

SCHOOL OF MATERIALS AND MINERAL RESOURCES ENGINEERING
UNIVERSITI SAINS MALAYSIA

DEVELOPMENT OF BISMUTH PARTICLES MODIFIED ELECTRODES
FOR HEAVY METAL SENSORS

By

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DECLARATION

I hereby declare that I have conducted, completed the research work and written the dissertation entitled: "Development of Bismuth Particles Modified Electrodes for Heavy Metal Sensors". I also declare that it has not been previously submitted for the award of any degree or diploma or other similar title of this for any other examining body of University.

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LIST OF ABBREVIATIONS

AAS	Atomic absorption spectroscopy
AC	Alternating current
ALA	Aminolevulinic acid
ALAD	Aminolevulinic acid dehydratase
ASV	Anodic stripping voltammetry
ATSDR	Agency for Toxic Substances and Disease Registry
Bi	Bismuth
BiFEs	Bismuth film electrodes
CDC	Centers for Disease Control and Prevention
CE	Counter electrode
CV	Cyclic voltammetry
DNA	Deoxyribonucleic acid
DPASV	Differential pulse anodic stripping voltammetry
DPCSV	Differentia pulse cathodic stripping voltammetry
DTPA	Diethylenetriaminepentaacetic acid
ECS	Electrochemical sensor
emf	Electromotive force
EQS	Environmental Quality Standards
FAAS	Flameless atomic absorption spectrophotometry
FAO	Food and Agricultural Organization
FET	Field-effect transistor
FRET	Forster resonance energy transfer

FWHM	Full Width at Half Maximum
GCE	Glassy carbon electrode
GR	Glutathione reductase
HDME	Hanging drop mercury electrode
HM	Heavy metal
HMIIs	Heavy metal ions
ICP	Inductively coupled plasma
ICP-MS	Inductively coupled plasma mass spectrometry
ISE	Ion-selective electrode
IQ	Intelligence quotient
ISO	International Organization for Standardization
ITO	Indium tin oxide
ITO-PET	Indium tin oxide coated polyethylene terephthalate
LOD	Limit of Detection
LSV	Linear Sweep Voltammetry
MFEs	Mercury Film Electrodes
MMR	Mismatch repair
MNPs	Metal nanoparticles
MS	Mass spectroscopy
NMOFs	Nanometal organic frameworks
NPs	Nanoparticles
ppb	Parts per billion
ppm	Parts per million
PSS	poly(sodium 4-styrenesulfonate)
PVP	poly(vinyl pyrrolidone)

QDs	Quantum dots
RE	Reference electrode
RGO	Reduced graphene oxide
SCE	Saturated calomel electrode
SEM	Scanning Electron Microscopy
SIA-ASV	Sequential injection analysis-anodic stripping voltammetry
SOD	Superoxide dismutase
SPE	Screen Printed Electrode
SWASV	Square-wave anodic stripping voltammetry
SWV	Square wave voltammetry
TEM	Transmission Electron Microscope
USEPA	United States Environmental Protection Agency
UV-Vis	Ultraviolet visible
vs.	Versus
WE	Working electrode
WHO	World Health Organization
XPS	X-ray fluorescence spectroscopy
XRD	X-Ray Diffraction
ZELA	Zimbabwe Environmental Law Association

LIST OF SYMBOLS

A	Area
As	Arsenic
Cd	Cadmium
Cr	Chromium
cm	Centimetre
cm ²	Centimetre square
Cu	Copper
°C	Degree Celsius
D	Diffusion coefficient
e ⁻	Electron
g	Gram
g/mol	Gram per Mole
g/L	Gram per Litre
h	Hour
Fe	Iron
K α	K-alpha wavelength
kV	Kilovolt
λ	Lambda
Pb	Lead
Mn	Manganese
MHz	Megahertz
Hg	Mercury

mg/L	Milligram per Litre
mL	Millilitre
ms	Millisecond
mM	Millimolar
min	Minute
μ A	Microampere
μ g/L	Microgram per Litre
M	Molarity
mol/L	Mole per Litre
Mw	Molecular weight
nM	Nanomolar
nm	Nanometre
Ni	Nickel
n	Number of electron
%	Percentage
R-3m	Rhombohedral structure space group
s	Second
v	Scan rate
θ	Theta
V	Voltage
V/s	Voltage per second
W	Watt
wt. %	Weight percent
Zn	Zinc

PENGHASILAN ELEKTROD DIUBAHSUAI PARTIKEL BISMUT UNTUK APLIKASI PENGESAN LOGAM BERAT

ABSTRAK

Dalam kajian ini, partikel bismut (Bi) dan pertumbuhan partikel bismut terus pada elektrod indium timah oksida (ITO) telah dihasilkan dengan menggunakan kaedah hidroterma. Partikel Bi yang telah dihasilkan juga didepositkan di atas substrat polietilena terephthalat yang disadur dengan indium timah oksida (ITO-PET). Kesan masa tindak balas hidrothermal telah dikaji (5, 6, 7 and 9 jam). Pencirian partikel Bi dan substrat indium timah oksida yang diubahsuai dengan partikel bismut (Bi/ITO) telah dijalankan dengan menggunakan Pembelau Sinar-X (XRD) dan mikroskop imbasan elektron (SEM). Analisa XRD menunjukkan Bi tulen dengan struktur Rombohedron daripada kumpulan ruang R-3m (Nombor kad JCPDS: 00-005-0519) telah dihasilkan bagi semua sampel. Bagi elektrod Bi/ITO, analisa XRD menunjukkan kewujudan Bi tulen dengan struktur Rombohedron daripada kumpulan ruang R-3m (Nombor kad JCPDS: 00-005-0519) dan bismut oksida (Bi_2O_3) dengan struktur hablur monoklinik daripada kumpulan ruang P21/c. Keputusan kajian ini dibuktikan dengan mikroskop imbasan elektron yang menunjukkan partikel Bi hitam dan partikel Bi_2O_3 putih. Partikel Bi yang dihasilkan dengan perubahan tindak balas hidroterma mempunyai saiz antara 76 nm hingga 224 nm. Kemudian, partikel Bi dititis di atas permukaan substrat ITO-PET sebelum dianalisa dengan voltametri berkitar dan pengkamiran nadi – anodik pelucutan voltametri. Kawasan permukaan berkesan elektrod ITO-PET adalah lebih besar daripada kawasan permukaan berkesan elektrod ITO. Had pengesanan (LOD) adalah sebanyak $12.29 \mu\text{g/L}$ bagi Pb(II). Pb(II) telah dikesan pada voltan -0.736 V di dalam larutan piawai pelbagai unsur bagi kajian penggangguan.

