ELUCIDATING ELECTROCHEMICAL PROPERTIES OF SUITABLE POLYMER COATED ON CARBON ELECTRODE TO DETECT HEAVY METALS

NURUL HANISARINA BINTI ZAHARUM

UNIVERSITI SAINS MALAYSIA

2022

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by

NURUL HANISARINA BINTI ZAHARUM

Thesis submitted in fulfilment of the requirement for degree of

Bachelor of Chemical Engineering

July 2022

ACKNOWLEDGEMENT

First, I would like to express my deepest and sincere gratitude to my supervisor, Associate Professor Dr. Low Siew Chun and her post-graduate student, Mr. Ong Chyh Shyang for their precious time, guidance, inspiration and endless encouragement to complete this report successfully. Then, I want to express my gratitude to Prof. Dr. Mohd Roslee Othman, the Coordinator of Final Year Project and all lecturers that involved in guiding and sharing their knowledges and experiences.

Apart from that, my heartfelt gratitude to my parents for their supports and prayer that allowed me to pull through all the hardship and challenges. Last, I also would like to thank to my friends that help and give moral support though out my writing of this report. Once again, I would like to thank all the people that have helped me directly or indirectly in completing this report. Thank you very much.

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LIST OF SYMBOLS

Symbol	**Description**	**Unit**
E	Potential	V
E_{amp}	Potential amplitude	V
E_{step}	Potential step	V
i	Current	μA
R^2	Correlation coefficient	-
S	Slope of calibration curve	-
S_y	Standard deviation	ppm
t_{dep}	Time deposition	s

LIST OF ABBREVIATIONS

Symbol	**Description**
AAS	Atomic absorption spectroscopy
AdCSV	Adsorptive cathodic stripping voltammetry
AdASV	Adsorptive anodic stripping voltammetry
AdSV	Adsorptive stripping voltammetry
AFS	Atomic fluorescence spectroscopy
Ag	Silver
AgCl	Silver chloride
Ag/AgCl	Silver-silver chloride electrode
As	Arsenic
ASV	Anodic stripping voltammetry
Au/SPCE	Gold modified screen printed carbon electrode
AuNP/SPCE	Gold nanoparticle modified screen printed carbon electrode
Au-CTS/SPCE	Gold and chitosan modified screen printed carbon electrode
Bi	Bismuth
Bi^{3+}	Bismuth (III) ions
BiFE	Bismuth film electrode
BiNPs	Bismuth nanoparticles
BiNPs/SPCE	Bismuth nanoparticle modified screen printed carbon electrode
CA	Cellulose acetate

CA/SPCE	Cellulose acetate modified screen printed carbon electrode
CA/ZnO	Cellulose acetate modified zinc oxide
CE	Counter electrode
Cellulose-CME	Cellulose modified electrode
Cd	Cadmium
Cd^{2+}	Cadmium (II) ions
CGE-CA/ZnO	Cellulose acetate and zinc oxide modified carbon glass electrode
Cl^-	Chloride ions
CNS	Central nervous system
CNTs	Carbon nanotubes
CPE	Carbon paste electrode
Cr	Chromium
CS/GO-IIP	Chitosan-graphene oxide composites polymer-modified glassy carbon electrode
CSV	Cathodic stripping voltammetry
CTS	Chitosan
CTS/CCE	Chitosan modified carbon glass electrode
CTS/SPCE	Chitosan modified screen printed carbon electrode
CTS/GO-IIP	Chitosan-graphene oxide composites ion imprinted Polymers modified glassy carbon electrode
Cu	Copper
CV	Cyclic voltammetry
e^-	Electron

ECS	Electrochemical sensor
EIS	Electrochemical impedance spectroscopy
FTIR	Fourier transform infrared spectroscopy
GC	Glass carbon
GCE	Glassy carbon electrode
H_2	Hydrogen
H^+	Hydrogen ions
Hg	Mercury
RE	Reference electrode
HMDE	Hanging mercury drop electrodes
HMs	Heavy metals
IC-UV-vis	Ion chromatography ultraviolet visible spectroscopy
ICP-AES	Inductively coupled plasma/atomic emission spectrometry
ICP-MS	Inductively coupled plasma-mass spectroscopy
IIPs	Ion imprinted Polymers
KCl	Potassium chloride
$K_4[Fe\ (CN)_6]\cdot 3H_2O$	Potassium ferrocyanide
LOD	Limit of detection
LSV	Linear sweep voltammetry
M - SPCE	Modified screen-printed carbon electrode
Mn	Manganese
MP	Microprobes
MWCNTs – CTS	Multiwalled carbon nanotubes – based chitosan
Nafion/CNTs	Nafion modified carbon nanotubes

Ni	Nickel
Nylon 6/SPCE	Modified nylon 6 on screen printed carbon electrode
OEM	Original Equipment Products
OPFP	N-octylpyridinum hexafluorophosphate
Pb	Lead
Pb^{2+}	Lead (II) ions
PNS	Peripheral nervous system
ppb	Part-per-billion
PPF	Pyrolyzed photoresist film
ppm	Part-per-million
Pt	Platinum
PVC	Polyvinyl chloride
SCE	Saturated calomel
SDGs	Sustainable development goals
SEM	Scanning electron microscopy
SHE	Standard hydrogen electrode
SPE	Screen printed electrode
SPCE	Screen printed carbon electrode
SPE/SWCNHs	Single wall carbon nanohorns modified SPE
SPCE/ MWCNTs – CTS	Multiwalled carbon nanotubes – based chitosan coated screen printed carbon electrode
SWASV	Square wave anodic stripping voltammetry
SWSV	Square wave stripping voltammetry
WE	Working electrode
XFS	X-ray fluorescence spectroscopy

Zn	Zinc
Zn^{2+}	Zinc (II) ions
ZnO	Zinc Oxide

MENJELASKAN SIFAT-SIFAT ELEKTROKIMIA POLIMER YANG SESUAI UNTUK DISALUTKAN PADA ELEKTROD KARBON UNTUK MENGESAN LOGAM BERAT

