |
| 3.5.6 Pulse period | 42 |
| 3.6 Sensor validation..... | 43 |
| 3.6.1 Accuracy..... | 43 |
| 3.6.2 Precision | 43 |
| 3.6.3 Linearity, linear concentration range, limit of detection and limit of quantitation | 44 |
| 3.6.4 Effect of impurities..... | 45 |
| 3.7 Determination of trace Lead (II), Cadmium (II) and Cobalt (II) in tap water using the developed sensor | 45 |
| CHAPTER FOUR..... | 46 |
| RESULTS AND DISCUSSIONS..... | 46 |
| 4.1 Characterization of the working electrode | 46 |
| 4.2 Optimization of the sensor | 53 |
| 4.2.1 Effect of the amount of polyacrylic acid (PAA)..... | 53 |
| 4.2.2 Effect of the drying period of the polyacrylic acid film | 57 |
| 4.2.3 Stability of the polyacrylic acid film on glassy carbon | 59 |
| 4.2.4 Buffer pHs (acetate buffer)..... | 61 |
| 4.2.5 Deposition time..... | 65 |
| 4.2.6 Pulse amplitudes | 68 |
| 4.2.7 Sampling widths | 70 |
| 4.2.8 Pulse period | 71 |
| 4.2.9 Pulse width | 72 |

| | |
|---|-----------|
| 4.3 Results of sensor validation..... | 73 |
| 4.3.1 Accuracy..... | 74 |
| 4.3.2 Precision..... | 77 |
| 4.4 Calibration..... | 78 |
| 4.4.1 Linearity..... | 83 |
| 4.4.2 Limit of detection..... | 83 |
| 4.5 Effect of impurities..... | 84 |
| 4.6 Determination of Pb(II), Cd(II) and Co(II) in tap water sample..... | 85 |
| CHAPTER FIVE | 89 |
| CONCLUSIONS AND RECOMMENDATIONS..... | 89 |
| 5.1 Conclusions..... | 89 |
| 5.2 Recommendations..... | 90 |
| REFERENCES..... | 91 |

LIST OF FIGURES

| | |
|---|----|
| Figure 2.1: A summary of some electrochemical methods of analysis..... | 11 |
| Figure 2.2: A schematic representation of a universal electrochemical experimental setup (Source: Author, 2016)..... | 12 |
| Figure 2.3: A typical Cyclic voltammogram of (A) GC/PAA and (B) GC plain for potassium ferrocyanide/0.1 M potassium chloride at 0.005 V/s (Source: Author, 2017)..... | 16 |
| Figure 2.4: Common sources of heavy metal pollutants (Source; Author, 2016)..... | 33 |
| Figure 4.1: Cyclic voltammetry of 1.0 mM potassium ferricyanide on plain glassy carbon electrode at scan rate of 0.005 V/s to 0.02 V/s..... | 47 |
| Figure 4.2: Plot of Current versus square root of scan rates on plain glassy carbon electrodes... | 48 |
| Figure 4.3: Cyclic voltammetry of 1.0 mM potassium ferricyanide on polyacrylic acid/glassy carbon electrode (PAA/GCE) at scan rate of 0.005 V/s to 0.02 V/s. The electrolyte was 0.1M potassium chloride..... | 49 |
| Figure 4.4: Plot of current (A) versus square root of scan rates on polyacrylic acid/glassy carbon electrode (PAA/GCE)..... | 50 |
| Figure 4.5: Effects of the PAA concentration on the peak currents of 0.5 mM Pb(II), Cd(II) and Co(II) at the PAA/GC electrode. Deposition potential: -0.8 V, Accumulation time: 300 sec, amplitude: 0.06 V, pulse period: 0.02 sec, pulse width: 0.01 sec and sampling width: 0.0033 sec. The PAA amounts added were: B = 2 μ L, C = 4 μ L, D = 7 μ L, E = 10 μ L, F = 13 μ L of 4 mM PAA on GC electrode..... | 55 |
| Figure 4.6: Plots of currents (μ A) versus volumes (μ L) of 4 mM PAA..... | 56 |
| Figure 4.7: Effects of drying time of 10 μ L of 4 mM PAA on the peak currents of 0.5 mM Pb(II),Cd(II) and Co(II) at the PAA/GC electrode. Deposition potential: -0.8 V, Accumulation time: 300 sec, amplitude: 0.06 V, pulse period: 0.02 sec, pulse width: 0.01seconds and sampling width: 0.0033 sec. Drying time were: B = 1 min, C = 5 min, D = 10 min, E = 15 min, F = 20 min, G = 25 min of drying the PAA film on GC electrode..... | 57 |
| Figure 4.8: Plots of currents (μ A) versus drying time (min) of the PAA films..... | 58 |

| | |
|---|----|
| Figure 4.9: PAA/GC electrode stability in 2 mL of 1 mM Pb(II), Cd(II) and Co(II) in acetate buffer, pH 6. Deposition potential: -0.8 V, Accumulation time: 300 sec, amplitude: 0.06 V, pulse period: 0.02 sec, pulse width: 0.01 sec and sampling width: 0.0033 sec. The films were dried for 20 min before investigating their stability..... | 60 |
| Figure 4.10: Effects of pH on the peak currents of 2 mM Pb(II), 2 mM Cd(II) and 2 mM Co(II) at the..... | 62 |
| Figure 4.11: Differential Pulse Anodic Stripping Voltammograms of 2 mM Pb(II), Cd(II) and Co(II) at pH 6 on Plain GCE (<i>Blue</i>) and PAA/GCE (<i>Red</i>)..... | 64 |
| Figure 4.12: DPASVs - Effect of the deposition time on the peak currents of Pb, Cd and Co in acetate buffer, pH 6.0 containing 2 mM of Pb, Cd and Co at the PAA/GCE. Deposition time: 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 min..... | 66 |
| Figure 4.13: Current versus Time: Effect of the deposition time on the peak currents of Pb, Cd and Co in acetate buffer, pH 6.0 containing 2 mM of Pb, Cd and Co at the PAA/GCE. Deposition time: 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 min..... | 67 |
| Figure 4.14: Differential pulse stripping voltammetric responses at the PAA/GCE in buffer pH 6.0 containing 2 mM Pb(II), Cd(II) and Co(II). Amplitudes: 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 V..... | 69 |
| Figure 4.15: Differential pulse stripping voltammetric responses at the PAA/GCE in buffer pH 6.0 containing 2 mM Pb(II), Cd(II) and Co(II). Sampling widths: 0.005, 0.0067, 0.009, 0.02 and 0.03 sec..... | 70 |
| Figure 4.16: Differential pulse stripping voltammetric responses at the PAA/GCE in buffer pH 6.0 containing 2 mM Pb(II), Cd(II) and Co(II). Pulse periods: 0.05, 0.06, 0.1 and 0.8 sec..... | 71 |
| Figure 4.17: Differential pulse stripping voltammetric responses at the PAA/GCE in buffer pH 6.0 containing 2 mM Pb(II), Cd(II) and Co(II). Pulse widths: 0.02, 0.03, 0.04, 0.05 and 0.06 sec..... | 72 |
| Figure 4.18: DPASVs of Cd(II), Co(II) and Pb(II) having concentrations of 2.0, 1.0, 0.5 and 0.125 mM from bottom to top, respectively, which were measured using PAA/GCE. Also respective calibration curves for the determination of Cd(II), Co(II) and Pb(II) of different concentrations are shown..... | 79 |
| Figure 4.19: Calibration curve for the determination of Cd(II) of different concentrations..... | 80 |

Figure 4.20: Calibration curve for the determination of Co(II) of different concentrations.....81

Figure 4.21: Calibration curves for the determination of Pb(II) of different concentrations.....82

Figure 4.22: DPAS voltamograms of the Pb, Cd and Co obtained from standard addition technique (A) 3 mL acetate buffer (pH = 6.0) + 3 mL tap water (B) A + 100 μ L of 2 mM standard solution of Pb, Cd and Co (C) A + 200 μ L of 2 mM standard solution of Pb(II), Cd(II) and Co(II).....86

Figure 4.23: The calibration plots of Pb(II), Cd(II) and Co(II) were obtained from standard addition by DPASV technique.....87

LIST OF TABLES

| | |
|---|----|
| Table 2.1: A summary of some electrode modifiers..... | 29 |
| Table 4.1: Electrochemical parameters for the selected electrodes..... | 52 |
| Table 4.2: Optimal parameters for the simultaneous determination of Pb(II), Cd(II) and Co(II) on PAA/GCE electrode..... | 73 |
| Table 4.3: The recovery values of the added analyte from spiked standard solutions of Pb(II), Cd(II) and Co(II) on PAA/GC electrode..... | 75 |
| Table 4.4: Experimental results and statistical calculated results obtained when proving repeatability on standard samples for Pb(II), Cd(II) and Co(II) on PAA/GC electrode... | 77 |
| Table 4.5: The values of the LCR, LOD, LOQ, R ² and regression equation of Pb(II), Cd(II) and Co(II) on PAA/GC electrode..... | 84 |
| Table 4.6: Pb(II), Cd(II) and Co(II) allowed limit values (according to EU, WHO and EPA: (Nursel <i>et al.</i> , 2013; Yayintas <i>et al.</i> , 2007; Tang <i>et al.</i> , 2009) and the found values of Lead, Cadmium and Cobalt heavy metals in tap water of Nairobi City (Kenya)..... | 88 |

LIST OF SCHEMES

| | |
|--|----|
| Scheme 4.1: Mechanism on the electrochemical behavior of metals at the surface of PAA modified electrode..... | 61 |
|--|----|

LIST OF ABBREVIATIONS AND SYMBOLS

| | |
|-----------|--|
| AA | Acrylic Acid |
| AAS | Atomic absorption spectrometry |
| Adsv | Adsorptive stripping voltammetry |
| ASV | Anodic stripping voltmmetry |
| ATSDR | Agency for Toxic Substances and Disease Registry |
| Au | Gold |
| Cd | Cadmium |
| Co | Cobalt |
| Cr | Chromium |
| CSV | Cathodic stripping voltammetry |
| DPV | Differential Pulse Voltammetry |
| E_a | Anodic potential |
| E_p | Cathodic potential |
| E° | Formal redox potential |
| EFSA | European Food Safety Agency |
| EPA | Environmental Protection Agency |
| GC | Glass carbon |
| I_a | Anodic current |
| I_p | Cathodic current |
| IARC | International Agency for the Research on Cancer |
| ICP | Inductively coupled plasma |
| K° | Electrochemical rate constant |
| LOD | Limit of detection |
| LOQ | Limit of quantification |

| | |
|-------|--|
| MDL | Method detection limit |
| NaOH | Sodium hydroxide |
| PAA | Polyacrylic acid |
| Pb | Lead |
| PBS | Phosphate buffered saline |
| PG | Pyrolytic graphite |
| ppb | parts-per-billion |
| Pt | Platinum |
| CSTEE | Scientific Committee on Toxicity Ecotoxicity and the Environment |
| SV | Stripping voltammetry |
| SWV | Square wave voltammetry |
| UN | United Nations |
| WHO | World Health Organization |

CHAPTER ONE

INTRODUCTION

1.1 Background

Water is a basic necessity of life and plays a crucial role in human's overall well-being. It sustains life through meeting human varied and important purposes such as drinking, cooking and washing among others. For these uses to be successfully met, water must be of the acceptable quality and enough. But, water on planet Earth is limited and therefore must be protected from any form of contamination in order to adequately meet human needs. The total amount of water on earth is as much as it was formed and there has never been any more or less (Gleick, 1998; EPA, 2013). Water supply covers about 70 percent of the physical environment of the planet Earth. Whereas the larger portion of the planet is covered by water, most of it is salty and unsuitable for human consumption. Ninety seven percent (97%) of the total water supply is salty water and is not available for consumption to meet daily human needs. Two percent is frozen and exist as ice caps and glaciers. That leaves only one percent (1%) of the planet's entire water supply that is available in lakes and underground to meet all people needs. Thus, only a small proportion of the world's water supply is available for human consumption (EPA, 2013).

Pollutants coming from varied sources have made the available water unsuitable for human use. The pollutants gets into the water sources; lakes, rivers and ground aquifers thus making them unsafe. This pollution of water exist in many forms such as plain dirt and toxic chemicals. This pose a threat to both people health and environment in many ways. Worldwide, there are increased efforts to curb the water pollution. Despite these efforts, permanent solutions to water pollutions have not been realized. There are many underlying factors that need to be addressed to get solutions to water pollution. This has complicated efforts to improving water quality. Some of the factors

that contribute to water pollutions include; poor water management systems, poor water handling practices and increased presence of various pollutants in the environment.

Besides, many of the ways that water is used are responsible for affecting its quality. Some of these ways include; careless industrial use of the water that result in the contamination with toxic chemicals and use of recreational boats that inject gases and oils into the waters. Pollutants in both underground and surfaces water cause trouble to humans and all other living things that depends on it. Some of the contaminants can be seen like garbage and are less serious. But, the pollutants that cause serious challenges are not obvious like sediments, nutrients, pathogens and toxic chemicals. These substances when present in the water in elevated levels cause negative effects, impairs the welfare of the water, reduces the quality of life and may eventually cause death (UN-Water, 2009).

Increasing urban population has further complicated the efforts to control water pollutions. This increase does not give enough time for a country to plan. Thus, it is important to think of ways that are useful in protecting human beings from these contaminants. This includes early detection to verify quality of the water before consumption. This requires powerful and sensitive tools that have capability to detect presence of pollutants in the environment and in particular in the water (UN-Water, 2009).

Toxicity of trace and heavy metal increased demand for demand for new tools that are able to detect them at very low concentrations (Heitzmann *et al.*, 2005). In this work, differential-pulse anodic stripping voltammetry (DPASV) technique was explored. The differential-pulse anodic stripping voltammetry (DPASV) technique is widely used for detection of toxic trace heavy metals because it is affordable to many analysts and exhibits extremely high sensitivity as a result of

the preconcentration process involved (Jia *et al.*, 2008; Achterberg *et al.*, 1999; Li *et al.*, 2013; Somerset *et al.*, 2010; Florence, 1986; Olsen *et al.*, 1994). The working electrode was modified by a polymer, polyacrylic acid (PAA). Recently, polymer-film electrodes have attracted research interest. This is because they have shown potential to replace mercury and mercury-film based electrodes. Several polymer modified electrodes have shown excellent advantages over mercury film electrodes when applied to detect trace heavy metals using stripping voltammetry (March *et al.*, 2015; Pujol *et al.*, 2014). In this work, lead (Pb), cadmium (Cd) and cobalt (Co) were trace metals of prime environmental concern. This is because they are toxic and readily available in the environment as a result of their many applications (Ensafi & Zarei, 2000; Zen, *et al.*, 2002; Estevez-Hernandez *et al.*, 2007; Honeychurch *et al.*, 2002; Li *et al.*, 2013). Worldwide, millions of people are exposed to elevated levels of these toxic heavy metals through contaminated water (WHO, 2012; Somerset *et al.*, 2010).

Essentially, analytical methods require to be validated. Validation involves assessment of their performance indicators and this depends on the type of the method and the inherent characteristics (ICH, 2005; Riley & Rosanske, 1996). Equally important, all electroanalytical methods have to undergo same validation studies just like any other analytical method in spite of their uses (Chan *et al.*, 2004). The aim of the validation studies, is to ensure that the method suitability is proven for its intended application. The validation studies include limit of detection, limit of quantification, linearity, linear concentration range, accuracy and precision among others (ICH, 2005; Riley & Rosanske, 1996; Chan *et al.*, 2004; De Bievre & Gunzler, 2005; Thompson *et al.*, 2002; Gumustas & Ozkan, 2011).

Consequently, the developed polyacrylic acid/glassy carbon based electrochemical sensor was optimized using differential-pulse anodic stripping voltammetry. The sensor was then

successfully used to determine Pb(II), Cd(II) and Co(II) in tap water sample. The purpose of the validation study was to optimize the sensor in accordance with the guidelines of the International Organization for Standardization and the International Electrotechnical Commission 17025 (ISO/IEC 17025) (International standard ISO/IEC 17025, 2005; Validation of Analytical Methods, 2010).

Polyacrylic acid (PAA) is a solid (three-dimensional) cross-linked attached polymer matrix. The polymer has the ability to swell when it absorbs water, up to about hundred times its own weight (Liu *et al.*, 2009). Hence, the PAA has attracted many applications (Elviraa *et al.*, 2002; Anamul Haque *et al.*, 2012; Li *et al.*, 2004; Bekiari *et al.*, 2008; Tang *et al.*, 2009; Nursel, *et al.*, 2002).

The use of a potentiostat has an edge when compared to other methods, in the sense that it is relatively of low cost and can easily be made portable for field-related activities and the expected signal is enhanced via a pre-concentration step. The overall achievement is the development of a highly sensitive probe for heavy metals and related pollutants.

The choice of the electrode environment is based on the well-known results observed, there earlier, and the ability to pre-concentrate the pollutant “on a reduced volume” (Win *et al.*, 1981; Kounaves *et al.*, 1987; Stephen *et al.*, 1980; La Pera *et al.*, 2002;2003; Wang *et al.*, 2000; Carballeira *et al.*, 2000). It is hoped that, not only will the pollutants targeted in this work be detected but also other pollutants, which are detrimental to human health. This will ensure improved water quality assessment and management in Kenya and the world in general.

1.2 Problem statement

The amount of heavy metals in the environment and specifically in waters has increased significantly because of the increase in mining, industrial and urban activities (Onyatta & Huang, 1999). These metals are essential in many of the products we use. However, when they are incorporated in food and water they have negative effects on people health. they change certain enzymatic processes in the body and they are incorporated in the bones, in the liver and other organs, they affect the nervous system. Studies have shown that human health and the environment are exposed to dangerous levels of these heavy metal pollutants (Ilaria *et al.*, 2001). Thus, millions of people are exposed to dangers which come along with the contamination. This should prompt us to be proactive in the fight against having heavy metals in our food and waters (UNDP, 2006).

Effective tools which can detect and remove these metals have been developed and implemented in a number of places (Ilaria *et al.*, 2001). However, there is still need for tools which can detect low concentrations possibly to pico-molar range, can be minimally affected by interferants, can do simultaneous analysis of a number of analytes and most importantly are field deployable. Interest in the low concentration is because heavy metals do not biodegrade and thus get retained indefinitely in the ecological systems and in food chains. Overtime, they bio accumulate to levels which are lethal to the human health (Ilaria *et al.*, 2001).

1.3 Objectives

This study had the following major and specific objectives;

1.3.1 Major objective

The major objective of this study was to develop an electrochemical sensor for detecting and quantifying low concentrations of heavy metals in water, employing electrodes containing thin films of polyacrylic acid (PAA).

1.3.2 Specific objectives

The study had the following specific study objectives

1. To develop polymer-coated working electrode using polyacrylic acid (PAA).
2. To characterize the polymer-coated working electrode using cyclic voltammetry.
3. To optimize film formation protocols for test of the Cd(II), Pb(II) and Co(II) ions.
4. To validate the optimized sensor with respect to ISO/IEC 17025 guidelines
5. To investigate the effects of interferants in the sensor performance.
6. To apply the developed sensor to determine Cd(II), Pb(II) and Co(II) ions in tap water.

1.4 Justification and significance of the work

Heavy metals are of health concern. They are present in our waters and thus people are exposed to these heavy metals every day. Over the years, these heavy metals accumulate in the tissues slowly poisoning them. The toxic effects of these metals occur gradually. The metals are posing major negative impacts on human health and the environment (Ilaria *et al.*, 2001). Disease burden attributable to contaminated water pollution is significant. Some of these metals are toxic even when they are present in very low concentrations.

There is therefore a need for a sensor that can detect these metals even at low concentrations (Flanagan *et al.*, 2012; WHO, 2012; Mihaela *et al.*, 2012).

The developed sensor can be used to detect heavy metals in drinking or domestic waters. The method that has been developed does not require highly sophisticated or complicated facilities. The developed sensor has the ability to detect and quantify Cd(II), Co(II) and Pb(II) ions and has the following capabilities: The sensor can be used as many times as possible without losing its efficiency; the sensor is minimally affected by the interferrants and this will ultimately reduce the cost of sample treatments as it is done traditionally; the sensor can do simultaneous analysis of a Cd(II), Co(II) and Pb(II) ions; the sensor is field deployable, that is, simple, small and portable. It is fast, more sensitive and independent of solution turbidity and optical length, compatible with microfabrication, have lower power requirements and low cost detection. Poly (acrylic acid) film provides cost-effective, environmentally benign processes for the analysis of the heavy metal pollutants in waters.

CHAPTER TWO

LITERATURE REVIEW

2.1 Electrochemistry

Electrochemistry or electroanalytical chemistry has played a crucial role towards meeting fast changing scientific and industrial research demands. Electrochemistry is about relationships between chemical reactions and electricity. Techniques employing electrochemistry principles have an edge over other techniques because they offer cheap and efficient ways of dealing with common pollutants including heavy metals in the environment such water which is the interest of this study. Electroanalysis forms a major part of electrochemistry and it involves one or a combination of electrochemical techniques to measure and/or monitor species of specific interest (Wang *et al.*, 2006; Brennan *et al.*, 1996; D'Orazio , 2003).

During a chemical reaction, electrons move between electrodes immersed in a cell solution containing an analyte. As a result, information (magnitude of current and potential) is gathered and then analyzed to determine the identity and composition of the analyte. These chemical reactions happen through oxidation-reduction reactions (Redox). Here, electrons move between atoms. The flow of electrons between the two atoms in an oxidation-reduction reaction creates electricity. On the other hand, electricity may be used to force electrons to move between two atoms in order to make an oxidation-reduction reaction to happen. Thus the relationships between chemical reactions and electricity in electrochemistry. As the movement of electrons between two atoms happens, one atom gains electrons and is it said to have been reduced and the other loses electrons and is said to have been oxidized. This chemical reactions happens simultaneously and are thus referred to as oxidation-reduction reactions (Redox). Electrons keep moving into the electrode on one side and moving out of the electrode on the other side. Electrodes are named

based on whether reduction or oxidations reactions have happened (Wang *et al.*, 2006; Brennan *et al.*, 1996; D’Orazio, 2003).

Currently, scientists are implementing measurement systems that most analysts can afford and there is an increased demand for the development of new techniques (tools) that are field deployable; are portable, simple, easy-to-use, more sensitive, selective and capable of performing faster analyses among others (Wang *et al.*, 2006; Brennan *et al.*, 1996; D’Orazio, 2003).

2.2 Electrochemical techniques

Electrochemical techniques have found many applications in various areas (Bard and Faulkner, 1980; Orata *et al.*, 1994). This is because they are cost-effective and are capable of carrying out *in-situ* and real-time studies (Somerset *et al.*, 2010). Over the past four decades, there has been increased demand in the area of electrochemical methods that have capability to detect low concentrations of the analytes. The analyte recognition is among the major processes involved in any sensor system (Li *et al.*, 2013; Somerset *et al.*, 2010; Florence, 1986; Olsen *et al.*, 1994). Additionally, the increased demand is as a result of their speed, portability, specificity and low cost (Win *et al.*, 1981; Kounaves *et al.*, 1987; Stephen *et al.*, 1980; La Pera *et al.*, 2002; 2003; Wang *et al.*, 2000; Carballeira *et al.*, 2000).

Interfacial electrochemical techniques or methods are among electrochemical methods of analysis commonly used. These methods are used to measure chemical reactions involving movement of electric charges between an electrode and ionic species in a solution (Skoog *et al.*, 1992). Interfacial electrochemical methods of analysis are broadly sub-divided into two techniques; static and dynamic as shown in Figure 2.1. Static techniques do not allow current to pass through the analytes solution. Hence, amount of an analyte in a solution does not change.

Moreover, static techniques are combined with a potentiometric technique to determine an electrochemical cell potential.

Unlike static technique, a dynamic technique allows current to flow through the analyte's solution. This technique uses a potentiostat to determine the potential between two electrodes, that is, an electrode on which the reaction of interest is occurring (a working electrode) and a reference electrode. Essentially, the working and reference electrodes are mostly immersed into a solution in a cell containing the analyte under investigation. The potentiostat injects a current into the solution through a counter electrode (also called an auxiliary electrode). This allows measurement of current flow between the working and auxiliary electrodes (Joseph *et al.*, 2003). The dynamic technique comprises of the largest group of interfacial methods as shown in Figure 2.2. Here, the analyte undergoes a reduction-oxidation (redox) reaction that results in the change of its concentration (Willard *et al.*, 1986; Joseph *et al.*, 2003).

A typical experimental set up consists of a read out with a software installed in a computer where the signals are read and recorded, a potentiostat, electrodes connected to an analyte and an electrochemical cell (Figure 2.2). In this context, the electrochemical cell contains an ionic solution with analyte of specific interest. The number of electrodes used depends on the experimental setup. Most experimental setups uses a three electrode system, that is, the working electrode (WE), reference electrode (RE) and counter electrode (CE). Usually, the three electrodes are dipped inside a solution containing the analyte in such a manner that they are not in contact with each other to achieve best results (Wang *et al.*, 2006; Brennan *et al.*, 1996; D'Orazio, 2003).

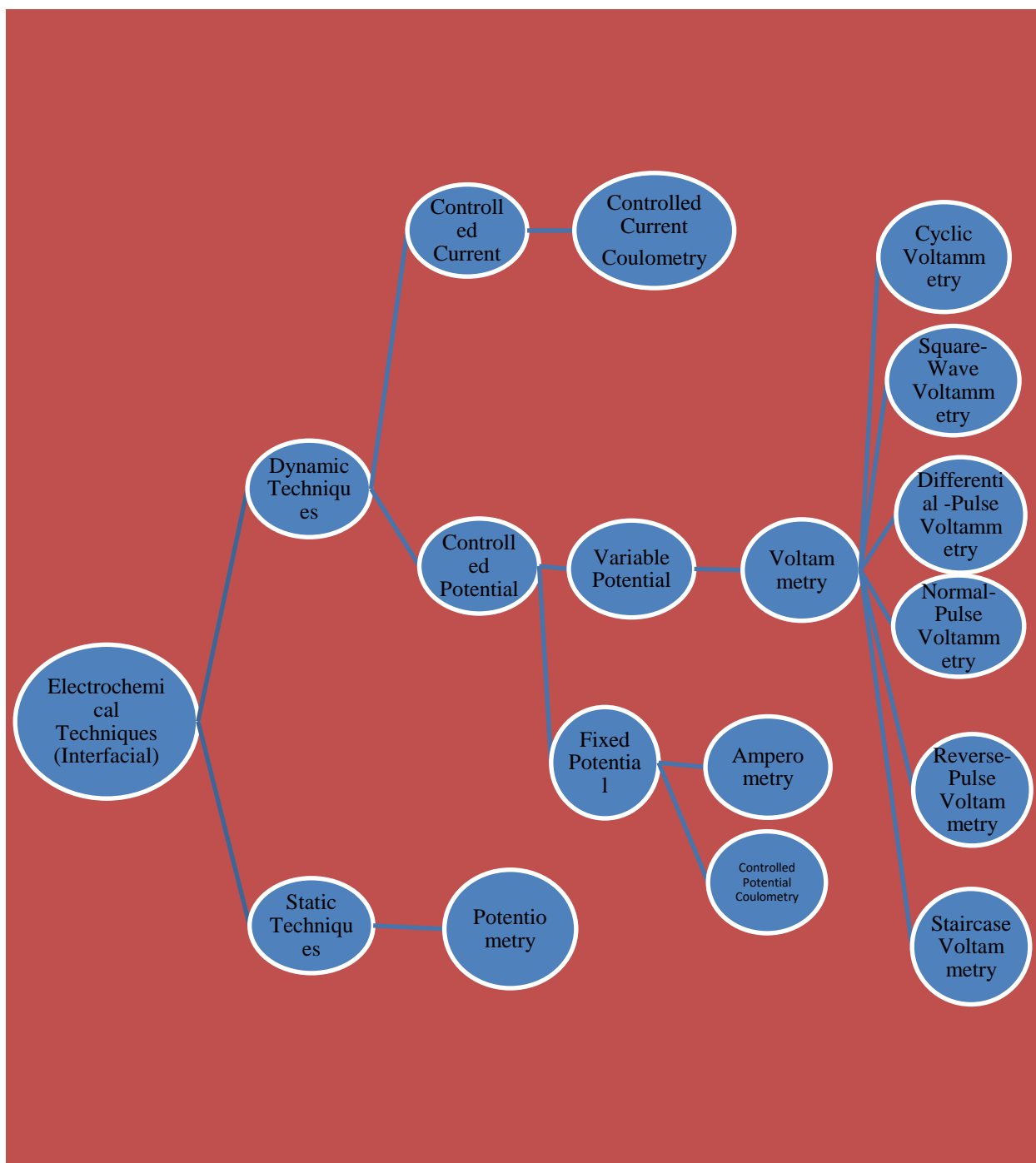


Figure 2.1: A summary of some electrochemical techniques. Retrieved on 20/06/17 from http://intranet.tdmu.edu.ua/data/kafedra/internal/pharma_2/classes_stud/en/pharm/prov_pharm/ptn/analytical%20chemistry/2%20course/22%20Electrochemical%20methods%20of%20analysis.htm.

