SCHOOL OF MATERIALS AND MINERAL RESOURCES ENGINEERING
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SELF ASSEMBLY GOLD NANOPARTICLES MODIFIED ELECTRODE FOR THE ELECTROCHEMICAL DETECTION OF MERCURY AND LEAD

By

NUR ASLEENA BINTI ALAUDIN

Supervisor: Professor Dr. Khairunisak Bt. Abdul Razak

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DECLARATION

I hereby declare that I have conducted, completed the research work and written the dissertation entitled: “Self Assembly Gold Nanoparticles Modified Electrode for the Electrochemical Detection of Mercury and Lead”. 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.

Name of student: Nur Asleena Binti Alaudin Signature:

Date:

Witnessed by

Supervisor: Professor Dr. Khairunisak Bt. Abdul Razak Signature:

Date:

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LIST OF ABBREVIATIONS

$[Fe(CN)6]^{3-}$	Ferrocyanide (III) Ion
AET	Aminoethanethiol hydrochloride
(AET)	3-Mercaptopropyl Trimethoxysilane
(MPTMS)	Aminopropyltriethoxysilane (APTES)
Ag^+	Silver ion
AuNPs	Gold Nanoparticles
Al^{3+}	Aluminium (III) Ion
As^+	Arsenic ion
ASV	Anodic Stripping Voltammetry
Bi	Bismuth
CE	Counter Electrode
CH_3COOH	Acetic Acid
CH_3COONa	Sodium Acetate
Cr^+	Chromium ion
Cr(II)	Chromium (II) Ion
Cr^{3+}	Chromium (III) Ion
Cu(II)	Copper (II) Ion
CV	Cyclic Voltammetry
DP	Differential Pulse

DPASV	Differential Pulse Anodic Stripping Voltammetry
DPV	Differential Pulse Voltammetry
Fe^{2+}	Iron (II) Ion
FETs	Field-Effect Transistors
GCEs	Glassy Carbon Electrodes
GO	Graphene Oxide
H^+	Hydrogen Ion
H_2	Hydrogen Gas
H_2O	Distilled Water
H_2O_2	Hydrogen Peroxide
HNO_3	Nitric Acid
HCl	Hydrochloric Acid
ISEs	Ion Selective Electrodes
K^+	Potassium (I) Ion
$K_3Fe(CN)^6$	Potassium Ferrocyanide (III)
KCl	Potassium Chloride
LOD	Limit of Detection
LSV	Linear Sweep Voltammetry
Mg^{2+}	Magnesium (II) Ion
Na^{2+}	Sodium (II) Ion

NaAc-HAc	Acetate Buffer Solution
Ni^{2+}	Nickel (II) Ion
NO^{3-}	Nitrate Ion
NPs	Nanoparticles
O_2	Oxygen Gas
OH^-	Hydroxide Ion
Pb	Lead
ppb	Part per billion
ppm	Part per million
RE	Reference Electrode
Sb	Antimony
SEM	Scanning Electron Microscope
SWAdCSV	Square Wave Adsorptive Cathodic Stripping Voltammetry
SWASV	Square Wave Anodic Stripping Voltammetry
SWV	Square Wave Voltammetry
TEM	Transmission Electron Microscope
WE	Working Electrode
XRD	X-Ray Diffraction
Zn(II) / Zn2+	Zn (II) Ion

LIST OF SYMBOLS

%	Percentage
°	Degree
°C	Degree Celsius
A	Area of Electrode
C	Concentration
cm	Centimeter
cm^2	Centimeter Square
D	Diffusion Coefficient
e^-	Electron
g	Gram
g/mol	Gram per Mole
h	Hour
i	Electric Current
M	Molarity
mg/l	Milligram per Liter
ml	Milliliter
mol/L	Mole per Liter
Mw	Molecular Weight
n	Number of Electron
nm	Nanometer
nM	Nanomolarity
v	Scan Rate
V	Voltage

V/s	Voltage per Second
μA	Microampere
μg/L	Microgram per Liter

PARTIKEL NANO EMAS SELF ASSEMBLY DIUBAHSUAI ELEKTROD UNTUK ELEKTROKIMIA PENGESANAN OLEH MERKURI DAN PLUMBUM

ABSTRAK

Dalam kajian ini, partikel nano emas yang mempunyai saiz 40 nm telah dihasilkan dengan menggunakan teknik pertumbuhan benih. Partikel nano emas yang telah dihasilkan telah dicirikan dengan menggunakan analisis Pembelauan Sinar X (XRD), analisa Zeta-sizer, Spektroskopi UV-Visible Near-Infrared (UV-Vis) and Mikroskop Transmisi Elektron (TEM). Partikel nano emas yang dihasilkan telah digunakan untuk *self assembly* menggunakan Aminopropyltriethoxysilane (APTES) atas substrate (indium tin oxide/polyethylene terephthalate) (ITO/PET) yang diubah suai bagi tujuan pengesanan elektrokimia logam merkuri dan plumbum. Kesan beberapa pemboleh ubah telah dikaji untuk ujian pengesanan logam merkuri dan plumbum iaitu kesan kepekatan (APTES), tempoh rendaman APTES dan tempoh rendaman AuNPs. Elektrod diubahsuai tertakluk kepada analisa voltametri berkitar bagi mengkaji tingkah laku elektrokimia. Analisis Square Wave Anodic Stripping Analysis (SWASV) telah dijalankan pada setiap pemboleh-ubah untuk mencari had terendah dalam pengesanan (LOD) untuk logam merkuri dan plumbum. Hasil kajian menunjukkan kepekatan APTES 1% memberikan nilai (LOD) paling rendah berbanding kalangan kepekatan lain iaitu 14.6 µg/L dan 0.78 µg/L, masing-masing untuk logam merkuri dan plumbum. Kemudian, untuk kesan tempoh rendaman APTES, 30 minit tempoh rendaman memberikan (LOD) sebanyak 13.79 µg/L dan 1.97 µg/L untuk logam merkuri dan plumbum, masing-masing. Akhirnya, 6 jam tempoh rendaman partikel nano emas memberikan (LOD) sebanyak 3.71 µg/L and 0.43 µg/L untuk logam merkuri dan plumbum, masing-masing.