DEVELOPMENT OF BISMUTH PARTICLES MODIFIED ELECTRODES FOR HEAVY METAL SENSORS

ABSTRACT

In this study, bismuth (Bi) particles were synthesised and grew directly on Indium Tin Oxide (ITO) electrode using the hydrothermal method. The synthesised Bi particles were deposited on indium tin oxide coated polyethylene terephthalate (ITO-PET) electrodes. The effect of hydrothermal reaction period was studied (5, 6, 7 and 8 hours). Characterisation of Bi particles were performed using X-ray Diffractometer (XRD) and Field Emission Scanning Electron Microscopy (FESEM). The properties of bismuth modified ITO electrodes (Bi/ITO) were characterised using XRD and Tabletop SEM. XRD analysis of Bi particles showed that Bi with Rhomboheral structure of R-3m space group (JCPDS card no.: 00-005-0519) were obtained for all samples. For Bi/ITO, the XRD results showed the present of Bi with Rhomboheral structure of R-3m space group (JCPDS card no.: 00-005-0519) and bismuth oxide (Bi_2O_3) of monoclinic crystal of P21/c space group (JCPDS card no.: 00-014-0699). The results were further proven by Tabletop SEM which showed the presence of black Bi particles and white Bi_2O_3 particles. The size of the synthesised Bi powders with varying hydrothermal reaction period were in the range of 76 nm to 224 nm. The synthesised Bi particles were then drop casted on ITO-PET electrode before subjected to cyclic voltammetry (CV) and differential pulse anodic stripping voltammetry (DPASV) analysis. The effective surface area of Bi/ ITO-PET electrode is larger than effective surface area of Bi/ITO, thus giving higher sensitivity. The limit of detection of Pb(II) was 12.29 $\mu\text{g/L}$. Pb(II) was detected at potential of -0.736 V in multi-element standard solution for interference studies.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Heavy metals are defined as naturally occurring metallic elements that have comparatively high density compared to water and is dangerous even though at very small quantity (Tchounwou et al., 2012). Heavy metals remain persistent in the environment once released. In even worse case, heavy metals continue to be virulent for as long as they exist (Bauer and Velde, 2014). Some basic metals such as iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) are required for most of the living things for growth. However, when exceeding the standard limits, all these metals give detrimental effects. The nonessential metals such as arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg) and nickel (Ni) are toxic even at relatively low concentration. Moreover, they are not important for metabolic activities (Hossen et al., 2015).

In recent years, there has been increasing global public health concern associated with environmental pollution by heavy metals. Environmental pollution can occur through metal corrosion, atmospheric deposition, soil erosion of metal ions and leaching of heavy metals, sediment re-suspension and metal evaporation from water resources to soil and ground water (Tchounwou et al., 2012). Wastewater which contain heavy metals indirectly functions as the medium for transportation of pollutants and gives serious negative impact to environment as well as living things (Fu and Wang, 2011). Table 1.1 shows some examples of major heavy metal disasters. In these heavy metal disasters, water was the transport medium of heavy metal ions and the disasters had taken many lives as a result of heavy metal poisoning.

Table 1.1: Examples of heavy metal disasters in the world.

Year	Location	Incident	Reference
1932	Minamata, Japan	Chisso Corporation released waste product of acetaldehyde manufacturing that contained mercury into Minamata Bay. The people nearby suffered from neurological disorder caused by mercury poisoning.	Juan, 2006
1952–1966	Hinkley, England	Water containing chromium (VI) which is used for corrosion resistance in cooling water system was discharged by Pacific Gas and Electric into unlined ponds, ultimately contaminating the groundwater which then supplied to the residents. The contaminant caused sickness and increased the occurrence of breast cancer, Hodgkin's lymphoma, miscarriages, kidney and ovarian tumours, and lung, brain and gastrointestinal cancers to the community.	Jacobs, 2011
1986	Sandoz, Switzerland	A fire at the agrochemical storehouse in an industrial complex had released toxic pesticides into the air and the Rhine river. An estimate of at least 20 tonnes of toxic agrochemicals together with mercury flown into the river and travelled more than 400 km downstream, killed around half a million fish and virtually wiping out the river's European eel population.	Capel and Giger, 1988
2012	Mutare, Zimbabwe	Wastewater from diamond mining was dumped into the river water, causing high levels of chromium, nickel, iron, fluoride and bacteria content in the river. In the area, children have developed rashes all over their bodies. Other alarmingly built up health threat in that area are cancer, dental and skeletal fluorosis, iron poisoning, and waterborne diseases like cholera, diarrhoea and typhoid.	ZELA, 2012; Chiketo, 2012

Unlike organic contaminants, heavy metals are dangerous because they tend to bioaccumulate in the environment (Kuyvenhoven, 2014). Bioaccumulation implies the concentration of a chemical in a body increases with time, as compared to the its concentration in the environment. These chemicals escalate in the body once they are taken up and accumulate very rapidly, exceeding the rate they are decomposed (Kumar et al., 2017). These heavy metals go in water system through industrial and residential waste, then discharge into ponds, streams, and groundwater, entering human bodies through major routes such as air, food and drinking water.