ABSTRAK

Pencemaran alam sekitar oleh logam berat (HM) telah mendapat perhatian global disebabkan oleh ketoksikan yang tinggi, beracun dan tidak terbiodegradasi dalam alam semula jadi walaupun pada kepekatan rendah, yang telah menimbulkan ancaman serius kepada kesihatan manusia dan alam sekitar. Oleh itu, pembangunan kaedah pengesanan yang mudah, sangat sensitif dan selektiviti yang baik dalam pengesanan HM adalah amat penting. Elektrod cetakan skrin (SPCE) digunakan secara meluas sebagai elektrod kerja (WE) dalam penderia elektrokimia kerana ia menawarkan beberapa kebaikan seperti mudah alih, sensitif, selektiviti dan dapat melakukan analisis pada masa yang singkat. Pengubahsuaian permukaan WE dengan menggunakan polimer juga telah mendapat perhatian dalam pembangunan penderia elektrokimia dalam pengesanan HM kerana sifatnya yang anti-kotoran dengan membentuk permukaan pelindung, kestabilan yang tinggi dan kinetic pemindahan electron yang cepat. Terdapat beberapa matlamat dalam Matlamat Pembangunan Lestari (SDGs) yang dapat dicapai dalam kajian ini iaitu Matlamat 6 (sir bersih dan sanitasi) dan Matlamat 9 (industri, inovasi dan infrastruktur).

Di dalam tesis ini, sifat elektrokimia polimer yang sesuai untuk disalut pada SPCE dalam pengesanan HM seperti plumbum (II) ion (Pb^{2+}) telah dikaji. Terdapat tiga jenis polimer yang digunakan untuk pengubahsuaikan SPCE iaitu kitosan, nilon 6 dan selulosa asetat dengan menggunakan kaedah salutan titisan. Kaedah voltammetri seperti voltammetri kitaran (CV) dan voltammetri pelucutan (SV) digunakan dalam penentuan tindak balas arus dan pengesanan Pb^{2+} kerana kaedah ini mempunyai

ketapatan dan kepekaan yang tinggi. Sifat fizikal dan kimia SPCE yang diubah suai (M – SPCE) telah dianalisis dengan menggunakan mkroskop cahaya dan spektroskopi inframerah transformasi fourier (FTIR). Berdasarkan analisis, terdapat perubahan yang ketara dalam struktur dan kumpulan befungsi di M – SPCE untuk semua polimer. Kekonduksian M–SPCE telah diperhatikan dengan menggunakan kaedah CV. Berdasarkan pemerhatian, kitosan yang diubah suai pada SPCE (CTS/SPCE) menunjukkan kekonduksian yang terbaik berbanding dengan nilon 6 yang diubah suai pada SPCE (Nylon 6/SPCE) dan sellulose acetate yang diubah usai pada SPCE (CA/SPCE). Ketabalan filem polimer juga dikaji dengan memanipulasi ketebalan filem polimer iaitu 20, 40 dan 60 μL untuk mengkaji kekonduksian dengan menggunakan kaedah CV. Ketabalan filem polimer iaitu 20 μL menunjukkan kekonduksian yang terbaik kerana menunjukkan puncak arus yang tertinggi berbanding 40 dan 60 μL.

Tahap kepekaan dalam pengesanan Pb^{2+} telah dikaji dengan memanipulasi kepekatan salutan polimer (1, 3 and 5 wt.%) dan kepekaan ion Pb^{2+} dalam analit (0.1 hingga 1.9 ppm) dengan menggunakan voltammetri pelucutan gelombang persegi (SWSV). Pada 1 dan 3 wt.% kepekataan polimer salutan, CTS/SPCE yang diubah suai menunjukkan kepekatan terbaik dengan nilai had pengesanan (LOD) yang rendah iaitu 0.0699 dan 0.01817 ppm, masing – masing. Sementara itu, CA/SPCE yang diubah suai menunjukkan kepekaan terbaik dengan nilai LOD iaitu 0.06598 ppm dan Nylon 6/SPCE yang diubah suai menunjukkan kepekaan terbaik dengan nilai LOD iaitu 0.09775 ppm pada kepekatan 5 wt.% polimer salutan. Dengan peningkatan kepekatan polimer salutan, prestasi Nylon 6/SPCE dan CA/SPCE yang diubah suai juga bertambah baik dalam pengesanan ion Pb^{2+}. Oleh itu, ini menunjukkan bahawa kepekatan polimer salutan mempengaruhi prestasi penderia elektrokimia.

Bagaimanapun, nilon 6 dan selulosa asetat tidak sesuai digunakan untuk pengubahsuaian SPCE kerana menunjukkan kepekaan yang rendah berbanding SPCE tanpa salutan polimer. Secara keseluruhannya, CTS/SPCE yang diubah suai menunjukkan sifar elektrokimia terbaik dengan kepekaan yang tinggi dan sesuai digunakan dalam penambahbaikan SPCE dalam pengesanan Pb^{2+}.

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ABSTRACT

Environmental pollution by heavy metals (HMs) have gained global attention due to high toxicity, poisonous and non-biodegradable in nature even at low concentration, which poses serious threats to human health and environment. Therefore, the development of detection methods that is simple, highly sensitive and good selectivity in detection of HMs is crucial. Screen printed electrode (SPCE) is widely used as working electrode (WE) in electrochemical sensor as it offers several advantages such as portability, excellent sensitivity, selectivity and on-site analysis with short detection time. Modification of the surface WE by using polymer also gained attention in development of electrochemical sensor in detection of HMs due to unique properties such as anti-fouling properties by forming a protective surface, high stability and fast electron transfer kinetics. From this work there are several goals in in Sustainable Development Goals (SDGs) is achieved which are Goal 6 (clean water and sanitation), Goal 7 (affordable and clean energy) and Goal 9 (industry, innovation and infrastructure).