SELF ASSEMBLY GOLD NANOPARTICLES MODIFIED ELECTRODE FOR THE ELECTROCHEMICAL DETECTION OF MERCURY AND LEAD

ABSTRACT

AuNPs with 40 nm particle size were synthesized using the seeding-growth method. The synthesized AuNPs were characterized using X-ray Diffractometer, Zetasizer analysis, Scanning Electron Microscopy, UV-Visible Near-Infrared Spectrophotometer and Transmission Electron Microscopy. The produced AuNPs were used to self-assembled with the aid of Aminopropyltriethoxysilane (APTES) on ITO/PET electrode for the purpose of electrochemical detection of Hg(II) and Pb(II). The effects of several parameters were investigated for the electrochemical detection of Hg and Pb: the effect of APTES concentration, APTES soaking period, and AuNPs soaking period. The modified electrodes were subjected to cyclic voltammetry (CV) analysis to study the electrochemical behavior. SWASV analysis was performed on each parameter investigated in order to find the lowest limit of detection (LOD) value for Hg and Pb. The results showed APTES concentration of 1% gave the lowest LOD value among the other concentration which were 14.6 µg/L and 0.78 µg/L for Hg and Pb, respectively. Then, for the effect of APTES soaking period, 30 minutes soaking period gave the LOD value of 13.79 µg/L and 1.97 µg/L for Hg and Pb accordingly. Finally 6 hours of AuNPs soaking period gives the LOD value of 3.71 µg/L and 0.43 µg/L for Hg and Pb, respectively.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Heavy metals are dense metallic chemical elements that have relatively high density and high atomic weight. Heavy metals are detrimental to human and environmental when the heavy metals are considered as “contaminant” or unwanted species (Singh et al., 2011). Heavy metals can be usually found in water and soil and they cannot be degraded or destroyed. Examples of heavy metals pollutants due to anthropogenic activities are mercury, lead, cadmium, arsenic, chromium, and thallium.

There are heavy metals that cause threat and hazard to the public health and environment, but there are also heavy metals that are essential for biochemical and physiological functions such as copper, manganese, iron, chromium, selenium, and zinc. Besides, heavy metals also act as trace elements because of their presence in trace concentration (ppb range to less than 10 ppm) in various environmental matrices. On the contrary, heavy metals like lead, mercury, cadmium, zinc that could cause threat and hazard to the public health and environment mainly due to its toxicity, bioaccumulation in the food chain, and persistence in nature (Gunjal et al., 2017).

Over the past few years, heavy metal pollution has attracted increasing attention and has been considered as serious environmental problems due to industrialization and technological advancement in our country. With the development of multiple industries, domestic, agriculture, medical and technology, waste that contain heavy metals are directly and indirectly being released into the environment at higher concentration than the prescribed limits by regulatory organizations such as World Health Organization (WHO) and United States Environmental Protection Agency (USEPA).

Among these toxic heavy metals, heavy metal ions such as Pb^{2+} and Hg^{2+} are the most severe toll on the brain development. The major sources of Pb are from industrial releases such as fossil fuels burnings, mining and manufacturing that release high concentrations of Pb. Furthermore, Pb has been widely used in many applications mainly in the manufacture of Pb storage batteries. Other uses of Pb including metal products, ammunitions, and devices to shield X-rays. Pb is not an essential element, but it is very toxic and dangerous chemical and harmful to human body. It has been reported that exposure to significant amount of Pb via inhalation and ingestion can accumulate in brain and cause poisoning (plumbism) or even death. Therefore, contamination of food, water, air, soil and consumer products result in the absorption of Pb into human body (Tchounwou et al., 2012).

Another major pollutant is mercury, Hg which mainly comes from human activities including industrial processes, coal burning, incineration, uses of mercury in chlor-alkali industry for chlorine production, zinc and steel production, cement production and mining (Pirrone et al., 2010). Humans are exposed to mercury through accidents, dental care, environmental pollutions, industrial and agricultural operations (Kwokal et al., 2012). The elemental mercury (Hg^0) and methyl mercury (MeHg) the two highly absorbed species mainly via inhalation in the form of vapour. Once the elemental mercury enters the blood, it can passes through the blood-brain barrier and the placenta barrier. Furthermore, the Hg easily oxidize and becomes highly reactive Hg^{2+}. Exposure to a small amount of Hg^{2+} can cause blindness and deafness, brain damage, digestive problems, kidney damage, reduce immunity and mental retardation (Guzzi et al., 2008).

Considering these heavy metals pollution that cause environmental problems, an accurate detection techniques and devices should be developed for the purpose of continuous environmental monitoring. These detection techniques of heavy metals ions at trace levels (from ppm to even in ppq range) have been established using various analytical instruments including, atomic absorption spectroscopy (AAS) and inductively coupled plasma optical mass spectroscopy (ICP-MS), ion chromatography, mass spectroscopy (MS), and X-ray fluorescence spectroscopy (XPS), high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry have been used to detect heavy metals (Bansod et al., 2017). The advantages of these techniques are their versatility which are suitable for many elements, highly sensitivity and low limit of detection (LOD) make them dominant for environmental monitoring (Pujol et al., 2014). However, these techniques require highly skilled workers, expensive apparatus, tedious procedure, low throughput and time consuming (Wen-Jing et al., 2012). Therefore, there is demand to develop a device and detection techniques with the low cost, simple and dependable techniques that can be used for real time field detection system.