The three most commonly found heavy metal pollutants are lead, cadmium, and mercury (Sloan et al., 2017). Mercury is highly toxic in its elemental form and is a known neurotoxin, while lead and cadmium are dangerous in their cation forms as ions are soluble in water and hence can be transported easily in the body. Lead is associated with neurological damage and intellectual disability, particularly among children, whereas cadmium is related with heart disease, high blood pressure, and lungs and prostate cancers. Other heavy metal such as arsenic may cause skin and other types of cancer (Kuyvenhoven, 2014). All these things happen because metal ions exhibit great affinity to thiol (sulfhydryl) groups that are found in proteins and enzymes (Dillon, 2017). These metal ions bind to the enzymes, preventing them from working properly. This leads to the improper functioning of the metabolic pathways and results in genetic disorders such as dementia, Parkinson's disease and overweight. All these disorders have been observed and detected as a result of exposure to numerous metal toxicants (Han et al., 2013).

Heavy metal pollution is transported globally in the atmosphere and in water. Generally, negative impacts on human health and environment associated with trace metal contamination have been well highlighted. Thus, it is of paramount importance to detect these heavy metal ions in the environment as well as determine their

concentration. As a result, several international organizations like World Health Organization (WHO), Joint Food and Agricultural Organization of the United Nations (FAO), the United States Environmental Protection Agency (USEPA), Centers for Disease Control and Prevention (CDC) and the European Union have considered heavy metals as the priority substances that need to be monitored tightly. They have set certain permissible limits for heavy metal concentrations in water following the environmental quality standards (EQS). This is not just to protect aquatic life and drinking water but also to minimize the health effects to human, caused by heavy metal pollution (Bansod et al., 2017). Although such guidelines are indispensable for public health shield, issues can rise when guidelines are not met. It is therefore of paramount importance to monitor the levels of major contaminants in the coastal area, where most fishery and farming activities are carried out (Lichtfouse et al., 2012).

By virtue of this, different methods utilising various concepts and approaches have been implemented for the purpose of heavy metal monitoring. These detection techniques are valued on their ability to quantify the amount of free and bound metal, and also to provide some understanding on the dynamic behaviour of the metal species. Conventional methods to detect heavy metal ions include atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectrometry (ICP-MS), and atomic fluorescence spectrometry (AFS), colourimetric analysis and ultraviolet visible (UV-Vis) spectrometry (Musameh et al., 2011). These methods usually involve time-consuming processes as well as costly and state-of-the-art instrumentation. They also usually involve complex chemical steps to extract metal ions from the as-sampled water which in turn causes the concentration change of metal ions inevitable (Zhou et al., 2014). Consequently, conventional methods are not suitable for on-field analysis and emergency detection. Therefore, it is crucial to have a mobile, cheap, and rapid heavy metal analysis system to detect heavy metal at contaminated sites (Long et al., 2013).

In recent years, chemical sensors which are small, portable and cheap are found in the market. They are useful alternative tools which enable on-site monitoring. These chemical sensors fall into three categories which are optical, biological and electrochemical. Apart from being small, portable and cheap, optical sensors are easy-operated and do not require complex sample pre-treatment. However, they can be only used once and hence not suitable for long-term use. Besides, they suffer from poor accuracy (Mayr, 2002). Having advantages of chemical sensors, the lifetime of biological sensors is limited by the stability of enzyme, thus they are not suitable for multiple use as optical sensors. The work to prepare biological sensors are tedious and also time-consuming (Vigneshvar et al., 2016).

In this situation, the weaknesses of the above methods can be overcome using electrochemical sensors. Electrochemical sensors operating electrically can help in improving the portability and give rapid response for screening heavy metal contaminated sites. Electrochemical sensors are low cost, easy to use, reliable and appropriate for in-field applications with minimal sample changes (Gumpu et al., 2015). Electrochemical methods only require simple procedures. The sensors are small, light and portable. As such, they are good candidates for in-situ monitoring of contaminated water samples since they can be fabricated into portable devices. Comparing electrochemical methods with spectroscopic methods that allow online water quality monitoring, electrochemical methods give rapid response as they only require short analytical time. Besides, these electrochemical methods give higher sensitivity and lower limits of detection (LOD). Most of the electrochemical sensors can be used multiple times and do not require complicated sample preparation process (Bansod et al., 2017).

Electrochemical sensing employs electrodes to pass current to the aqueous solution (electrolyte) which contains heavy metal ions and produce quantifiable electrical signal. Electrical signal implies that there are electrochemical reactions happen in the

solution due to existence of heavy metal ions. The electrochemical instrumentation setup is in general light, portable, easy and convenient due to the mini-size electrodes and easy electrode modifications. This makes the method beneficial and to a great extent useful for heavy metal detection (Bansod et al., 2017). An electrochemical detection setup normally consists of three electrodes: a working electrode (WE), a counter electrode (CE) and a reference electrode (RE). The working electrode is the most important component of an electrochemical cell as it is at the interface between the WE and solution that transfer electron. The working electrode can be remodelled using different materials for specific determination of heavy metal ions (Cui et al., 2015a).

To selectively detect heavy metal ions, potentiometric, voltammetric and galvanostatic techniques are the most popular among several electrochemical methods which has high sensitivity and give low detection limit (Aragay and Merkoci, 2012). The key point to obtain a good and reliable electrochemical sensor is based on the kind of material that constitutes the detection platform. Traditional electrodes such as glassy carbon, graphite, screen printed, mercury film and hanging mercury drop electrodes have been widely modified with a variety of interface materials including carbon based materials, polymers, metal oxides, nanomaterials and biomaterials like deoxyribonucleic acid (DNA) and enzymes to enhance the sensitivity, selectivity and lower limits of detection (Bansod et al., 2017).