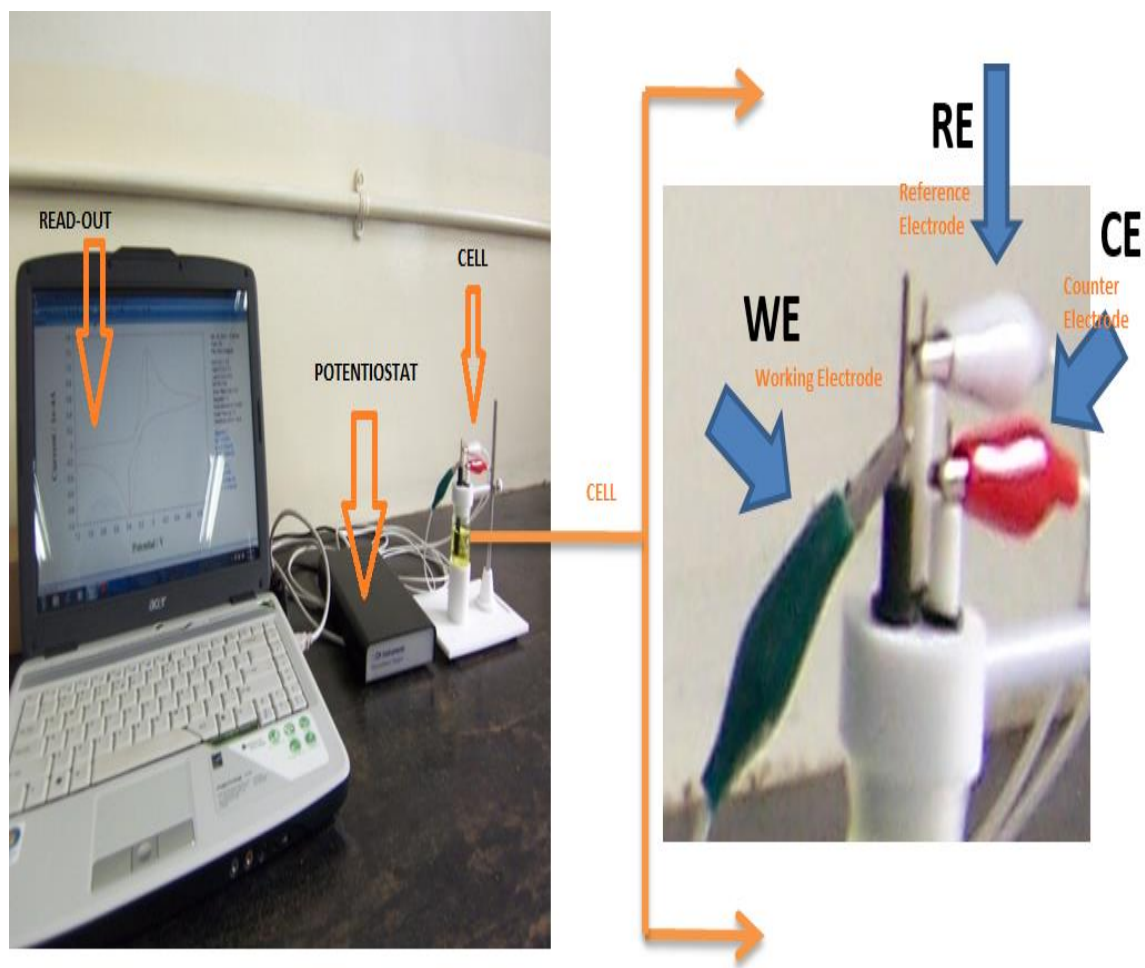


Figure 2.2: A schematic representation of a universal electrochemical experimental setup (Source: Author, 2016).

The section that follows describes some of the electrochemical methods of analysis as shown in Figure 2.2.

2.2.1 Voltammetry

Voltammetry is applied in the determination of trace components such toxic heavy metals in drinking water, effluents and sea waters. Voltammetric technique analyzes small proportions of a given solution at a solid working electrode (Lesney, 2002). Under this technique, a potential is applied between working and reference electrodes and metals in a solution that are being investigated are either oxidized or reduced. In connection with this, the potential forces an electron transfer to occur and results in a current flow. The magnitude of the current produced is used to estimate the amount or concentration of the analyte being studied (Grady *et al.*, 2004). Normally, the working electrode has a small surface area. This is preferred as it enhances polarization that results into the current (Eggins, 2002; Katz & Willner, 2003; Pei *et al.*, 2001). Measured current (y-axis) is plotted against voltage (x-axis) resulting to a plot known as a voltammogram (Gosser, 1994; Pei *et al.*, 2001; Liu *et al.*, 2006; Patolsky *et al.*, 2006; Li *et al.*, 2003). As the reaction progresses, the electroactive species (the analyte under investigation) move towards the surface of the electrode where an electron transfer takes place (Bockris and Reddy, 1970).

The chemical nature of the reactants, intermediates, products and the electrode surface determines the kind of interactions between the parameters mentioned above (Bard & Faulkner, 1980). When the interactions are strong, the species concerned get adsorbed or bonded to the electrode surface. This kind of behavior leads to quasi-reversible or totally reversible systems. (Bard & Falkner, 1980). Under certain conditions, peaks of a pair of oxidizing and reducing agents

(redox couples) may shift with the increasing scan rates. Those couples whose peak shift more are grouped as quasi-reversible or irreversible (Mabbot, 1983).

The choice of the solvent and Supporting Electrolyte (SE) is determined by factors like conductance, solubility of the electrolyte and electroactive substance and reactivity with electrolyte products. The common solvent properties are dielectric constant and acceptor/donor ability which are the indicators of solvent polarity, solubilizing power and the ability to participate in electron-pair donor-acceptor interactions respectively. A good solvent is one which allows satisfactory dissolution and dissociation of the SE with low toxicity (Gosser, 1994). The following are some of the different ways a solvent/SE system influences an electrochemical activity:

- (i) The diffusion of an electroactive species will be affected by viscosity of the medium and strength of the solute-solvent interactions
- (ii) The solvent is responsible for proton mobility. Water and other protic solvents display a high proton mobility because of the fast solvent proton exchange, unlike aprotic solvents
- (iii) The medium affects structure of the electrical double layer where electron transfer occurs

The use of inert SE is indispensable in electrochemistry. It regulates the cell resistance and mass transport by electrical migration. Organic solvents are non-conductors (high resistance) hence the absence electrolyte requires very impractical high voltages. The primary function of the SE is to enhance conductivity. The solvent/SE combination chosen should therefore give resistance that is as small as possible to minimize iR drop which leads to potential control error and ohmic heating of the solution. Fry and Britton (1984), recommended acetonitrile (ACN), ethanol, methanol, and

dichloromethane as good oxidative (anodic) electrochemical solvents, while ACN and dimethylsulphoxide (DMSO) are suggested for reductive (cathodic) electrochemistry. However, ACN is suggested as the best overall non-aqueous solvent on the basis of its electrochemical properties and its relative non-toxicity.

An electrical field on an electrolyte solution initiates a migration of the ions. These migrations constitutes the current flow in an electrochemical cell. Here, each ion involved carries a proportion of the total current. The current is proportional to mobility and amount of the ions. Addition of inert electrolyte for a given current therefore reduces solution resistance, thus decreasing the electric field ($E=iR$). Mass transport of an ionic electroactive species that is caused by migration in an electric field can be reduced to a negligible level by ‘swamping’ the solution with inert electrolyte. Most of the current will then be carried by the ions of the SE. The SE in polarography and voltammetry experiments is made 50-100 times (high ionic strength) the concentration of electro active species to suppress electrical migration. This will justify the use of quantitative equations in calculating limiting or peak currents which are based on the assumption that mass transport is controlled by pure diffusion. Below are a few voltammetric techniques that are commonly used.

2.2.2 Forms of Voltammetry

By varying potential, voltammetry gives information about an analyte under investigation by measuring current. There are many ways the potential is varied. The different ways of varying the potential have resulted in many forms of voltammetry such as potential sweep voltammetry, pulse voltammetry, stripping voltammetry (Heyrovsky, 1956; Eggins, 2002; Katz & Willner, 2003).

2.2.2.1 Potential Sweep Voltammetry

Potential sweep voltammetric techniques have been developed to measure and give more information about an analyte under investigation in a single experiment by sweeping (rather than steps) the potential with time and recording potential and current. Linear Sweep Voltammetry (LSV) is the simplest of these techniques; the potential/voltage is varied linearly with time (Lesney, 2002; Adams, 1969). Similar to the linear sweep technique, Cyclic Voltammetry (another example of this technique) is swept linearly. However, at the end of the linear sweep, the potential scan is reversed unlike in the LSV. Cyclic Voltammetry (CV) is always used to investigate the chemical reactivity of a species of choice for the first time (Lesney, 2002). The potential scan may be reversed as many times as possible to giving rise to several cycles. Figure 2.3 shows an example of a cyclic voltammogram for one complete cycle.

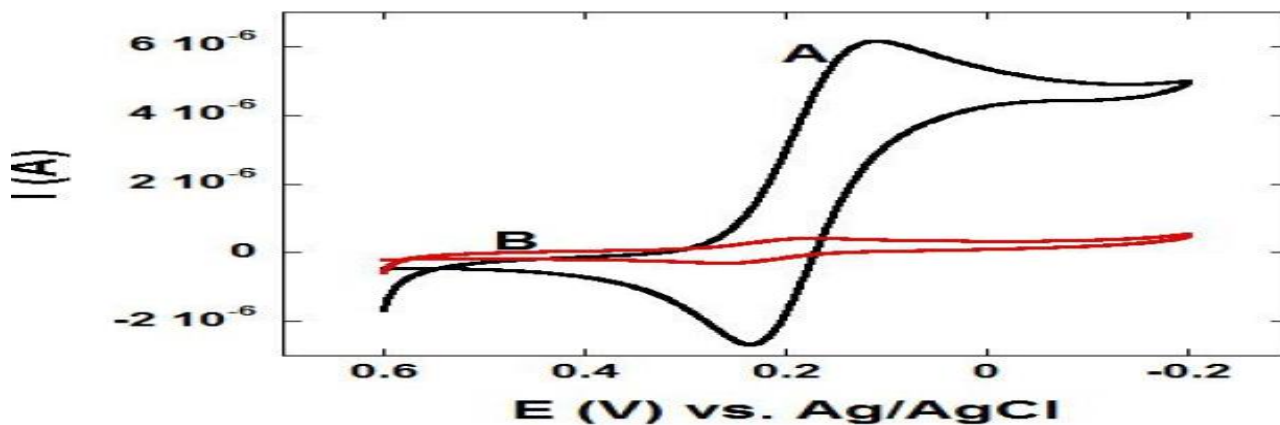


Figure 2.3: A typical Cyclic voltammogram of (A) GC/PAA and (B) GC plain for potassium ferrocyanide/0.1 M potassium chloride at 0.005 V/s (Source: Author, 2017).

The CV makes it possible to carry out quantitative experiments with an electrochemical system. This allows one to predict the composition of the system before proceeding with further studies. Voltammograms are recorded for a range of scan rates and potential. These leads to many

peaks of similar shape on either sides of the voltammogram. However, the magnitude of full reversible reactions can be identified. These peaks indicate either oxidation or reduction taking place at the forward or reverse scan (Lesney, 2002).

2.2.2.2 Differential-Pulse Voltammetry

Differential-pulse voltammetry (DPV) was developed to lower the detection limits down to 10^{-8} M (Lesney, 2002). The differential pulse voltammetry (DPV) improves the resolution between the species with similar potential through allowing current to be sampled twice.

2.2.2.3 Stripping Voltammetry

Interest in the development of techniques that are capable of detecting analytes at low concentrations that could not have been possible with the other electrochemical methods of analysis has led to the emergence of stripping voltammetric techniques. Stripping voltammetric technique is among the highly sensitive and selective techniques, hence the technique of choice for this study. With this technique, it has been possible to detect and analyze trace metals in solutions (Tercier & Buffle, 1993; Wang, 1985). This technique has attracted a lot of attention because it requires minimal sample preparation for analysis to be done and thus eliminates possible contaminations. Forms of stripping voltammetric techniques follow various steps but all the forms have two common steps. First, the analyte of specific interest in a sample solution is accumulated (preconcentrated) into (or onto) the surface of the working electrode. This constitutes a very important step that leads to an exceptionally high sensitive devices (sensors). Secondly, the analyte that accumulated into (or onto) the surface of the electrode is measured or stripped off back into the solution by the application of a potential scan. Importantly, stripping analysis is used with any

of the potential waveforms such differential-pulse, square-wave, linear-sweep or staircase but the most common are differential-pulse and square-wave due to their excellent ability to discriminate against charging current.

Stripping analysis is a technique of choice as it provides important information on metal speciation (Florence & Batley, 1980; Grady *et al.*, 2004). The following sub-section describes the three forms of stripping voltammetric techniques.

2.2.2.3.1 Anodic-Stripping Analysis

This form of stripping voltammetry analysis is among the more sensitive and reproducible techniques for metal ion analyses. It has the ability to analyze trace metal ions in aqueous media with concentration ranging from as low as 10^{-6} to 10^{-12} M. This compares favorably with atomic absorption spectrometry (AAS) or inductively coupled plasma (ICP) analysis but has advantage since it has a simple set-up and can be used onsite with ease. Also, it has the capability for simultaneous multi-element determination (Tercier & Buffle, 1993; Wang, 1985). The following steps are involved during anodic stripping analysis.

Step 1: Electrodeposition

During the first step (the electrodeposition step), metal ions (M^{n+}) under investigation are deposited (pre-concentrated) through electrochemical means into (or onto) the working electrode's surface. Initially, mercury working electrode used, also referred to as hanging mercury-drop electrode in the form of amalgam, $M(Hg)$:



Where M^{n+} is metal ion, n is number of moles, e is electrons, M is metal and Hg mercury.

Electrodeposition requires short-time electrolysis of about 0.5 to 5 minutes. The electrolysis is done after stirring the solution while maintaining appropriate potential. This process leads to gaining of electrons (reduction) by the trace metal ions that are under investigation to obtain a fairly concentrated amalgam.

Step 2: Rest period

Normally, the allowed duration is about 30 seconds. During this period, the applied potential is not changed. This ensures the amalgam is uniform and re-oxidation of the metal-ions under investigation does not take place.

Step 3: Stripping

This happens after the pre-concentration process, here the metal (M) that is now deposited into (or onto) the working electrode's surface (mercury electrode) is stripped off back into the solution. This involves an oxidation process since the metal gives off the electrons and is pushed back into the solution in an ionic form. This takes place under the conditions of the experiment.

Normally, this are diffusion controlled conditions and the stripping is done using any of the voltammetric techniques (or methods).



The amount (concentration) of the metal (M) is easily estimated by the anodic diffusion current of the experiment. This current is directly proportional to time the electrolysis took. Moreover, the current is directly proportional to the stirring rate and amount of metal ion (M^{n+}) in the aqueous solution.

2.2.2.3.2 Cathodic-Stripping Analysis

This technique is similar to anodic-stripping analysis, it is among the more sensitive and reproducible techniques for metal ions and follows similar steps. Cathodic-stripping analysis is commonly used to analyze ionic species that form insoluble salts.

2.2.2.3.3 Adsorptive-Stripping Voltammetry

Like the anodic stripping voltammetry (ASV), the Adsorptive stripping voltammetry (AdSV) also consists of two main steps; that is, the deposition and stripping steps.

Step 1: Deposition step

During this process, the metal ion is accumulated and adsorbed as a complex on the working electrode's surface. Normally, an active surface-complexing agent (ligand) is added in the solution. As soon as the ligand is introduced into the solution, it forms an adsorbed layer on

the working electrode's surface. The metal ion under investigation quickly reacts with the adsorbed ligand resulting to the formation of an adsorbed complex.



Where M^{n+} is metal ion, n is number of moles, L is ligand, M^{n+} is ligand ion and M is metal.

This activity (process) is carried out under the following conditions; that is, in a solution that is stirred and under a voltage (potential) control. Similar to the pre-concentration process of anodic stripping voltammetry, the analyte under investigation moves to the working electrode by the same convection.

Step 2: Stripping step

This process is achieved through the electro-reduction of the complex agent adsorbed on the surface of the working electrode. The concentration (amount) of adsorbed metal is easily estimated from the recorded current corresponding to the current peak (Wang, 1985; Heineman, 1984).

2.3 Electrochemical sensors

Electrochemical techniques have led to emergence of different types of sensors that are commonly referred to as electrochemical sensors. The development of these electrochemical sensors involved combination collaboration of engineering and electrochemistry concepts to create modern, top-of-the-line technologies and inventions. As a result, the technology has ensured that developed devices are easy to use, ease to interpret and most of them are portable and can be used

even at homes. Basically, the sensor detect, measure and record a physical property or information, mainly chemical in nature, about the analyte under investigation into a signal that is used to identify the type of analytes in samples under study and giving information about concentration of the analyte. Among the chemical sensors, electrochemical sensors forms the oldest and largest group of sensors (Wang, 1991; Karl –Heinz & Kurt, 2010).

2.3.1 History of electrochemical sensors

Electrochemical sensors have been in existence for over six decades. The technology was first used in 1950s to monitor oxygen concentration in the environment. Cremer, a famous researcher and scientist, developed the first group of electrochemical sensors in 1906 using glassy electrodes. The invention by Cremer has since informed development of electrochemical sensors for many analytical applications (Karl –Heinz & Kurt, 2010). Since mid-1980s, there has been improvements to Cremer’s invention aimed at making sensors more sensitive and selective to address challenges of analytes or environmental pollutants that are still toxic even at very low concentrations. To date, diversity in the application and stiff market competition have been among the key drivers of technological advancements in the electrochemical sensors.

2.3.1.1 Technological advancements in electrochemical sensors

These are inventions made to improve electrochemical sensors and are aimed at making them more effective, cost-effective and easy-to-use among other important parameters. Improving these technologies have led to increased interest in electrochemical sensors. This has expanded their potential applications for determination of many analytes since they are now simple, rapid and economical. Their impact is evident in many current activities where they are widely used

(Murray *et al.*, 1987; Wang, 1991; Karl –Heinz & Kurt, 2010). Currently, the electrochemical sensors play a crucial role in the analysis of samples in different fields. Some of the fields include environment for the applications are used for its conservation and monitoring, health sector for prevention of disasters and diseases and industrial use for analysis of samples (Murray *et al.*, 1987; Wang, 1991).

Among the technological advancements in this field is the miniaturization and microfabrication. As a result, the sensors have eventually found many applications (Grady *et al.*, 2004; Karl –Heinz & Kurt, 2010). The miniaturization of electrochemical devices is highly important due to convenient handling, portability and reduced sample preparation (Popovtzer and Neufeld, 2006; Karl –Heinz & Kurt, 2010). The miniaturized electrochemical devices are available for detection of many different toxic gases and have been reported to be exhibiting high sensitivity and selectivity. More recently, the interest to have toxic and combustible gases in a confined space monitored has increased. This requires use of miniaturized electrochemical devices (OSHA, 1992). It is increasingly clear that the demand for miniaturized electrochemical devices has led to an emergency of new and better electrochemical sensors which can be employed either as stationary applications or as portable applications (Wang, 1991; Karl –Heinz & Kurt, 2010). Microfabrication involves modifications of the working electrode's active surface. This is aimed at enhancing sensitivity and selectivity of the electrode surface by lowering its detection limits. Here, the surface of the working electrode is most cases coated with a metal or complex which has properties sought to make the surface of the electrode more sensitive and selective. There exists many ways of depositing the coat on the working electrode's active surface such as drop coating among others. The method used to deposit a surface coat ensures the sensitivity and selectivity achieved are

sustained and that the coat remains on the surface of the electrode for a longer period. Thus, allowing the electrode to be used many times without losing its effectiveness.

2.3.2 Main types of electrochemical sensors methods

Electrochemical sensors methods are classified according to the nature of a quantity they measure. Normally, a chemical reaction would lead to generation of current or potential which is then measured and recorded as the output of the reaction. The main types of electrochemical sensors methods are amperometric, potentiometric and conductometric. These methods are distinguished by their mode of measurement. For instance, amperometric sensors generates a current which is then measured to give an idea of the identity and amount of an analyte under investigation. On the other hand, potentiometric sensors generates a potential which is then measured to give identity and concentration of the analyte. Regarding conductometric sensors, they are used to measure resistance of a medium between electrodes (Chaubey and Malhotra, 2002). Other forms of electrochemical sensors include impedimetric and field-effect based sensors among others (Mirsky *et al.*, 1997; Guiseppi-Elie & Lingerfelt, 2005; Thevenot *et al.*, 2001).

These reactions are effectively sensed near the surface of the working electrode. Usually, electrochemical sensors are composed of a three electrode system. Importantly, the both electrodes are made from materials which are conductive and chemically stable. For this reason, the commonly used materials include; platinum, gold, carbon and silicon. Use of these compounds depends on an analyte under study (Chaubey & Malhotra, 2002; Ben *et al.*, 2006).

Nano-objects are known to have higher surface area to volume ratio and therefore higher sensitivity measures (Patolsky *et al.*, 2006; Nair & Alam, 2007). Therefore nanostructures have introduced new and crucial components in electrochemical sensors development. Such

components include use of nanoparticles to enhance sensitivity (Merkoci, 2007; Park *et al.*, 2002). For instance, nanowires, carbon nanotubes, nanoparticles and nanorods are emerging. They are poised to be crucial elements of future electrochemical devices (Wanekaya *et al.*, 2006; Stadler *et al.*, 2007).

The following sub-section broadly describes four types of electrochemical sensors based on their electrochemical principle and/or the technological advancements involved. The four types of sensors includes; amperometry-based sensors, potentiometry-based sensors, conductometric sensors and biosensors.

2.3.2.1 Amperometry-based sensors

These are devices that measure current as a result of an electroactive substance losing (oxidation) or gaining (reduction) an electron while undergoing an electrochemical reaction (Thevenot, 2001; Lippa *et al.*, 2005). Clark oxygen electrode is simple form of an amperometric sensor which is used to measure oxygen concentration (Chaubey & Malhotra, 2002).

The cell potential of the electrochemical sensor is held constant while measuring the current, and thus referred to as amperometry. There exist different variations on how the current is produced and this has led to the emergency of different names as used by different scientists. Use of the correlations of the generated current and concentrations of the analyte to estimate the amount of an analyte or electroactive species under study is done through current plot. The current plot is commonly referred to as a voltammogram and its highest peak gives an indication of highest amount of current generated (Thevenot *et al.*, 2001; Eggins, 2002; Chaubey & Malhotra, 2002; Lippa *et al.*, 2005; Santandreu, 1999).

2.3.2.2 Potentiometry-based sensors

These types of electrochemical sensors measure changes in potential/voltage as the current is kept constant. The potential (charge) is normally accumulated at the working electrode. Potentiometry-based sensors provide data about activity of an ion in an electrochemical reaction (Bakker & Pretsch, 2005; Xu, 2005). Here, the relationship between the amount of the analyte and the cell's potential is controlled by an equation referred to as a Nernst equation (Eggins, 2002; D'Orazio, 2003).

Ion-selective electrodes (ISE) have led to improved detection limits for the potentiometry-based sensors. Thus, this has rendered potentiometric devices more suitable for measuring low concentrations. The devices have capability of performing these measurements with very small sample volumes. Additionally, potentiometric sensors do not chemically influence a sample. They can also be used to measure selective ion concentrations. It is also possible to use them to measure the kinetics of other reactions such as biocatalytic reactions which involve enzymes among others (Caras and Janata, 1980; Hafeman *et al.*, 1988; Mourzina *et al.*, 2001; Poghossian *et al.*, 2001; Xu *et al.*, 2008; Kloock *et al.*, 2006; Stein *et al.*, 2004).

2.3.2.3 Conductometric sensors

These types of electrochemical sensors measure the current conducted through the two electrodes. They measure and detect changes in resistance (ability of an analyte to restrict charge flow through it). There is a clear increased demand for conductometric sensors due to the wide uses associated with them. Among other uses, they have been used to measure quality of many analytes such as that of oil. Normally, in an electrochemical set up, an analyte has ability to allow current to pass between two electrodes. Analytes with high conductance (low resistance) will

allow more electrical current to pass through and vice versa. So, with this sensors, it is possible to distinguish two analytes based on their conductance i.e. clean and dirty oil since the two restrict charge flow differently. This explains why conductometric sensors have found wide applications today (D'Orazio, 2003; Thevenot *et al.*, 2001). Further improvements on these sensors have been carried out to improve their sensitivity. As a result, it is possible to directly observe the changes in conductance that occur on the surface an electrode. Additionally, these sensors have a capability of measuring conductance of multiple analytes as a result of these advancements in technology such fabricating the sensitive surface of the electrode with materials which enhances its sensitivity. Other technologies include use of semiconductors and integrating sensors with microelectronics devices such as field effect transistors (FET) devices (Cullen *et al.*, 1999; Contractor *et al.*, 1994). Currently, nano structures have also been used to improve the performance of the sensors and have made them attract many potential uses (Patolsky *et al.*, 2006; Stadler *et al.*, 2007; Eggins, 2002). These type of sensors have been successful wherever they have been used. Some of the applications where these type of sensors are utilized include, detection of drugs in human urine, detection of pollutants in environments among others (Yagiuda, 1996; Chouteau *et al.*, 2004).

2.3.2.4 Biosensors

Biosensors are type of electrochemical sensors with a biological component incorporated whose role is to sense the analyte and produce a signal which is converted to a readable format. What distinguishes this type of sensor from other electrochemical sensors is that it incorporates a biological material. Such biological materials includes enzymes, cells and whole organisms among others. The biological material interacts with the analyte and produces a signal that is measured. The trend in the development of electrochemical sensors during the past two decades is more on the design of biosensors. These types of sensors are either potentiometric or amperometric but the

large percentage of them are amperometric. Based on the type of the biological material used, the more predominant sensors reported in literature are enzyme based. Some of enzyme based sensors includes immunosensors and deoxyribonucleic acid (DNA) sensors (Lesney, 2002). The first biosensor was invented by “Clark” (1962). He used his oxygen electrode to determine oxygen depletion. Oxygen depletion was as a result of an action by an enzyme called glucose oxidase on glucose (Magdy *et al.*, 2012). Further improvement were carried on the glucose sensor by MediSense (1987). He launched screen printed electrodes. The screen electrodes were then used for medical applications. There is exist a synergy between the biosensors and electronics as well. The screen printed carbon electrodes came from electronics. For electronics, the screen printed electrodes were already being used for printing electric connections. Additionally, sensors modified with deoxyribonucleic acid (DNA) have got major applications and continuous to be an area of a major interest (Gerard *et al.*, 2002; Elamathi *et al.*, 2013; Magdy *et al.*, 2012).

2.3.3 Polymers

Electrodes modified with polymer-films have become a major subject of interest for electroanalytical investigations. They are viewed as being capable of replacing mercury based modified electrodes (were electrodes of choice). The polymers have shown many advantages over mercury film (March *et al.*, 2015; Pujol *et al.*, 2014). They are preferred over mercury because mercury is a toxic element.

Polymers on the surface of electrodes have been used for improving selectivity of the electrochemical transducers. Basically, the modification of an electrode surface would involve immobilizing of reagents onto its surface. The role of the immobilized material is to enhance electrochemical properties of the bare electrode (Murray *et al.*, 1987; Wang, 1991).

Table 2.1: A summary of some electrode modifiers.

| No. | Electrode | Modifier | LOD (Cd) (ppb) | LOD (Pb) (ppb) | Reference |
|-----|-----------|--|----------------|----------------|-------------------------------|
| 1. | SPCE | Bi film | 0.34 | 0.03 | Chen <i>et al.</i> , 2013 |
| 2. | SPCE | Bi salts | 1.1 | 0.9 | Lezi <i>et al.</i> , 2012 |
| 3. | GCE | Nafion/Graphene/Bi film | 0.02 | 0.02 | Li <i>et al.</i> , 2009 |
| 4. | Graphite | rGO + Bi film | 0.09 | 0.12 | Pokpas <i>et al.</i> , 2014 |
| 5. | SPCE | Bi ₂ O ₃ NPs | 0.2 | 0.2 | Riman <i>et al.</i> , 2015 |
| 6. | SPCE | Bi NPs | 1.7 | 1.3 | Rico <i>et al.</i> , 2009 |
| 7. | Bi SPE | Bi film | 0.1 | 0.16 | Sosa <i>et al.</i> , 2014 |
| 8. | SPCE | Functional porous SiO ₂ NPs | - | 0.1 | Sánchez <i>et al.</i> , 2010 |
| 9. | GCE | Polymeric Thiadizole film | 0.05 | 0.3 | Zhao <i>et al.</i> , 2012 |
| 10. | CPE | Cd(II) ion imprinted polymer | 0.06 | - | Alizadeh <i>et al.</i> , 2011 |
| 11. | GCE | Graphene | 1.08 | 1.82 | Liu <i>et al.</i> , 2014 |
| 12. | CNTs film | CNTs | 2.2 | 0.6 | Bui <i>et al.</i> , 2012 |
| 13. | GCE | MgSiO ₃ Nanospheres | 0.02 | 0.05 | Xu <i>et al.</i> , 2013 |

Table 2.1 summarizes common electrode modifiers.