Sensors have great potential to produce high throughput detection of multiple heavy metals on-site. The development of nanotechnology at present, gave an opportunity for sensors improvement in terms of sensitivity, limit of detection, selectivity and reproducibility. Sensors are categorized based on different signal transduction mechanism for heavy metals detection such as optical, electrochemical, field effect transistor (FET) and biological sensor. Apart from all the mentioned techniques, electrochemical sensors are of interest for detection of heavy metal ions and as substitution for spectroscopic techniques. Electrochemical devices are user friendly as these techniques requires simple procedures. These techniques are also reagent less, low cost, and suitable for minituarization, appropriate for selective detection of heavy metals and good sensitivity.

In addition, electrochemical technique requires minimum sample changes, and allows fast detection of heavy metal ions (Pujol et al., 2014)

For the detection of heavy metal ions in an aqueous solution, electrochemical techniques are categorized based on different electrical signals generated in the solution that contains heavy metals: amperometric, voltammetric, potentiometric, impedance measurement, coulometric, and electrochemicaluminescent techniques (Bansod et al., 2017). For instance, anodic stripping voltammetry (ASV) is a well known analysis among the voltammetric techniques that provide a powerful tool for heavy metal detection, which is portable, high selectivity and sensitivity (Aragay and Merkoçi, 2012). Thus, different electrochemical techniques have been used for environmental monitoring, depending on suitability of the technique used for the analysis, the sensitivity or selectivity requirements.

In the current era of rapid advancement in technology, nanomaterial becomes useful in analytical sensing application owing to their unique optical, electronic, chemical, mechanical properties, easy to synthesize and functionalize. Nanomaterial can be easily assembled on the surface of electrode to fabricate electrochemical sensors for highly sensitive heavy metal detection (Cui et al., 2015). There are many types of nanomaterials have been used as electrode modifiers for the high sensitivity heavy metal ions detection such as carbon nanomaterials, metal nanoparticles and other natural nanostructured adsorbents (Aragay and Merkoçi, 2012).

Apart from that, metallic nanostructured materials exhibit good physical and chemical properties. Metallic nanoparticles have advantages over other nanomaterials including large surface-to-volume ratio, excellent conductivity, stability, magnetic and optical properties, electrochemical sensor with high sensitivity, selectivity and efficiency can be developed (Wang, 2012). In addition, metallic nanoparticles also have the ability to activate specific reactions, high electron transfer rate between analytes and electrode, and is able to eliminate unwanted products (Hoffmann et al., 1995)

There are different techniques that have been used to modify the electrode surfaces by using metallic nanoparticles such as through dip coating, drop coating, electrodeposition, electropolymerization, self-assembled monolayers (SAMs) techniques. The distribution of nanoparticles that adsorbed onto the surface of the electrode are dependent to the techniques to modify the electrode surfaces.

In recent years, assembling the nanoparticles onto electrode surface are commonly used to modify electrode. The main advantage of the self assembly technique over the mentioned techniques to modify electrode using metal nanoparticles is the surface of nanoparticles is tightly bound to the electrode by functionalized organic molecule. This technique uses bifunctional molecules which bind to the substrate and then the metal nanoparticles are bound to the bifunctional molecules modified electrode by ligand exchange. Therefore, the modified electrode with good stability can be achieved (Liu et al., 2018)

The coupling agents that are usually used as a linker to bind nanoparticles to substrates through their terminal groups are thiol-terminated, amine-terminated, alkyl-terminated and phenyl-terminated silanes. Among these coupling agents, 3-aminopropyltriethoxysilane (APTES) that is amine-terminated is preferable for surface functionalization on the substrate. By using electrochemical sensors or devices that are chemically modified with APTES, the nanoparticles could be arranged in orderly manner and strongly bind to the surfaces resulting in high sensitivity and low limit of detection (LOD) of heavy metal ions.

1.2 Problem Statement

Many efforts have been performed to monitor heavy metals in the environment due to heavy metals pollution. Electrochemical sensors are the great choice among various types of sensor and as substitution for spectroscopic techniques. In addition, electrochemical devices are suitable to detect the selective heavy metals, good sensitivity, rapid, accurate and portable, and suitable to be used for *in situ* applications and measurement (Wen-Jing et al., 2012).

In recent years, many works have been done on unmodified and modified electrodes that are used for heavy metal ions detection as working electrodes for electrochemical measurements. However, unmodified electrodes are lacked of some characteristics in terms of sensitivity, selectivity towards electroactive species and efficiency that is very important for a good electrochemical sensor. Therefore, in order to obtain high sensitivity for heavy metal ions detection, the modified electrodes becomes of interest (Xuan et al., 2016).