With the development of nanotechnology, nanomaterials are being used to modify and improve the analytical capability of numerous electrodes (Guo et al., 2017). Nanomaterials are attractive owing to their peculiar chemical and physical properties such as size, composition, conductivity, magnetism, mechanical strength, light absorbing, and emitting properties. Besides, nanomaterials have large specific surface area, large number of absorption-active sites, high electronic conductivity, good thermal stability, selectivity, and reproducibility (Zhang and Fang, 2010). Incorporation of nanoparticles

such as nanowires, nanorods and nanospheres assists in rising the potential to activate specific reactions, electron transfer rate between analytes and electrode, and also aids in preventing impermissible outcome (Gumpu et al., 2015).

Development of electrochemical sensor by coupling with the nanomaterials offer distinguish merits such as high sensitivity, real time detection in non-destruction manner and high dimensional resolution for localized detection. The designing of a sensor with the suitable structure helps in selective identification of metal ions from any kind of matrix such as water, saliva, urine and blood (Gumpu et al., 2015). By customizing functionalisation and assembly, nanomaterials can be simply used on electrode surface for the fabrication of sensing electrodes and the sensitive and selective detection of heavy metal ions. The assembly of different nanomaterials can further enhance the electrochemical performance (Cui et al., 2015a). Inorganic nanomaterials such as metal, metal oxide, carbon and silica-based nanomaterials are often used for electrode modification in electrochemical detection of heavy metal ions. This is because inorganic nanomaterials has intrinsic advantages of even structure, chemical and thermal stability, high surface reaction activity and catalytic efficiency, large surface-to-volume ratio and strong adsorption ability (Cui et al., 2015a).

For many years, mercury has been used to modify electrode for electrochemical detection of heavy metal ions. The two basic electrode systems are the mercury film electrode (MFE) and hanging mercury drop electrode (HMDE). Although these mercury electrodes offer excellent performance in detection of heavy metals, there is growing concerns regarding the toxicity, handling, volatility, and disposal of mercury. The toxicity of mercury, with concentrations as little of 1 $\mu\text{g/L}$ has the ability to cause serious harm, as defined by the World Health Organisation (WHO), has become an issue which the use of mercury is strictly restricted as an electrode material (Pandey et al., 2016). As a result, new alternative electrodes materials with similar performance are urgently needed.

Different bare carbon, gold, silver or iridium electrodes have been used as possible alternatives to mercury. While offering useful signals for several metals, the overall performance of these non-mercury electrodes has not been better than that of mercury, due to low cathodic potential limit, multiple distorted peaks, large background contributions or poor precision and resolution (Wang, 2005). Hence, the development of reliable non-mercury electrodes is considered a major challenge.

In recent years, the use of mercury-free solid-state electrodes such as bismuth based electrodes has arisen for heavy metals detection because they have the behaviour similar with electrolytical performance of mercury and are obviously being more environmental friendly (Zhang et al., 2009). Bismuth has also the ability to form “fused alloy” with numerous heavy metals including lead, cadmium, thallium, antimony, indium or gallium. Such formation of alloys at low temperature facilitates the nucleation process during the deposition of heavy metal ions, leading to sensitivity similar to mercury electrodes (Barón-Jaimez et al., 2014).

Bismuth electrodes offer a well-defined, undistorted, and highly sensitive stripping signals, excellent resolution of neighbouring peaks (eg. Cd, Pb, Zn), high hydrogen evolution, insensitive to dissolved oxygen and simple preparation, in contrast with mercury electrodes (Aragay et al., 2011; Legeai et al., 2005). To date, the research of the bismuth electrode mainly focuses on the bismuth film electrodes (BiFEs) which was produced *in situ*. However, the various surfactants in real samples can be absorbed on the surface of BiFE which can result in serious interference and bad performance.

The development of Bi nanoparticles (BiNPs) modified electrodes which have large electrochemical active surface area has aroused much attention in recent years (Zhang et al., 2009). Additionally, these electrodes are directly applicable to trace metal analysis without a pre-deposition step of bismuth in a bismuth-containing solution which can reduce the chance of interference by surfactants in real samples (Wu et al., 2012).

Electrodes modified by bismuth nanoparticles is believed to be the favourable for its large electrochemical active surface area and the exclusion of a pre-treatment in Bi-containing solution.

1.2 Problem Statement

Bismuth nanostructures can be synthesised by electrode reduction, soft templating, thermal decomposition, gas phase transportation or levitational gas condensation method (Yang et al., 2013a). These methods can produce bismuth nanostructures with ideal sizes. Unfortunately, all these reported synthesis methods till now are associated to high temperature reaction, toxic precursors, special equipment or troublesome post treatment, which are unsuitable and impractical for mass production. Lee and his team produced bismuth nanopowder that is spherical in shape with a size of nearly 50 nm synthesised by the levitational gas condensation method using a micro powder feeding system. The apparatus consisted of a high frequency induction generator of 2 kW, a levitation and evaporation chamber and an oxygen concentration control limit. Argon gas was flowed into the chamber with a rate of 1.4 L/min in order to maintain the pressure in the chamber at 70 torr (Lee et al., 2007). However, this method needs special equipment to produce bismuth particles.