In this work, polyacrylic acid (PAA) was employed as a polymer of choice in the development of the sensor under the study. Polyacrylic acid (PAA), a polymer, has ability to absorb water and swells thus retaining the water (Liu *et al.*, 2009; Tang *et al.*, 2009; Nursel, *et al.*, 2002). As a result, PAA has found applications as a complexing agent for heavy metals and proved effective. The applications are based on its ability to adsorb and retain water, dissolved substances and heavy metal ions. The polymer adsorbs metal ions and swells up removing them from the water or aqueous solutions (Elamathi *et al.*, 2013; Magdy *et al.*, 2012; Gerard *et al.*, 2002; Elviraa *et al.*, 2002; Anamul Haque *et al.*, 2012; Li *et al.*, 2004; Bekiari *et al.*, 2008).

2.4 Heavy metal pollutants

Industrial and urban activities have led to increased pollution of the environment with heavy metals being among the pollutants (Onyatta & Huang, 1999). As a result people are exposed to elevated levels of the heavy metals (Ilaria *et al.*, 2001). According to UNDP, 2006, millions of people worldwide are exposed to dangers which come along with these contamination. Effective tools which can detect and remove these metals have been implemented (Ilaria *et al.*, 2001). However, there is still need for tools which can detect low concentrations possibly to pico-molar range; can be minimally affected by interferants, can do simultaneous analysis of a number of analytes and most importantly are field deployable. In this work, our interest in the low concentration was because heavy metals, overtime, bio accumulate to levels which are lethal to the human health (Ilaria *et al.*, 2001).

Heavy metals are metals with elemental densities above four grams per centimeter cubic (4 g/cm^3). They are not easily broken down by microorganisms and are therefore retained in the environment for longer time. It is therefore important to have the knowledge of the content of these metals in various environmental matrices (Wang, 1985).

2.4.1 Sources of heavy metal pollutants

Heavy metal pollutants, also referred to as contaminants, are introduced to the environment such waters by either anthropogenic (human) or natural activities. Globally, industrial activities are known to contribute more to contamination of waters by heavy metal (Al-Musharafi *et al.*, 2014). The common ways in which the heavy metal contaminants get into the environment is through waste disposal, fumes and exhausts from industries, corrosions, agricultural activities and mineral exploitation among others. Mineral exploitation on the other hand contributes more to the

waste disposal challenges. Through mining exploitations, a lot of waste materials and by- products are produced. Heavy metals at mining sites are produced and carried to other sites through runoffs and thus contaminating soil, waters and air as they are transported. Small particles are carried by wind to other sites and where they get their way to water bodies. Fumes and exhausts produced as a result of fossil combustions also results in small particles of the heavy metals which are carried and spread to other sites. During rains, run off water washes the heavy metals off the soil and thus gets their way to the water bodies. While in the water, the heavy metals or particles with adsorbed heavy metals get to the bed of the water bodies where they settle over time as they get accumulated as sediments. Some of the agricultural activities are source of the heavy metals includes use of pesticides and fungicides and burning wood among others (Guha-Sapir *et al.*, 2011). These heavy metals gets into the food web through microorganisms that consume the sediments and ultimately to the human beings getting in contact with contaminated water or food endangering their life. Figure 2.4 summarizes common sources of heavy metal contaminants.

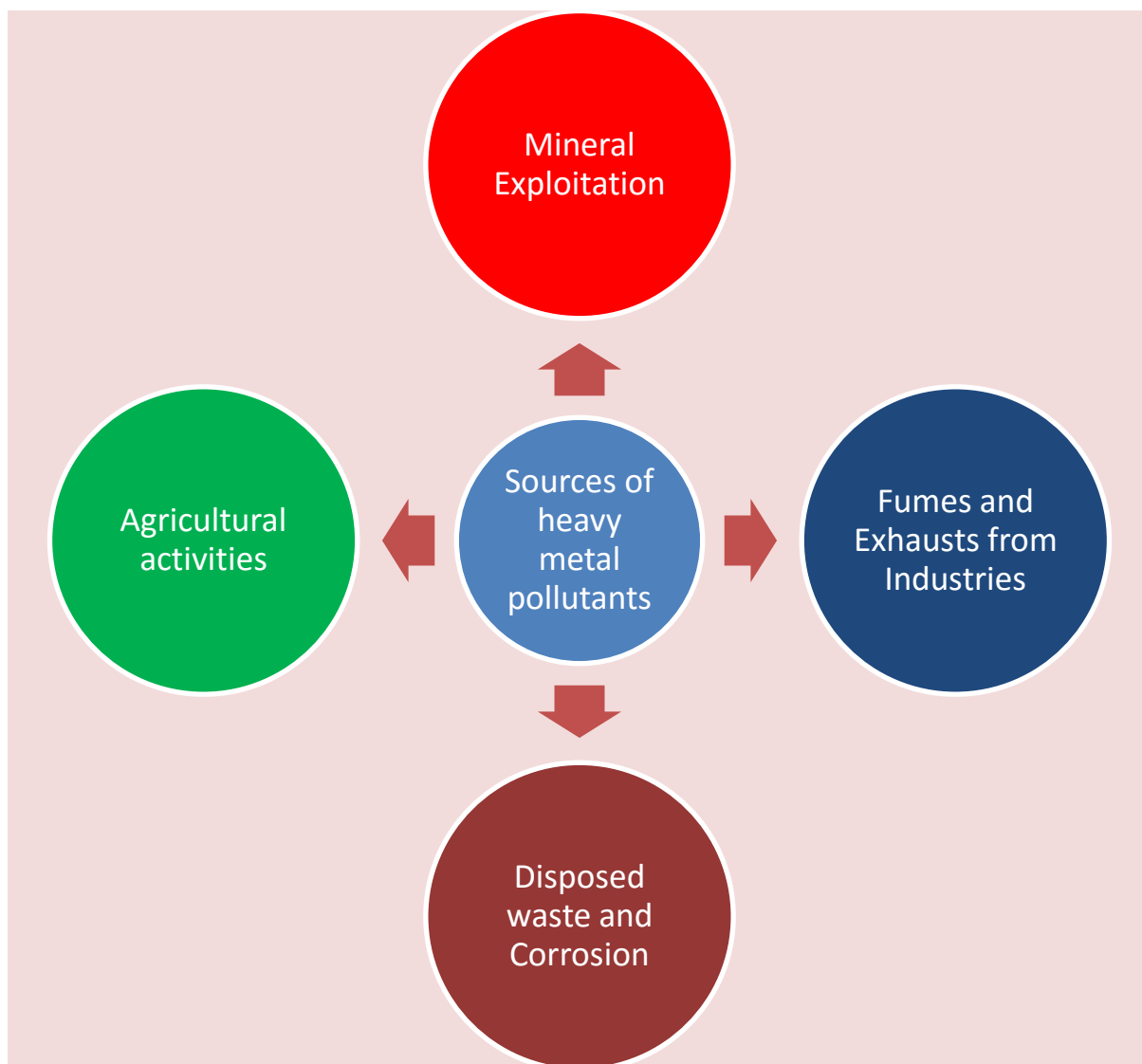


Figure 2.4: Common sources of heavy metal pollutants (Source; Author, 2016)

2.4.2 Implication of heavy metals pollutants on human health

Heavy metals are responsible for emergence of many health conditions some of which have no cure. Most of the diseases caused by the heavy metals are not detected in time for prevention. Instead they manifest at late stages when little can be done to cure or prevent the disease. Some heavy metals such lead exhibit toxic effects at any concentration (Jain, 1978; Otake, 1984; Pilsner, 2009). But others becomes dangerous to human health when there levels are elevated beyond

acceptable levels. However, there is no level of exposure to heavy metals that can be considered safe. This is because, the heavy metals have a tendency to accumulate in the human system over time and may eventually exceed the acceptable levels. Thus, even those heavy metals that have a role in human bodies may end up building up to levels that are lethal if they are continually consumed.

The section below discusses health implications of some of the heavy metals; lead (Pb), cadmium (Cd) and cobalt (Co). Detection of these three heavy metals in water was chosen for this study because they are dangerous to human health and are widely used and thus readily available in the environment.

2.4.2.1 Lead

Lead is the most common poisoning heavy metal available in the environment. It is available in materials widely available in the environment and gets its way to our waters and thus increasing human exposure (Shilu *et al.*, 2000). It is considered as one of the most deadly substances because of its potential to harm our bodies in any exposure to any amount (Landrigan, 1989). Lead is retained for a long time in human body organs especially the teeth and bones and thus slowly weakening them out (Amitai *et al.*, 1987; UNEP, 2008; Rosner & Markowitz, 1985; Landrigan, 2002). While in our bodies, lead and calcium compete for the same location, that is teeth and bones, as a result calcium is eventually replaced. A small portion of lead has ability to replace a very large portion of calcium. This results in weak bones (osteoporosis) and damaged teeth. Lead has high affinity over calcium binding sites. Thus calcium is unlikely to replace lead. Lead poisoning also attacks the brain and the nervous system where it results in many dangerous effects. Some are adversely affected and are often left with mental retardations and behavioural

disruptions. Additionally, lead poisoning causes injuries that may lead to shorter attention span, lack of cognition, hypertension, renal impairment and immunotoxicity among others. These conditions are in most cases not treatable and may lead to loss of life (WHO, 2009; Gibson, 1904).

The intake of lead through drinking water and food is the most common route of exposure. Once swallowed, the substance enters our bodies by absorption from the gastrointestinal tract leading to lead body burden. Effects of lead exposure is more predominant in young children (EPA, 2002; Wu, 2008). This is because, children do not have safe blood lead level (Landrigan *et al.*, 2007; Basha, 2005).

Removing lead from our environment is the first step in treating these conditions. This is important because the approach reduces the likelihood of lead causing problems later. Additionally, we need tools with excellent sensitivity for detecting the presence of lead in our environment. Since lead is toxic at any level, there is need to develop tools which have capability of detecting these metals even at very low concentrations. Currently, there exist several techniques aimed at detecting and removing lead from aqueous solutions but not at very low concentrations (WHO, 2010; WHO, 2009; Gibson 1904; Byers, 1943).

2.4.2.2 Cadmium

Cadmium is a natural element that is abundantly available in the environment and because it does not corrode easily it has found many applications that includes use in the manufacture of batteries, electroplating, pigment works, textile printing and in chemical industries and therefore increasing exposure levels. The effects of cadmium are many but it has more effects on the kidneys, the cardiovascular system and has a potential of causing cancer. Aging of the skin has also been linked to cadmium poisoning. Cadmium also contributes to mental illness such violence, mental

disorder and attitude. Cadmium is linked to many diseases such as kidney diseases, heart diseases, and diabetes among others. Cadmium competes for locations with zinc in human bodies and thus replacing zinc. Zinc is a major component of some critical enzymes. These includes enzymes that are involved in activities enhancing food digestion, immune system and heart health (ATSDR, 2008; UNEP, 2008; IARC, 1992 & 1993; CSTE, 2004; EFSA, 2009). Cadmium is very similar to magnesium and calcium and thus affects biological activities that require magnesium and calcium as well.

Cadmium is one of the heavy metals that has caused major health concerns. It has no role in human bodies and is very toxic at low concentrations. While in the environment, it does not break down and overtime it bio accumulate in organisms and ecosystems. Complex interaction of the metal with biological systems is responsible for its carcinogenic effects. Cadmium follows metabolic path ways of similar essential metals. Exposure to any amount of cadmium is toxic.

The main route of exposure for Cadmium is through ingestion of water and food (Friedberg *et al.*, 1971). While in the body, some of it is accumulated in the kidneys where it bio accumulates over time giving rise to kidney damage (Tsuchiya *et al.*, 1972). It destroys proximal tubules, glomeruli, Henle's loop and distal tubules in the kidney (Nandini *et al.*, 2007; Friedberg *et al.*, 1971). Most the metal remains in the plasma where it stays for few hours after administration (Guha-Sapir *et al.*, 2011; Kabata-Pendias *et al.*, 2011; Environment Agency, 2009; Friberg *et al.*, 1986).

The concerns related to the toxicity of cadmium have led to a need for tools that have a capability of detecting the presence of the metal in waters and other environmental samples at low concentrations. Since the cadmium is toxic even at low concentrations, the tools should be able to

detect it at trace levels. This will prevent consumption of much of the metals into our body systems that poses health risks.

2.4.2.3 Cobalt

Studies have linked cobalt exposure to many health effects. The main route of exposure is through water and food. Cobalt when leaked from industrial plants is settled on soils and transported by water. While in the environment, cobalt is retained for longer periods of time just like other heavy metals. And thus, cobalt cannot be destroyed in the environment. It is fairly widespread in the environment and is almost contained in all land based deposits where it is available in combination with other elements such as nickel and copper. Major cobalt poisoning occurs when toxic levels of the metal build up in the body when exposed to higher concentrations. However, there are reported cases of cobalt poisoning even at low concentrations due to nano particles. Cobalt poisoning affects human beings, terrestrial and aquatic animals and plants (Holm-Hansen *et al.*, 1954; Hamilton, 1994; Bruland *et al.*, 1991).

Cobalt is common in dust as it is readily available in the environment. It is possible to inhale the dust laden cobalt. Once inhaled, its effects are quickly felt. Mostly affected organs are the respiratory tract system and the kidney. The cobalt poisoning is linked to fatal kidney problems which are often from renal failure. Additionally, ingesting high amount of cobalt causes immediate poisoning and damage to the liver and the kidneys.

The most common way human beings are exposed to excess cobalt is through drinking of water which has been cobalt contaminated. The amount of cobalt absorbed into the blood system depends on solubility of the particles. The cobalt will pass into the pass stream much faster if the particles are dissolving faster and do not stay longer in the lungs. But if the particles are dissolving

slowly, the cobalt will remain in the lungs for a longer duration and thus causing complications. The cleansing of the particles from the body system will involve some of the particles leaving the lungs. Not all the cobalt particles are may get out of the lungs, some may get their way into the stomach.

Similarly, the major concern of cobalt is that it does not break down and will therefore accumulates over time to levels that are toxic in the environment and thus potential exposure to levels which are toxic. This therefore means there is need to keep monitoring levels of cobalt so that it do not get to levels which are toxic. This calls for analytical methods for detection of this metal so that human beings are not exposed to levels that have exceeded acceptable levels.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Chemicals and solutions

All the chemicals and acid used were of analytical grade quality and were used without further purification. The chemicals used include; Poly(acrylic acid), 25 wt% solution in water, Cobalt(II) chloride (AR), cadmium nitrate (AR), lead nitrate (AR), acetic acid glacial (AR) and sodium acetate anhydrous (AR) were from fisher scientific and were used as received. For voltammetry, the electrolyte used was acetate buffer. Water was purified to specific resistivity >15 M Ω cm. All other chemicals were reagent grade.

3.2 Apparatus

All the electrochemical experiments were performed with a CHI 1232B Electrochemical Station (CH Instruments, Inc., USA). A three-electrode system (CH Instrument In., USA) consisted of a modified glassy carbon working electrode with diameter of 3 mm, a platinum wire auxiliary electrode and a Ag/AgCl reference electrode. A pH meter Bench – Model CyberScan pH Tutor (Eutech Instruments) was used for all pH measurement. All experiments were carried out in a 10.0 mL electrochemical cell at room temperature. All data were analyzed using Kaleidagraph software, version 4.1.1.

3.3 Cleaning of the glassware

All the glassware used in this work were thoroughly cleaned by soaking them in nitric acid and sulphuric acid mixtures for 48 h, followed by cleaning with detergent, ordinary water and finally rinsing them with de-ionized water.

3.4 PAA film preparation and optimization

Glassy carbon disk electrodes (3 mm diameter) were abraded on wet silicon carbide paper (400 grit, Buehler) followed by (600 grit, Buehler) (Guto & Rusling, 2005). Rinsed in water, then polished thoroughly with 0.05 micron micropolish (CH Instruments) slurry on a soft cloth before sonicated in ethanol and distilled water for 3 min each to remove particles and other possible contaminants (Guto & Kamau, 2010). The actual surface area was 0.071 cm². Varying amounts of freshly prepared 4 mM polyacrylic acid were deposited on the polished electrode and allowed to react at room temperature for varying times. Then the electrodes were rinsed with de-ionized water before use.

3.4.1 Procedure

All measurements were carried out in the differential pulse anodic stripping voltammetric (DPASV) mode. The differential pulse stripping voltammograms were recorded from 0V to -1.45 V followed by a 10 sec rest period. Prior to the next determination, the modified electrode was activated for 60 sec in a pH 6.0 acetate buffer to remove the previous deposits completely.

3.4.2 Electrochemical characterization of the PAA/GCE

Standard potassium ferricyanide solution was used to characterize the plain GC, Pt and Au and PAA/GC, PAA/Pt and PAA/Au electrodes. Diffusion coefficients for the standard ferricyanide redox couples were determined by cyclic voltammetry at the electrodes using Randles-Sevcik equation. Then Nicholson method was used to calculate the rate constant (k). Anodic and cathodic peak separations from a background subtracted voltammogram were used to evaluate ψ from which k was obtained using the expression:

$$\psi = \frac{k}{(aD_o)^{1/2}} \quad (3.1)$$

Where D_o is the diffusion coefficient and $a = nFv/RT$ (v being the scan rate).

3.5 Optimization of the sensor

The following conditions were optimized for the DPV-SV studies. Optimal conditions were used for subsequent studies. 2 mM of Cd(II), Pb(II) and CdII) ions were used for this experiments.

3.5.1 Buffer pHs (acetate buffer)

A series of buffer pHs (2-12) were prepared by mixing appropriate ratios of 0.2 M Acetic acid and 0.2 M Sodium acetate. Effects of pH on the peak currents of 2 mM Pb(II), 2 mM Cd(II) and 2 mM Co(II) at the PAA/GCE in acetate buffer of pH 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 values were determined. The electrochemical responses of 2 mM Pb(II), Cd(II) and Co(II) in acetate buffer with different pH values at the PAA/GCE were studied by DPASV. To determine the optimal buffer pH, a graph of Current against pH, was plotted.

3.5.2 Pulse amplitude

The effect of pulse height on the sensitivity of the stripping peak currents of Pb, Cd and Co in acetate buffer, pH 6.0 containing 2 mM of Pb(II), Cd(II) and Co(II) at the PAA/GCE, was also checked using different pulse amplitude in the range of 10 to 100.0 mV.

3.5.3 Deposition time

The Effect of the deposition time on the stripping peak currents of Pb(II), Cd(II) and Co(II) in acetate buffer, pH 6.0 containing 2 mM of Pb(II), Cd(II) and Co(II) at the PAA/GCE, were studied from 0 minutes to 12 min. To determine the best accumulation time, a graph of current against time was plotted. The values were obtained from the voltammograms.

3.5.4 Pulse width

The influence of potential scan rate or (pulse width) on the peak current of Pb(II), Cd(II) and Co(II) was also investigated in the range of 1 to 25 mV/s with optimum conditions. The pulse width were gradually varied as follows; 0, 0.02, 0.04, 0.06, 0.08, 0.1 and 0.12. To determine the optimal pulse width of the system, a plot of pulse width verse time was made.

3.5.5 Sampling widths

Differential pulse stripping voltammetric responses at the PAA/GCE in buffer pH 6.0 containing 2 mM Pb(II), Cd(II) and Co(II) were done by varying sampling widths: 0.005, 0.0067, 0.009, 0.02 and 0.03 sec were used for the study.

3.5.6 Pulse period

The influence of pulse period on the peak current of Pb(II), Cd(II) and Co(II) was also investigated in the range of 0, 0.2, 0.4, 0.6, 0.8, 1 and 1.2 sec.

3.6 Sensor validation

3.6.1 Accuracy

The accuracy of an electrochemical sensor/method expresses the nearness between the expected value and the value found. It is expressed by calculating the percent recovery (%R) of the analyte recovered. In this case, to evaluate the accuracy of the developed electrochemical sensor, successive analysis of three different standard concentrations of the analyte (Pb(II), Cd(II) and Co(II)) solutions were added to the samples in different experiments. The standard solutions added were 50, 100 and 150% of the expected working sample concentration. The data of the experiment were statistically analyzed using equation 3.2 (Gumustas & Ozkan, 2011) to study the recovery and validity of the sensor.

$$\frac{\text{Recovered}}{\text{Added}} \times 100\% \quad (3.2)$$

3.6.2 Precision

Precision of an analytical method is showing the closeness, matching or concordance degree of a measurement series obtained from several samples derived from the same homogeneous sample under specific conditions. Precision of the current method was considered at repeatability level only. Analysis was done on ten identical samples (reference material of Pb, Cd and Co standard solutions) and expressed as RSD% amongst responses using equation 3.3 (Gumustas & Ozkan, 2011).

$$RSD\% = \frac{SD}{Mean} \times 100\% \quad (3.3)$$

3.6.3 Linearity, linear concentration range, limit of detection and limit of quantitation

Measurements of six replicates were made for the detection of Pb(II), Cd(II) and Co(II) with the concentrations varying from 2 mM to 0.1 nM each in the acetate buffer, pH 6.0 for 300 sec deposition time on PAA/GC electrode. The linearity of the sensor was evaluated by using calibration curve to calculate coefficient of correlation, slope and intercept values. Based on three times the standard deviation of the baseline (equation 3.4) (Gumustas & Ozkan, 2011; Analytical Method Committee, 1988), the limits of detection (LOD) were estimated for Pb(II), Cd(II) and Co(II).

$$C_{LOD} = \frac{3.s}{m} \quad (3.4)$$

While limit of quantitation (LOQ) being the lowest concentration of analyte that can be measured in the sample at an acceptable level of accuracy and precision was calculated using the standard deviation of the response and the slope method expressed as shown in equation 3.5 (Gumustas & Ozkan, 2011):

$$C_{LOQ} = \frac{10.s}{m} \quad (3.5)$$

3.6.4 Effect of impurities

The effect of interference on the performance of the electrochemical sensor was evaluated to ensure that there was no interference from other components present in the samples. This was studied by adding the expected possible interferants like Cu^{2+} , SO_4^{2-} , K^+ , Na^+ , Cl^- , NH_4^+ and O_2 . These interferants were added first independently, then in combinations and their effect on the stripping currents of the analyte was monitored.

3.7 Determination of trace Lead (II), Cadmium (II) and Cobalt (II) in tap water using the developed sensor

Tap water sample was collected from our research laboratory (Nairobi city, Kenya) for the determination of Pb(II), Cd(II) and Co(II). 3 mL of this water sample and 3mL of acetate buffer (pH 6) were put into voltammetric cell. After the solution was de-aerated by purging with nitrogen, Pb, Cd and Co heavy metals were analyzed at the following condition: Deposition potential: -0.8 V, Accumulation time: 300 sec, amplitude: 0.06 V, pulse period: 0.02 sec, pulse width: 0.01 sec and sampling width: 0.0033 sec. After a rest period of 10 sec, the potential scan was started in the anodic direction using the differential pulse mode. After the addition of 100 μl of the mixture standard solution, the procedure was repeated three times. Voltammetric current readings were taken and used for quantitative analysis.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Characterization of the working electrode

The typical electrodes and relevant electrochemical cell used in this research work were standardized using potassium ferricyanide that gave well defined voltammograms as shown in Figures 4.1 and 4.3. Cyclic voltammetry of potassium ferricyanide on all the electrodes used in this work gave one reduction peak and one oxidation peak as it is evident in the Figures 4.1 and 4.3. It is interesting to note that the reduction and oxidation potentials for the plain electrodes compares relatively well with those of polyacrylic acid modified electrodes. The slight difference, particularly with respect to the shape of the voltammogram, can be attributed to the slight change in the surface chemistry of the modified electrodes. The shape of the voltammograms, can be attributed to many experimental factors. That is, it depends on how current is measured. Figure 4.1 shows a cyclic voltammetry of 1.0 mM potassium ferricyanide on plain glassy carbon electrode at scan rate of 0.005 to 0.02 V/s and using an electrolyte of 0.1 M potassium chloride. Looking at the three voltammograms, they appear to differ slightly. The oxidative and reductive potentials have shifted to the positive values as the scan rate increased. Under similar conditions as it is evident in Figure 4.3, similar observations were made with the glassy electrodes modified with the polyacrylic acid.

This experiments for the characterization of the working electrode were important in that, they led to the selection of an appropriate electrode for the construction of a sensitive sensor in this work. It helped establish an electrode that gave optimal sensor sensitivity. In addition, they ensured that the selected electrode for the sensor are stable and can be used to determine presence of heavy metals in different matrices without losing its sensitivity. The characterization of the

electrode gave information related to the selected electrode such as area and activity of the active surface.

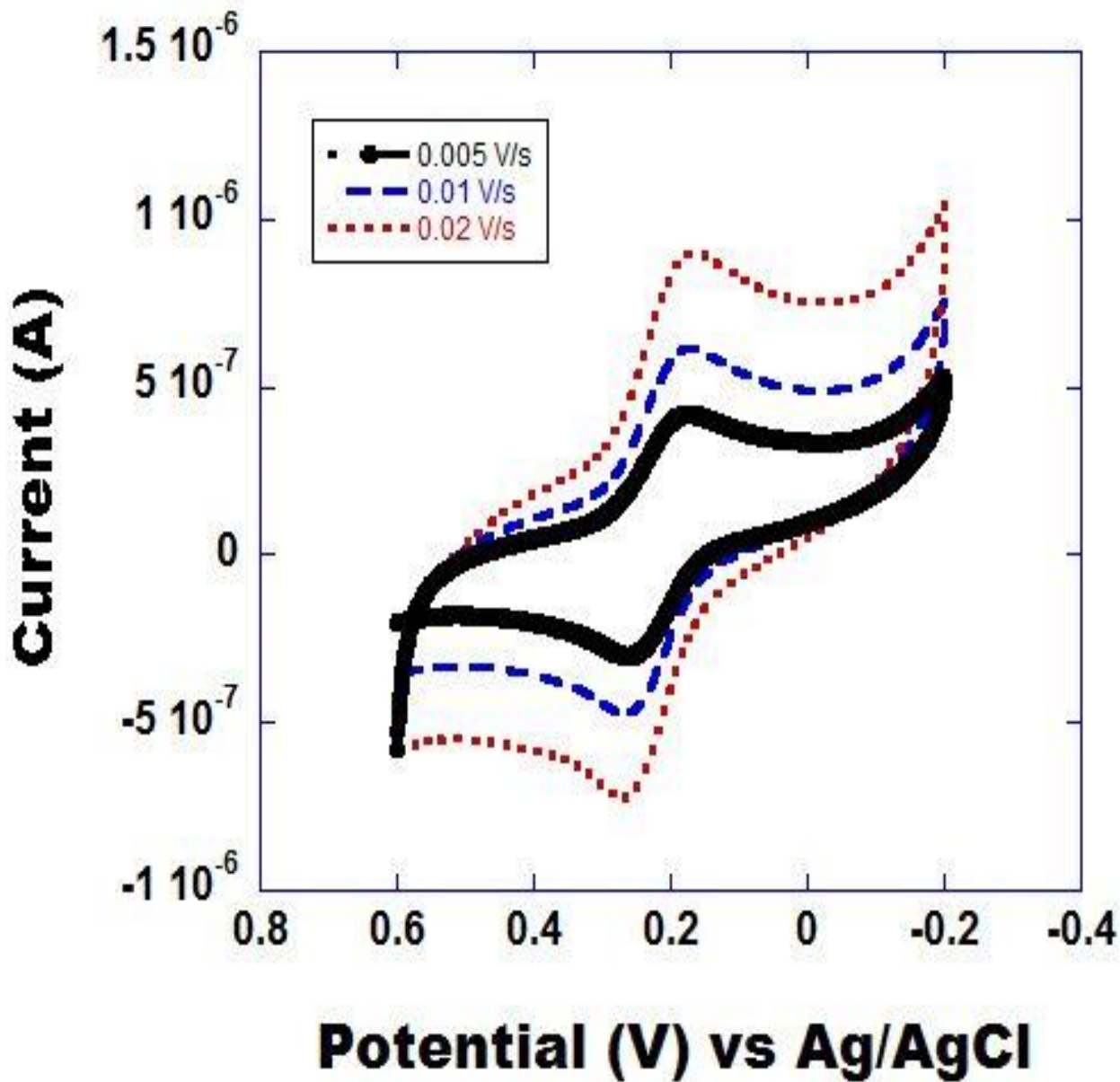


Figure 4.1: Cyclic voltammetry of 1.0 mM potassium ferricyanide on plain glassy carbon electrode at scan rate of 0.005 to 0.02 V/s.

Current readings from the voltammograms in Figure 4.1 were taken and then plotted against the square root of the scan rate. The purpose of the plot was to find out whether the system is diffusion or adsorption controlled or both. As shown in Figure 4.2, the linearity indicates that the electrochemical reaction is diffusion controlled.