In this thesis, the electrochemical properties of suitable polymer coated on SPCE in detection of HM such as lead (II) ions (Pb^{2+}) was studied. There are three types polymer were used for modification of SPCE which are chitosan, nylon 6 and cellulose acetate by using drop coating method. Voltammetry method such as cyclic voltammetry (CV) and stripping voltammetry (SV) were used in determination of current response and detection of Pb^{2+} ions due to its high precision and sensitivity. The physical and chemical properties of modified SPCE (M – SPCE) was

characterized by using light microscope and fourier transform infrared spectroscopy (FTIR). It observed that there are significant changes of the structure and functional groups of the M – SPCEs for all polymers. The conductivity of M – SPCE was observed by using CV method. It was observed that the modified chitosan on SPCE (CTS/SPCE) showed the best conductivity compared to modified nylon 6 on SPCE (Nylon 6/SPCE) and modified cellulose acetate on SPCE (CA/SPCE). The thickness of polymer film also studied by manipulating the thickness film which are 20, 40 and 60 μL to study the conductivity by using CV method. The thickness of polymer film of 20 μL seems to best conditions to coating as the peak of current showed the highest followed by 40 and 60 μL.

The sensitivity of detection of Pb^{2+} ions was studied by manipulating the concentration of coating polymers (1, 3 and 5 wt.%) and concentration of Pb^{2+} ions in analyte (0.1 to 1.9 ppm) by using square wave stripping voltammetry (SWSV). At 1 and 3 wt.% concentration of coating polymer, modified CTS/SPCE showed the best sensitivity with low limit of detection (LOD) value of 0.0699 and 0.01817 ppm, respectively. Meanwhile, modified CA/SPCE showed the best sensitivity with LOD value of 0.06598 ppm and modified Nylon 6/SPCE showed the best sensitivity with LOD value of 0.09775 ppm at 5 wt.% concentration of coating polymer. With increasing the concentration of coating polymers, the performance of modified Nylon 6/SPCE and CA/SPCE also improved in detection of Pb^{2+} ions. Thus, this indicated that the concentration of coating polymers affected the performance of electrochemical sensor. However, nylon 6 and cellulose acetate is not suitable to be used for modification of SPCE as it showed low sensitivity compared to bare SPCE. Overall, the modified CTS/SPCE showed the best electrochemical properties with high sensitivity and suitable to be used in development of SPCE in detection of Pb^{2+} ions.

CHAPTER 1

INTRODUCTION

1.1 Research Background

The continuous increase of heavy meals (HMs) ions in the environmental along with the increasing population, urbanization and economic development need to be concerned. HMs are metallic chemical elements that has a relatively high density more than 5 gcm^{-3}, high toxicity, poisonous and non-biodegradable in nature even at low concentration. The examples of heavy metals are cadmium (Cd), arsenic (As), lead (Pb), copper (Cu), mercury (Hg), boron (B), silver (Ag), nickel (Ni), and zinc (Zn). These heavy metals lead several adverse effects to human health and environmental such as water and soil. Antwi et al. (2021) summarises that the top sources of water pollution in China is dumping from industry (41%) followed by sewage and wastewater (13%), HMs from fertilizer and pesticides (29%), leakages (11%) and animal waste (6%). It is indicating that the heavy metals are one of the main sources of water solution. The main sources of HMs contamination are from anthropogenic activities such as mining activities, sewage, wastewater, fertilizer and others. These HMs can lead many adverse effects to human health due to its toxicity and non-biodegradable in nature. The most dangerous HMs are cadmium (Cd) that come from pain sludge and zinc smelting can cause itai-itai disease, chromium (Cr) comes from mining can cause lung cancer and damage DNA and mercury (Hg) arises from thermal power plant can lead to Minamata disease and damage kidneys.

There are various techniques have been established to detect and monitor the heavy metal (HMs) ions. The conventional techniques of HMs detection are atomic absorption spectroscopy (AAS), atomic fluorescence spectroscopy (AFS) and inductively coupled plasma-mass spectroscopy (ICP-MS) (March et al., 2015;

Mohamad et al., 2021a; Waheed et al., 2018). These techniques are highly sensitive and selective. However, there are several limitations such as expensive equipment, complex preparation and procedure which require trained personnel, long detection time of response and not suitable for on-site measurement and applications. Therefore, electrochemical sensor technique has received a huge attention for detection of HMs. This is because electrochemical offers simple operational procedure, low cost, excellent sensitivity and selectivity, portability and on-site detection ability.

Electrochemical sensor system mainly consists of three type of electrodes which are working electrode (WE), counter electrode (CE) and reference electrode (RE). Its sensor performance is depending on the reaction that take place between WE and solution interface. The higher the reaction take place on the WE, the higher sensitivity of heavy metals ion detection. Thus, the selection of the materials of WE and its surface structure are important to enhance the rate of reaction take place onto WE and sensitivity towards HMs ion. Stripping voltammetry is the most used in electrochemical sensor to detect HMs ions due to its low limit of detection and can simultaneously detect different type of HMs ions. Historically, hanging mercury drop electrode (HMDE) and mercury film thin was commonly used as working electrode in HMs ions detection due to its ability to form amalgams with HMs ions and wide cathodic potential range. However, its toxicity has limited its application in electrochemical sensor (Garciá-Miranda et al., 2020).

Throughout the years followed with the trend of miniaturization of sensors, there are various type of electrodes have been studied in electrochemical sensor for on-site measurement and detection of HMs. For the example, screen printed carbon electrodes (SPCEs) which knowns as disposable sensor have obtained extensive attentions due to its portability, rapid reaction, simple and low cost compared to the conventional bulky electrodes. Figure 1.1 shows the variety of fabrication techniques of SPCE which are screen printing (61.5%), inkjet printing (19.2%), combination of screen and inkjet printing (11.5%) and coating (7.7%) (Wahyuni, Riza Putra, et al., 2021).