The use of nanomaterial as modified electrode gives advantages such as increase electrode surface area, enhance mass-transfer rate and fast electron transfer resulted to excellent conductivity compared to bulk material. Examples of nanomaterials that have been used in electrochemical sensing application in environmental monitoring are carbon nanotube (CNT), bismuth nanoparticles (BiNPs), antimony nanoparticles (SbNPs), silver nanoparticles (AgNPs), and gold nanoparticles (AuNPs) modified electrode. However, BiNPs modified electrode tend to have lower potential window and tend to easily oxidized and critical method of deposition to the electrode surface. Generally, the nanomaterial based sensor should be field-portable, low cost, inexpensive and straightforward method of preparation. In this case, AuNPs are chemically stable, easy to synthesize and with well- control size, composition, shape, and surface chemistry to develop powerful sensing application in environmental monitoring. The main reason of choosing AuNPS was due to their chemical inertness and low oxidation rate compared to other nanomaterials modified electrode especially metal nanoparticles modified electrode makes AuNPs viable replacements of other nanomaterial modified electrodes and has been widely used in electrochemical technique for heavy metal ions detection (Paul and Tiwari, 2015).

Surface modification of AuNPs can be achieved by the previously mentioned techniques such as dip coating, drop coating, electrodeposition, self-assembled monolayers (SAMs) techniques. Among the various modification approaches, SAMs techniques which using bifunctional coupling agent using silane based coupling agent as surface modifier. Generally, the substrate surface is covered using a self-assembly technique by soaking in a silane solution forming a strong covalent adhesion, resulting to have high affinity to AuNPs. Therefore, among the different types of surface modifier available such as Aminoethanethiol Hydrochloride (AET), 3-Mercaptopropyl Trimethoxysilane (MPTMS), Aminopropyltriethoxysilane (APTES) is the most surface modifier to form amine terminated silane layer used for the surface functionalization of substrate interact with AuNPs (Matcheswala, 2010).

Previously, the AuNPs functionalized by APTES-modified ITO electrodes have been developed for detecting biotinylated antimouse Immunoglobulin G (IgG) using electrochemical immunosensor by (Aziz et al., 2007). They found out that the modification allowed easy immobilization of AuNPs to the surface and the proteins adsorbed onto AuNPs surface remained active for longer period. To our knowledge, most works have been done using AuNPs functionalized by APTES-modified electrode biosensor applications such as protein detection, DNA detection and others. There is limited published works on using AuNPs functionalized by APTES-modified electrode for the electrochemical detection of heavy metal ions. Therefore, this work is engaged in the reliable and facile pathway of the self-assembly technique, which can be used for the production of the working electrode material for the development of electrochemical sensor to detect heavy metal ions.

In this work, AuNPs were synthesized using seeding-growth method. APTES was used as surface modifier agent for the substrate interact with the produced AuNPs. The novelty of works lies on the parameter for the purpose of optimizing the electrodes that have a good electrochemical property and to study the aspect of limit of detection (LOD) and sensitivity for the heavy metal ions detection. In order to achieve that, the produces AuNPs/APTES/ITO/PET electrode were tested using cyclic voltammetry (CV) and square wave anodic stripping voltammetry (SWASV).

1.3 Research Objectives

The main objectives of this research are :

i) To synthesize and characterize gold nanoparticles (AuNPs) by using seeded-growth method.

ii) To study the effect of APTES concentration, APTES soaking period, and AuNPs soaking period on the AuNPs/APTES/ITO/PET electrode.

iii) To investigate the performance of modified electrode in the detection of Hg and Pb.

1.4 Scope of Study

This study was aimed to synthesize AuNPs using seeding-growth method to produce 40 nm particle size. In this study, different parameters for the modification of electrodes were investigated, which were the effect of APTES concentration, APTES soaking period, and AuNPs soaking period. The properties of AuNPs were studied using Zetasizer, UV-Vis, Transmission Electron Microscopy (TEM), and X-Ray Diffraction (XRD). Then, the APTES was used as the modifier agent for the substrate interact with the produced AuNPs, thereby forming AuNPs/APTES/ITO/PET electrode. Scanning Electron Microscopy (SEM) was used to observe the morphology and distribution of AuNPs on the electrode. The modified AuNPs/APTES/ITO/PET electrodes were then analyzed using electrochemical analyses called cyclic voltammetry (CV) and square wave anodic stripping voltammetry (SWASV).

1.5 Research Outline

This thesis is organized into five chapters. Chapter 1 outline the background of the study, identifies the problem statement, objectives, and scope of study for this research. Chapter 2 presents the literature review of the works related to the concept of heavy metals and the techniques to modify electrode. Moreover, experimental procedures, parameters conducted and characterization method are described in Chapter 3. Chapter 4 focuses on the results and discussion of the research. Finally, Chapter 5 presents conclusion and recommendations for future work.

CHAPTER 2

LITERATURE REVIEW

2.1 Heavy Metals

Heavy metals can be categorized as metallic elements with high atomic weight as compared to water and a minimum density of 5 times greater than that of water (Khalil et al., 2017). Heavy metals, are of interest among many researchers because they are widely used in various manufacturing processes in industry field (Michal et al., 2013). A certain amount of heavy metals are discharged into the environment through natural sources directly or indirectly from irresponsible human activities. These heavy metals can enter the body via food, air, soil, and water. As a result, heavy metals cause toxic effects if the concentration of heavy metals exceed the permitted limit. Above their permitted levels, this can cause risk to human health such as respiratory and cardiovascular problem.

There are heavy metals that considered as harmful and hazardous compounds present even at lower concentrations such as mercury, cadmium, lead, arsenic and chromium (Gumpu et al., 2015). This is due to the hazardous effects of heavy metals that are non-biodegradable and these heavy metals can eventually accumulate in human body (Tchounwou et al., 2012). For an example, heavy metals like zinc and lead cause kidney and nervous damage as they accumulate in human body through the exposure at industrial area (Kinder et al., 2018)

2.2 Sources and Toxicity of Heavy Metals

Heavy metals are well described as hazardous elements to human and other living organisms due to their increasing toxicity and non-biodegradable nature. It has becoming a major concern to many people as it cause detrimental effect to human health. Although the metals are significant to perform its cellular functions, its concentration level gives a massive impact on human health. Table 2.1 shows the sources of heavy metals in the environment presently.