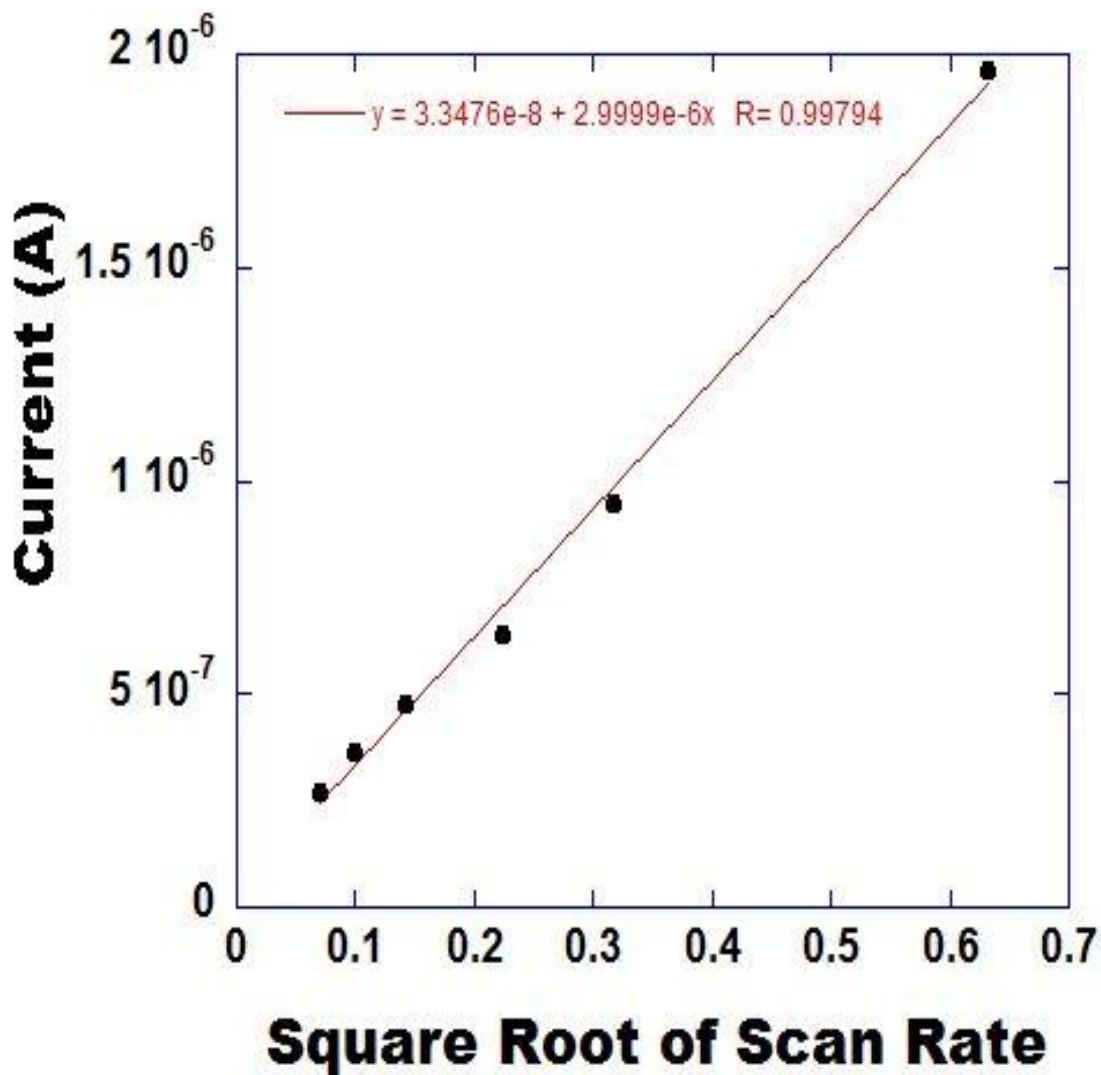


Figure 4.2: Plot of Current versus square root of scan rates on plain glassy carbon electrodes.

Figure 4.3 shows a cyclic voltammogram of a similar experiment conducted using the glassy carbon electrode with polyacrylic acid (PAA/GCE).

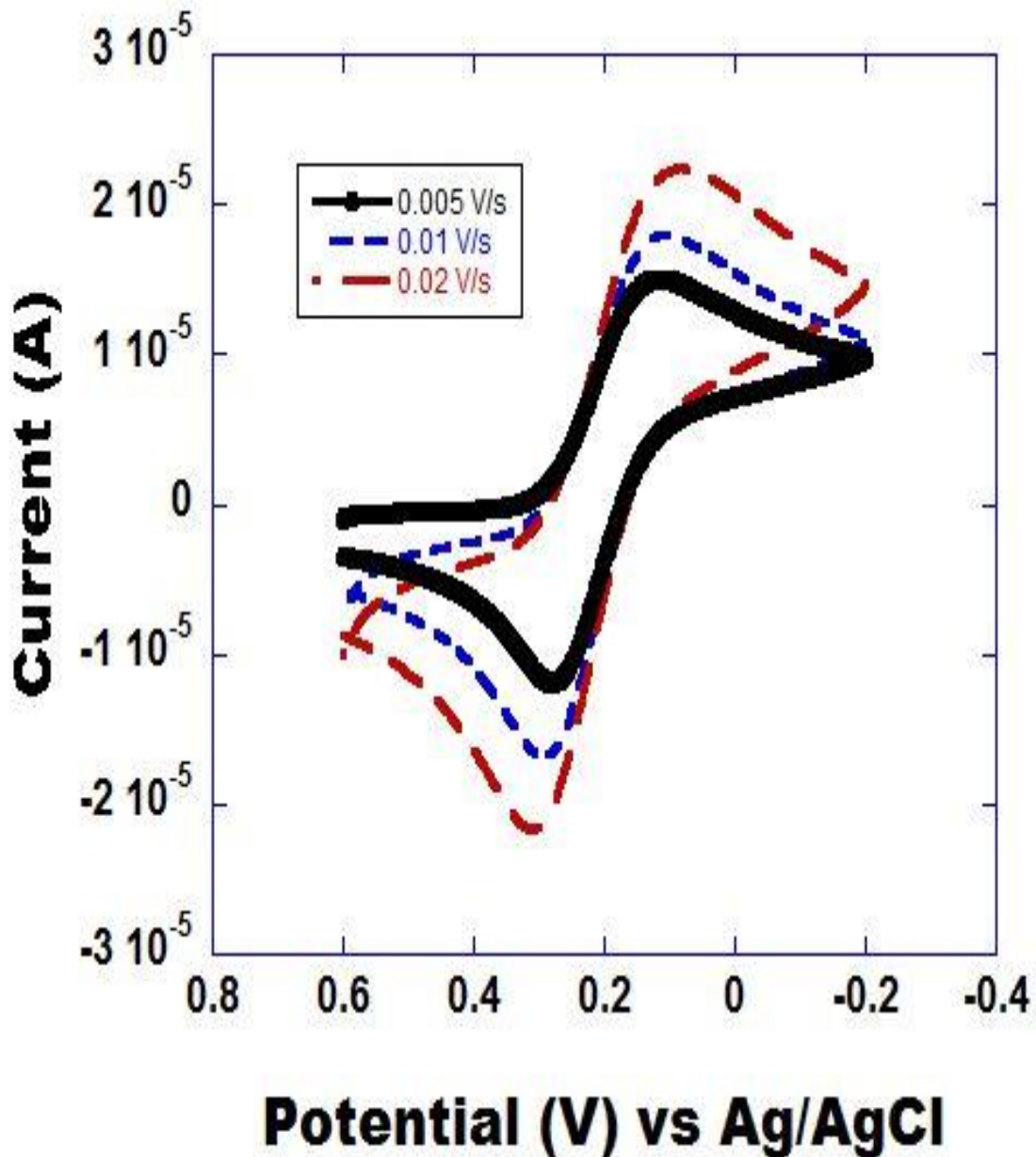


Figure 4.3: Cyclic voltammetry of 1.0 mM potassium ferricyanide on polyacrylic acid/glassy carbon electrode (PAA/GCE) at scan rate of 0.005 to 0.02 V/s.

Now using the glassy carbon electrode modified at their surfaces with polyacrylic acid, current readings were taken from the voltammograms in Figure 4.3 and then plotted against the square root of the scan rate. Similarly, the purpose of the plot was to find out whether the electrochemical reaction is diffusion or adsorption controlled or both. As shown in Figure 4.4, the linearity indicates that the electrochemical reaction for the modified electrode is diffusion controlled as well.

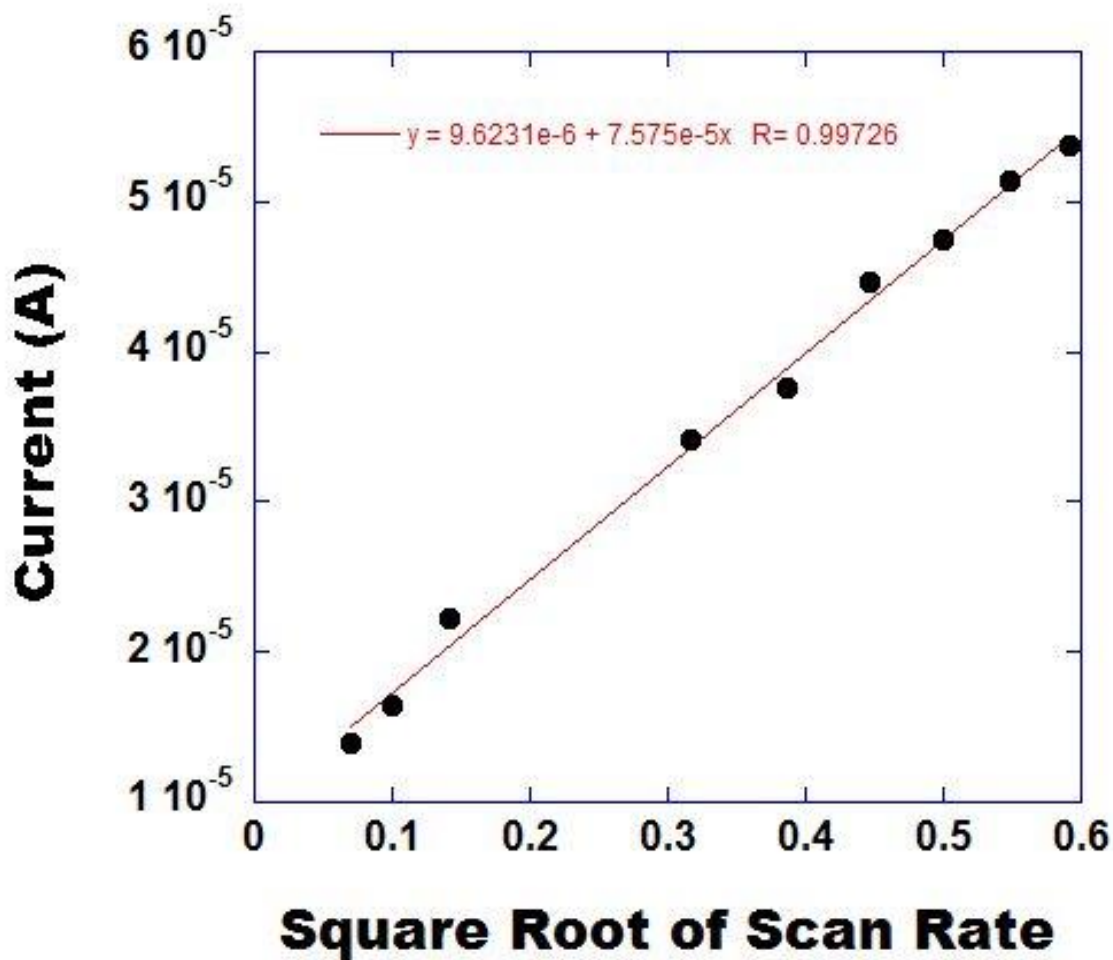


Figure 4.4: Plot of current (A) versus square root of scan rates on polyacrylic acid/glassy carbon electrode (PAA/GCE).

Following Randles-Sevcik equation 4.1 (Bard & Faulkner, 2004), plots of reduction currents (i_{pc}) versus the square root of scan rates ($v^{1/2}$) between 0.005 V/s and 0.1 V/s were made as shown in Figures 4.2 and 4.4. Linear plots were obtained whose slopes were used to obtain the diffusion coefficients on all the electrodes.

$$i_{pc} = (2.69 \times 10^5) n^{3/2} C^* A D^{1/2} v^{1/2} \dots\dots\dots(4.1)$$

Where i_{pc} is the diffusion peak current, n is the number of electrons exchanged in the redox process, C^* is the concentration of potassium ferricyanide, A is the area of the electrode, D is the diffusion coefficient and v is the scan rate. The linear plots obtained in Figures 4.2 and 4.4 indicates that the electrode processes are all diffusion controlled. From Table 4.1, the diffusion coefficient (D_o) for Au was $0.000275 \times 10^{-7} \text{cm}^2/\text{s}$ while Au/PAA was $1.7910^{-7} \text{cm}^2/\text{s}$. For Pt gave $0.0036810^{-7} \text{cm}^2/\text{s}$ while Pt/PAA afforded $0.01610^{-7} \text{cm}^2/\text{s}$. On the other hand, GC gave $0.0012210^{-7} \text{cm}^2/\text{s}$ while that of GC/PAA was $0.031810^{-7} \text{cm}^2/\text{s}$. Overly, oxidation currents had higher D_o values compared to reduction currents.

Formal redox potential (E^o) for a reversible system is taken to be the mid-point between the reduction (E_c) and oxidation (E_a) potentials. As shown from Table 4.1, the E^o obtained were 0.142 V for Au, 0.058 V for Au/PAA, 0.0567 V for Pt, 0.0168 V for Pt/PAA, 0.222 V for GC and 0.063V for GC/PAA versus Ag/AgCl. This data shows that the mid-point potential for the PAA modified electrodes are more negative while those of plain electrodes were more positive.

Table 4.1: Electrochemical parameters for the selected electrodes.

| <i>No.</i> | <i>ELECTRODE</i> | <i>E^o</i> (V) | <i>D_c</i> , $\times 10^{-7}$ (<i>cm²/s</i>) | <i>D_a</i> , $\times 10^{-7}$ (<i>cm²/s</i>) | <i>k^o_c</i> , $\times 10^{-3}$ (<i>cm/s</i>) | <i>k^o_a</i> , $\times 10^{-3}$ (<i>cm/s</i>) |
|------------|------------------|--------------------------|--|--|--|--|
| 1 | Au | 0.142 | 0.000275 | 0.0122 | 0.028 | 0.186 |
| 2 | Au/PAA | 0.233 | 1.79 | 6.57 | 3.87 | 2.02 |
| 3 | Pt | 0.0567 | 0.00368 | - | 2.35 | - |
| 4 | Pt /PAA | 0.0168 | 0.016 | 3.57 | 1.64 | 24.511 |
| 5 | GC | 0.222 | 0.00122 | 0.000781 | 0.0883 | 0.0705 |
| 6 | GC/PAA | 0.063 | 0.0318 | 7.07 | 9.074 | 135.38 |

D_c = diffusion coefficient (cathodic); *E^o* = formal redox potential and *k^o_c* = heterogeneous rate constant (cathodic).

Methods developed by Laviron (1979) and Hirst and Armstrong (1998) were applied to compute the rate of electron transfer between the electrode and the analyte. By compensating for the constant peak separations (ΔE_p) at low scan rates, the resulting data for the electron transfer between electrode and the diffusing analyte species were plotted. Assuming the electron transfer coefficient to be $\alpha = 0.5$, the apparent electrochemical electron transfer rate constants was estimated (Feeney & Kounaves, 1998). The results obtained in Table 4.1 show that the cathodic current k_s value for the Au/PAA and GC/PAA modified electrodes were much higher by almost 100 times compared to plain Au and GC electrodes. The trend for Pt and Pt/PAA electrodes was not clear. Overly, for anodic currents, the k_s are highest in GC/PAA electrodes.

4.2 Optimization of the sensor

Sensor optimization is a process of maximizing or minimizing some functions of the sensor or its components. This allows comparison of different choices for determining which might give the best performance. Optimization of electrochemical sensor depends on the electrode material, electrode surface modification, geometry of the electrodes and other conditions essential for designing integrated system. In order to further improve sensitivity of the developed sensor, effect of the amount of polyacrylic acid, drying period of the polyacrylic acid film, stability of the polyacrylic acid film on glassy carbon, pH buffer, deposition time, pulse amplitudes, sampling width, pulse period and pulse width were systematically studied.

4.2.1 Effect of the amount of polyacrylic acid (PAA)

To investigate the effect of the amount of PAA on the stripping responses, different amounts of the 4 mM PAA were deposited on the glassy carbon (GC) electrode. Voltammograms

in Figure 4.5 shows the effects of varying PAA concentrations on peak currents of 0.5mM Pb(II), Cd(II) and Co(II) at the PAA/GC electrode. Increasing the amount of PAA on the GC electrode caused the sensitivity to increase linearly up to 10 μ L, whereas beyond 10 μ L caused the sensitivity of the electrode to decline due to the over-thick of the film, which hampered the electron transfer between metal ions and base electrode and also increased the background current. Similar observation was made by Tian *et al.*, (2009) on MWCNTs-NADBS modified stannum film electrodes. Therefore, 10 μ L of the 4 mM PAA was used on the GC electrode surface in all subsequent work.

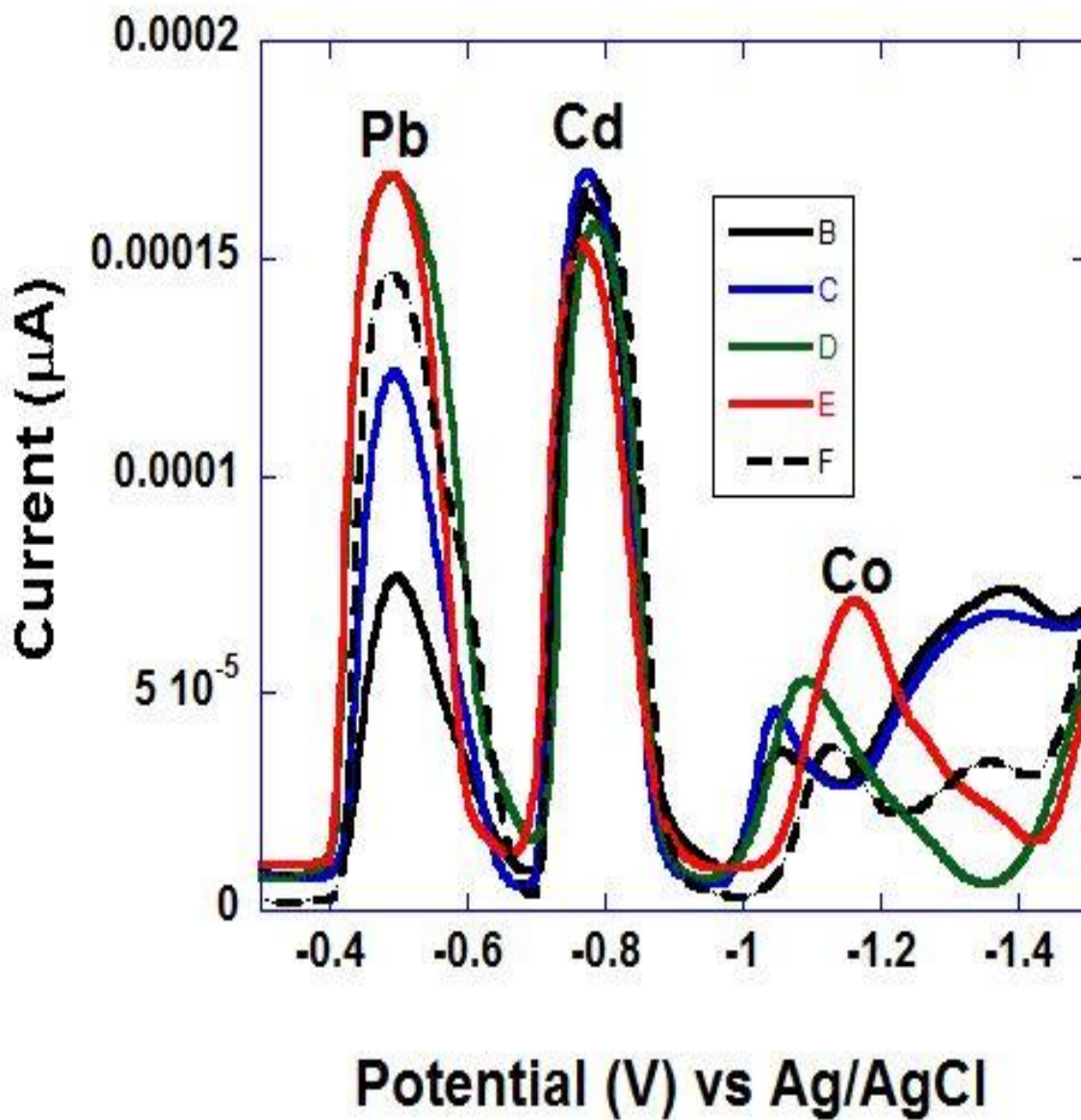


Figure 4.5: Effects of the PAA concentration on the peak currents of 0.5 mM Pb(II), Cd(II) and Co(II) at the PAA/GC electrode. Deposition potential: -0.8 V, Accumulation time: 300 sec, amplitude: 0.06 V, pulse period: 0.02 sec, pulse width: 0.01 sec and sampling width: 0.0033 sec. The PAA amounts added were: B = 2 μ L, C = 4 μ L, D = 7 μ L, E = 10 μ L, F = 13 μ L of 4 mM PAA on GC electrode.

To determine amount of polyacrylic acid on the surface of glassy carbon electrode that enables the electrode to give maximum peak current, current readings were taken from the voltammograms in Figure 4.5 and plotted against the amount (volume) of PAA as shown in Figure.4.6.

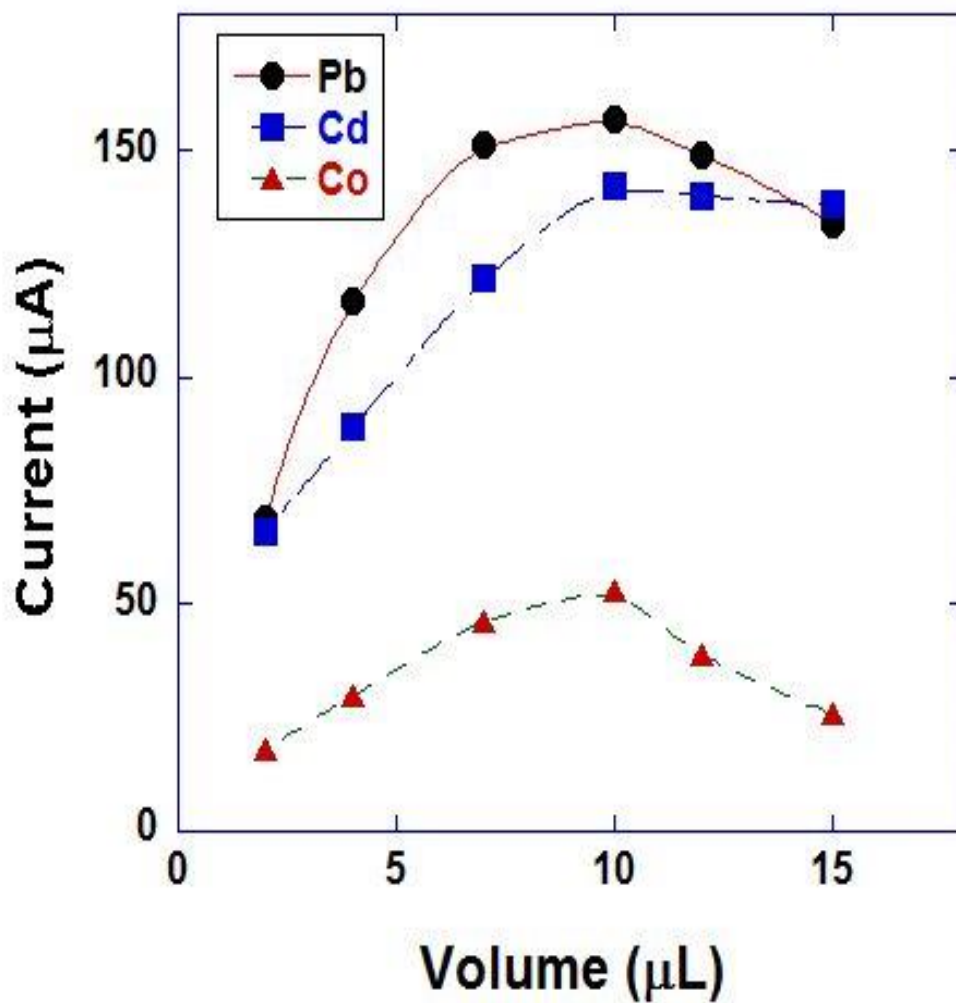


Figure 4.6: Plots of currents (µA) versus volumes (µL) of 4 mM PAA.

4.2.2 Effect of the drying period of the polyacrylic acid film

The electrochemical responses of 0.5 mM Pb(II), Cd(II) and Co(II) at the PAA/GC electrode containing 10 μ L of 4 mM PAA film drying times were studied by differential pulse anodic stripping voltammetry (DPASV). The results are shown in Figure 4.7. It can be seen from Figure 4.7 that the maximum peak currents for Pb(II), Cd(II) and Co(II) were obtained when the films were dried for 20 min. There was no observable change in the current responses when the PAA films were dried beyond 20 minutes. Consequently, a 20 min drying period was selected for this work.

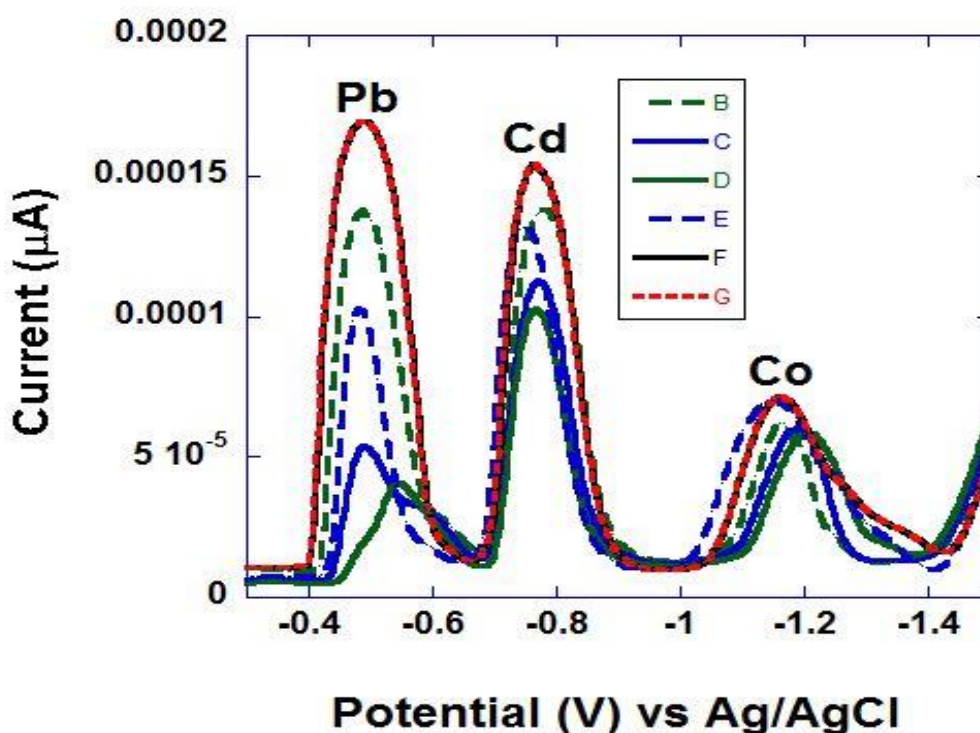


Figure 4.7: Effects of drying time of 10 μ L of 4 mM PAA on the peak currents of 0.5 mM Pb(II), Cd(II) and Co(II) at the PAA/GC electrode. Deposition potential: -0.8V, Accumulation time: 300 sec, amplitude: 0.06 V, pulse period: 0.02 sec, pulse width: 0.01sec and sampling width: 0.0033 sec. Drying time were: B = 1 min, C = 5 min, D = 10 min, E = 15 min, F = 20 min, G = 25 min of drying the PAA film on GC electrode.

To determine the optimal drying period, current readings for Co(II), Pb(II) and Cd(II) were taken from the voltammograms shown in Figure 4.7 and plotted against drying period (mins) of poly acrylic acid (PAA) on the surface of glassy carbon electrode. As indicated in Figure 4.8, the developed sensor gave a high response (current) and thus more sensitive at a 20 minute drying period.