A team of researchers from the Tokyo Institute of Technology, Japan have prepared powder for ceramic application using hydrothermal method. The powder produced showed no agglomeration and is fine-grained, high purity and with controlled morphology, small size and consists of single crystal (Byrappa and Yoshimura, 2012). Other advantages of hydrothermal method is it does not require expensive and sophisticated equipment as well as being able to produce bismuth at low temperature. Yang and his team had synthesised bismuth nanoparticles with the dimension of about 80 – 90 nm using hydrothermal method at 160 °C for 6 hours. The limit of detection for

Pb(II) is around 10 $\mu\text{g/L}$ (Yang et al., 2013a). In this work, varying hydrothermal reaction period was studied to understand the growing process of bismuth particles. This is because the varying size of bismuth particles could improve the sensitivity of the heavy metal sensor.

To the best of current knowledge, no work has been done on direct growth of bismuth particles on flexible indium tin oxide electrode for heavy metal sensors application. As such, in this work, Bi/ITO electrodes of different hydrothermal reaction period were produced. Also, the produced bismuth particles were used to modify indium tin oxide (ITO) coated polyethylene terephthalate (PET) film to compare the performance with Bi/ITO electrodes produced. The performance of these electrodes were tested using cyclic voltammetry (CV) and differential pulse anodic stripping voltammetry (DPASV). Lastly, the electrodes will be tested on its detection of heavy metals.

1.3 Scope of Research

This work aims to produce bismuth particles and electrode modified by direct grown of bismuth using hydrothermal method. The effect of hydrothermal reaction period was studied to understand the growing process of bismuth particles. The phase of Bi particles and directly grown Bi particles electrodes produced were investigated using X-ray diffraction (XRD). The morphology of the Bi particles produced was observed by Scanning Electron Microscope (SEM). Bi particles were immobilized on indium tin oxide coated polyethylene terephthalate (ITO-PET) substrate by using drop casting layer-by-layer as modified electrodes, forming Bi/ITO-PET. Both Bi/ITO and Bi/ITO-PET electrodes were then analysed by electrochemical analyses called cyclic voltammetry (CV) and differential pulse stripping voltammetry (DPASV) for their heavy metal ions sensing properties.

1.4 Objectives

- i. To synthesise bismuth particles by means of hydrothermal with varying hydrothermal reaction period.
- ii. To study the properties of bismuth synthesised with varying hydrothermal reaction period directly grown on ITO electrodes.
- iii. To optimise the performance of bismuth particles drop-casted electrodes and bismuth particles directly grown electrodes for detection of heavy metal ions by voltammetry.

1.5 Dissertation Outline

This dissertation consists of five chapters. Chapter 1 discusses the background of the study, problem statement, scope of this research and the objectives for this work. Chapter 2 discusses literature review on heavy metals, heavy metal sensors, electrochemical sensing methods and bismuth nanoparticles. Research methodology, parameters conducted and characterisation methods are described in Chapter 3. Chapter 4 focuses on the results and discussion of the research. Finally, conclusion and recommendations for future work are discussed in Chapter 5.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Heavy metals (HMs) are the elements having metallic characteristic and belong to the family of transition metals, metalloids, lanthanides, and actinides. Also, heavy metals are defined as metals with atomic weights between 63.5 and 200.6 g mol⁻¹ and a specific gravity greater than 5 g cm⁻³ (Pandey et al., 2016). There are over 50 elements which are classified as heavy metals. Seventeen of them are considered to be both very toxic and quite obtainable. Lead (Pb), mercury (Hg), arsenic (As), and cadmium (Cd) are typically considered as main elements in human poisoning even at trace level (Pujol et al., 2014). These heavy metals exist as natural constituents of the Earth's crust. Heavy metals are non-biodegradable, widely distributed and lead to extensive adverse effects to human health and environment.

Human activities are the major contributing factors that lead to environmental imbalance. Although heavy metals are naturally present, they become major pollutants as a consequence of industrial and mining activities, use of fertilisers and pesticides during agricultural processes, mining activities, and release from fuels and microelectronic products (Khan and Abdullah, 2013). Heavy metals do not decompose and get accumulated in the environment and enter living organisms through the alimentary chain, thus threaten human health. Its toxicity is as a result of the bond formation of metals with thiol group of proteins and when goes in the cell, changes the biochemical life cycle. The toxicity mechanism of heavy metal ions is through enzyme inhibition, oxidative stress and impaired antioxidant metabolism. These mechanisms

present detrimental health consequences through free radical generation that leads to DNA damage, lipid peroxidation and depletion of protein sulfhydryl (Gumpu et al., 2015).

Large numbers of metal are essential for proper functioning of the human body. A deficiency of heavy metals on one side can lead to serious symptoms. Accumulation of heavy metals which are toxic, non-biodegradable in the human body for a long time can potentially cause death, even at minute amount. Exposure to heavy metals is associated with carcinogenesis in the lung, ovary, and intestine, nervous system interruption, genotoxicity and metabolic disorders (Bae et al., 2018). Water being the main sources for growth of living things is the main route to receive large amount of pollutants. Regulatory organisations across the world have implemented regulations through the maximum permissible limits for the discharge of heavy metal in the aquatic environment and intervention through ISO 14000 in order to control contamination.

In water, heavy metals are being rapidly degraded and sediment in the form of hard soluble carbons, sulphates and sulphides on the bottom. At the time when the absorptive capacity of sediment is fatigued, the concentration of metal ions in water is increasing. Heavy metals in water appear in different chemical forms and in different oxidising conditions, so their toxicity is changing depending on chemical form in which they are found (Odobasic, 2012). Table 2.1 outlines the parameter limits and health hazard of heavy metal toxicity. The parameter limits for heavy metal by the international organisations of WHO and USEPA are mostly lower compared to that from the Malaysia Standards of EQA and INWQS. This may be due to strict regulations applied by WHO and USEPA when compared to Malaysia just to safeguards human health and the environment (Tay, 2012).