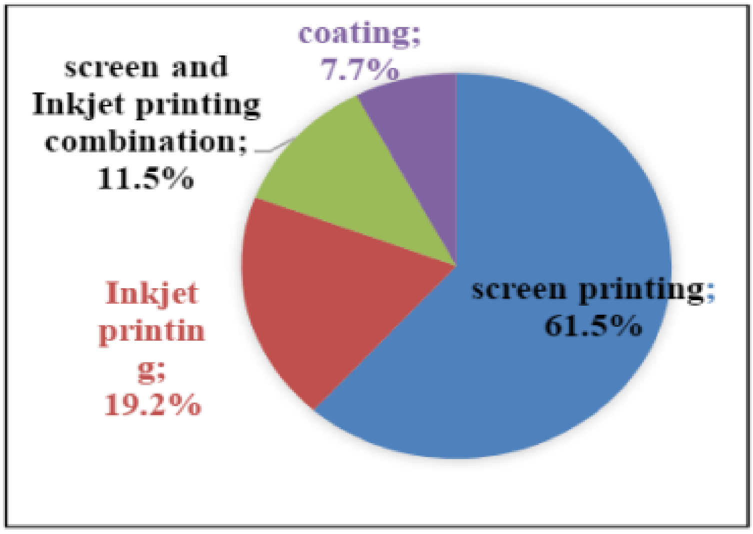

Figure 1.1: Type fabrication techniques of SPCEs

In this research project, screen printing technique is used as fabrication technique onto the surface WE of SPCEs. Other than that, modification of WE electrodes of different interface of materials like metal oxides, nanoparticles and polymer such as chitosan, cellulose acetate and nylon also have gained great attention to increase the performance of electrochemical sensor analysis. This report will focus on the electrochemical properties of suitable polymer coated on carbon electrode to detect heavy metals.

1.2 Problem Statement

The developments of conventional electrochemical sensors in HMs ions detection are required due to complicated and multiple operational procedures that consume time, high fabrication cost, low sensitivity and potential range. Garciá-Miranda et al. (2020) stated that conventional solid electrodes such as glassy carbon (GC) need to be rigorously polished and cleaned before undertaking the readings and the presence of external RE and CE are necessary. In the past, the uses of hanging mercury drop electrode (HMDE) and mercury film thin as working electrode in HMs ions detection is limited due to high toxicity. Thus, screen printed carbon electrode (SPCE) was introduced to overcome these problems. SPCE has gained extensive attentions due its advantages such as offering a reduction in fabrication cost, portability, simple and ready to use, reproducible, can be used as single shot, miniaturization possibility, excellent sensitivity and on-site analysis due to short detection time compared to the conventional bulky electrodes (Dai et al., 2018; Jadon et al., 2016).

Next, electrochemical sensor also facing problems of low sensitivity and conductivity of stripping voltammetry in HMs ion detection, especially in low concentration of analyte. This is because of low deposition or accumulation of HMs ions onto the surface of electrode which results on slow rate of electron transfer. Other than that, the ability to detect HMs ion also limited due to some challenges in simultaneous detection such as the possibility of overlapping peaks due to narrow potential range during oxidation or reduction process, the presence of intermetallic interference, low cathodic potential limit that give large background of current and the generation of hydrogen bubbles during deposition process that affect the enrichment of HMs ions (Mohamad et al., 2021; Thiruppathi et al., 2017). These challenges can be overcome through modification of electrode by using polymer coating such as nylon, chitosan and cellulose acetate. The advantages of modified electrode by using polymer coating are excellent conductivities and adhesion properties, enhance the selectivity toward HMs, high stability and fast electron transfer kinetics. Moreover, polymer coating also can help to prevent agglomeration and enhances physical and chemical properties which results in increasing catalytic and sensing activities.

Lastly, the operating conditions of modified SPCE with polymer coating such as the type of polymers, thickness of coated polymer film and analyte concentration of HMs ions give several impacts to the performance of electrochemical sensor. Thus, the evaluation of the electrochemical response of carbon electrode on heavy metal will be done by cyclic voltammetry (CV) and stripping voltammetry (SV). The purpose of this evaluation is to study the electrochemical properties of suitable operating conditions for polymer coating on carbon electrode based on their sensitivity and conductivity on HMs ions detection in electrochemical sensors.

1.3 Research Objectives

1. To produce and characterize a high electrochemical screen-printed carbon electrode.

2. To surface coating or blending of heavy metal-attracted polymer on carbon electrode.

3. To evaluate electrochemical response of carbon electrode on heavy metal by cyclic voltammetry (CV) and stripping voltammetry (SV).

1.4 Scope of Thesis

Application of polymers in modification of WE electrodes have gained great attention in enhancement of the performance of electrochemical sensor analysis and increase the sensitivity of sensor in detection of HMs. Thus, it is important to understand the electrochemical properties that effect the performance of the electrochemical sensor analysis. The scope of this research project is to determine the electrochemical properties of suitable polymer coated on the carbon electrode to detect heavy metals. Firstly, fabrication of SPCE is conducted by using printing technique and used as WE in electrochemical sensor. Then, CV and SV test are run to determine the conductivity and sensitivity of bare SPCE. After that, modification of SPCEs (M – SPCEs) of different polymers (chitosan, nylon 6 and cellulose acetate) at 1 wt.% concentration of polymers and 4 μL of coating polymer thickness are conducted by using drop coating method. The CV test is run to study the conductivity of M – SPCEs and SV test is run to observe the sensitivity of M – SPCEs. The light microscope and fourier transform infrared spectroscopy (FTIR) are used to study the changes of surface structure and functional groups of the modified SPCEs, respectively. Then, the thickness of coating polymer is manipulated from 20 to 60 μL to study the effect of different thickness of coating polymer on the current response and conductivity of M – SPCE by using CV method.