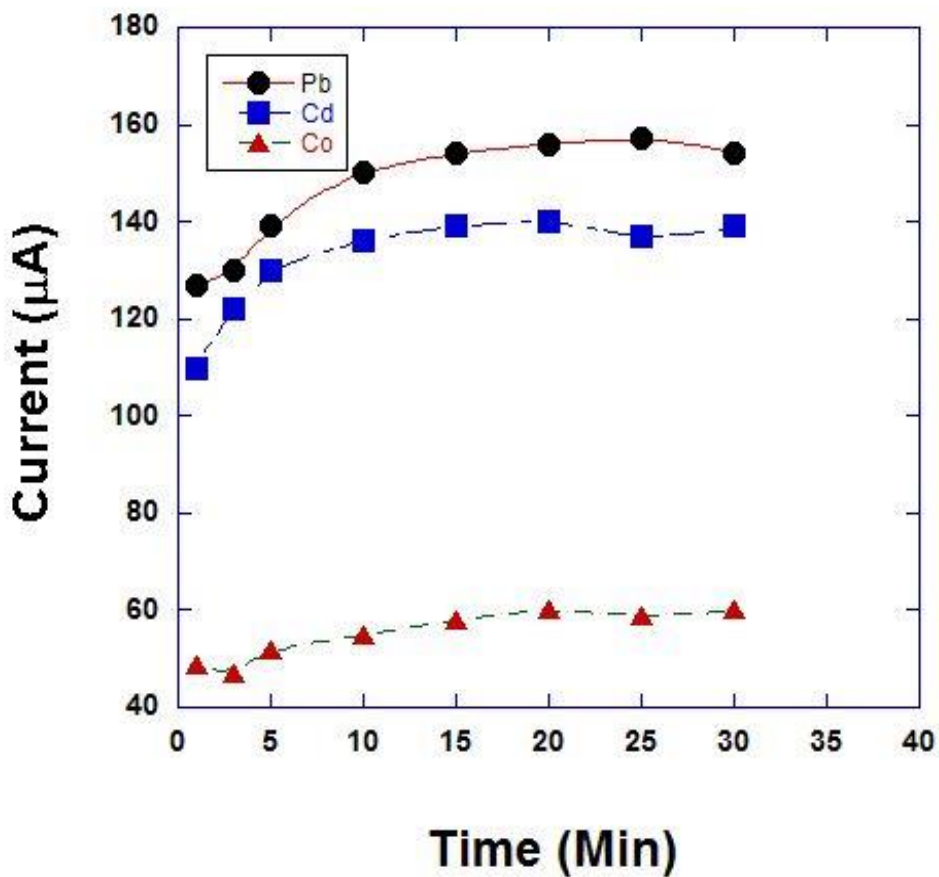


Figure 4.8: Plots of currents (μA) versus drying time (min) of the PAA films.

4.2.3 Stability of the polyacrylic acid film on glassy carbon

Stability of the polyacrylic acid film on glassy carbon electrode refers to the number of times the sensor can be used while giving consistent results or performance within the universally acceptable range. It also ensures that the sensor will remain unaffected by the surrounding environment and assures that the results obtained by the sensor are true. The method to attach the polymer film to be selected will depend on the type of film and thus determines the stability of the film on the surface of the electrode. This ensures that the film remains on the surface of the electrode for a longer period of time to giving the sensor the desired performance.

Under this study, the stability of the PAA/GCEs were tested for six weeks in a solution containing 1.0 mM Pb(II), Cd(II) and Co(II) dissolved in acetate buffer, pH 6. After six weeks, the percentage decrease in the PAA film activity were 6.3% for Pb(II), 4.8% for Cd(II) and 17% for Co(II) as shown in Figure 4.9. Thus, the stability of the PAA/GCE is satisfactory. The small decrease can be attributed to the fouling of the electrode surfaces. The mechanical robustness of the PAA/GCEs is excellent compared to many of the reported polymer films in literature (Pujol *et al.*, 2014; Ali *et al.*, 2014; Nourifard *et al.*, 2015; Wang *et al.*, 2001).

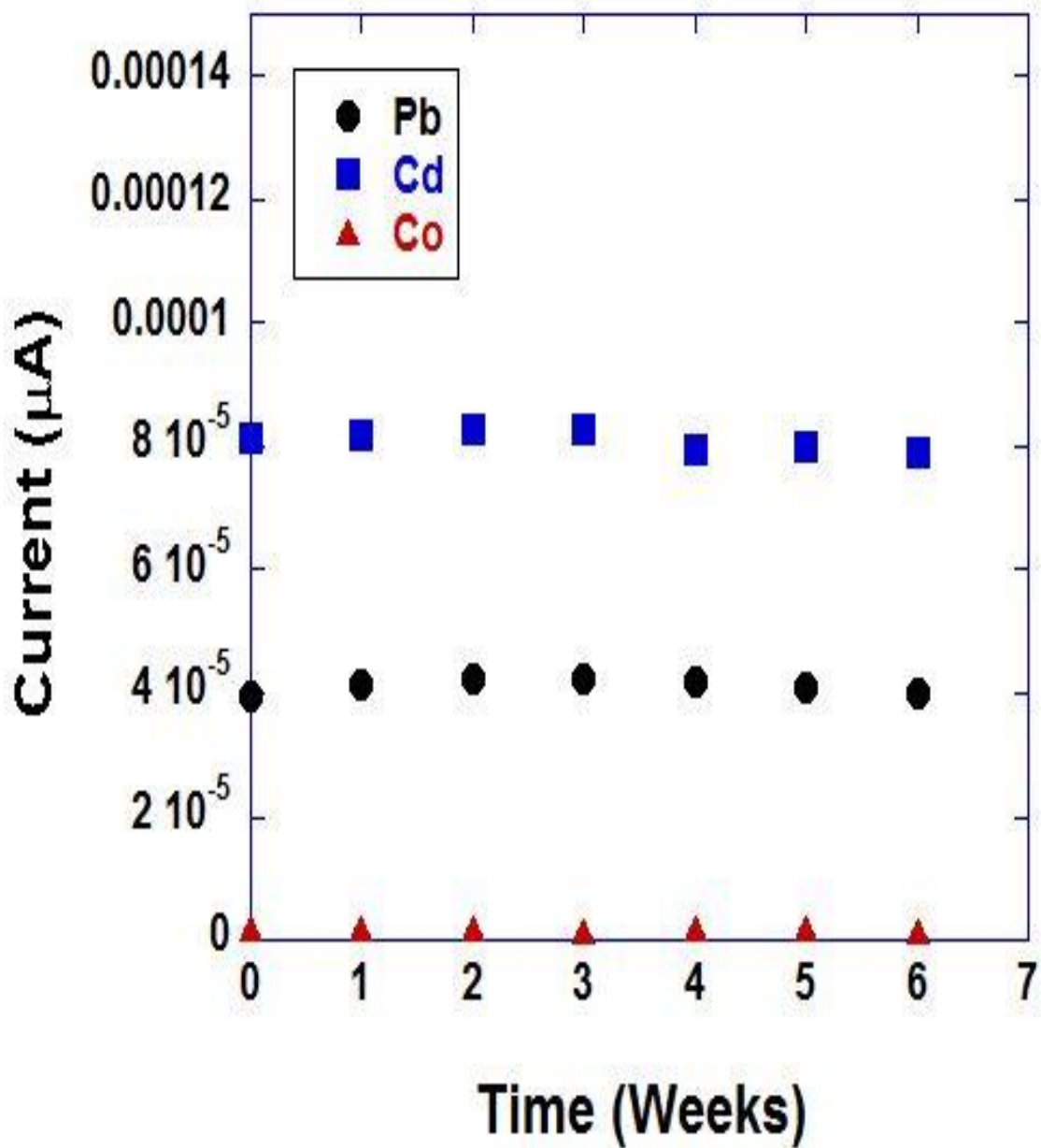
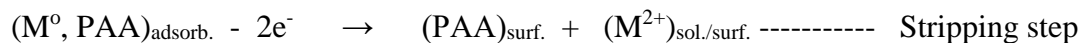
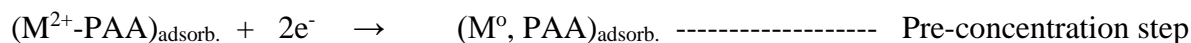
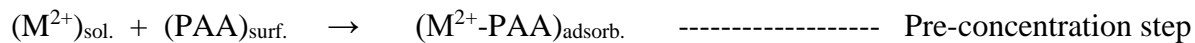


Figure 4.9: PAA/GC electrode stability in 2 mL of 1mM Pb(II), Cd(II) and Co(II) in acetate buffer, pH 6. Deposition potential: -0.8 V, Accumulation time: 300seconds, amplitude: 0.06 V, pulse period: 0.02 sec, pulse width: 0.01 sec and sampling width: 0.0033 sec. The films were dried for 20 min before investigating their stability.

As per above results for the reduction of metal ions (Pb(II), Cd(II) and Co(II)) at modified electrodes, the following scheme highlight the expected mechanism:



Scheme 4.1: Mechanism on the electrochemical behavior of metals at the surface of PAA modified electrode.

Where *M* is the metal and *PAA* is the polyacrylic acid. Subscripts “*sol*” is solution, “*surf*” is surface, “*adsorb.*” is adsorbed and “*sol/surf*” is solution/surface. Comparing the differential pulse anodic stripping voltammetric currents of the modified and unmodified electrodes observed in both this work indicates that modification of the GC electrode with PAA caused easier and faster charge transfer at the electrode surface.

4.2.4 Buffer pHs (acetate buffer)

The electrochemical responses of 2 mM Pb(II), Cd(II) and Co(II) in acetate buffer with different pH values at the PAA/GCE were studied by differential pulse anodic stripping voltammetry (DPASV), and the results are shown in Figure 4.10. It can be seen from Figure 4.10 that the maximum peak current responses for both Pb(II), Cd(II) and Co(II) were obtained when pH was 6.0. When the pH value increased to 7.0 and above the peak current began to gradually decrease. Consequently, a pH 6.0 acetate buffer was selected for this work. Note that the maximum

peak current corresponds to high sensitivity. This therefore means that the sensor is more sensitive at the selected pH value. This is evident in all the three heavy metals that were used to test sensitivity of the developed sensor.

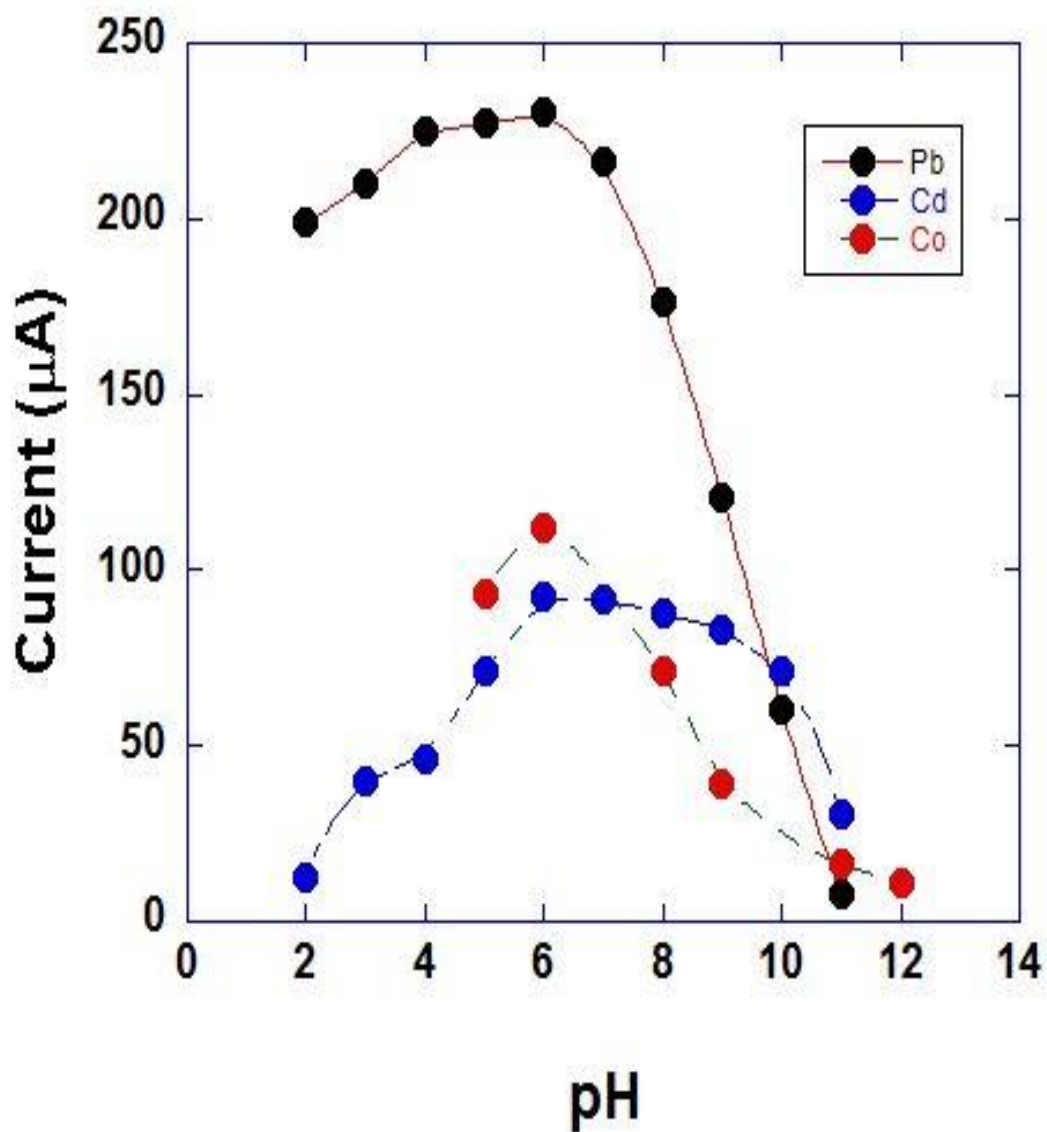


Figure 4.10: Effects of pH on the peak currents of 2 mM Pb(II), 2 mM Cd(II) and 2 mM Co(II) at the PAA/GCE in acetate buffer of pH 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 values.

In Figure 4.10, we plotted a graph of current against buffer pH and the graph was used to determine the optimal buffer pH for the developed sensor.

Figure 4.11 shows the differential pulse anodic stripping voltammetric responses of Pb(II), Cd(II) and Co(II) at the GCE plain and PAA/GCE at pH 6.0. The optimal buffer pH value at 6.0 was used in all the subsequent studies. The response signals on the bare GCE were at potentials -0.495 V for Pb(II), -0.720 V for Cd(II) and -1.215 V for Co(II) while for PAA/GCE were at -0.480 V for Pb(II), -0.745 V for Cd(II) and -1.190 V for Co(II). The peak potential maxima results obtained have shown that the peaks for the different metal ions can be identified as Pb(II), Cd(II) and Co(II). With PAA/GCE, the stripping current peaks for Pb(II), Cd(II) and Co(II) are remarkably higher compared to plain GCE under similar conditions. This further indicate that the PAA/GCE sensor can greatly promote the pre-concentration of Pb(II), Cd(II) and Co(II) ions at the electrode and significantly increase the sensitivity for the determination of Pb(II), Cd(II) and Co(II) ions. This indicates that PAA possibly increases the adsorption of metal ions onto the electrode surface. Similar behavior was observed previously by Elamathi *et al.*, (2013) and Magdy *et al.*, (2012).

Performance of the plain electrode with glassy carbon electrode with a film of polyacrylic acid on its surface were compared. According to Figure 4.11 using differential Pulse Anodic Stripping Voltammograms of 2 mM Pb(II), Cd(II) and Co(II) at pH 6 on Plain GCE and PAA/GCE

there was a tremendous improvement for the sensor that had a film on it is surface.

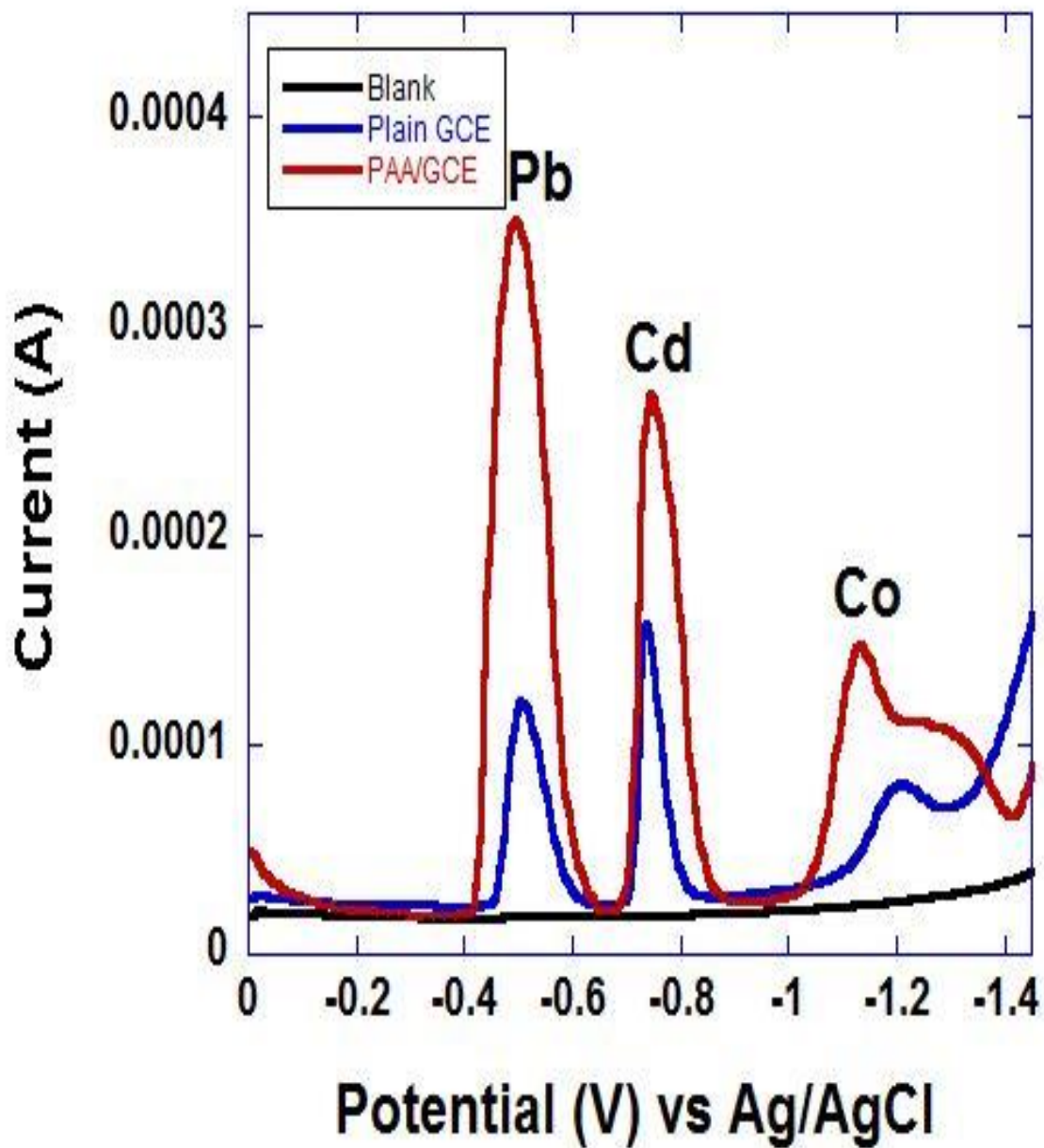


Figure 4.11: Differential Pulse Anodic Stripping Voltammograms of 2 mM Pb(II), Cd(II) and Co(II) at pH 6 on Plain GCE (*Blue*) and PAA/GCE (*Red*).

4.2.5 Deposition time

Deposition time is the time the sensor is allowed to preconcentrate metal ions from the solution and have them deposited on the surface of the electrode. Here, the metal ions extracted from the solution are allowed to accumulate on the surface of the electrode where they form a layer on top of each other. The deposition time of the metal ions allows the metal ions which exist in the solution in small concentrations to build up to levels which are capable to be detected and give a signal.

The effects of the deposition time on the stripping peak currents of Pb(II), Cd(II) and Co(II) were studied from 0 to 10 min and the results are shown in Figure 4.12. It can be seen from Figure 4.12 that the peak currents increased linearly from 0 to 5 min. As deposition time increased, more and more metals accumulated at the surface of modified electrode, so the peak currents greatly increased. However, when the deposition time was beyond 5 minutes, the peak currents started to level off, indicating that the amount of metals at the electrode surface tends to saturation. In view of both sensitivity and deposition time in this work, 5 min was selected as the optimal deposition time.

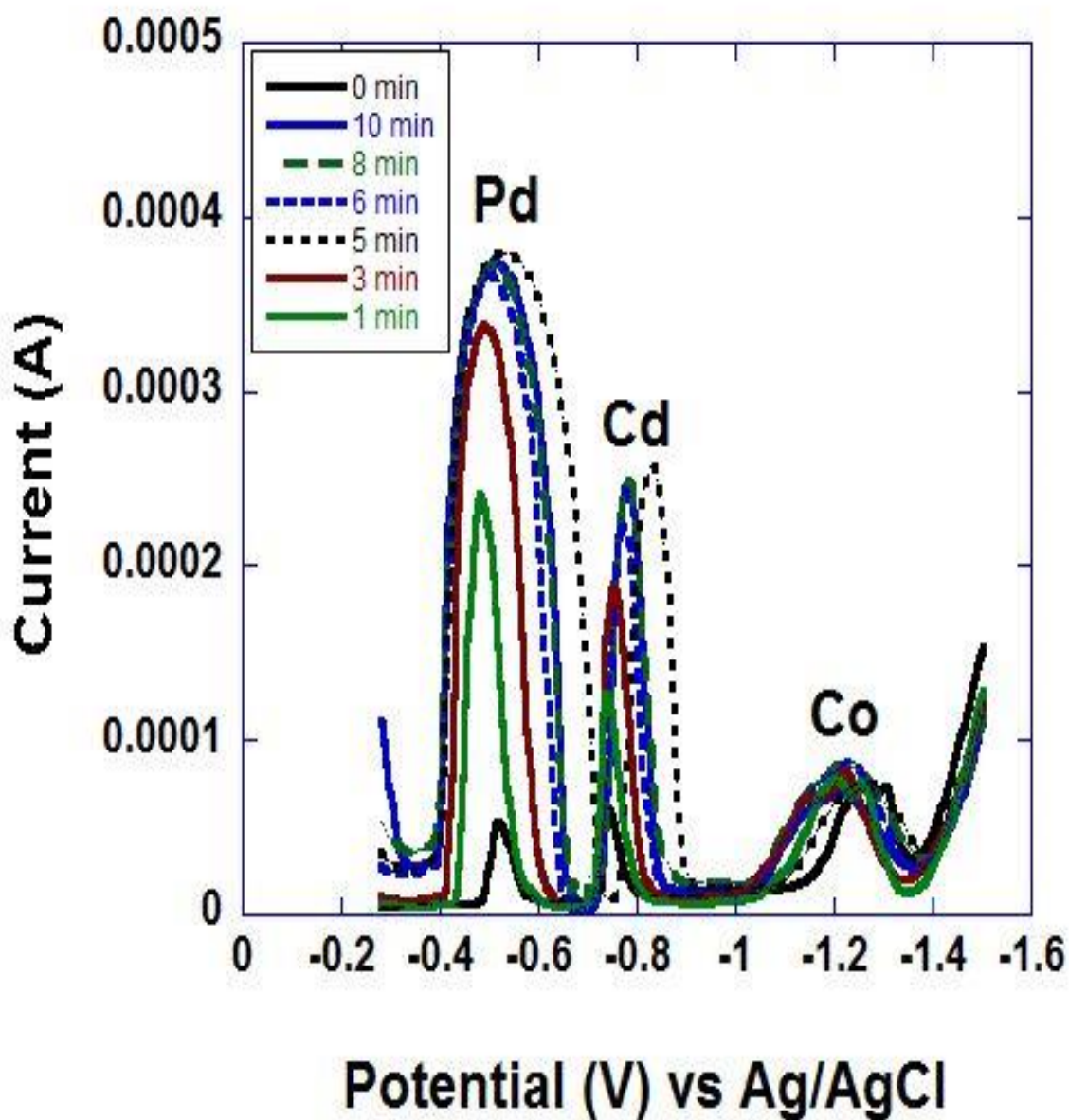


Figure 4.12: DPASVs - Effect of the deposition time on the peak currents of Pb(II), Cd(II) and Co(II) in acetate buffer, pH 6.0 containing 2 mM of Pb, Cd and Co at the PAA/GCE. Deposition time: 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 min.

To determine the optimal depositional time that will give maximum current shown in Figure 4.13, current readings were taken from the voltammograms in Figure 4.12 then plotted against deposition time.

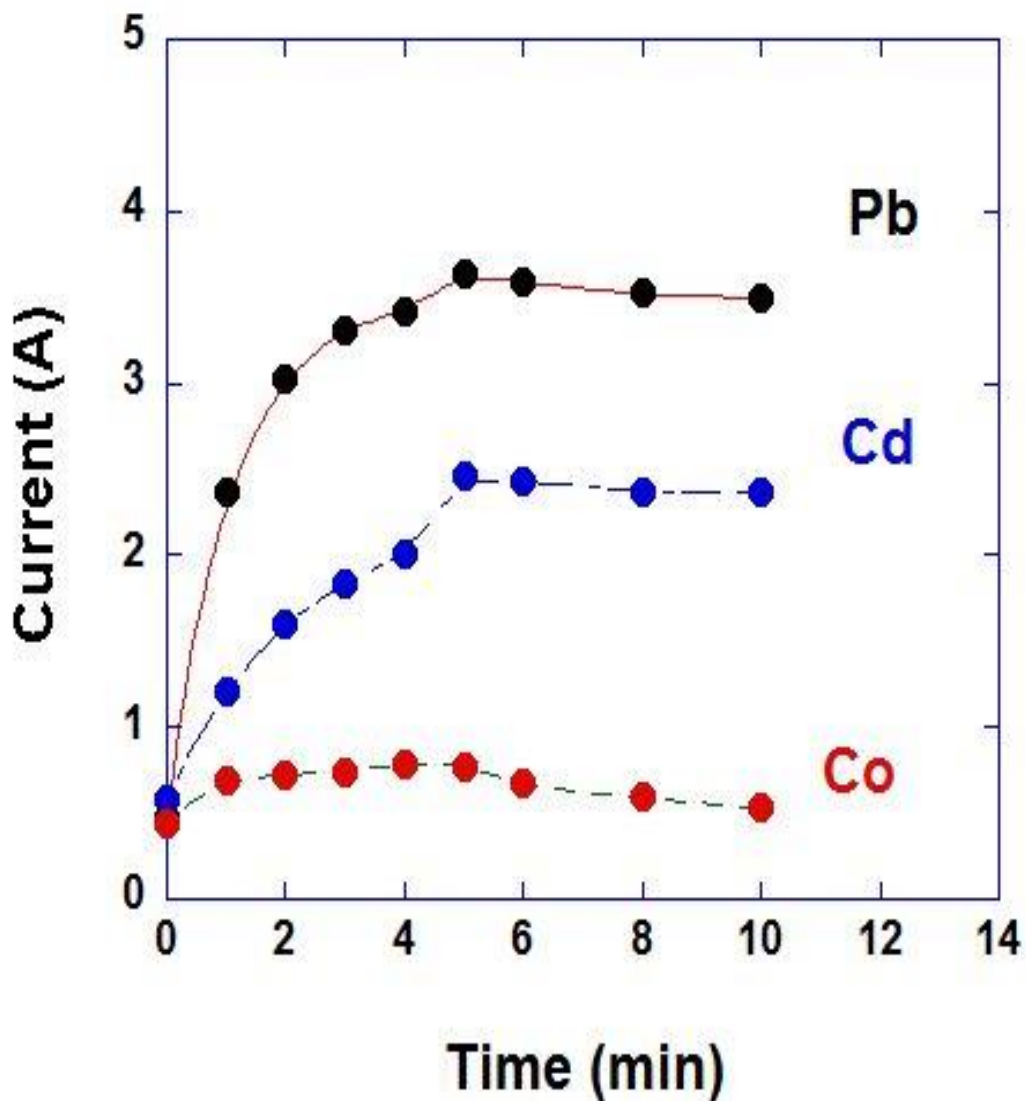


Figure 4.13: Current versus Time: Effect of the deposition time on the peak currents of Pb(II), Cd(II) and Co(II) in acetate buffer, pH 6.0 containing 2 mM of Pb(II), Cd(II) and Co(II) at the PAA/GCE. Deposition time: 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 min.

4.2.6 Pulse amplitudes

Pulse amplitude refers to the height of a potential pulse. It determines the height of a current peak. Larger pulse amplitude results in larger and broader peaks. This may be or may not be constant depending upon the technique.

The basis of pulse techniques is the difference in the rate of the charging and the faradaic currents following a potential step (pulse) and current is measured at the end of the potential pulse allowing discrimination between faradaic and charging currents. The rate of decay of charging current is considered to be faster compared to that of the faradaic current. It means the current measured at the end of the potential pulse is that of the faradaic current.

The influence of pulse amplitudes on the peak current of Pb(II), Cd(II) and Co(II) were investigated by varying pulse widths in a range between 0.01 and 0.06 V with optimum conditions. According to Figure 4.14 the optimal pulse width at 0.06 V gave maximum current and were selected for subsequent work.

