TIN NANOPARTICLES MODIFIED ELECTRODE FOR SIMULTANEOUS DETECTION OF CADMIUM (Cd) AND LEAD (Pb) IONS

LOO JI YU

UNIVERSITI SAINS MALAYSIA

2022

TIN NANOPARTICLES MODIFIED ELECTRODE FOR SIMULTANEOUS DETECTION OF CADMIUM (Cd) AND LEAD (Pb) IONS

by

LOO JI YU
Supervisor: Prof. Dr. Khairunisak Abdul Razak

**Thesis submitted in fulfilment of the requirements
for the degree of
Bachelor of Engineering with Honours
(Materials Engineering)**

JULY 2022

DECLARATION

I hereby declare that I have conducted, completed the research work and written the dissertation entitled "**Tin Nanoparticles Modified Electrode for Simultaneous Detection of Cadmium (Cd) and Lead (Pb) Ions**". I also declare that it has not been previously submitted for an award of any degree or diploma or other similar title of this for any other examining body or university.

Name of student: LOO JI YU   Signature:

Date: 12nd August 2022

Witnessed by,

Supervisor: Prof. Dr. Khairunisak Abdul Razak   Signature:

Date: 12nd August 2022

ACKNOWLEDGEMENT

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

I_{pa} Anodic peak current

E_{pa} Anodic peak potential

θ Bragg diffraction angle

d   Crystallite size

β Full width at half maximum

m/e   Mass-to-Charge ratio

i_p Peak current

E_p Peak potential

$\triangle E_p$ Peak-to-peak separation

η Refractive index

κ Shape factor constant

λ Wavelength of X-ray

LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
ABS	Acetate Buffer Solution
AgNPs	Silver Nanoparticles
ASV	Anodic Stripping Voltammetry
AuNPs	Gold Nanoparticles
BiNPs	Bismuth Nanoparticles
CE	Counter Electrode
CV	Cyclic Voltammetry
DNA	Deoxyribonucleic Acid
DPASV	Differential Pulse Anodic Stripping Voltammetry
DPV	Differential Pulse Voltammetry
EHDA	Electrohydrodynamic Atomization
EMF	Electromotive force
E-tongue	Electronic tongue
FWHM	Full Width at Half Maximum
GCS	Glassy Carbon Sheet
GSPE	Graphite Screen-Printed Electrodes
ICDD	International Centre for Diffraction Data
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
Ig	Immunoglobulins
ITO	Indium Tin Oxide
LOD	Limit of Detection
LSASV	Linear Sweep Anodic Stripping Voltammetry
LSV	Linear Sweep Voltammetry
MS	Mass Spectroscopy
ppb	Parts Per Billion
ppm	Parts Per Million
ppq	Parts Per Quadrillion
PtNPs	Platinum Nanoparticles
RE	Reference Electrode
RGO	Reduced Graphene Oxide

ROS	Reactive Oxygen Species
SnNPs	Tin Nanoparticles
SnNPs WOPVP	Tin Nanoparticles without Polyvinylpyrrolidone
SnNPs WPVP	Tin Nanoparticles with Polyvinylpyrrolidone
SnO_2HSs	Tin Oxide Hollow Spheres
SnO_2NMs	Tin Oxide Nanomaterials
SPE	Screen-Printed Electrode
ss-DNA	Single Stranded Deoxyribonucleic Acid
SWASV	Square Wave Anodic Stripping Voltammetry
TEM	Transmission Electron Microscopy
WE	Working Electrode
XRD	X-ray Diffraction
XRF	X-ray Fluorescence

PENGUBAHSUAIAN ELEKTROD ZARAH-NANO TIMAH UNTUK PENGESANAN SERENTAK ION KADMIUM (Cd) DAN PLUMBUM (Pb)

ABSTRAK

Pencemaran logam berat dalam alam sekitar telah membahayakan kesihatan manusia dengan ketara kerana pengumpulan ion logam berat dalam rantai makanan. Oleh itu, pengesanan logam berat adalah penting untuk pemantauan alam sekitar. Dalam penyelidikan ini, zarah-nano timah (SnNPs) telah disintesis menggunakan kaedah penurunan kimia dan digunakan untuk pengubahsuaian elektrod Indium Timah Oksida (ITO) untuk pengesanan ion logam berat. SnNPs telah disintesis dalam dua cara, iaitu dengan dan tanpa penambahan polivinilpirolidon (PVP), iaitu SnNPs WPVP, dan SnNPs WOPVP, masing-masing. SnNPs yang disintesis telah diletakkan di atas elektrod ITO dengan mempelbagaikan kepekatan. Seterusnya, Nafion diletakkan di atas lapisan terakhir di atas elektrod SnNPs/ITO. Elektrod yang diubahsuai telah dikaji dengan menggunakan voltammetri kitaran (CV) dan voltammetri pelucutan anodik gelombang persegi (SWASV). Elektrod Nafion/SnNPs WPVP/ITO menunjukkan prestasi yang baik dalam pengesanan serentak ion Cd^{2+} dan Pb^{2+} dengan julat linear 10-100 ppb, nilai had pengesanan (LOD) untuk pengesanan serentak ion Cd^{2+} dan Pb^{2+} ialah 1.44 ppb dan 1.97 ppb. Sensitiviti elektrod Nafion/SnNPs WPVP/ITO terhadap ion Cd^{2+} dan Pb^{2+} ialah 0.25 μA/ppb dan 0.20 μA/ppb. Elektrod yang diubah menunjukkan selektiviti yang tinggi terhadap pengesanan ion Cd^{2+} dan Pb^{2+} dalam analisis interferens. Kebolehgunaan elektrod yang diubahsuai telah dikaji dalam sampel air laut untuk pengesanan ion Cd^{2+} dan Pb^{2+} dan berjaya mengesan kehadiran ion Pb^{2+} dalam sampel air laut. Oleh itu, keputusan mendedahkan bahawa elektrod SnNPs/ITO boleh digunakan sebagai pengesan ion logam berat.