Then, the electrochemical response of SPCE in HMs detection is carried out to study the performance of electrochemical sensors. There are two parameters, which are concentration of coating polymers and concentration of HMs in analyte are varied to study the performance of sensor in detection of HMs. The sensitivity of bare SPCE and M – SPCE is determined by manipulating the concentration of coating polymers such as 1, 3 and 5 wt.% and concentration of HMs in analyte from 0.1 to 1.9 ppm of Pb^{2+} ions. The limit of detection also calculated to observe the sensitivity of sensor in detection of Pb^{2+} ions. The best M – SPCE is determined based on high sensitivity with low LOD value of the performance electrochemical sensor in Pb^{2+} ions detection.

CHAPTER 2

LITERATURE REVIEW

2.1 Heavy Metals

Heavy metals (HMs) are the elements with metallic properties, with atomic weights between 63.5 and 200.6 and specific gravity greater than 5 (Pandey et al., 2016). HM is one of the challenging pollutants due to their toxicity, nonbiodegradable nature and bioaccumulation in ecological system, such as cadmium (Cd), arsenic (As), lead (Pb), copper (Cu), mercury (Hg), arsenic (As), silver (Ag), nickel (Ni), Chromium (Cr) and zinc (Zn). The properties of HMs lead to several issues in industry, with adverse effect in environment and human health. Thus, advanced techniques for analyzing HMs are employed to monitor the concentration HMs.

2.1.1 Issues Related to Heavy Metals in Industry

HMs are defined as a metallic element that is relatively dense compared to water and capable of inducing toxicity at low exposure level. HMs exits in various environments such as wastewater, river water, seawater, soil, mud, and food. With the rapid development of industrial and agricultural production, HMs contamination has been become a serious problem. HMs contamination is mainly caused by the anthropogenic activities such as mining activities, industrial processing, sewage discharge, wastewater, domestic effluents, fertilizer and agricultural use of metals and metal-containing compounds. HMs are a naturally occurring elements found in the earth’s crust. However, HMs contamination occurs highly in the anthropogenic industry. The anthropogenic factors can generate a drastically increased concentration levels of HMs in water and sediment. Thus, resulting in higher concentrations of HMs and higher pollution to environment. HMs enter the environment through different industrial sources, including 1) mercury (Hg) through coal burning, mining and municipal solid waste incineration, 2) lead (Pb) arises from automobile exhausts, old paints, mining wastes and, incinerator ash, 3) cadmium (Cd) arises from electroplating, batteries and mining industries, 4) chromium (Cr) from leather industry and chrome plating industries and 5) arsenic (As) comes from herbicides, fertilizer and agriculture (March et al., 2015).

Contamination of HMs in natural waters is a global epidemic. Natural water sources are important to living organism because of their use in drinking water supplies, where even trace amounts of HMs such as Pb, Cu and Cd, represent as a real threat and degrade the quality of drinking water. Since 2009, more than 30 serious water pollution incidents due to HM pollution have been reported in China every year (Lo et al., 2020). In addition to this, contamination of HMs also occurs due to leaching of the existing water infrastructure. A severe problem has arisen in many countries due to inadequate and aging water infrastructure for monitoring HMs concentration, resulting in the leaking of HMs into household water systems. HMs commonly found as contaminants in water supplies are mercury, lead, copper, and cadmium (Garciá-Miranda et al., 2020). Therefore, rapid industrialization has become a global concern due to the high contamination of HMs.

2.1.2 Adverse Effect of Heavy Metals in Environment and Human Health

HMs are considered to be one of the most dangerous elements of water pollutants and are extremely destructive to nature with adverse effects on human health (Bansod et al., 2017). These metals are toxic, nonbiodegradable and ubiquitously distributed, albeit in very low concentrations, posing high risks to the environment and human health. These HMs are ranked in the top 10 of the "Agency for Toxic Substances and Disease Registry Priority List of Hazardous Substance" and considered as the "Environmental health hazards" due to their high toxicity of substance and potential exposure to contaminated air, water, and soil (Gumpu et al., 2015).

HMs pollutants are highly persistent in environment such as water and soils because they cannot be degraded naturally, which will accumulate in the biosphere and enter living organisms through the alimentary chain. These HMs possess a high risk of entering the human body the food chain, posing a threat to human health. HMs can trigger serious health disorders and symptoms when ingested above threshold. This is because these HMs have the ability to bind to protein sites and irreversibly change the protein structure, thereby affecting the normal function of cells and alter the biochemical life cycle, which is highly toxic and harmful to human health (Almustapha et al., 2014; Gumpu et al., 2015). The most hazardous metal ions are Hg, Cr, Cd, As and Pb, as they are poisonous and highly undesirable even at low concentrations (<2 ppb).

Excessive levels of these metals can affect human health, including damage to multiple organs, kidney diseases and nervous system. The short-term damage of heavy metals to human are skin discoloration, stomach pain, headache and vomiting. Long term exposure can lead to serious illness and cancer. The bioaccumulation of HMs in soil is also of concern because it can lead to reduced crop yields and may cause risk to human health when transferred to the food chain. A report indicated the detection of HMs, such as Cd, Pb, Mn, Cu and Zn, in the urine samples from patients aged 15–70 years old with kidney-, liver- and lung-related diseases. The same team also reported the detection of HMs in pathologically abnormal organs from cadavers aged 21–50 during autopsy (Lo et al., 2020; Ogunfowokan et al., 2019). Other than that, a serious heavy metal pollution incident occurred in Japan in 1950s, resulting in more than 10 thousand people infected with Minamata disease caused organic mercury poisoning, and about 500 of them died (Dai et al., 2018). These reports indicate that HMs have become as serious issues for the environment and human health. Table 2.1 shows the health effects of long-term exposure to HMs concentration based on World Health Organization (WHO) limits (Gumpu et al., 2015; Pandey et al., 2016).