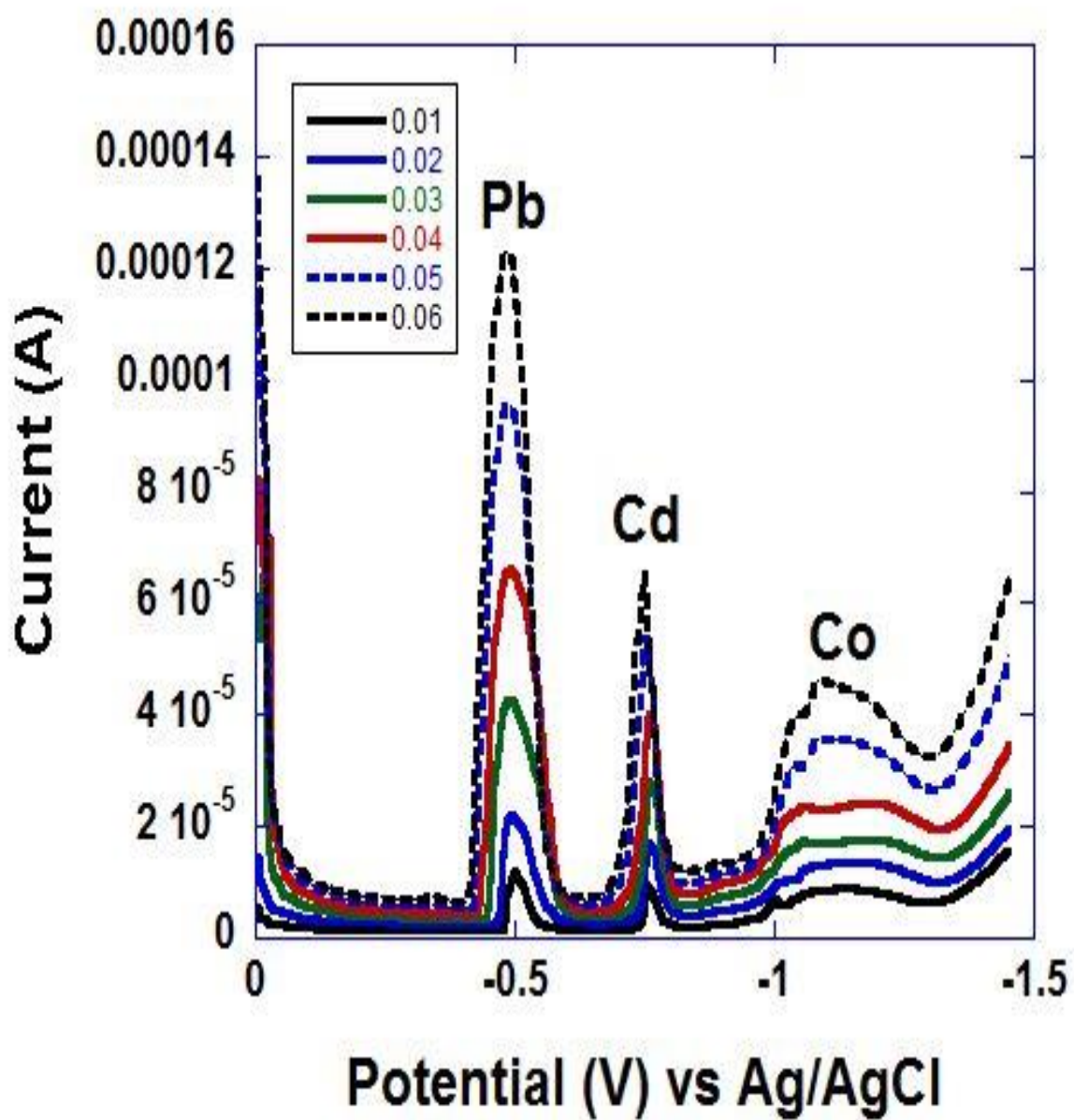


Figure 4.14: Differential pulse stripping voltammetric responses at the PAA/GCE in buffer pH 6.0 containing 2 mM Pb(II), Cd(II) and Co(II). Amplitudes: 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 V.

4.2.7 Sampling widths

Sampling width is the time at which the current is measured after each application of the potential pulse. This parameter defines the time required to have a potential cycle and it is therefore important that this parameter is determined and specified.

Differential pulse stripping voltammetric responses at the PAA/GCE in buffer pH 6.0 containing 2 mM Pb(II), Cd(II) and Co(II) were studied by varying sampling widths from 0.005 to 0.03 sec. A sampling width at 0.0033 sec resulted in a highest response and was selected for future work as it evident in Figure 4.15.

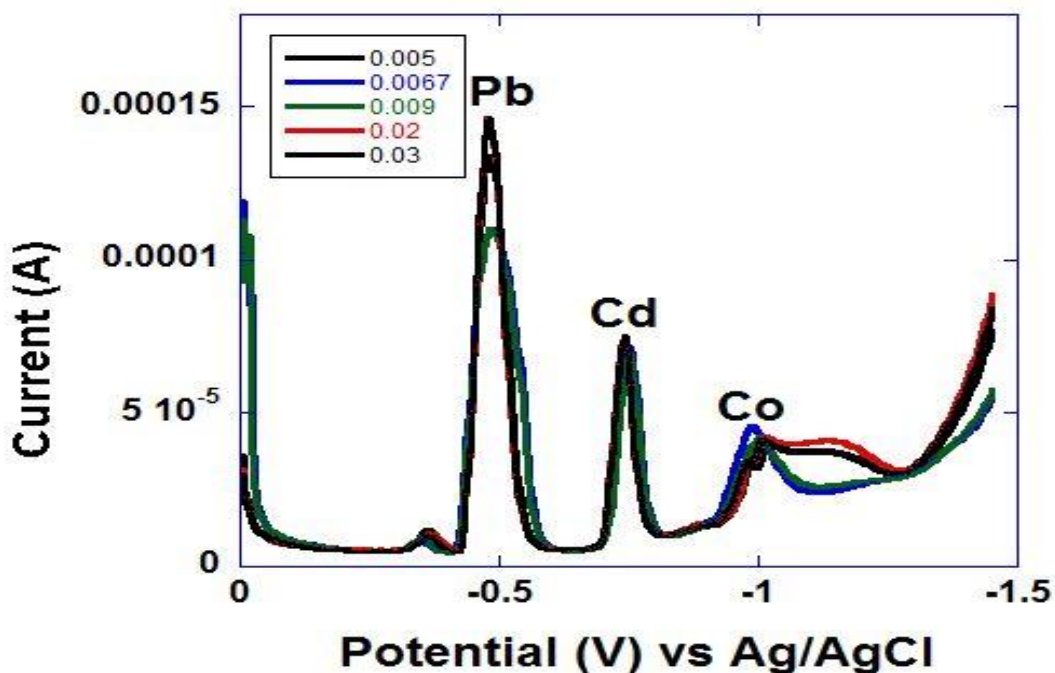


Figure 4.15: Differential pulse stripping voltammetric responses at the PAA/GCE in buffer pH 6.0 containing 2mM Pb(II), Cd(II) and Co(II). Sampling widths: 0.005, 0.0067, 0.009, 0.02 and 0.03 sec.

4.2.8 Pulse period

Pulse period is the time at the end of the pulse during which the current is measured. This parameter is fixed prior to running the experiment.

The influence of pulse period on the peak current of Pb(II), Cd(II) and Co(II) was also investigated in the range of 0 to 1.2 sec. A pulse period of 0.02 sec gave maximum current and was selected for subsequent work.

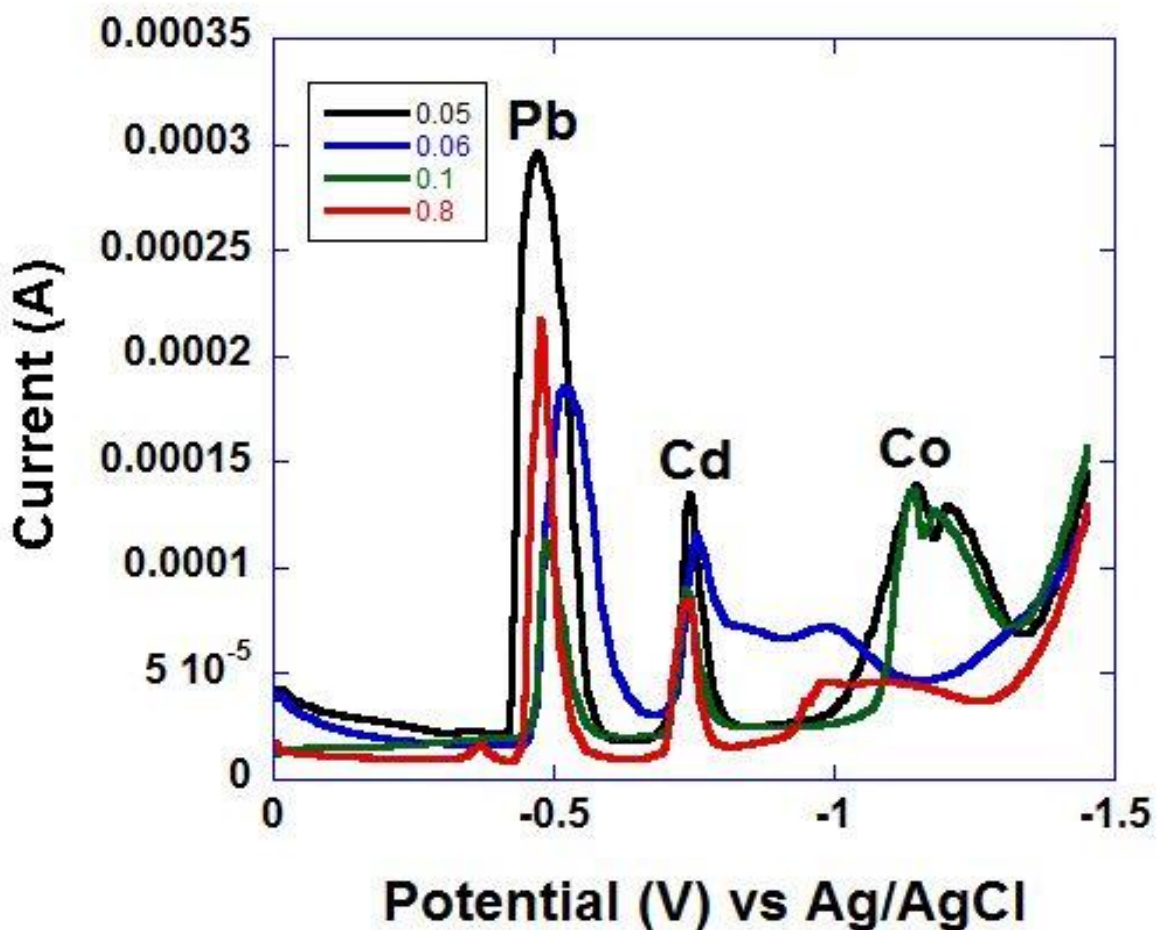


Figure 4.16: Differential pulse stripping voltammetric responses at the PAA/GCE in buffer pH 6.0 containing 2 mM Pb(II), Cd(II) and Co(II). Pulse periods: 0.05, 0.06, 0.1 and 0.8 sec.

4.2.9 Pulse width

Pulse width is the duration of the potential pulse. This parameter defines the time required to have a potential cycle and it is therefore important that this parameter is determined and specified.

The influence of potential scan rate or (pulse width) on the peak current of Pb(II), Cd(II) and Co(II) was also investigated in the range of 1 to 25 mV/s with optimum conditions. The pulse width were gradually varied in the range of 0 to 0.12 sec. A pulse width at 0.01 sec gave the maximum current and was selected for subsequent work.

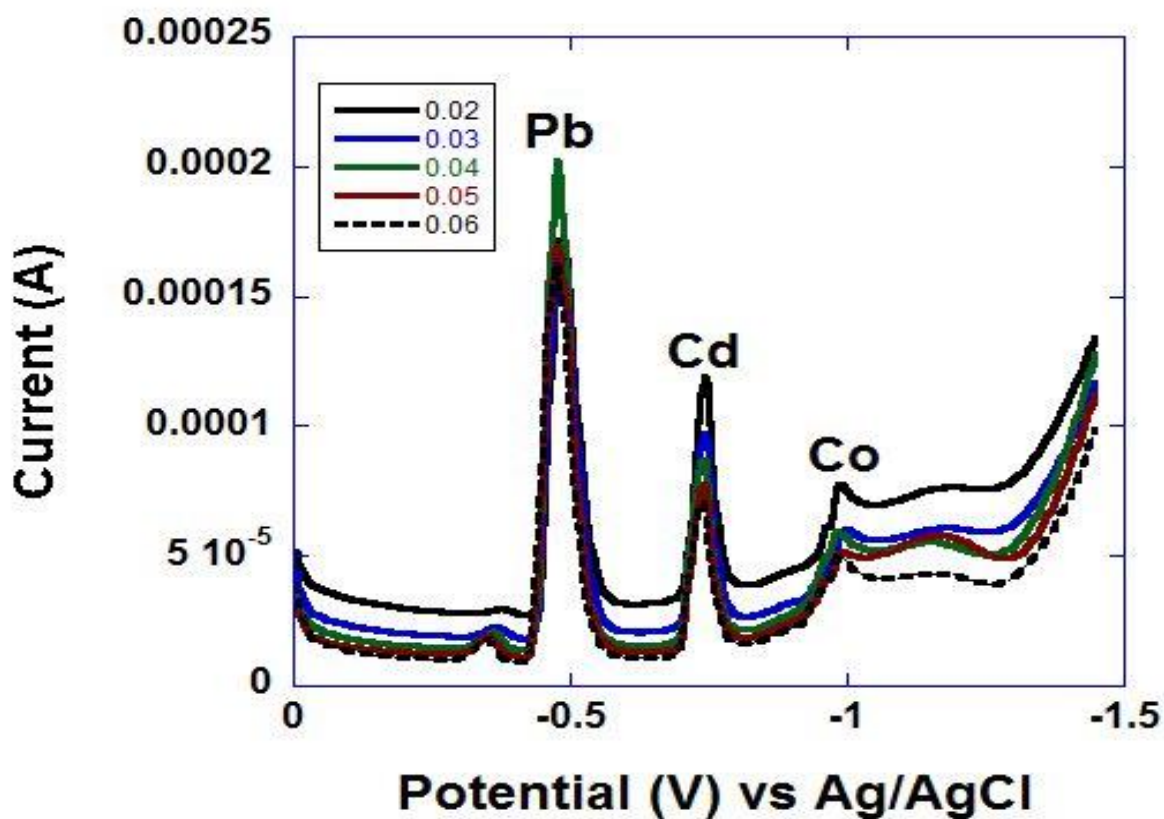


Figure 4.17: Differential pulse stripping voltammetric responses at the PAA/GCE in buffer pH 6.0 containing 2 mM Pb(II), Cd(II) and Co(II). Pulse widths: 0.02, 0.03, 0.04, 0.05 and 0.06 sec.

Table 4.2 shows optimal conditions obtained from the differential pulse anodic stripping voltammograms obtained when the amplitude, sampling width, pulse period and pulse width were varied. The best optimal conditions which provide the highest response currents and well defined peaks are summarized in the table below.

Table 4.2: Optimal parameters for the simultaneous determination of Pb, Cd and Co on PAA/GCE electrode.

| PAA/GCE Electrode | Optimal Parameter Determined |
|--------------------------|-------------------------------------|
| 1. Deposition potential | -0.8V |
| 2. Accumulation time | 300seconds |
| 3. Amplitude | 0.06V |
| 4. Pulse period | 0.02seconds |
| 5. Pulse width | 0.01seconds |
| 6. Sampling width | 0.0033seconds |
| 7. pH | 6.0 |

4.3 Results of sensor validation

Sensor validation involves assessment of the performance indicators which depend on the type of the method used and the characteristic of the sensor. They require same validation studies regardless of their application. The goal is to ensure they are suitable for their intended use. Some of the sensor performance indicators include accuracy, precision, effects of impurities among others.

4.3.1 Accuracy

Accuracy of an analytical method is showing the closeness of the value accepted as conventional true value or as reference value and the measured value (Thompson *et al.*, 2002; De Bievre *et al.*, 2005; Chan *et al.*, 2004; Riley & Rosanske, 1996; ICH, 2005). The accuracy was evaluated by adding a known amount of pure active constituent to the known sample concentration, then analysing the mixture and comparison of the obtained and the expected results. The experimental results obtained and the calculated values are presented in Table 4.3.

Table 4.3: The recovery values of the added analyte from spiked standard solutions of Pb(II), Cd(II) and Co(II) on PAA/GC electrode.

| | Sample | Original (mM) | Current (A) | Added (mM) | Current (A) | Found (mM) | Recovery, % |
|----|----------------|------------------|-----------------------|---------------|-----------------------|------------|-------------|
| 1. | Sample (Pb) | 3.0 | 2.16×10^{-4} | 2.5 | 1.82×10^{-4} | 2.53 | 101% |
| | | 3.0 | 2.16×10^{-4} | 3.0 | 2.38×10^{-4} | 3.30 | 110% |
| | | 3.0 | 2.16×10^{-4} | 3.5 | 2.70×10^{-4} | 3.75 | 107% |
| 2. | Sample (Cd) | 3.0 | 2.05×10^{-4} | 2.5 | 1.76×10^{-4} | 2.57 | 103% |
| | | 3.0 | 2.05×10^{-4} | 3.0 | 2.15×10^{-4} | 3.15 | 105% |
| | | 3.0 | 2.05×10^{-4} | 3.5 | 2.38×10^{-4} | 3.48 | 100% |
| 3. | Sample (Co) | 3.0 | 2.79×10^{-5} | 2.5 | 2.60×10^{-5} | 2.79 | 93% |
| | | 3.0 | 2.79×10^{-5} | 3.0 | 2.81×10^{-5} | 3.02 | 101% |
| | | 3.0 | 2.79×10^{-5} | 3.5 | 3.40×10^{-5} | 3.66 | 104% |

Overly, the recovery degree obtained in this study were in the range of 101 to 110% for Pb(II), 100 to 105% for Cd(II) and 93 to 104% for Co(II). These lies between the imposed limits

of 90 to 110% (Thompson *et al.*, 2002; De Bièvre *et al.*, 2005; Chan *et al.*, 2004; Riley & Rosanske, 1996; ICH, 2005). This indicates that the developed sensor is sensitive enough and accurate for the determination of Pb(II), Cd(II) and Co(II) ions in real samples.

4.3.2 Precision

Precision of an analytical method is showing the closeness, matching or concordance degree of a measurement series obtained from several samples derived from the same homogeneous sample under specific conditions. Precision of the current method was considered at repeatability level only. Repeatability is obtained when test/measurement is realized in one laboratory, by one operator, using only one type of measuring equipment and only one method in a short time period. Analysis was done on 10 identical reference material of Pb(II), Cd(II) and Co(II) standard solutions. Experimental and calculated results are shown in table 4.4. The precision was found to be less than 10%. These were within the acceptable range (Thompson *et al.*, 2002; De Bievre *et al.*, 2005; Chan *et al.*, 2004; Riley & Rosanske, 1996; ICH, 2005).

Table 4.4: Experimental results and statistical calculated results obtained when for repeatability on standard samples for Pb(II), Cd(II) and Co(II) on PAA/GC electrode.

| Calculated Statistical Parameter | Pb Standard Sample | Cd Standard Sample | Co Standard Sample |
|-------------------------------------|------------------------|------------------------|------------------------|
| 1. Number of replicate Sample | 10 | 10 | 10 |
| 2. Average Value | 4.18×10^{-5} | 8.12×10^{-5} | 2.24×10^{-6} |
| 3. Standard Deviation (SD) | 3.967×10^{-6} | 5.802×10^{-6} | 1.946×10^{-7} |
| 4. RSD% | 9.0% | 7.0% | 8.7% |

4.4 Calibration

Calibration are a set of operations that are aimed at providing corrections required to make the sensor accurate at a time of their use to ensure that the sensor is giving accurate measurements. It is a comparison with a known value resulting in determining corrections and the results of the calibration may be used to adjust the sensor. Calibration of the sensor results in the determination of range of values which will be used for the comparison to correct the sensor.

Calibration curves (plot of current against analyte concentrations) were plotted to determine sensitivity (obtained from the slope of the graph) and detection limits of the sensor. Figure 4.18 shows the DPASVs measured from the PAA/GCEs for the detection of Pb(II), Cd(II) and Co(II) with the concentrations varying from 2 mM to 0.9 nM each in the acetate buffer, pH 6.0 for 300 seconds deposition time. The calibration curves for the Pb(II), Cd(II) and Co(II) are linear in the range of 125 – 7.8 μM for Pb(II), 16 – 2 μM for Cd(II), and 2 – 0.125 mM for Co(II) as shown in Figures 4.19, 4.20 and 4.21. The detection limits of Pb(II), Cd(II) and Co(II) were found to be about 0.9 nM, 1.9 nM and 11.0 μM , respectively. Detection limits depends on the background current exhibited by polyacrylic acid and importance of their estimation is to know the least amount the sensor can detect (Grennan *et al.*, 2006). The sensor showed increased sensitivity resulting from the presence of polyacrylic acid on the surface of glassy carbon electrode.

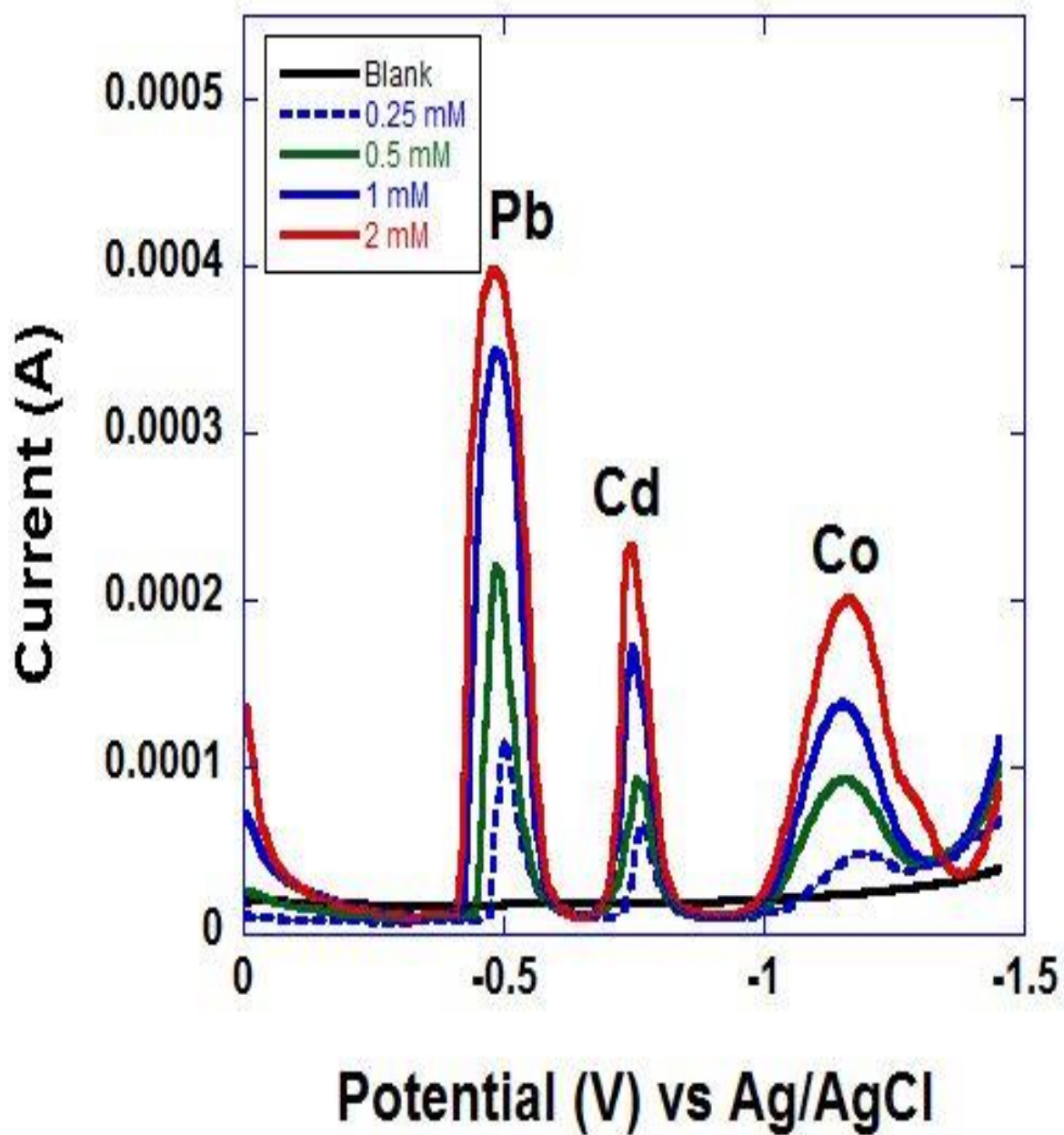


Figure 4.18: DPASVs of Cd(II), Co(II) and Pb(II) having concentrations of 2.0, 1.0, 0.5 and 0.125 mM from bottom to top, respectively, which were measured using PAA/GCE. Also respective calibration curves for the determination of Cd(II), Co(II) and Pb(II) of different concentrations are shown.

Now using the modified electrode, we took current readings from the first voltammograms in Figure 4.18 then plotted the current versus concentration. The purpose of the plot was to determine the linear range for each of the heavy metals that were detected by the developed sensor. Figure 4.19, shows the linear range for determination of Cd(II) ions

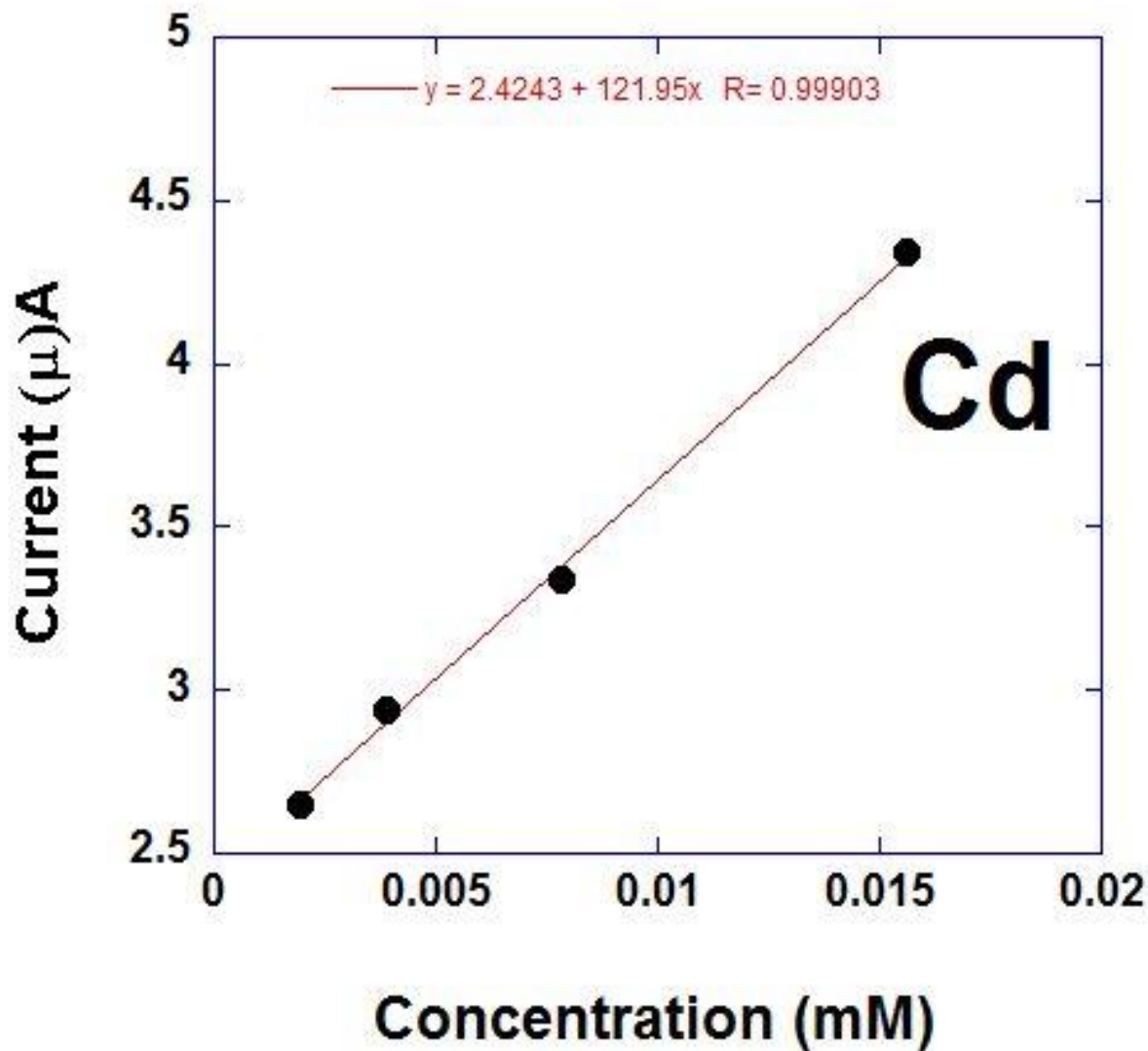


Figure 4.19: Calibration curve for the determination of Cd(II) of different concentrations.