Table 2.1:Sources of Heavy Metals in the Environment (Ayaz et al., 2014)

Heavy Metals	**Sources**
Arsenic	Pesticides and wood preservative
Cadmium	Paints and pigments plastic stabilizers, electroplating incineration of cadmium containing plastics, phosphate fertilizers.
Chromium	Tanneries, steel industries, fly ash
Copper	Pesticides, fertilizers
Mercury	Release from Au-Ag mining and coal combustion, medical waste
Nickel	Industrial effluents, kitchen appliances, surgical instruments, steel alloys, automobile batteries
Lead	Aerial emission from combustion of leaded petrol, battery manufacture, herbicides and insecticides

2.3 Heavy Metals Pollution

Heavy metals pollution is a serious problem due to their toxicity and ability to accumulate in the biota, and causes various health problems in humans and animals. Even though there are some heavy metals are significant trace elements, but most of them are toxic to all forms of life at high concentrations. Table 2.2 lists the parameter limits of heavy metals toxicity set by World Health Organization (WHO) and United States Environmental Protection Agency (USEPA) for safe drinking water whereas Table 2.3 lists heavy metals and their toxicity that could endanger life forms when they are above permitted levels. As a result, heavy metals generate many of their adverse health effects through the formation of free radicals, resulting in DNA damage, cardiovascular and respiratory problems (Kumar, et al., 2017).

Table 2.2 Parameter limits of heavy metals toxicity for safe drinking water (Opaluwa et al., 2012)

Heavy Metal	**Parameter Limits (mg/L)**	
	World Health Organization	**US Environmental Protection Agency**
Arsenic	0.01	0.05
Cadmium	0.003	0.005
Copper	-	1.3
Lead	0.01	0.005
Mercury	0.001	0.002
Nickel	0.02	0.1

Table 2.3 Toxicity of heavy metals to life forms

Heavy Metals	Sources	Effects on Human Health	References
Arsenic	Atmospheric deposition, mining, pesticides, rock sedimentation, medicine, preservative for wood	Brain damage, cardiovascular and respiratory disorder, various of disorders, skin lesions problems with respiratory and nervous system	(Chung et al., 2014)
Antimony	Sea spray, forest fires, and biogenic sources, windblown dust, soil erosion, volcanic eruption	Respiratory, cardiovascular, gastrointestinal, dermal, reproductive, carcinogenicity and genotoxicity effects.	(Sundar and Chakravarty, 2010)
Beryllium	Coal and oil combustion, volcanic dust	Pneumonitis, cough, chest pain, berylliosis, lung cancer, heart diseases.	(Cooper and Harrison, 2009)
Cadmium	Fertilizer, anticorrosive agent, stabilizer in PVC products, colour pigment, and batteries	Respiratory distress syndromes (ARDS), kidney damage, bone damage, itai-itai disease, cancer,	(Godt et al., 2006)
Chromium	Dyeing, electroplating, paints production, steel fabrication, tanning, textile	Bronchopneumonia, chronic bronchitis, diarrhea, emphysema, headache, irritation of the skin, itching of respiratory tract, liver diseases, lung cancer, nausea, renal failure, reproductive toxicity, vomiting	(Ayangbenro and Babalola, 2017)
Copper	Mining, pesticide production, chemical industry, metal piping	Anemia, liver and kidney damage, stomach and intestinal imitation	(Singh et al., 2011)
Mercury	Batteries, fossil fuel emissions, commercial products, incineration of medical waste, geothermal activities,	Death, mental retardation, dysarthria, blindness, neurological deficits, blindness, deafness, decrease rate of fertility, dementia, dizziness, kidney problem,	(Rice et al., 2014)

	mining, paint industries, volcanic eruption.	loss of memory, pulmonary edema, reduced immunity, sclerosis	
Lead	Gasoline, water distribution system, food, coal combustion, electroplating, manufacturing of batteries	Kidney and nervous system damage, premature infant and low births, circulatory system damage, kidney malfunction.	(Tchounwou et al., 2012)
Nickel	Electroplating, metallurgical, food processing and chemical industries.	Allergic skin disease, asthma, conjunctivitis, lung fibrosis, cancer of the respiratory tract and iatrogenic nickel poisoning	(Cempel and Nikel, 2006)
Selenium	Soil	Nail discoloration and brittleness, diarrhea, fatigue, hair loss, joint pain, fatigue, irritability, and foul breath odour.	(MacFarquhar et al., 2010)
Silver	Battery manufacture, mining, photographic processing, smelting, window coatings and mirror, medicine, lubrication, catalyst and electroplating.	Argyria and argyrosis, liver and kidney damage, eyes, skin, respiratory and intestinal tract irritation, and changes in blood cell.	(Drake and Hazelwood, 2005)
Thallium	Photoelectric cells, lamps, electronics, low-temperature thermometers, imaging procedures, scintillation counters, and optical lenses semiconductor.	Alopecia, ataxia, burning feet syndrome, coma, convulsions, delirium, fatigue, gastroenteritis, hair fall, hallucinations, headache, hypotension, insomnia, nausea, tachycardia, vomiting	(Saha et al., 2004)
Zinc	Brass manufacturing, refinery, plumbing, and metal plating	Zinc fumes have corrosive on skin, and cause damage to nervous membrane.	(Wuana and Okieimen, 2011)

Heavy metals can cause pollution to the environment through wide range of mediums such as air during combustion, extraction and processing, and water through runoff and releases from storage and also other mediums such as soil and food. Among these mediums, atmospheric emissions are the greatest concern because of the quantities of involved and the widespread dispersion and potential for exposure that often ensues (Jarup et al., 2003).