Table 2.1: The health effects from long term exposure of HMs concentration based on limit by World Health Organization (WHO)

Metals	**WHO limits (mgL^{-1})**	**Effect**
Lead (Pb)	0.05	Risk for Alzheimer, mutagenic, anaemia, abdominal pain, gradual paralysis in the muscles, convulsions, coma, renal failure and cancer.
Cadmium (Cd)	0.005	Renal toxicity, hypertension, weight loss, fatigue, microcytic hypochromic anaemia, lymphocytosis, pulmonary fibrosis, atherosclerosis, peripheral neuropathy, lung cancer, osteoporosis, and hyperuricemia.
Mercury (Hg)	0.001	Lung damage, paraesthesia, Minamata, hypertension, severe kidney and nervous system disorder and coma
Arsenic (Ar)	0.05	Causes effects on central nervous system (CNS), peripheral nervous system (PNS), skin cancer, carcinogenic, problems with circulatory systems and hyperkeratosis.
Chromium (Cr)	0.05	Reproductive toxicity, mutagenicity, carcinogenicity, lung and stomach cancer, dermatitis, skin ulcers, perforation of septum, and irritant contact dermatitis
Zinc (Zn)	5	Respiratory disorders, metal fume fever, bronchiolar leukocytes, neuronal disorder, prostate cancer risks, macular degeneration, and impotence
Copper (Cu)	1.3	Gastrointestinal distress, allergies, anaemia, alopecia, arthritis, autism, cystic fibrosis, diabetes, haemorrhaging, and kidney disorders

2.1.3 Methods Used to Detect Heavy Metals

HMs detection requires a simple, highly sensitive, good selectivity and portable analytical technique. In view of their accuracy and sensitivity, conventional methods for detecting HMs include atomic absorption spectroscopy (AAS), inductively coupled plasma/atomic emission spectrometry (ICP-AES), ion chromatography ultraviolet visible spectroscopy (IC-UV-vis), inductively coupled plasma-mass spectroscopy (ICP-MS), microprobes (MP) and X-ray fluorescence spectroscopy (XFS) (Y. Lu et al., 2018; Waheed et al., 2018; Yao et al., 2019). These techniques are highly sensitive and selective techniques. However, these techniques have several limitations, such as they require relatively expensive equipment, larger size equipment that requires more workspace and operating procedures are complicated and complex, such as lasers and photodetectors that require professional and trained personnel. Other than that, these techniques are time-consuming operation and multiple preparation, requiring long detection time and restriction of single composition detection. Therefore, these conventional techniques are not suitable for on-site and continuous monitoring application to achieve rapid detection of HMs.

To overcome the limitations of these conventional HMs detection techniques, electrochemical sensor (ECS) technique was applied for analytical purposes. Compared to other spectroscopic and optical techniques, ECS technique is widely known as a highly sensitive technique for the detection of various HMs at extremely low concentrations. ECS technique offers simple procedures, possibility of transportability and portability, on-site workability, fast time response, and suitability for miniaturization (Kumar et al., 2020; Mohamad et al., 2021). Other than that, ECS also has low detection limit (LOD), high sensitivity, high surface area, highly specific, good reproducibility, good signal-to-noise ratio and effectiveness in the multielemental detection, because of its selective sensing ability for more than one metal ion. Moreover, ECS is more economical, cost effective, user friendly, reliable and easy installation. It is well suited for fabrication on small circuits in the form of portable devices for in-situ monitoring of HMs contaminated samples (Bansod et al., 2017). Therefore, the electrochemical sensor techniques have aroused great interest in HMs detection based on its unique advantages.

2.2 Electrochemical Sensor (ECS)

This section will discuss the working mechanism and configuration of electrochemical sensor (ECS), type of electrode such as working, counter and reference electrode and type of voltammetry such as stripping voltammetry, square wave anodic stripping voltammetry and cyclic voltammetry.

2.2.1 Working Mechanism and Configuration

A standard analytical electrochemical sensor system mainly consists of three part: electrochemical sensing device, electrochemical detecting instrument and electrolyte (Y. Lu et al., 2018). Electrochemical measurement was conducted using a working electrode (WE), counter electrode (CE) and reference electrode (RE) three-electrode system. Generally, ECS consist of two basic functional units, such as chemical recognition system called receptor and physicochemical transducer (Dai et al., 2018). The role of the receptor is to provide the sensor with a high degree of selectivity for analyte analysis and known as WE. The transducer is responsible for the sensitivity of the device, which converts chemical information of molecular or ionic recognition into a detectable analytical signal such as current, potential, charge, phase or frequency. Figure 2.1 shows the configuration of electrochemical sensor and the three-electrode cell setup (Bansod et al., 2017).