Table 2.1: Parameter limits and health effects of heavy metals (Tay, 2012)

Metal	Parameter limits by Malaysian organisation (mg/L)				Parameter limits by international organisations (mg/L)		Health Hazards
	EQA		INWQS		WHO	USEPA	
	Standard A	Standard B	Classes IIA/IIB	Classes III			
Arsenic	0.05	0.10	0.05	0.4 (0.05)	0.01	0.01	Carcinogenic, producing liver tumours, skin and gastrointestinal effects.
Cadmium	0.01	0.02	0.01	0.01* (0.001)	0.003	0.005	Carcinogenic, cause lung fibrosis, dyspnoea and weight loss.
Copper	0.20	1.0	0.02	-	2	1.30	Long term exposure causes irritation of nose, mouth, eyes, headache, stomach ache, dizziness, and diarrhoea.
Lead	0.10	0.5	0.05	0.02* (0.001)	0.01	0.015	Suspected carcinogen, loss of appetite, anaemia, muscle and joint pain, diminishing IQ, cause sterility. Kidney problem and high blood pressure.
Mercury	0.005	0.05	0.001	0.004 (0.0001)	0.001	0.002	Corrosive to skin, eyes and muscle membrane, dermatitis, anorexia, kidney damage and muscle pain.
Nickel	0.2	1.0	0.05	0.9*	0.07	0.04	Carcinogenic, dermatitis, allergic sensitization, lung and nervous system damages.

* – at hardness 50 mg/L

- – not available

Standard A – Effluent that is discharged upstream of water supply intake

Standard B – Effluent that is discharged downstream of water supply intake

2.2 Sources of Heavy Metals

The main sources for heavy metal exposures come from anthropogenic activities such as mining, smelting, or different kind of wastes. The increasing development of industries occur throughout the world by the extraction and distribution of mineral substances from raw. Many of these undergo chemical processes and finally, they enter as effluent into water, earth, air and thus into the food chain. It is impossible that they can be removed by washing. Besides, the contamination by fertilisers and insecticides contributes to this problem as their widespread use results in their existence in the environment and foods (Barón-Jaimez et al., 2014). Controlling the levels of these pollutants in natural waters, drinking water, sediments and industrial waste has become a subject undergoing intense study as its effects to human and environment has become public concern. It is necessary to develop analytical methodologies for heavy metal pollution determination (March et al., 2015). The following section discusses some major heavy metal pollutants.

2.2.1 Arsenic

Natural phenomena such as eruption of volcanoes, soil erosion and anthropogenic activities releases arsenic to the environment, causing environmental pollution. Industrially, arsenic is produced and used for agricultural and medical applications (Tchounwou et al., 2012). Severe acute arsenic toxicity in humans include diarrhoea, vomiting, bloody urine, convulsions and death while chronic toxicity effects from arsenic are skin lesions and Blackfoot disease which leads to gangrene of hands and feet, reported in Taiwan (Hughes, 2002). Populations exposed to arsenic through drinking water shows risk of lung, bladder and kidney cancer while those who are exposed to arsenic by inhalation demonstrate lung cancer (Järup, 2003).

2.2.2 Cadmium

Generally, cadmium accumulates in human body by cigarette smoke, being the major source of cadmium exposure, through inhalation of contaminated air and dust particles and via uptake of contaminated food (Johri et al., 2010). Cadmium is bio-persistent. This means that it tends to remain inside a biological organism for many years although it is eventually excreted. Acute exposures may cause diarrhoea, nausea, muscle cramps, convulsions, liver injury and renal failure. Long-term exposure is associated with renal dysfunction, liver, bone and blood damage. High exposure can lead to lung cancer (Berkowitz et al., 2014). The most significant uses of cadmium are in the manufacture of batteries and in electroplating as it provides good corrosion resistance. Other uses of cadmium are as pigments, stabilisers for PVC, in alloys and electronic compounds. Cadmium also exists in some products, including phosphate fertilisers, refined petroleum commodities and soap as an impurity (Berkowitz et al., 2014).

2.2.3 Chromium

Chromium is used for chrome laminate, colour and pigments, leather manufacturing and wood treatment. Chromium gets into the air, soil and water primarily in the form of chromium (III) and chromium (IV). Chromium (III) exists naturally in the environment and is an essential nutrient while chromium (VI) is generally produced by industrial processes. Inhaling high levels of chromium (VI) can cause nose irritation. Taking substantial amounts can cause stomach-aches and ulcers, convulsions, kidney and liver damage, damage to circulatory and nerve tissue and even death. Skin contact with certain chromium (VI) compounds can cause skin ulcers. The World Health Organisations (WHO) has determined that chromium (VI) is carcinogenic to human (Berkowitz et al., 2014).

2.2.4 Lead

Lead is the most significant toxicant affects the organs and systems in the body. Its toxic biochemical effects in human cause problems in the synthesis of haemoglobin, dysfunctions in the kidneys, liver, gastrointestinal tract, joints, central nervous system, endocrine system and reproductive system (Duruibe et al., 2007). Lead poisoning targets the most vulnerable nervous system. Early symptoms of lead exposure effects on the central nervous system include headache, poor attention, irritability, reduced consciousness, memory deterioration and prolonged reaction (Järup, 2003). Lead exposure is of particular concern especially among pregnant women as lead absorbed by the pregnant mother is readily transferred to the developing foetus, causing neuro-developmental abnormalities in children. These children are associated with lower intelligence quotient (IQ), delayed neurobehavioral development, decreased hearing and speech ability, growth retardation and anti-social (Tchounwou et al., 2012).

Lead in the environment arises from both natural and anthropogenic activities such as mining and fossil fuels burning. It is widely used in many different industrial, agricultural and domestic. Exposure can occur mainly through breathing in of lead-contaminated dust particles, and ingestion of lead-contaminated drinking water, food and old paint. Lead is even exists in some traditional medicines and cosmetics (Tchounwou et al., 2012).