In Malaysia, heavy metal water pollution issues have grown in large magnitude with higher complexity as compared to 30 years ago. This is attributed due to the shift of the Malaysia economy from agricultural to industry based since 1990s. From the data collected by the Department of Environment (Malaysia), it tells us that poor quality of river water. Heavy metals highly exists in the Malaysian aquatic environment and should be monitored because the manufacturing sector is the major contributor of heavy metal pollution in the environment. In the metal finishing processes, such as electroplating, etching, and preparation of metal components for various industries that at the end turned up to wastes that containing high concentrations of Cd, Cu, Zn, Nickel (Ni),Iron (Fe), Aluminum (Al), Zinc (Zn), Manganese (Mn), Chromium (Cr) and Stanum (Sn) (Shazili et al., 2006).

On top of that, heavy metals essentially become contaminants in our environments because of their rates of generation from manmade cycles are more faster compare to natural ones, and also the heavy metals easily transferred from one place to another. For example, heavy metals transferred from mines to random environmental place where there were higher tendency direct exposure to mankind. Therefore, the society needs to be addressed the necessity for development of strategies to decrease exposure to these metals as well as create the awareness to this issue earlier in order to overcome their hazardous effects within the body of living organisms.

2.4 Techniques for Heavy Metal Ions Detection

Heavy metals becomes growing environmental and health problems since these metals are not biodegradable. By delaying in detection and monitoring of these metal contaminants could cause major human health problems. High sensitivity, rapid and good selectivity currently one of the main requirements for the determination of trace levels subparts per billion (ppb) of these toxic heavy metal ions in various complex matrices like biological samples (blood, serum, saliva), natural and waste water, food, air and soil (Bansod et al., 2017).

At early stages, spectroscopic techniques are mainly used to target heavy metal ions which is atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectroscopy (ICP-MS), X-ray Fluorescence and neutron activation analysis. These techniques can be used for large amount of elements, have good sensitivity and gives limit of detection (LOD) in the femtomolar range. However, these detection techniques are complicated to perform as they require multi-step sample preparation, tedious analytical procedures, and require high-skilled workers that are not suitable for on-site applications (Pujol et al., 2014). Therefore, there is demand to develop a device and detection techniques with the low cost, simple and dependable techniques that can be used for real time field detection system.

There should be an urgent requirement for in-situ, real-time and highly sensitive sensors. Hence, sensor has been an alternative analytical tools to be used for heavy metals detection. There are many efforts have been made to develop sensor based on nanomaterial based materials. The incorporation of nanomaterials in the sensor has brought many possible applications in biological sensor, drug delivery, bio-imaging, environmental monitoring, and more. There are different type of sensor including

Surface-enhanced Raman Spectroscopy (SERS) sensor, Field-Effect Transistor (FET) sensor, fluorescent sensor, plasmonic sensor and optical sensor . Among the various type of sensor, electrochemical sensor received great interest for heavy metal detection. These devices offer efficient method to target heavy metal ions because of their high sensitivity and selectivity, rapid analysis, portable and inexpensive (Shiquan Xiong et al., 2013).

Based on the studies conducted by farahi et al., (2012), a sensor is described as a combination of two components: the recognition element and the transducer. Typically, an electrochemical sensor is a device that transforms electrochemical information into an analytically useful signal. Consequently, the major part is the recognition element (molecular), while the physicochemical transducer converts the chemical response into a signal that can be detected by modern electrical instrumentations. The combination of these two components form a working (sensing) electrode that able to detect substances such as heavy metal ions. A reference electrode and a counter electrode are also used in electrical measurements (Farnoush Faridbod, 2011).

Electrochemical techniques offer several benefits in term of cost, simplicity, portability, and the possibility of usage in-field application compared to the other detection techniques. There are several techniques available in the electrochemical sensing based on different signals produced, including voltammetric, potentiometric, amperommetric, electrochemical impedance, capacitance, and electrochemiluminescent methods. Based on the studies, the most reported electrochemical techniques for heavy metals sensing are voltammetric and potentiometric (Cui et al., 2015). Another studies carried out by Zhang et al., (2010) depicts that stripping voltammetry has been successfully used for detection of lead, cadmium and copper in seawater as it require simple measurement, high sensitivity, and can be able to monitor variety of heavy metals simultaneously.

The key element to obtain a good electrochemical sensor depends on the kind of material that develop the detection platform. At present, nanomaterials have shown excellent behavior on the development of new electrochemical sensor that is expecting to bring many advantages in the area of heavy metals sensor (Aragay et al., 2012). Nanomaterials that have been used in sensor fields are metal nanoparticles (gold, silicon, magnetic composite, polymer), nanowires (gold, polymer, composite), nanoporous surfaces, carbon allotropes, and quantum dots (farahi et al., 2012). Among various nanostructured materials, metal nanoparticles have large surface-to-volume ratio, good optical, electronic and magnetic properties that depends on their core materials. Furthermore, metal nanoparticles can be designed and surface modified with many small organic ligands and large biomacromolecules by using many approaches (Agasti et al., 2010). Therefore, by having these capabilities, it is expected to have a detection system that have advantages in terms of sensitivity, selectivity, reliability and practicality as shown in Figure 2.1 the example of metal nanoparticles based detection system.