Similarly, Figure 4.20 shows the linear range for determination of Co(II) ions.

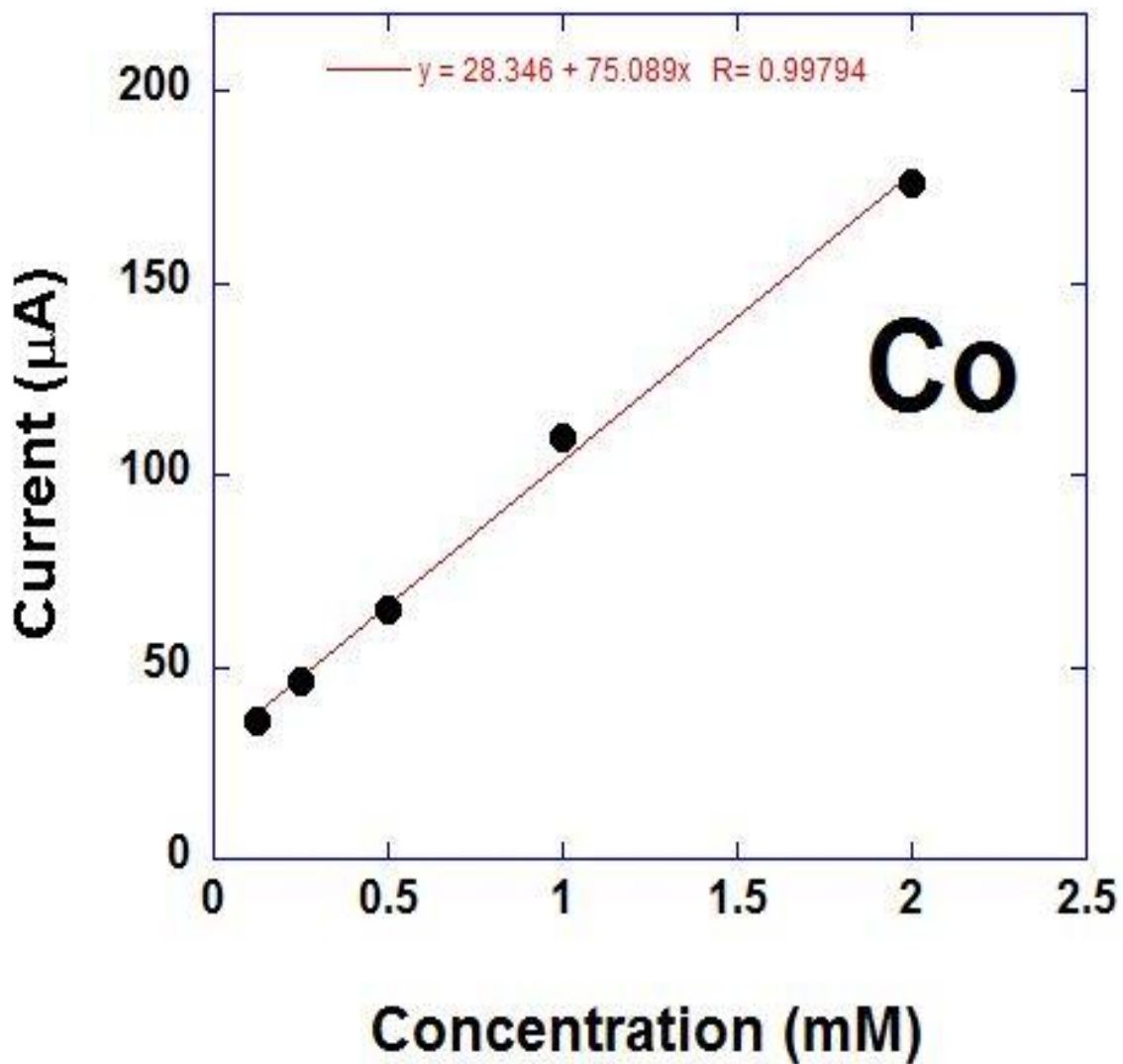


Figure 4.20: Calibration curve for the determination of Co(II) of different concentrations.

Finally, Figure 4.21 shows the linear range for determination of Pb(II) ions.

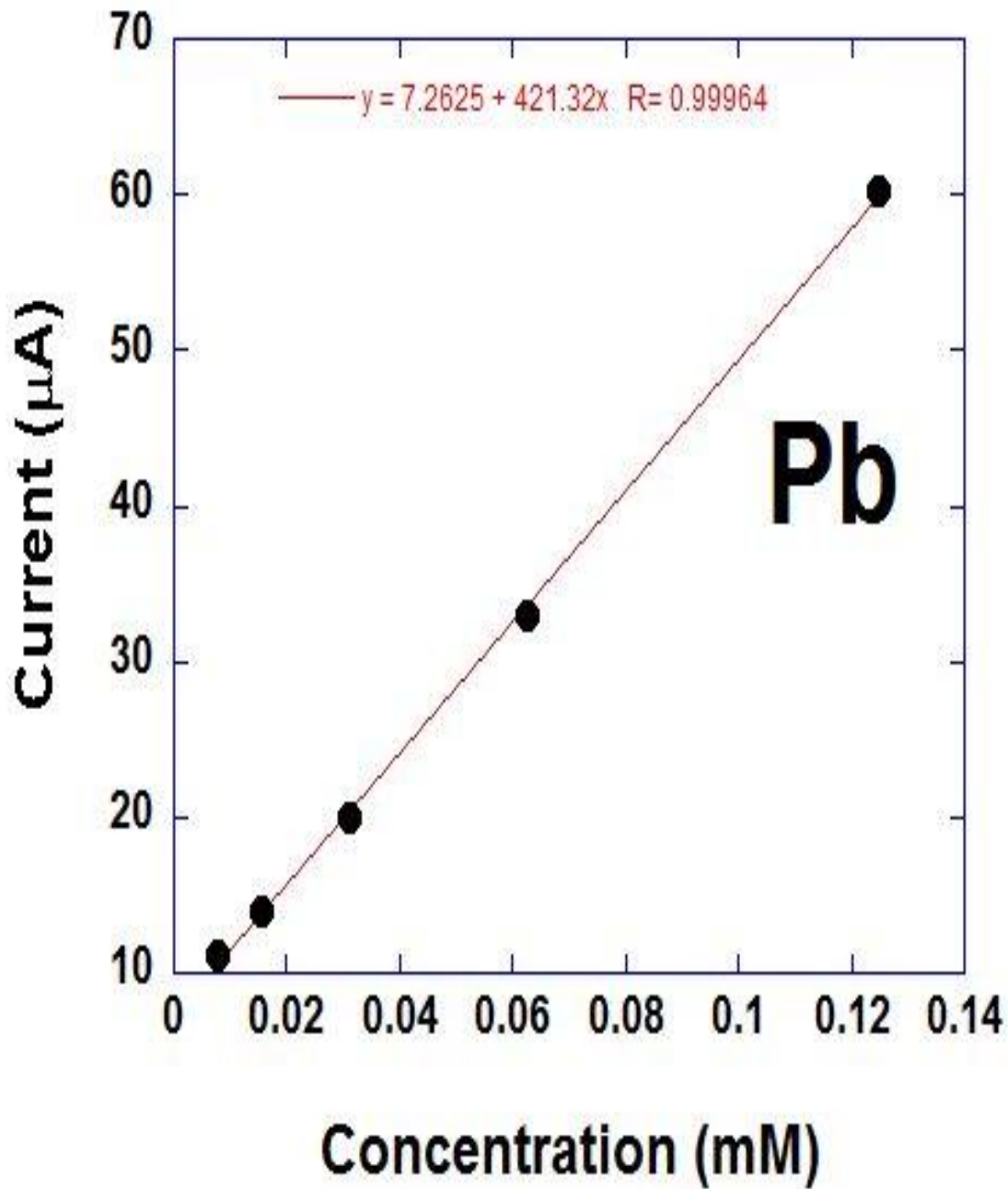


Figure 4.21: Calibration curves for the determination of Pb(II) of different concentrations.

4.4.1 Linearity

Linearity of an electroanalytical method is a measure of how well calibration plot of response versus concentration approximates a straight line. Linearity was checked by plotting a response factor (RF) versus concentration. The RF is obtained as shown in equation 4.2 (Gumustas & Ozkan, 2011).

$$RF = \frac{\text{Signal} - y^{\text{intercept}}}{\text{Concentration}} \quad (4.2)$$

The changes in the RF factor for Pb(II), Cd(II) and Co(II) were found to range from 1.5 to 2.5%. This was considered acceptable linearity (Thompson *et al.*, 2002; De Bievre *et al.*, 2005; Chan *et al.*, 2004; Riley & Rosanske, 1996; ICH, 2005). The linear range was observed to vary from one metal to another.

4.4.2 Limit of detection

Limit of detection (LOD) is the lowest concentration level that can be determined to be statistically different from a blank and with 99% confidence level. The LOD of the sensor is typically determined to be in the region where the signal to noise ratio is greater than three.

Measurements were made for the detection of Pb(II), Cd(II) and Co(II) with the concentrations varying from 2 mM to 0.1nM each in the acetate buffer, pH 6.0 for 300 sec deposition time on PAA/GC electrode. Based on three times the standard deviation of the baseline, the limits of detection were estimated for Pb(II), Cd(II) and Co(II). The limits of detection were recorded in Table 4.5. They were found to vary from one metal to another.

Table 4.5: The values of the LCR, LOD, LOQ, R² and regression equation of Pb(II), Cd(II) and Co(II) on PAA/GC electrode.

| | Analyte | LCR | LOD | LOQ | RE | R ² (N = 6) |
|----|---------|-------------|--------|---------|------------------|------------------------|
| 1. | Pb | 125 – 7.8μM | 0.9nM | 3.0 nM | y=7.2625+421.32x | 0.99964 |
| 2. | Cd | 16 – 2μM | 1.9nM | 6.3 nM | y=2.4243+121.95x | 0.99903 |
| 3. | Co | 2 – 0.125mM | 11.0μM | 36.7 μM | y=28.346+75.089x | 0.99794 |

LCR = Linear concentration range, LOD = Limit of detection, LOQ = Limit of quantitation, RE = Regression equation. NOTE: Regression equation $y = bx + a$, $y = i(\mu A)$, $x = Conc$, $b = Calibration curve slope$, $a = y\text{-intercept}$.

Limit of quantitation (LOQ) being the lowest concentration of analyte that can be measured in the sample at an acceptable level of accuracy and precision was calculated using the standard deviation of the response and the slope method expressed as shown in equation 3.5 where the voltammetric current “S” was estimated from six replicate determinations of the blank signals. The linear calibration, concentration range, linear correlation coefficient, regression equation and detection limits were investigated and summarized in Table 4.5.

4.5 Effect of impurities

Large numbers of easily oxidizable/reducible substances are normally present in most natural samples. Serious interference in metal ion determination occurs when there is competitive adsorption of the interferants into the polymer film(s) at uncontrolled concentrations. Possible interferences arise when the foreign substances reduce/oxidize at potentials close to the target ions and/or due to poor specificity of the adsorbing polymer film(s) on the electrode surface. The effects of Cu²⁺, SO₄²⁻, K⁺, Na⁺, Cl⁻, NH₄⁺ and O₂ foreign substances were tested in the determination of

Pb(II), Cd(II) and Co(II) heavy metals at concentrations exceeding 50 fold. These foreign substances were found to have insignificant effect on the stripping current responses of the target heavy metals. However, in case of trace levels of these heavy metal ions in less polluted natural samples, their interference(s) will not be of paramount consequence (Wang *et al.*, 2001).

4.6 Determination of Pb(II), Cd(II) and Co(II) in tap water sample

Tap water sample collected from our research laboratory (Nairobi city, Kenya) were used for the determination of Pb(II), Cd(II) and Co(II). The heavy metals were analyzed at the following condition: Deposition potential: -0.8 V, Accumulation time: 300 sec, amplitude: 0.06 V, pulse period: 0.02 sec, pulse width: 0.01 sec and sampling width: 0.0033 sec as shown in Figure 4.22.

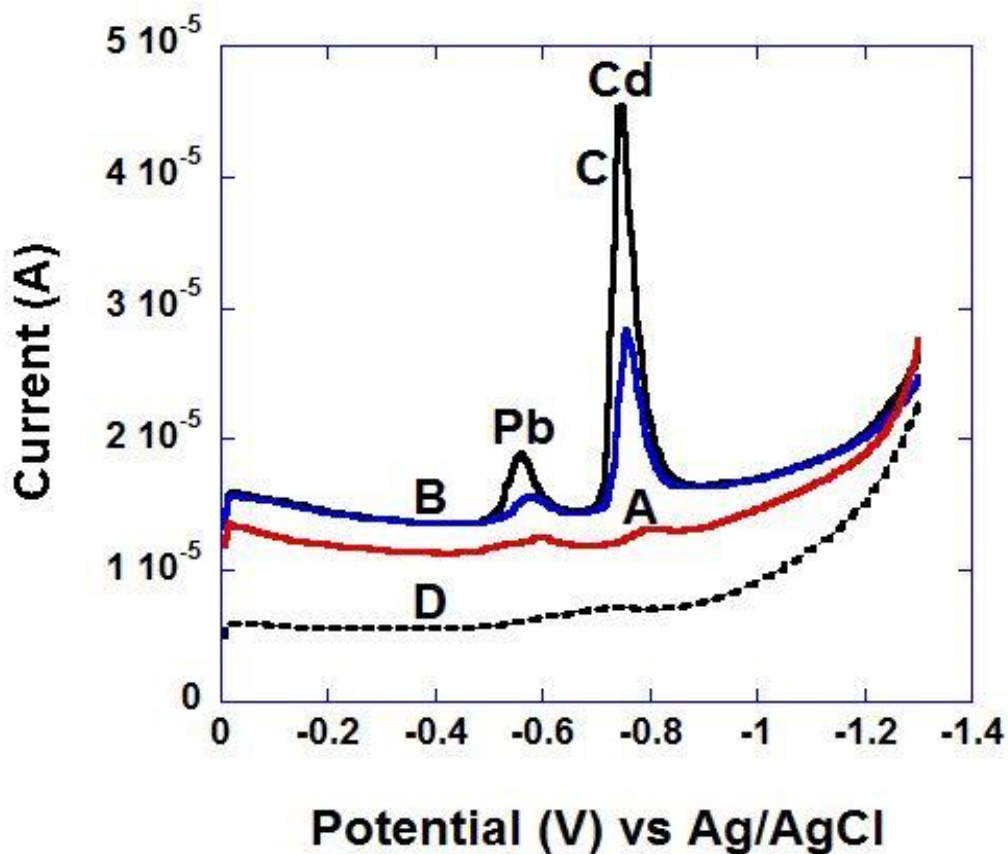


Figure 4.22: DPAS voltammograms of the Pb(II), Cd(II) and Co(II) obtained from standard addition technique (A) 3 mL acetate buffer (pH = 6.0) + 3 mL tap water (B) A + 100 μ L of 2 mM standard solution of Pb(II), Cd(II) and Co(II) (C) A + 200 μ L of 2 mM standard solution of Pb(II), Cd(II) and Co(II).

Current readings were taken from the voltammograms in Figure 4.22 and plotted against the amount (volume) of added standard solutions as shown in Figure 4.23.

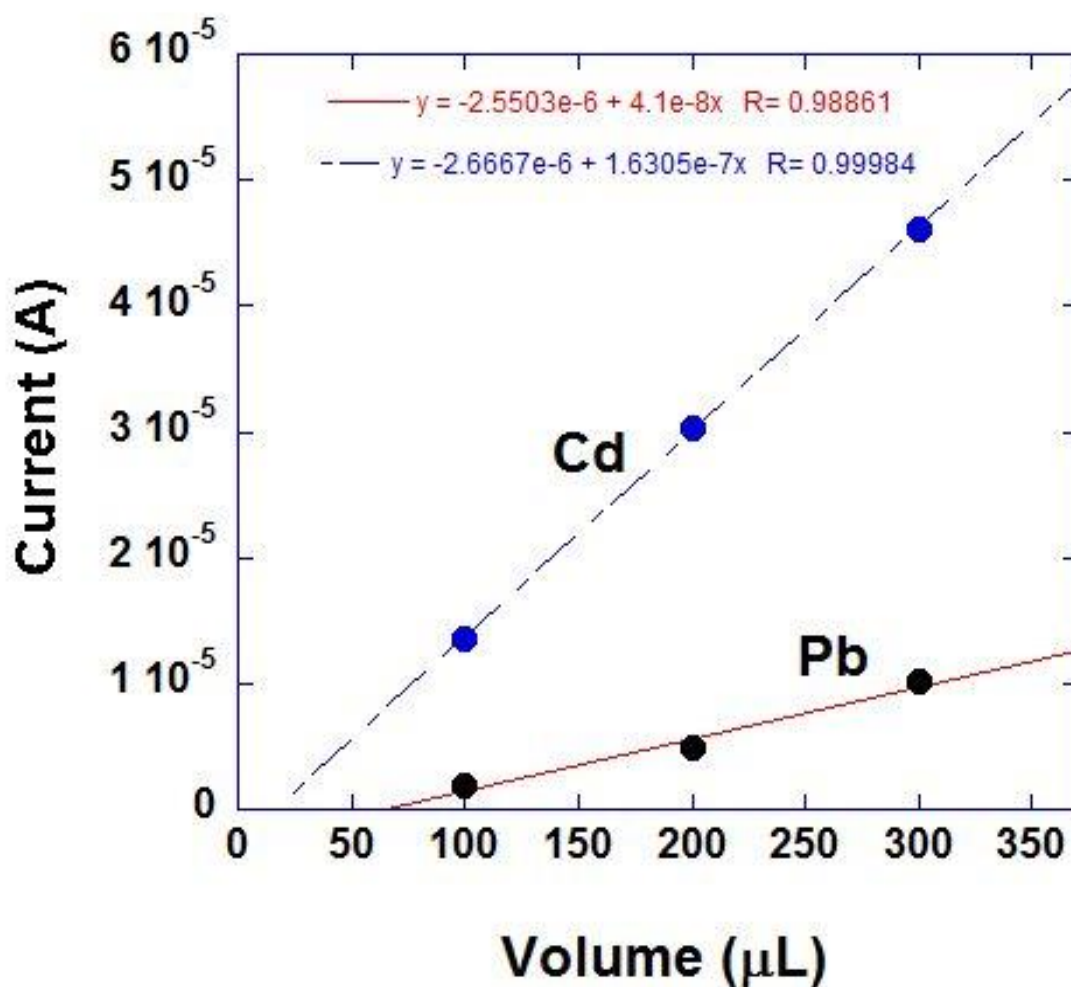


Figure 4.23: The calibration plots of Pb(II), Cd(II) and Co(II) were obtained from standard addition by DPASV technique.

As can be seen from the Figure 4.23, the current peaks of Lead and Cadmium increased by the addition of the standard solution. Under the optimal conditions established above, the concentration of Pb(II), Cd(II) and Co(II) in tap water was found to be 0.008 mg L^{-1} for Lead(II) and 0.001 mgL^{-1} for Cadmium(II). Cobalt(II) was not detected in the tap water. These values are within the limit values stipulated by WHO, EU and EPA (Table 4.6). In addition, the

concentrations of Pb(II) and Cd(II) found indicates to “the first quality water” of the tap water according to the inland water quality classification (WHO, 2003; Magdy *et al.*, 2012; Bekiari *et al.*, 2008; Li *et al.*, 2004).

Table 4.6: Pb, Cd and Co allowed limit values according to EU, WHO and EPA: (Nursel *et al.*, 2013; Yayintas *et al.*, 2007; Tang *et al.*, 2009) and the found value of Lead, Cadmium and Cobalt heavy metals in tap water of Nairobi City (Kenya)

| Heavy Metal | WHO (mg/L) | EPA (mg/L) | EU (mg/L) | Values obtained (mg/L) |
|-------------|---------------|---------------|--------------|---------------------------|
| Pb | 0.01 | 0.015 | 0.01 | 0.008±0.001 |
| Cd | 0.003 | 0.005 | 0.005 | 0.001±0.0002 |
| Co | 0.05 | - | - | Not found |

* **WHO:** World Health Organization, **EPA:** Environmental Protection Agency, and **EU:** European Union.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

A simple differential pulse anodic stripping (DPAS) voltammetric sensor based on polyacrylic acid/glassy carbon electrode (PAA/GCE) has been developed for the simultaneous determination of Pb(II), Cd(II) and Co(II) ions. Electrochemical characterization of the Polyacrylic acid/Glassy carbon electrode (PAA/GCE) using potassium ferricyanide standard revealed that the diffusion coefficients (D_0) for the PAA/GC electrode was $0.0318 \times 10^{-7} \text{ cm}^2/\text{s}$ and for GC plain electrode was $0.00122 \times 10^{-7} \text{ cm}^2/\text{s}$. The apparent electrochemical electron transfer rate constants (k^0) were $9.074 \times 10^{-3} \text{ cm/s}$ and $0.0883 \times 10^{-3} \text{ cm/s}$ for PAA/GC and GC plain electrodes respectively. Comparison of the D_0 and k^0 for the modified and unmodified electrodes indicates that modification of GC electrode with PAA led to easier and faster charge transfer at the electrode surface.

The optimal conditions for the DPAS voltammetry found were: Deposition potential: -0.8 V, Accumulation time: 300 sec, amplitude: 0.06 V, pulse period: 0.02 sec, pulse width: 0.01 sec, sampling width: 0.0033 sec and pH: 6.0.

This DPASV sensor has been validated according to ISO/IEC 17025 with respect to accuracy, precision, linearity, range and limits of detection and quantification. The detection limits of Pb(II), Cd(II) and Co(II) were found to be about 0.9 nM, 1.9 nM and 11.0 μM while limits of quantification were 3 nM, 6.3 nM and 36.7 μM respectively.

This electrochemical sensor successfully determined Pb(II), Cd(II) and Co(II) ions simultaneously in tap water. In summary, this sensor provides accurate, reliable and consistent

results for the simultaneously determination of Pb(II), Cd(II) and Co(II) heavy metals in waters and possibly other environmental and clinical samples.

5.2 Recommendations

It is recommended that further studies on the application of the sensor be expanded to heavy metals in other samples for instance wastewater, geological samples, soil samples, air samples, clinical samples and related environmental samples. Further studies are also recommended on the characterization of the Polyacrylic acid/Glassy carbon (PAA/GC) electrode with atomic force microscopy (AFM), scanning electron microscopy (SEM), tunneling electron microscopy (TEM) and Raman resonance spectroscopy (RRS). This information will be helpful in elucidating the actual mechanism by which the current film enhances the rate of electron transfer at the electrode interface. Finally, it is recommended that further studies on other types of polymers that can be employed either singly or in combinations aimed at developing a more sensitive sensor capable of detecting wide variety of pollutants.

REFERENCES

- Achterberg, E. P., & Braungardt, C. (1999). Stripping voltammetry for the determination of trace metal speciation and in-situ measurements of trace metal distributions in marine waters. *Anal. Chim. Acta*, **400**, 381–397.
- Adams, R.W. (1969). *Electrochemistry at solid Electrodes*. Marcel Dekker, New York, pp144.
- Ali, T. A., Aglan, R. F., Mohamed, G. G., Mourad, M. A. (2014). New Chemically Modified Screen-Printed Electrode for Co(II) Determination in Different Water Samples. *Int. J. Electrochem. Sci*, **9**, 1812-1826.
- Alizadeh, T., Ganjali M. R., Parviz, N., Mashaalah, Z., Mahmoud, H. (2011). A carbon paste electrode impregnated with Cd²⁺ imprinted polymer as a new and high selective electrochemical sensor for determination of ultra-trace Cd²⁺ in water samples. *Journal of Electroanalytical Chemistry*, **657**, 98–106.
- Al-Musharafi, S. K., Mahmoud, I. Y., & Al-Bahry, S. N. (2014). Environmental Contamination by Industrial Effluents and Sludge Relative to Heavy Metals. *Journal of Geoscience and Environment Protection*, **2**, 14-18.
- Amitai Y, (1987). Hazards of ‘deleading’ works of children with lead poisoning. *A merican Journal of Diseases of children*, 141,758-760.
- Analytical Method Committee, (1988). Use (Proper and Improper) of correlation coefficients. *Analyst*, **113**, 1469-1471.
- Anamul Haque, M. D., Kurokawa, T., & Gong, J. P. (2012). Super tough double network hydrogels and their application as biomaterials. *Polymer*, **53**, 1805–1822.
- Bakker, E., & Pretsch, E. (2005). Potentiometric sensors for trace-level analysis. *Trac-Trends in Analytical Chemistry*, **24**, 199 – 207.
- Bard, A. J., & Faulkner, L. R. (2004). *Electrochemical Methods: Fundamentals and Applications*. John Wiley & Sons, New York: 2nd Ed. ISBN-10: 0471043729
- Bard, A. J., and Faulkner, L. R. 1980. *Electrochemical methods-Fundamentals and Application*. John Wiley, New York and references therein. ISBN-13: 978-0471043720
- Basha, M. R. (2005). The fetal basis of amyloidogenesis, exposure to lead and latent over expression of amyloid precursor protein and amyloid in the aging brain. *Journal of Neuroscience*, **25**, 823-829.

- Bekiari, V., Sotiropoulou, M., Bokias, G., Lianos, P. (2008). Use of poly(*N, N* dimethylacrylamide *co*-sodium acrylate) hydrogel to extract cationic dyes and metals from water. *Colloids Surf. A*, **312**, 214 – 218.
- Ben, A. M., Korpan, Y., Gonchar, M., El'skaya, A., Maaref, M. A., Jaffrezic-Renault, N., Martelet, C. (2006). Formaldehyde assay by capacitance versus voltage and impedance measurements using bi-layer bio-recognition membrane. *Biosensors & Bioelectronics*, **22**, 575–581.
- Bockris, J. O., M., & Reddy, A. K. W. (1970). *Modern Electrochemistry*. Plenum, Press, New, York. Vol. 2.
- Brennan, S., (1996). Comparative Studies of organic and aqueous phase reactivities of phthalic anhydride-modified hydrogen peroxide biosensor, Ph.D Thesis, Dublin City University.
- Bruland, K.W., Donat, J.R., & Hutchins, D.A. (1991). Interactive influences of bioactive trace metals on biological production in oceanic waters. *Limnol Oceaogr*, **36**, 1555-1577.
- Bui, M., Li, C., & Han, K. (2012). Electrochemical determination of cadmium and lead on pristine single-walled carbon nanotube electrodes. *Anal. Sci*, **28**, 699-704.
- Byers, R. K., & Lord, E. E. (1943). Late effects of lead poisoning on mental development. *American Journal of Diseases of children*, **66**, 471- 494.
- Caras, S., & Janata, J. (1980). Field-effect transistor sensitive to penicillin. *Analytical Chemistry*, **52**, 1935–1937.
- Carballeira J.L., Antelo J.M., & Arce F. (2000). Analysis of the Cu²⁺-Soil Fulvic Acid Complexation by Anodic Stripping Voltammetry Using an Electrostatic Model. *Environ. Sci. Technol*, **34**, 4969 - 4973.
- Chan, C. C., Lam, H., Lee, T. C., Zhang, X. M. (2004). *Analytical Method Validation and Instrument Performance Verification*. Wiley Interscience Pub. Eds. New Jersey. pp 1-304.
- Chaubey, A., & Malhotra, B. D. (2002). Mediated biosensors. *Biosensors & Bioelectronics*, **17**, 441 - 456.
- Chen, C., Niu, X., Chai, Y., Zhao, H., Lan, M. (2013). Bismuth based porous screen-printed electrode with enhanced sensitivity for trace heavy metal detection by stripping voltammetry. *Sens Actuators B Chem*, **178**, 339 - 342.
- Chouteau, C., Dzyadevych, S., Chovelon, J. M., Durrieu, C. (2004). Development of novel conductometric biosensors based on immobilised whole cell *Chlorella vulgaris* microalgae. *Biosensors & Bioelectronics*, **19**, 1089 – 1096.