2.2.5 Mercury

Mercury is a neurotoxin which creates detriment to the brain and the central nervous system, while foetal and postnatal exposure have brought on to miscarriage, inherent abnormality and growth changes in young children. Large amount of mercury cause lungs and kidney illness, angina pectoris and breathing difficulty (Fu and Wang, 2011). Inorganic mercury poisoning is relayed to trembling, gum inflammation and minor

psychological changes like panic, melancholy and sleep disorders, together with unplanned miscarriage and inherent abnormality (Duruibe et al., 2007).

Mercury is used in major as an electrode in the electrochemical process of chlorine production (Järup, 2003). It is also widely utilised in electrical industry as switches, thermostats and batteries, in dentistry as an amalgam for fillings and by the pharmaceutical industry. The main sources of mercury which human are exposed are from industrial and agricultural, environmental pollution, food contamination, dental care and preventive medical practices. Major sources of low level mercury exposure are dental amalgams and fish consumption. Mercury enters water through degassing of the Earth's crust, emissions from volcanoes and evaporation from natural bodies of water (Tchounwou et al., 2012). The Minamata catastrophe in Japan as shown in Table 1.1 was caused by mercury poisoning from mercury-contaminated fish.

2.2.6 Nickel

Nickel occurs naturally in the earth's crust, found in all soil and is also emitted into the atmosphere from volcanoes, during nickel mining and by industries. Human are exposed to nickel through breathing, drinking water or smoking cigarettes containing nickel (ATSDR, 2005). Nickel exceeding its critical level rises chronic lung and kidney problems aside from gastrointestinal distress, pulmonary fibrosis and skin rashes (Fu and Wang, 2011). Lung and sinus cancers occur in those who are exposed to high concentration of nickel. Nickel is used mostly to produce stainless steel and other nickel alloys with high-corrosion and temperature resistance. Nickel alloys and nickel plating are used in vehicles, processing machinery, armaments, tools, electrical equipment, household appliances, and coins. Nickel compounds also are used as catalysts, pigments and in batteries (Berkowitz et al., 2014).

2.3 Water Pollution by Heavy Metals

Throughout the years, rapid development activities have been driving the economic growth in Malaysia. The rapid industrialization has helped in achieving a number of social objectives such as employment, poverty eradication, gender equality, labour standards and greater access. Unfortunately, such indiscriminate growth has resulted in water pollution in Malaysia (Idriss and Ahmad, 2013). The water drainage from various origins such as landfill, glass manufacturing process, electronic parts, paints and used batteries are the primary causes of heavy metal pollution. Unwanted residues and mud from electroplating industries, wood processing units, manufacturing of printed circuit board (PCB) and petroleum refining are the major generators of heavy metal contamination in surface and ground water (Pandey et al., 2016). Effluent from the industrial area is released into drains without undergoing appropriate water treatment. As heavy metal ions are constant environmental pollutants, they can be absorbed by the body through food, air, soil, and water. Metal ions in seawater are readily absorbed by ocean animals, and they directly enter the food chain, contributing health and environmental danger (Kumar et al., 2017).

A total of 1035 samples from 229 monitoring stations were analysed by Department of Environment (DOE) in 2006, heavy metal pollution was found with lead (Pb) as the most prominent heavy metal detected in the marine waters, exceeding the Interim National Water Quality Standard (INWQS) by 20%, followed by mercury (18%) and copper (6%). Total chromium and cadmium pollution were recorded at 2% and arsenic (As) at 1% was detected in 2006 (IEM, 2014).

The impact of industrial wastewater discharge with high concentrations of heavy metal is of great worry which guarantees appropriate remediation. Therefore, the society needs to deal with the development of approaches to reduce exposure to these metals

as well as creating the awareness to this issue urgently and seriously in order to overcome their hazardous effects to human and the environment.

Heavy metals exist in human body by entering through various medium and channels which can cause functioning disorder in cellular levels and lead to cytotoxicity. They get excreted only either through liver, kidney or spleen. Once they are absorbed by the body, these metals get excreted very slowly unlike the other molecules. Longer storage of these metals in excretory organs leads to organ damage by changing cellular functioning (Ochiai, 1987).

Cadmium acts as a carcinogen that inhibits the enzyme activities that are involved in DNA repair system such as correcting the DNA mismatches. When DNA mismatch repair (MMR) system fails to happen, gene mutations occur and lead to abnormal proliferation of cells – cancer. Cadmium can also inhibit the first line defence antioxidants which include superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase. Inhibition of these antioxidants lead to the formation of free OH radicals in the body (Ighodaro and Akinloye, 2017). Cadmium cannot produce free radicals by itself but it replaces copper and iron in cytoplasmic as well as in membrane proteins. As free ion concentrations of Cu and Fe get increased, they induce oxidative stress by Fenton reactions and produce hydrogen peroxide (Gumpu et al., 2015).

Lead causes the down-regulation of delta- aminolevulinic acid dehydratase (ALAD) in heme synthesis pathway and glutathione reductase (GR) in intracellular regulation. ALAD is an important molecule in the second step in production of heme. Inactivation of ALAD leads to the accumulation of delta-aminolevulinic acid (ALA) which can cause the formation of reactive oxygen species and is associated with oxidative damage (La-Llave-León et al., 2017). Inhibition of glutathione reductase fails to detoxify the peroxide in the

body and can cause accumulation of peroxides which will lead to harmful effects to the body such as oxidative stresses (Giblin and McCready, 1983).