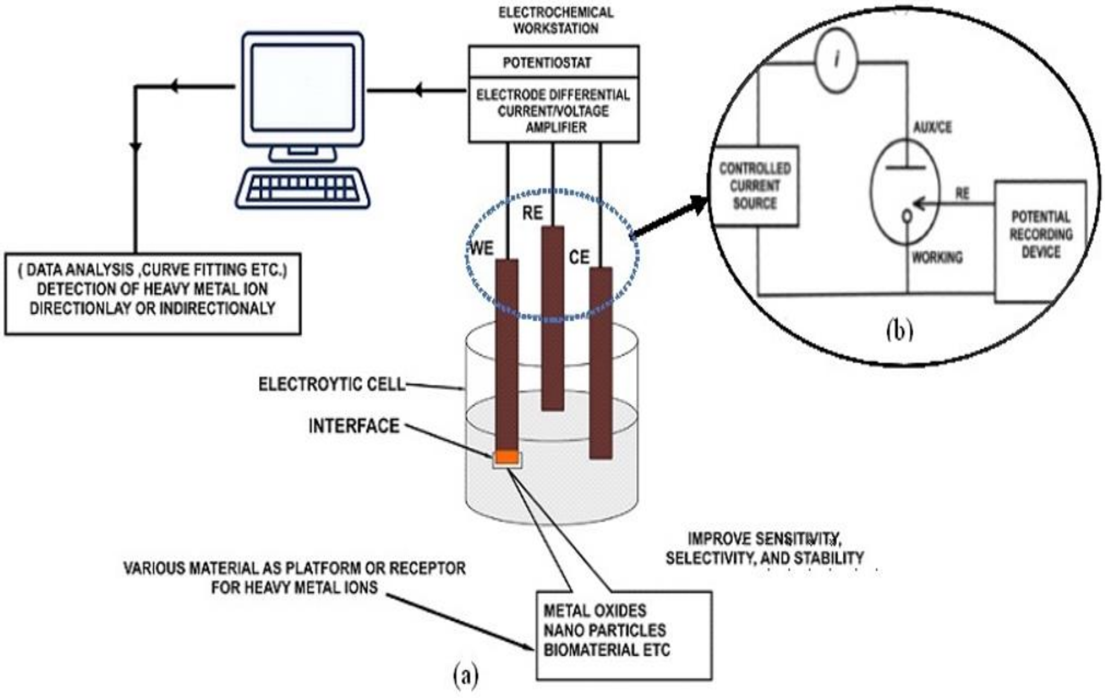

Figure 2.1: (a) The configuration of electrochemical sensor and (b) The three-electrode cell setup.

The device was placed in an electrolytic cell with modified WE of different interface of materials, such as metal oxides, nanoparticles, polymer, etc., as a platform for HMs detection. Generally, current is passed between WE and CE, and the cell potential is measured at the interface of the electrode and electrolyte solution. CE is separated from WE by some glass separator and the potential between WE and RE must be measured with a high input impedance device to prevent any current being drawn from RE. ECS is a sensor that adjusts the chemical information of a sample based on the redox reaction of the analyte due to the presence of HMs ions in the receptor unit. These electrodes are electrically connected to measure and monitor electrical signal or output-transducer signal, wherein the potential difference is measured by using a potentiostat. A potentiostat is an electronic hardware used to control and monitor electroanalytical response of experiment such as current, voltage and resistance (Garciá-Miranda et al., 2020). It is widely used due to its low-cost, small size, portability and stability for on-site analysis.

ECS is widely used for the detection of heavy metals ion in water. For example, Pujol et al. (2014) analyzed the performance of ECS by using bismuth film electrode (BiFE) in acidified tap water to detect HMs. It was reported that the ECS exhibited an excellent performance toward Cd^{2+} ions with a linear concentration range between 10^{-8} to 10^{-6} M, respectively. The ECS also shows a successful tested for simultaneous detection of Cd^{2+}, Zn^{2+} and Pb^{2+} ions at 10^{-5} M. Thus, this indicates that ECS is suitable and can be used for HMs ions detection.

2.2.2 Types of Electrodes

In electrochemical sensor (ECS), there are three types of electrodes involved, which are working electrode (WE), counter electrode (CE) and reference electrode (RE).

2.2.2(a) Working Electrode (WE)

The working electrode (WE) is the most important component in electrochemistry, as the electron transfer of greatest interest occurs at the interface between the WE and the electrolyte solution (Ong et al., 2021). Apart from that, the ions stripped from the surface of WE showed the highest peak of current (S. Li et al., 2018). The sensitivity of electrochemical sensor depends on the type of WE, and the sensitivity can be optimized by modifying the WE. Thus, the selection of working electrode material is critical to achieve good analytical performance and response. There are several factors to consider: 1) an efficient electron transporter, 2) high surface-to-volume-ratio, 3) a wide potential window of the electrolyte solution to provide the greatest degree of analyte characterization, 4) low background current, 5) low toxicity and 6) chemical stability, 7) electrochemically and interference-free, and 8) those with surface functional groups to enhance metal bonding and selectivity toward a specific target metal ion (Garciá-Miranda et al., 2020; Hou et al., 2018). The most commonly material used as WE in ECS is carbon – based electrode.

Carbon – based electrode is widely used in ECS as it can detect high negative potentials. Carbon – based electrode has a good cathodic potential window and exist in a variety of configuration and forms. Carbon – based electrode is widely used in electrochemical sensor due to its competence as a conductor, high electron transfer, chemical stability and abundant surface groups and robust carbon – to – carbon bonds for both internally and externally (Garciá-Miranda et al., 2020). It also has a high melting point (3652 – 3697 °C), which indicates that it can be used to facilitate a wide range of different reactions. The most common forms of carbon – based electrodes are glassy carbon electrode, carbon paste electrodes, graphite, pencil graphite and multiwalled carbon nanotubes (H. Hou et al., 2018; Ong et al., 2021). Carbon paste electrode has a wide potential range and low background current but cannot be used in organic solvents and are prone to mechanical damage during operation.

Historically, the hanging mercury drop electrodes (HMDE) is the most widely used WE material to detect HMs in electrochemical sensors because of its ability to form amalgams with HMs (Garciá-Miranda et al., 2020; Ong et al., 2021). Mercury electrode has a wide and excellent cathodic potential range, which enables the analysis of HMs with high sensitivity, efficient electron transporter, high surface-to-volume-ratio, low background current, chemical stability and reproducibility. However, the toxicity of mercury limits its use as an electrode in ECS due to its many adverse effects on the environment and human health. Next, bismuth (Bi) electrode has been called "green" alternative to mercury electrodes due to their low toxicity properties similar to mercury (Garciá-Miranda et al., 2020). Bismuth electrode offers highly reproducible stripping responses, excellent resolution of adjacent peaks, good hydrogen evolution and wide linear dynamic range, and signal-to-background characteristics.