- Contractor, A. Q., Sureshkumar, T. N., Narayanan, R., Sukeerthi, S., Lal, R., Srinivasa, R. S. (1994). Conducting polymer-based biosensors. *Electrochimica Acta*, **39**, 1321–1324.
- CSTEE, (2004). Opinion on the results of the risk assessment of: cadmium metal human health and cadmium oxide human health carried out in the framework of Council Regulation (EEC) 793/93 on the evaluation and control of the risks of existing substances.
- Cullen, D. C., Sethi, R. S., & Lowe, C. R. (1990). Multianalyte miniature conductance biosensor. *Analytica Chimica Acta*, **231**, 33 – 40.
- D’Orazio, P. (2003). Biosensors in clinical chemistry. *Clinica Chimica Acta*, **334**, 41 – 69.
- De Bievre, P., & Gunzler, H. (2005). *Validation in Chemical Measurement*, Springer-Verlag pub. Heidelberg. Eds. DOI 10.1007/b138530
- EFSA, (2009). Cadmium in food. Scientific opinion of the Panel on Contaminants in the Food Chain. Question No. EFSA-Q-138. Adopted on 30 Jan 2009. *The EFSA Journal*, **980**, 1 - 139.
- Eggins, B. (2002). Chemical sensors and biosensors. *Analytical Techniques in the Sciences*. John Wiley & Sons, West Sussex. ISBN10: 0471899143
- Elamathi, S., Saravanan, N., Padmavathi, R., Sangeetha, D. (2013). Separation of Ni²⁺, Cu²⁺ and Cr⁶⁺ Metal Ions from Water by Complexation Micro Filtration Technique Using Synthetic Polymer Membranes. *Progress in nanotechnology and Nanomaterials*, **2**, 47-54.
- Elviraa, C., Manoa, J. F., San Romanb, J., Reisa, R. L. (2002). Starch-based biodegradable hydrogels with potential biomedical applications as drug delivery systems. *Biomaterials*, **23**, 1955 – 1966.
- Ensafi, A. A., & Zarei, K. (2000). Simultaneous determination of trace amounts of cadmium, nickel and cobalt in water samples by adsorptive voltammetry using ammonium 2-amino cyclopentene dithiocarboxylate as a chelating agent. *Talanta*, **52**, 435 – 440.
- Environment Agency (EA) (2009). Soil guideline values for cadmium in soil. Science report SC050021 / Cadmium SGV. Environment Agency. Bristol.
- Estevez-Hernandez, O., Naranjo-Rodriguez, I., Hidalgo-Hidalgo de Cisneros, J. L., Reguera, E. (2007). Evaluation of carbon paste electrodes modified with 1-furoylthioureas for the analysis of cadmium by differential pulse anodic stripping voltammetry. *Sensors and Actuators B*, **123**, 488 – 494.
- Feeney, R., Kounaves, S. P. (1999). *Electrochemistry Communications*, **1**, 453 – 458.

- Flanagan, S.V., Johnston R.B., Zheng, Y. (2012). Arsenic in tube well water in Bangladesh. Health and economic impacts and implications for arsenic mitigation. *Bull World Health Organ*, **90**, 839 - 846.
- Florence, T. M. and Batley, G. (1980). Scheme for classification of heavy metal species in natural waters. *Anal. Chem*, **52**, 1962 – 1963.
- Florence, T.M. (1986). Electrochemical Approaches to Trace Element Speciation in Waters: A Review. *Analyst*, **111**, 489 – 505.
- Friedberg, L., M. Piscato & Nordberg, G. (1971). Cadmium in the environment. CRC Press, Cleveland.
- Fry, A. and Britton, W.E. (1984). Laboratory techniques in Electroanalytical Chemistry, First Ed. Kissinger, P. t., Hinman, W.R. Eds., Marcel Dekker, New York, Chapter 13.
- Gerard, M., Chaubey, A., (2002). Application of conducting polymers to biosensor. *Biosensors and Bioelectronics*, **17**, 346 - 347.
- Gibson J. L. (1904). A plea for painted railings and Painted walls of rooms as the source of lead poisoning amongst queensland children. Australian medical Gazette. *Public health Reports*, **120**, 301-304.
- Gleick, P.H. (1998). The World's Water 1998-1999. *Washington, D.C*: Island Press, 319.
- Gosser, D. K. (1994). Cyclic voltammetry: Simulation and analysis of reaction mechanisms, VCH publishers, New York.
- Grady, H., Deepa G. P. and Wang, J. (2004). Electrochemical sensors for environmental monitoring: design, development and applications. *J. Environ. Monit*, **6**, 657 – 664.
- Grennan, K., Killard, A.J., Hanson, C.J., Cafolla, A.A., & Smyth, M.R., (2006). Optimization and characterization of biosensors based on polyaniline. *Talanta*, **68**, 1591-1600.
- Guha-Sapir D., Vos F., Below R., Ponserre, S. (2011). Annual Disaster Statistical Review 2010. *The Numbers and Trends*, **63**, 479-492.
- Guisseppi-Elie, A., Lingerfelt, L. (2005). Impedimetric detection of DNA hybridization: Towards near patient DNA diagnostics. *In Immobilisation of DNA on Chips I*, **260**, 161–186.
- Gumustas, M., & Ozkan, S. (2011). The Role of and the Place of Method Validation in Drug Analysis Using. Electroanalytical Techniques. *The Open Analytical Chemistry Journal*, **5**, 1-21.

- Guto, P. M. & Kamau, G. N. (2010). Electrochemical Characterization of Myoglobin-Polylysine Films at a Temperature Range of 6–80 °C. *Electroanalysis*, **22**, 1186 -1190.
- Guto, P. M., & Rusling, J. F. (2005). Enzyme-like Kinetics of Ferryl-oxo Myoglobin Formation in Films on Electrodes in Microemulsions. *J. Phys. Chem. B*, **109**, 24457-24464.
- Hafeman, D. G., Parce, J. W., McConnell, H. M. (1988). Light-addressable potentiometric sensor for biochemical systems. *Science*, **240**, 1182–1185.
- Hamilton, E.I., (1994). The geobiochemistry of cobalt. *The Science of the Total Environment*, **150**, 7-39.
- Heineman, W.R., Mark, J.A., Wise & Roston, D.A., (1984). Laboratory Techniques. In: Kissinger, P.T. and Heineman, W.R. Eds. *Electroanalytical chemistry*. New York: Decker, 499 - 538.
- Heitzmann, M., Basaez, L., Brovelli, F., Bucher, C., Limosin, D., Pereira, E., Rivas, B. L., Yal, G., Saint-Aman, E., Moutet, J. C. (2005). Voltammetric Sensing of Trace Metals at a Poly (pyrrole-malonic acid) Film Modified Carbon Electrode. *Electroanalysis*, **17**, 1970 – 1976.
- Heyrovsky, J. (1956). The development of polarographic analysis. *Analyst*, **81**, 189 – 190.
- Hirst, J., Armstrong, A. (1998). *Inorganic Chemistry Laboratory*, South Parks Road, Oxford, OX1 3QR, England. *Anal. Chem*, **70**, 5062 - 5071.
- Holm-Hansen, O., Gebloff, G.C., Skoog, F. (1954) Cobalt as an essential element for blue-green algae. *Physiologia Plantarum*, **7**, 665 - 675.
- Honeychurch, D., Hawkins, M. Hart, J. P., Cowell, D. C. (2002). Voltammetric behaviour and trace determination of copper at a mercury-free screen-printed carbon electrode. *Talanta*, **57**, 565 – 574.
- ICH Guideline Q2A (RI), Validation of Analytical Procedures: Text and methodology. (2005). Ilaria, P., Giovanna M. and Marco, M. (2001). New procedures to obtain Electrochemical Sensors for heavy metal detection. *Analytical letters*, **34**, 813 – 824.
- International Standard (ISO/IEC 17025), (2005). General requirements for the Competence of Testing and Calibration Laboratories. 2nd Edition.
- Jain, V. K., (1978). Studies on effect of cadmium on the growth. *Environ. Contam. Tox*, **67**, 489 - 495.

- Jia, J. B., Cao, L. Y., Wang, Z. H., Wang, T. X. (2008). Properties of Poly(sodium 4 styrenesulfonate)-Ionic Liquid Composite Film and Its Application in the Determination of Trace Metals Combined with Bismuth Film Electrode. *Electroanalysis*, **20**, 5542 – 549.
- Joseph, R.S., William, R.P., & Sheng, Y. (2003). Sensors, Chemical Sensors, Electrochemical Sensors, and ECS. *Journal of the Electrochemical Society*, **150**, 11-16.
- Kabata-Pendias, A., Pendias, H., (2011). Trace elements in soils and plants. Boca Raton: CRC Press, pp. 181-300.
- Karl-Heinz, Kurt, K., (2010). History of Electroanalytical methods. *Electroanalysis*, **22**, 1937-1946
- Katz, E. Willner, I. (2003). Probing biomolecular interactions at conductive and semiconductive surfaces by impedance spectroscopy: Routes to impedimetric immunosensors, DNA sensors, and enzyme biosensors. *Electroanalysis*, **15**, 913 – 947.
- Kloock, J. P., Moreno, L., Bratov, A., Huachupoma, S., Xu, J., Wagner, T....Schoning, M. J. (2006). Pld-prepared cadmium sensors based on chalcogenide glasses- isfet, laps and mu ise semiconductor structures. *Sensors and Actuators B-Chemical*, **118**, 149 – 155.
- Kounaves, S.P., O'Dea J.J., Chandrasekhar, P. & Janet, O. (1987). Correction-Square Wave Anodic Stripping Voltammetry at the Mercury Film Electrode. Theoretical Treatment. *Anal. Chem*, **59**, 1888 – 1888.
- La Pera, L., Lo Curto, S., Visco, A., La Torre, L., Dugo, G. (2002). Derivative Potentiometric Stripping Analysis (dPSA) Used for the Determination of Cadmium, Copper, Lead, and Zinc in Sicilian Olive Oils. *J. Agric. Food Chem*, **50**, 3079 - 3358.
- La Pera, L., Saitta, M., Di Bella, G., Dugo, G. (2003). Simultaneous Determination of Cd(II), Cu(II), Pb(II), and Zn(II) in Citrus Essential Oils by Derivative Potentiometric Stripping Analysis. *J. Agric. Food Chem*, **51**, 1125-1129.
- Landrigan P. J. (1989). Toxicity of lead at low dose. *British Journal of industrial medicines*, **46**, 593-596.
- Landrigan, P. J. (2002). Environmental pollutants and disease in American children; estimates of morbidity, mortality and costs of lead poisoning, asthma, cancer and developmental disabilities; *Environmental Health perspectives*, **110**, 721-728.
- Landrigan, P. J. (2007). The declaration of Brescia on Prevention of the neurotoxicity of metals. *American Journal of Industrial medicine*, **50**, 709-711.
- Laviron, E. J. (1979). General expression of the linear potential sweep voltammogram in the case of diffusionless electrochemical systems. *Electroanal. Chem*, **101**, 19 - 28.

- Lesney M. S. (2002). Today's Chemistry at Work. *American Chemical Society*, **1**, 33 - 34.
- Lezi, N., Economou, A., Dimovasilis, P.A., Trikalitis, P.N., Prodromidis, M., I. (2012). Disposable screen-printed sensors modified with bismuth precursor compounds for the rapid voltammetric screening of trace Pb(II) and Cd(II). *Anal Chim Acta*, **728**, 1 - 8.
- Li, A., Wang, A., & Chen, J. (2004). Studies on Poly(acrylic acid)/Attapulgate Superabsorbent Composites. II. Swelling Behaviors of Superabsorbent Composites in Saline Solutions and Hydrophilic Solvent–Water Mixtures. *J. Appl. Polym. Sci.*, **94**, 1869 – 1876.
- Li, J., Guo, S., Zhai, Y., Wang, E. (2009). High-sensitivity determination of lead and cadmium based on the Nafion-graphene composite film. *Anal Chim Acta*, **649**, 196-201.
- Li, J. P., Peng, T. Z., & Peng, Y. Q. (2003). A cholesterol biosensor based on entrapment of cholesterol oxidase in a silicic sol-gel matrix at a prussian blue modified electrode. *Electroanalysis*, **15**, 1031–1037.
- Li, M., Gou, H., Al-Ogaidi, I. and Wu, N. (2013). Nanostructured Sensors for Detection of Heavy Metals: A Review. *ACS Sustainable Chem. Eng*, **1**, 713–723.
- Liu, G., Chen, J., Hou., X., Huang, W. (2014). A highly-sensitive electrochemical sensor for simultaneous detection of Cd²⁺ and Pb²⁺ using liquid phase-exfoliated graphene. *Analytical methods*, **6**, 5760 - 5765.
- Liu, Y., Yuan, R., Chai, Y. Q., Tang, D. P., Dai, J. Y., Zhong, X. (2006). Direct electrochemistry of horseradish peroxidase immobilized on gold colloid/cysteine/nafion-modified platinum disk electrode. *Sensors and Actuators B-Chemical*, **115**, 109 – 115.
- Liu, Z., Miao, Y., Wang, Z., Yin, G., (2009). Synthesis and characterization of a novel super absorbent based on chemically modified pulverized wheat straw and acrylic acid. *Carbohydr. Polym.*, **77**, 131–135.
- Luppa, P. B., Sokoll, L. J., & Chan, D. W. (2005). Immunosensors - principles and applications to clinical chemistry. *Clinica Chimica Acta*, **314**, 1 – 26.
- Mabbot, G. A. (1983). *An introduction to cyclic voltammetry*. J. Chem. Edu, 697 - 701.
- Magdy, Y. A., Mohammad, S.I. M., & Tariq, R. A. S. (2012). Modification and Characterization of Polyacrylic Acid for Metal Ion Recovery. *American Journal of Polymer Science*, **2**, 73 - 78.
- March, G., Nguyen, T. D. and Piro, B. (2015). Modified Electrodes Used for Electrochemical Detection of Metal Ions in Environmental Analysis. *Biosensors*, **5**, 241-275.
- Merkoci, A. (2007). Electrochemical biosensing with nanoparticles. *Febs Journal*, **274**, 310 – 316.

- Mihaela, D. T., Igor, C., Doina, S., Corneliu, S. (2012). Electrochemical Sensors for heavy metal ions Detection from aqueous solutions. *Environmental Engineering and Management Journal*, **11**, 463 - 470.
- Mirsky, V. M., Riepl, M., & Wolfbeis, O. S. (1997). Capacitive monitoring of protein immobilization and antigen-antibody reactions on monomolecular alkylthiol films on gold electrodes. *Biosensors & Bioelectronics*, **12**, 977– 989.
- Mourzina, Y., Yoshinobu, T., Schubert, J., Luth, H., Iwasaki, H., Schoning, M. J. (2001). Ion selective light-addressable potentiometric sensor (IAPS) with chalcogenide thin film prepared by pulsed laser deposition. *Sensors and Actuators B-Chemical*, **80**, 136 – 140.
- Murray, R.W., Ewing, A.G., Durst, R., (1987) Chemically Modified Electrodes: Molecular Design for Electroanalysis. *Anal. Chem*, **59**, 379-390.
- Nair, P., Alam, M. (2007). Dimensionally frustrated diffusion towards fractal adsorbers. *Physical Review Letters*, **99**, 256101- 256106.
- Nandini, S., D. J. Chaparro-Herrera, CaRdenar- Arriola, Sava Leticia (2007). Population growth of *Brachionus macracanthus* (Rotifera) in relation to cadmium toxicity; influence on algal (*Chlorella vulgaris*) density. Part A, Toxic/Hazardous Substances and Environmental engineering, *Journal of Environmental Science and Health*, **42**, 1467-1472.
- Nandini, S., E. A. Picazo-paez and S.S.S Sarma (2007). The combined effects of heavy metals (copper and Zinc), temperature and food (*Chlorella vulgaris*) level on the demographic characters of *Moina macrocopia* (Crustaca cladocera OU). Part A. toxic/Hazardous Substances and Environmental Engineering, *Journal of environmental science and health*, **42**, 1433-1442.
- Nourifard, F., Payehghadr, M., Kalhor, M., Nejadali, A. (2015). An Electrochemical Sensor for Determination of Ultratrace Cd, Cu and Hg in Water Samples by Modified Carbon Paste Electrode Base on a New Schiff Base Ligand. *Electroanalysis*, **27**, 2479-2485.
- Nursel, P., G€uner, A., G€uven, O. (2002). Conductometric and Viscometric Investigation of Poly (*N*-Vinylimidazole)–Metal Ion Complex Formation. *J. Appl. Polym. Sci.*, **85**, 376-384.
- Olsen, K., Wang, J., Setiadji, R., Lu, J. (1994). Field Screening of Chromium, Cadmium, Zinc, Copper, and Lead in Sediments by Stripping Analysis. *Environ. Sci. Technol*, **28**, 2074 - 2079.
- Onyatta, J. O, Huang, P. M. (1999). Chemical speciation and bioavailability index of cadmium for selected tropical soils in Kenya. *Geoderma*, **91**, 87-107.

- Orata, D. O., Matheka, A. and Munge, B. (1994). Quantitative Aspects of Charge Transfer in polyaniline during its electrodeposition and electrochemical degradation. *Macromol. Chem. Phys.*, **195**, 3002-3007.
- OSHA (Occupational Health and Safety Administration), (1992). Occupational exposure to cadmium. Department of Labour, 42102 - 42427.
- Otake, M., Schull, W. (1984). Mental retardation in children exposed in utero to the atomic bombs: A reassessment. Technical Report RERF TR 1-83, Radiation Effects Research Foundation, Japan.
- Park, S. J., Taton, T. A., Mirkin, C. A. (2002). Array-based electrical detection of DNA with nanoparticle probes. *Science*, **295**, 1503 – 1506.
- Patolsky, F., Zheng, G. F., Lieber, C. M. (2006). Nanowire-based biosensors. *Anal. Chem.*, **78**, 4260 – 4269.
- Pei, R. J., Cheng, Z. L., Wang, E. K., Yang, X. R. (2001). Amplification of antigen-antibody interactions based on biotin labeled protein-streptavidin network complex using impedance spectroscopy. *Biosensors & Bioelectronics*, **16**, 355 – 361.
- Pilsner, J. R. (2009). Influence of prenatal lead exposure on genomic methylation of cord blood DNA. *Environmental Health perspectives*, **117**, 1466 - 1471.
- Poghossian, A., Yoshinobu, T., Simonis, A., Ecken, H., Luth, H., Schoning, M. J. (2001). Penicillin detection by means of field-effect based sensors: ENFET, capacitive EIS sensor or IAPS? *Sensors and Actuators B-Chemical*, **78**, 237 – 242.
- Pokpas, K., Zbeda, S., Nazeem J., Mohamed, N., Baker, G., P. Iwuoha I., E. (2014). Electrochemically Reduced Graphene Oxide Pencil-Graphite in situ Plated Bismuth-film Electrode for the Determination of Trace Metals by Anodic Stripping Voltammetry. *Int. J. Electrochem. Sci.*, **9**, 736 – 759.
- Popovtzer, R. and Neufeld, T. (2006). Electrochemical detection of biological reactions using novel nano-bio-chip array. *Sensors and Actuators*, **119**, 664 - 665.
- Pujol, L., Evrard, D., Groenen-Serrano, K., Freyssinier, M., Ruffien-Cizsak, A., Gros, P. (2014). Electrochemical sensors and devices for heavy metal assay in Water: the French group's contribution. *Frontiers in Chemistry*, **2**, 1-24.
- Rico MAG, Olivares-Marín, M., Gil EP (2009). Modification of carbon screen-printed electrodes by adsorption of chemically synthesized Bi nanoparticles for the voltammetric stripping detection of Zn(II), Cd(II) and Pb(II). *Talanta*, **80**, 63 - 635.
- Riley, C. M., Rosanske, T. W. (1996). Development and Validation of Analytical Methods. Pergamon Press; Eds. Oxford.

- Riman, D., Jirovsky, D., Hrbac, J., Prodromidis, MI. (2015). Green and facile electrode modification by spark discharge: bismuth oxide-screen printed electrodes for the screening of ultra-trace Cd(II) and Pb(II). *Electrochem Commun.*, **50**, 20-23.
- Rosner, D. and Markowitz, G. (1985). A gift of God? The public health controversy over leaded gasoline during the 1920s. *American Journal of Public Health* **75**, 344-352.
- Sánchez, A., Morante-Zarcelero, S., Pérez-Quintanilla, D., Sierra, I., del Hierro, I. (2010). Development of Screen-Printed Carbon Electrodes Modified with Functionalized Mesoporous Silica Nanoparticles: Application to Voltammetric Stripping Determination of Pb(II) in Nonpretreated Natural Waters. *Electrochimica Acta*, **55**, 6983 - 6990.
- Santandreu, M., Alegret, S., Fabregas, E. (1999). Determination of beta-hcg using amperometric immunosensors based on a conducting immunocomposite. *Analytica Chimica Acta*, **396**, 181–188.
- Shilu, T. Yasmin, E. S., & Tippawan. P., (2000). Environmental lead exposure: a public health problem of global dimensions. *Bulletin of the world Health organization*, **9**, 78.
- Skoog, D. A., West, D. M., & Holler, F. J. (1992). *Fundamentals of Analytical Chemistry*. Fort Worth: Saunders College Publishing, 100-200.
- Somerset, V., Leaner, J., Mason, R., Iwuoha, E., Morrin, A. (2010) Determination of inorganic mercury using a polyaniline and polyaniline-methylene blue coated screen-printed carbon electrode. *International Journal of Environmental Analytical Chemistry*, **90**, 480-482.
- Sosa, V., Serrano, N., Ariño, C., Díaz-Cruz, JM., Esteban, M. (2014). Sputtered bismuth screen printed electrode: a promising alternative to other bismuth modifications in the voltammetric determination of Cd(II) and Pb(II) ions in groundwater. *Talanta*, **119**, 348-352.
- Stadler, B., Solak, H. H., Frerker, S., Bonroy, K., Frederix, F., Voros, J., Grandin, H. M. (2007). Nanopatterning of gold colloids for label-free biosensing. *Nanotechnology*, **18**, 1- 6.
- Stein, B., George, M., Gaub, H. E., Parak, W. J. (2004). Extracellular measurements of averaged ionic currents with the light-addressable potentiometric sensor (laps). *Sensors and Actuators B-Chemical*, **98**, 299–304.
- Stephen, D., Brown Bruce R., K., (1980). Correction-Pseudopolarographic Determination of Metal Complex Stability Constants in Dilute Solution by Rapid Scan Anodic Stripping Voltammetry. *Anal. Chem*, **52**, 2240 – 2240.

- Tang, Q., Wu, J., Sun, H., Fan, S., Hu, D., Lin. (2009). Synthesis of Polyacrylate/Poly (ethylene glycol) Hydrogel and its Absorption Properties for Heavy Metal Ions and Dye. *J. Polym. Compos*, **30**, 1183 – 1189.
- Tercier, M.-L. & Buffle, J. (1993). In situ voltammetric measurements in natural waters: future prospects and challenges. *Electroanalysis*, **5**, 187–200.
- Thevenot, D. R., Toth, K., Durst, R. A., Wilson, G. S. (2001). Electrochemical biosensors: recommended definitions and classification. *Biosensors & Bioelectronics*, **16**, 121–131.
- Thompson, M., Ellison, S. L. R., & Wood, R. (2002). Harmonized Guidelines for Single Laboratory Validation of Methods of Analysis (AUPAC technical Report). *Pure Appl. Chem*, **74**, 835-855.
- Tian, Y.Q., Li, N.B., & Luo, H.Q. (2009). Simultaneous determination of trace zinc(II) and cadmium(II) by differential pulse anodic stripping voltammetry using a MWCNTs-NaDBS modified stannum film electrode. *Electroanalysis*, **21**, 2584-2589.
- Tsuchiya, K., M. Sugita and Y. Seki (1972). Proceedings of the 17th International Congress on Occupational Health.
- Wanekaya, A. K., Chen, W., Myung, N. V., Mulchandani, A. (2006). Nanowire-based electrochemical biosensors. *Electroanalysis*, **18**, 533–550.
- Wang, J., (2006). Electrochemical biosensors towards point-of-care cancer diagnostics. *Biosensors and Bioelectronics*, **21**, 1889-1891.
- Wang, J. (1991). Modified Electrodes for Electrochemical Sensors. *Electroanalysis*, **3**, 255-255.
- Wang, J., (1985). Stripping analysis: Principles, applications, instrumentations and applications. *VCH Publishers*, **98**, 764-764.
- Wang, J., Grundler, P., Flechsig, G-U., Jasinski, M., Rivas, G., Sahlin, E., Lopez P.L.J. (2000). Stripping Analysis of Nucleic Acids at a Heated Carbon Paste Electrode. *Anal. Chem*, **72**, 3752-3756.
- Wang, J., Lu, J. M., Kirgoz, U. A., Hocevar, S. B., Ogorevc, B. (2001). Insights into the anodic stripping voltammetric behavior of bismuth film electrodes, *Anal. Chim. Acta*, **434**, 29 – 34.
- WHO, (2010). World Health Organization Report, Childhood lead Poisoning, WHO Document production services, Geneva. ISBN: 9789241500333.
- WHO, (2012). Weekly Epidemiological Report. Cholera annual report 2011. *Cholera articles*, **87**, 289-304.

- Willard, H. H., L.L. Merrit, J. A. Dean, and F. A. Settle, (1986). *Instrumental methods of analysis*, 6th edition, CBS, Delhi, India. ISBN 10: 8123909438.
- Win, S., Lee Jean C. M. (1981). Determination of total arsenic species by anodic stripping voltammetry. *Anal. Chem.*, **53**, 130 – 131.
- Wu, J. (2008). Alzheimer's disease (AD) like pathology in aged monkeys after infertile exposure to environmental metal lead. Evidence for the developmental origin and environmental link for AD. *Journal of Neuroscience*, **28**, 3-9.
- Xu, G., Ye, X., Qin, L., Xu, Y., Li, Y., Li, R., Wang, P. (2005). Addressable potentiometric sensors for single cell monitoring. *Biosensors & Bioelectronics*, **20**, 1757-1763.
- Xu, G., Ye, X., Qin, L., Xu, Y., Li, Y., Li, R., Wang, P. (2008). Cell-based biosensors based on light. *Sensors*, **8**, 1442-1448.
- Xu, R., Yu, X., Gao, C., Jiang, Y., Han, D., Liu, J. (2013). Non-conductive Nanomaterial Enhanced Electrochemical Response in Stripping Voltammetry: The Use of Nanostructured Magnesium Silicate Hollow Spheres for Heavy Metal Ions Detection. *Analytical Chimica Acta*, **790**, 31-38.
- Yagiuda, K., Hemmi, A., Ito, S., Asano, Y., Fushinuki, Y., Chen, C. Y., Karube, I. (1996). Development of a conductivity-based immunosensor for sensitive detection of methamphetamine (stimulant drug) in human urine. *Biosensors & Bioelectronics*, **11**, 703-707.
- Yayintas, O., Yılmaz, S., Turkoglu, M., Dilgin, Y. (2007). Determination of heavy metal pollution with environmental physicochemical parameters in waste water of Kocabas Stream (Biga, Canakkale, Turkey) by ICP-AES. *Environ Monit Assess*, **127**, 389–397.
- Zhao, Changzhi, Liu, Hui, Wang, Li. (2012). Simultaneous determination of Pb(II) and Cd(II) using an electrode modified with electropolymerized thiadiazole film. *Anal. Methods*, **4**, 3586-3592.
- Zen, J., Yang, C., & Kumar, A. (2002). Voltammetric behavior and trace determination of Pb²⁺ at a mercury-free screen-printed silver electrode” *Anal. Chim. Acta*, **464**, 229–235.

Internent References

- ATSDR, (2008). Toxicological Profile for Cadmium. Draft for public comment. US Department of Health and Human Services. Atlanta, US. <https://www.atsdr.cdc.gov/toxfaqs/tf.asp?id=47&tid=15>. Link valid on 24th April 2016.

- EPA, (2002). Child specific exposure factors handbook (interim report). Washington, DC, National Center for Environmental Assessment. <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=55145>. Link valid on 25th November 2013.
- EPA, (2013). Environmental Protection Agency report, Earth Day 2013. *Water statistics*. <http://www.afcec.af.mil/shared/media/document/AFD-130322-056.pdf>, Link valid on 4th March 2014.
- IARC, (1992). Cadmium in the human environment: toxicity and carcinogenicity. IARC Scientific publications. No 118. Lyon. <https://monographs.iarc.fr/ENG/Monographs/vol58/mono58-7F.pdf> Link valid on 4th October 2016.
- IARC, (1993). Beryllium, cadmium, mercury and exposures in the glass manufacturing industry. IARC monographs on the evaluation of carcinogenic risk to humans, vol 58. Lyon. the report was extracted from the website below <http://monographs.iarc.fr/htdocs/monographs/vol58/mono58-2.htm> Link valid on 4th October 2016.
- UNDP, (2006). United Nations Development Programme, Human Development Report 2006. Beyond Scarcity: Power, poverty and the global water crisis. Link valid on 5th October 2013.
- UNEP, (2008). Target 2008; global elimination of leaded petrol. NAIROBI, United Nations Environmental Programme (http://www.unep.org/pcfr/PDF/lead_Report-Brochure.pdf), Link valid on 5th October 2013.
- UN-Water, (2009). World Water Day brochure, <http://www.unwater.org/worlwater/downloads> Varma, M.M., Sardandy, S.C., Katz H.M. (1976) Environ. Health, 30, 90-100. Link valid on 4th March 2014.
- Validation of Analytical Methods, (2010). Agilent Technologies, Publication Number 5990 5140EN. Official Website: www.agilent.com/Chem/. Link valid on 4th March 2014.
- WHO, (2003). International year of fresh water. *General Assembly Resolution A/RES/55/196*. Official website: www.wateryear2003.org. Link valid on 16th December 2013.
- WHO, (2009). Global health risks; mortality and burden of disease attributable to selected major risks. Generally, world Health Organization report extracted from the website indicated below (https://whqliboc.who.int/publications/2009/9789241563871_eng.pdf). Link valid on 16th December 2013.