2.4 Conventional and Nanomaterials Heavy Metal Sensor

The continuous and acute exposure to toxic heavy metal ions such as Cd^{2+} , Pb^{2+} and Hg^{2+} can bring direct consequences on human health condition and are related to critical human diseases such as cancer and cardiovascular illness. The development of highly sensitive and selective methods for detection of metal ions not only can provide insight into the physiological activity of metal ions but also are in high interest for food safety screening (Yin et al., 2011). So far, approaches to detect heavy metal ions at trace levels (ppb range to less than 10 ppm) have been well-established using advanced analytical instruments, including atomic absorption spectroscopy (AAS), inductively coupled plasma-mass spectrometry (ICP-MS), mass spectroscopy (MS), flameless atomic absorption spectrophotometry (FAAS) and X-ray fluorescence spectroscopy (XPS). Nevertheless, these techniques have drawbacks including high cost, immobility, low throughput, laborious pre-treatment procedures, and the need for well-trained operators (Zawisza et al., 2011, Lu et al., 2018a).

Current breakthroughs in new materials, peculiarly the development of nano- and bio- materials, have initiated a new generation of analytical techniques (Zhang and Fang, 2010). Many approaches have been accomplished in the evolution of nanomaterial-based technologies to monitor heavy metal ions in standards and real-world samples based on (a) optical, (b) biological, and (c) electrochemical sensing strategies. These strategies have been set up with various kind of nanomaterials such as metal nanoparticles (MNPs), quantum dots (QDs), nanometal organic frameworks (NMOFs), magnetic nanoparticles, carbon nanotubes, and nano-composites. The development of multiple nanomaterials has not only assisted in enhancing the selectivity, sensibility, and

reproducibility of platforms for sensing HMIs, but also has provided opportunities to miniaturize sensing devices (Kumar et al., 2017).

2.4.1 Optical Sensing

Comparing with traditional instrumental analysis methods, optical sensing shows merits at easy operation and does not require complex sample pre-treatment procedures. Optical sensing methods like colorimetric assay has become particularly popular due to high sensitivity. Colorimetric assay allows signalling the target event into colour changes, which could be visually quantified by naked eyes and make onsite, real-time detection possible (Wang and Chen, 2015). Organic dyes are usually used as the fluorophores in Forster resonance energy transfer (FRET) sensors due to their commercial availability, established labelling protocols, and small size to minimize possible steric hindrance. However, the organic dyes are also found to be subject to photobleaching and narrow excitation spectra and is not suitable for aqueous environment (Kumar et al., 2017).

2.4.2 Biological Sensing

Recently, the use of biological sensing approaches has raised noticeably in the detection of heavy metals ions given their facile interaction with various biomolecules such as proteins, enzymes, antibodies, metalloenzymes, and metalloproteins. These biosensing approaches have advocated notable progress in the selectivity and detectability of heavy metals ions in real samples. Even though, they also suffer from several limitations such as tedious and time-consuming enzyme purification. Purified biomolecules used for biosensing application have a high specificity for their substrates or inhibitors. Otherwise, heavy metal ions may act as either catalysts or cofactors. (Kumar et al., 2017).

2.5 Electrochemical sensing

Over the last few years, study has been focused on creating chemical sensor with a lower limit of detection for analysis of metal ions. Particularly, electrochemical sensor (ECS) provides low detection limit, high sensitivity, high surface area, excellent reproducibility, better signal-to noise ratio and selective sensing of more than one metal ion (Pandey et al., 2016). Electrochemical sensor can detect trace pollutants which can hardly be detected by using conventional electrodes (Zhang and Fang, 2010). This sensor is a device that converts electrochemical information into an interpretively useful signal. Electrochemical sensors normally encompassing two fundamental parts, a chemical recognition platform which is the most crucial part of a sensor and a physiochemical transducer which is a device that turns the chemical response into a signal which can be identified by potentiostat/galvanostat based electrochemical workstation (Faridbod et al., 2011). The final analytical useful outcomes can be deliberated by implementing a number of signal amplifications techniques such as potentiometry, amperometry, conductometry and voltammetry as the presence of heavy metal ions can cause changes in various electrical parameters such as current, voltage, electrochemical impedance, charge and electroluminescence (Bansod et al., 2017).

Potentiometric sensors are frequently used to carry out quantitative analysis of ions in solutions by employing selective electrodes. Potentiometry covers the measurement of emf (E) at zero current, which means, no current is applied for this measurement. This approach has been effectively employed due to its low cost, fast response, high selectivity and wide range of response in determination of heavy metal ions. Despite that, there are disadvantages to this method in terms of high detection limits, reduced sensitivity and difficulties in electrode miniaturization (Bakker and Telting-Diaz, 2002).

Amperometric sensors involve the use of a very small current which is under controlled and a fixed potential applied between a reference and a working electrode to

promote the oxidation or reduction of an electroactive species. The fixed potential applied causes the flow of very large current which is proportional to the concentration gradient at the electrode surface. The current is recorded as the function of time. This technique detects one selected components only from an electrochemically reducible species due to a fixed potential of the working electrode. The analyte to be detected experiences a faradaic reaction at some desired polarity and magnitude of the potential applied. Anyhow, due to less surface area of the working electrode, this faradaic reaction is incomplete and only a fraction of analyte reacts (Stradiotto et al., 2003).

In contrast, conductometry is subjected to the current flow initiated by movement of ions of opposite charge, when an electric field is established between two electrodes immersed in the electrolyte solution (Blanco-López et al., 2004). The sensing outcome is dependent on transition of the number of mobile charge carriers in the electrolyte. The electrolyte exhibits ohmic behaviour if the electrodes are halted from polarizing. Conductivity measurements are in general accomplished with AC supply. The conductivity is a linear function of the ion concentration and thus it can be utilised for sensor applications. Yet, it is not particular for given ion type. Apart from that, both the polarisation and limiting current operation mode must be avoided (Wang et al., 2008). However, voltammetry is the only electrochemical technique that has high sensitivity and can be employed for the *in situ* identification and detection of HMIs pollution. Figure 2.1: Schematic illustration of general principle of electrochemical sensing of heavy metal ions