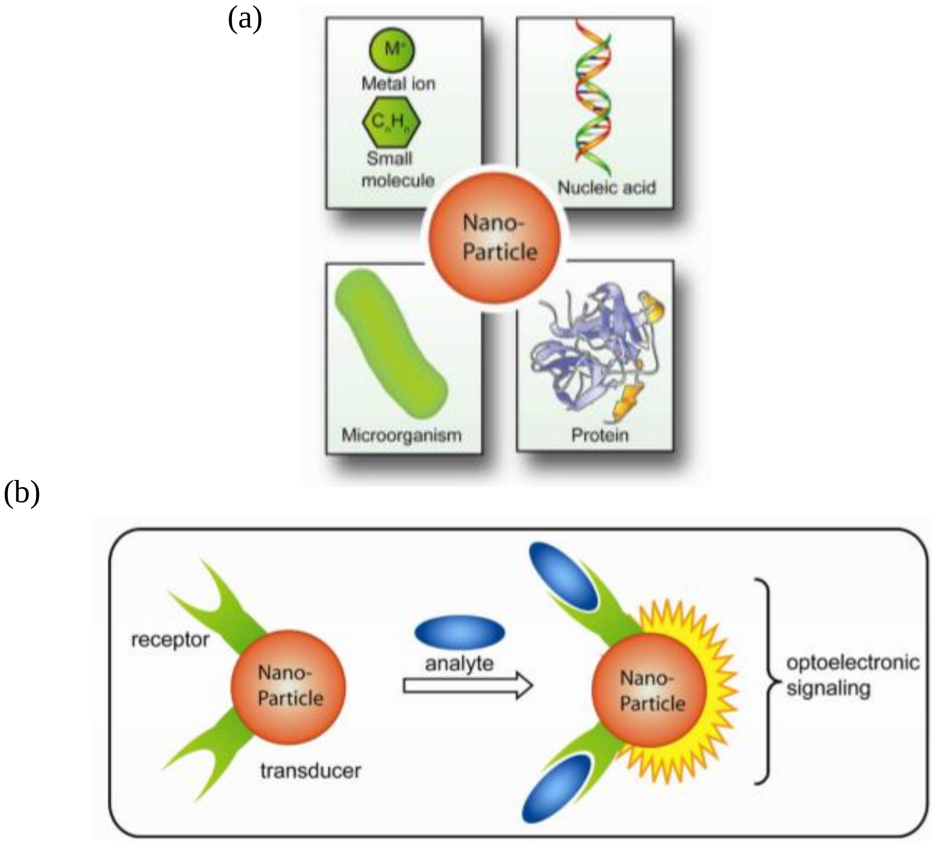

Figure 2.1: Example of nanoparticle based sensing system: (a) Examples of analyte for the detection (b) Schematic of nanoparticle based sensing system (Agasti et al., 2010)

2.4.1 Electrochemical Detection Techniques

Electrochemical detection techniques basically transforms electrochemical information into useful signal and can be used to detect heavy metal ions which have advantages including fast analysis, good selectivity and sensitivity. There are some criteria should be fit for metal ions analysis using electrochemical measurements: (1) selective adsorption of metal ions, (2) intensifying electron exchange between the working electrode and the metal ions, (3) strong electrochemical response.

Based on various electrical signals, electrochemical techniques are classified into voltammetric, amperometric, potentiometric, impedance measurement, and electrochemiluminescent techniques (Bansod et al., 2017). In most of the heavy metal ions determination, voltammetry techniques is frequently used in various complex environmental matrices. Moreover, voltammetry techniques is used for trace metal detection due to its high accuracy and sensitivity.

Voltammetry technique involves measurement of current at various potential points in a current-voltage curve whereas Amperometric technique used the fixed potential point. These techniques are suitable to partially suppress the background current and improve the limit of detection. Qualitative information can be obtained by studying the reversibility of reactions in the electrochemical cell setup (Bansod et al., 2017). Voltammetry techniques is further classified as linear sweep voltammetry (LSV), pulse voltammetry techniques, cyclic voltammetry (CV), hydrodynamic voltammetry and stripping voltammetry. Among voltammetric techniques, stripping techniques such as anodic stripping voltammetry (ASV) is favourable for trace heavy metal detection due to the high sensitivity and selectivity owing the combination of the separation, pre-concentration and determination steps in one single process.

Stripping voltammetry technique can be divided into Anodic Stripping Voltammetry (ASV), Cathodic Stripping Voltammetry (CSV), Cyclic Voltammetry (CV), Adsorptive Stripping Voltammetry (AdSV) in which the two-step method that allows simultaneous detection of various inorganic and organic species in the sub-nanomolar range (March et al., 2015). Stripping voltammetry is capable to identify and quantify trace components from 10^{-5}M to 10^{-9}M with good sensitivity and selectivity (Mousa et al., 2014).

The remarkable sensitivity of stripping voltammetry is attributed to the pre-concentration step in which the chemical species are electrolytic deposited onto an inert electrode surface at a fixed potential. Stripping technique can be either an anodic or cathodic reaction. In general, stripping voltammetry that involves a cathodic reaction is more favourable as deposition of metal ionic species are reduced from the solution to the electrode surface. The next step in stripping voltammetry comprises the application of a voltage scan to the electrode. At a specific potential, it causes the stripping of a specific species accumulated onto the electrode surface as amalgam or thin films, into the solution. Figure 2.2 shows the faradic current is proportional to the concentration of the chemical species (March et al., 2015). The stripping waveform plot of potential applied to the working electrode vs. time consist of several discrete steps: (a) deposition, (b) equilibration, (c) stripping.