The sensitivity of ECS can be optimized by modifying different WEs with different interfaces of materials such as metal oxide and nanoparticle. Nanostructured electrodes help to enhance the sensor's response amplitude (260%), lower the limit of detection (LOD) (~22mg/m^3) and provide higher accuracy (98%) (Waheed et al., 2018). For the example, bismuth nanoparticles (BiNPs) are superior to bismuth film electrode because of their enhanced electroanalytical capability, excellent catalytic activity, reduced effect of solution resistance, large surface area that can increase mass transfer rate and signal-to-noise ratio due to an extensive range of active sites (Pandey et al., 2016; Waheed et al., 2018). BiNPs have been shown to have high sensitivity and are reliable modifier for the detection of trace HMs by using square wave anodic stripping voltammetry (SWASV) with low LOD of 8.0 nM (Zn^{2+}), 4.0 nM (Cd^{2+}) and 2.0 nM (Pb^{2+}) (Liu et al., 2019). The lower LOD, the higher the sensitivity of the electrochemical sensor. Another example is that gold nanoparticles (AuNPs) modified electrode also known to have a unique ability to promote faster electron transfer kinetic between the WE and the active site of desired species. AuNPs also help to improve the LOD of Pb^{2+} and Cu^{2+}, which are 2.7 and 9.6 nM, respectively (Zhao et al., 2017).

Carbon nanotubes (CNTs) have also been valued in ECS due to their numerous advantages, such as large effective detection surface, excellent electron transfer rate compared to bulk carbon electrodes, high electrocatalytic activity, decreased influence from solution resistance and low electrode fouling. CNTs enhance high electrical conductivity because of their inherent size and hollow geometry, which makes them attractive as excellent electrochemical transducer for various applications. The conductivity of the sensor also depends on the thickness of the film electrode. The film thickness on the electrode surface must be optimized for optimal stripping performance to achieve high conductivity and high electron transfer rate. Yao et al. (2019) demonstrated that the thickness of the BiFE is controlled by the concentration of Bi^{3+} ions, which strongly affects the stripping responses of Cd^{2+} and Pb^{2+} ions. The higher the concentration of Bi^{3+} ions, the thicker the electrode film. It was found that the peak current response of the target HMs ions increased rapidly when the Bi^{3+} ions concentration increased from 0.7 to 1.0 mgL^{-1}. Then, with the further increase of Bi^{3+} ions concentration, there is a downward trend. This indicates that the mass transfer and diffusion rates are slower during the stripping process of the thick-film electrode. This is because when the thickness increases, so does the resistance, which slows down the electron transfer rate. Thus, thin film electrode can enhance a greater conductivity than thick film electrode. Furthermore, diffusion profile electrodes also play a role in the electron transport rate in ECS. The mass transfer to and from the WE surface can be improved by reducing the working area of the sensing platform, such as the change in the diffusion profiles from linear array (macroelectrode) to radical array (microelectrode) (Ong et al., 2021).

2.2.2(b) Counter Electrode (CE) and Reference Electrode (RE)

The counter electrode (CE) is also known as the auxiliary electrode (AE) in electrochemical sensor. The function of CE is to act as an electrode, completing the current circuit by providing the path for the current flow in the electrochemical cell without passing through RE. The redox reaction that occurs at WE is the opposite of CE, such as if reduction occurs at WE, then oxidation occurs at CE. The CE and WE can be setup separately using a fritted tube or some glass separator. This is to avoid the products generated at CE will not interfere with the WE reaction. There are no specific material requirements for this electrode, as long as it does not adversely affect the reactions taking place in WE (Ong et al., 2021). The most commonly used electrochemical sensor material for CE is platinum (Thanalechumi et al., 2019; Zhao et al., 2017). This is because platinum has excellent stability, inertness, high conductivity, and high transfer electron. However, its material cost has been a concern and less expensive material such as carbon, copper and stainless steel also can be considered as CE.

The reference electrode (RE) is used as a reference point in the electrochemical sensor system to quantitatively compare the potential induced by WE without the need for current to pass through. RE should be constructed by using half-cell assemblies that are stable over time, temperature and exist at well-defined potentials established through a redox system. The most common reference half-cells are standard hydrogen electrode (SHE), saturated calomel (SCE) and silver-silver chloride electrode (Ag/AgCl). SHE consists of an inert solid, such as platinum, in which hydrogen gas is absorbed by immersion in a solution containing hydrogen ions at unit activity. The half-cell reaction of SHE is $2\mathrm{H}^{+}(\mathrm{aq}) + 2\mathrm{e}^{-} \leftrightarrow \mathrm{H}_2(\mathrm{g})$, and the potential value is $\mathrm{E}^{0} =$

0.000 V (Ong et al., 2021). However, the limitation of SHE is that it is difficult to prepare H^+ solution and keep H_2 (g) at unit activity.

Silver-silver chloride electrode (Ag/AgCl) are also widely used as RE in electrochemical sensor. The Ag/AgCl reference electrode consists of a silver (Ag) wire coated with a layer of solid silver chloride (AgCl) and immersed in a saturated solution of AgCl and potassium chloride (KCl), known as a double-junction arrangement. The advantages of Ag/AgCl-based solid-state quasi-reference electrode is that there is no drawback such as leakage of internal filling solution and storage problem of the internal filling solution problem, since no internal filling solution is needed (Zhao et al., 2017). The half-cell reaction of Ag/AgCl is $\mathrm{AgCl(s) + e^- \leftrightarrow Ag(s) + Cl^-(sat'd)}$ with a potential value of 0.197 V (Ong et al., 2021).

2.2.3 Voltammetry Electrochemical Sensor

Voltammetry has been widely used in the determination and measurement of HMs ions in various complex environmental matrices due to its high precision and high sensitivity. These techniques are used by measuring the current-potential or potential-time curves (Dai et al., 2018). There are many types of voltammetry, such as stripping voltammetry (SV) and cyclic voltammetry (CV).

2.2.3(a) Stripping Voltammetry

Stripping voltammetry is known as an ultrasensitive detection method, is widely used in electrochemical sensor to detect HMs ions. Stripping voltammetry is popular due to its low LOD, high sensitivity, excellent selectivity, small size and its ability to detect multiple HMs ions simultaneously (Lo et al., 2020; Mohamad et al.,