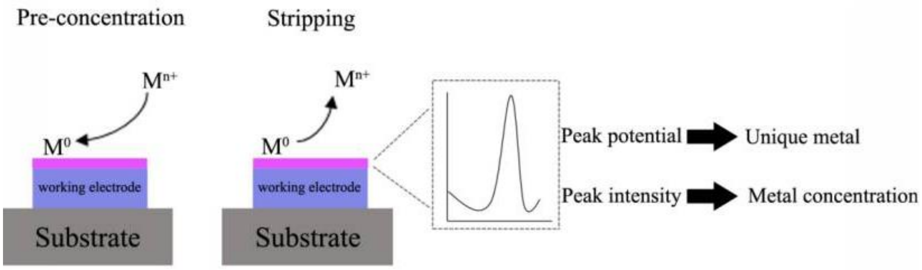

Figure 2.2: Anodic Stripping Voltammetry (ASV) principle (March et al., 2015)

ASV technique is known as a very sensitive and reproducible (RSD< 5%) technique for trace metal ion analysis in aqueous media. Furthemore, ASV method is capable to identify and quantify trace components from 10^{-5} to 10^{-9} M with superior sensitivity and selectivity (Mousa et al., 2014). In the same way, potentiometric techniques involves the agglomeration of a charge potential at the working electrode differ to the reference electrode in an electrochemical measurement when there are no significant current flows between. In short, potentiometric techniques gives information regarding the ion activity in an electrochemical reaction. Furthemore, Nernst equation as shown in Equation 2.1 is used for potentiometric measurement that enlighten the relationship between the concentration and the potential.

$$\text{EMF or Ecell} = E_o\,\text{cell} - RT\ nF\ \ln Q \qquad \text{(Equation 2.1)}$$

E cell is the cell potential at zero current and known as the electromotive force (EMF), E_o cell is a constant potential distribution to the cell, R is the universal gas constant, T is the absolute temperature, n is the charge number of the electrode reaction, F is the Faraday constant and Q is the ratio of ion concentration at the anode to ion concentration at the cathode. Therefore, potentiometric technique is suitable for measuring and analysing small sample in low concentrations, yet presently quite limited for sensing some important analytes such as nickel, manganese, mercury and arsenic ions (Grieshaber, et al., 2008).

Table 2.4 Nanomaterials application in relation to voltammetric techniques for heavy metals detection

Electrochemical Platform	**Technique**	**Analyte**	**Detection Limit**	**References**
Au Nanoparticle Modified GCE	LSV	As(III)	0.024 µM (1.8ppb)	Zhang and Fang ., 2010
Bi Nanopowder electrode	SWASV	Cd(II) and Pb(II)	Cd(II): 0.15µg/L Pb(II): 0.07µg/L	
Nano-TiO_2/chitosan (CS) composite film modified GCE	ASV	Cd(II)	2.0 x 10^{-10} mol/L	
solid gold electrode (SGE) and a gold nanoparticle-modified glassy carbon electrode (AuNPs-GCE)	SWASV	Hg (II)	LOQ in fish-matrix of 0.1 µg/l	Abollino et al., 2008
Screen-printed carbon electrodes (SPCEs) modified with gold films		Hg (II)	0.9 µg/l	Meucci et al., 2009
RGO-CS/PLL modified glassy carbon electrode (RGO-CS/PLL/GCE)	DPASV	Cd(II), Pb(II), and Cu(II)	Cd(II):0.01 µg L^{-1} Pb(II)):0.02 µg L^{-1} Cu(II): 0.02 µg L^{-1}	Guo et al., 2016
Au/MPS-(PDDA-AuNPs)	DPASV	As(III)	0.48µM	Ottakam Thotiyl etal.,2012
GC+AuNPs	SWASV	Hg(II)	0.42nM	Hezzard et al., 2012
Nanostructured Au modified GCE	ASV	Cr(VI)	0.01 µg/L	Zhang and Fang ., 2010
Pt Nanoparticle-modified GCE		As(III)	0.12 ppb	

2.5 Modified and Unmodified Electrode for Heavy Metal Ions Detection

Generally, there are many types of electrodes such as Pt, Au, C and Hg amalgams and semiconductor (indium-tin oxide) used in sensor applications. However, the sensitivity and reproducibility of these bulky electrodes were decreasing and getting poisoned quickly (Khairy et al., 2017). For example, previously, hanging mercury drop and mercury film were used to detect heavy metal ions due to their advantages of having wide cathodic potential range, good sensitivity and repeatability. On the contrary, mercury-film based electrode contains high toxicity of mercury and harmful to the environment, difficulty in handling and storage. That is why mercury based electrode was banned and the use of mercury-free solid-state electrodes for heavy metals sensing demanded (Cui et al., 2015). Therefore, other electrodes made of other materials are sought after being used in modified forms to improve the electroanalytical performance.

For heavy metal ions sensing, the modification of the working electrode plays a significant role especially during the pre-concentration step, by having good selective and increased accumulation, higher sensitivities and lower limit of detections (LOD) achieved. Besides, it will also enhances the electron transfer movements (Pujol et al., 2014). This is in agreement with the results reported by some researchers. Figure 2.3 shows the analytical performance of sensor during the cyclic voltammetry (CV) analysis in which modified thermally reduced graphite oxide with gold nanoparticles (TRGO/Au) electrode and Au electrode used in heavy metal detection. By observing the shaped and position of the redox peak current, the Au electrode shows a weak redox peaks current while the TRGO/Au electrode shows significantly high redox peak currents (Xuan et al., 2016). Therefore, modified electrodes are used to improve the electroanalytical performance.