TIN NANOPARTICLES MODIFIED ELECTRODE FOR SIMULTANEOUS DETECTION OF CADMIUM (Cd) AND LEAD (Pb) IONS

ABSTRACT

Heavy metal pollution in the environment has significantly endangered human health by accumulation of heavy metal ions within the food chain. Therefore, heavy metal detection is important for environmental monitoring. In this research, the tin nanoparticles (SnNPs) were synthesized using chemical reduction method and used for modification of Indium Tin Oxide (ITO) electrode for detection of heavy metal ions. The SnNPs were synthesized using two approaches, with and without the addition of polyvinylpyrrolidone (PVP), namely SnNPs WPVP, and SnNPs WOPVP, respectively. The as-synthesized SnNPs were drop casted on the ITO electrode with varying concentration, and with and without Nafion. The modified electrode was subjected to cyclic voltammetry (CV) and square wave anodic stripping voltammetry (SWASV). Nafion/SnNPs WPVP/ITO modified electrode showed good performance in simultaneous detection of Cd^{2+} and Pb^{2+} ions with linear range of 10-100 ppb, the limit of detections (LOD) for simultaneous detection of Cd^{2+} and Pb^{2+} ions were 1.44 ppb and 1.97 ppb, respectively. The sensitivity of the Nafion/SnNPs WPVP/ITO modified electrode was 0.25 µA/ppb and 0.20 µA/ppb towards Cd^{2+} and Pb^{2+} ions, respectively. The modified electrode showed high selectivity towards detection of Cd^{2+} and Pb^{2+} ions in interference study. The feasibility of the modified electrode was studied in real seawater sample for detection of Cd^{2+} and Pb^{2+} ions and successfully detected the presence of Pb^{2+} ions in the seawater sample. Thus, the results revealed that the SnNPs/ITO electrode can be used as heavy metal ions sensor.

CHAPTER 1

INTRODUCTION

1.1   Introduction

Metals are materials with high electrical conductivity that easily lose electrons to form cations. The term heavy metals are generally defined as natural existing elements that possess high specific density more than 5 g/cm^3 with atomic weight greater than 40.04 such as antimony, arsenic, bismuth, cadmium, cerium, chromium, cobalt, copper, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, thallium, tin, tellurium, uranium, vanadium, zinc. Some heavy metals such as chromium, cobalt, copper, iron, magnesium, manganese, molybdenum, nickel, selenium, and zinc are essential elements that humans need for a variety of physiological and biochemical reactions in the body and can lead to deficiency disorders or syndromes if they are insufficient. However, overdose amounts may cause adverse effects (Engwa et al., 2019). Anthropogenic activities, urbanization, and industrialization may slowly increase the toxicity of heavy metals in the environment because heavy metals are non-biodegradable and will remain in the environment for a long period of time (Briffa et al., 2020).

Heavy metals can be easily absorbed by living organisms in marine ecosystems because heavy metals are soluble in water. There are studies show that the presence of heavy metals among various aquatic lives in contaminated marine ecosystems (Kinuthia et al., 2020). Humans can be in direct contact with heavy metals via eating contaminated foods, drinking contaminated water, inhaling polluted air as dust fumes, or being exposed to heavy metals at work (Engwa et al., 2019). Commonly, heavy metals enter the human body via the gastrointestinal route, which is through ingesting contaminated foods, fruits, vegetables, seafood, or drinking contaminated water source,

1

but some heavy metals can enter the human body via inhalation or being absorbed through the skin when in contact. Once heavy metals enter human body, majority of the heavy metals are transported from blood to tissues in the body. For example, arsenic is transported in the blood and accumulates in various vital organs in humans. Lead is usually transported to the liver and kidney by red blood cells and then redistributed as phosphate salt to teeth, bone, and hair. Moreover, cadmium will bind to blood cells and albumin at first then bind with metallothionein in kidney and liver tissue (Engwa et al., 2019). Therefore, it is crucial to monitor the level of heavy metals in the environment to protect humans.

In order to detect heavy metals at a trace level with high accuracy and selectivity, conventional detection techniques such as atomic absorption spectroscopy (AAS), mass spectroscopy (MS), inductively coupled plasma mass spectrometry (ICP-MS), and X-ray fluorescence (XRF) are often used to detect heavy metal ions, but conventional detection techniques have many limitations such as high equipment cost, huge and bulky equipment or instrument, and complex procedure for detection that required experienced operator which is unfavorable for on-site monitoring application (Kumar et al., 2017). Therefore, a small, simple, easy, and cost-effective detection method is crucial for monitoring the heavy metal ions in the environment effectively.

A sensor refers to a device that can be stimulated by a physical stimulus which then transmits a resulting electrical response that corresponds to a change of any intrinsic properties of the constituent material (Simões and Xavier, 2017). A chemical sensor refers to a device that can convert the chemical reactions of the analyte into a useful analytical signal. Typically, chemical sensors include two main functional units which are receptor and physico-chemical transducer (Dai et al., 2018). The receptor in the chemical sensor is the chemical recognition system that aims to provide high

selectivity for the analyte to be determined and the physico-chemical transducer is served to transform the signal from receptor into the useful analytical data and this transducer is responsible for the sensitivity of the chemical sensor. An electrochemical sensor is an appealing device which able to convert physical and chemical reactions that occurred in the sample into quantifiable electrical signals like potential, current, and resistance changes. Generally, an electrochemical sensor usually consists of a working electrode (WE), counter electrode (CE), and reference electrode (RE). The working electrode is commonly modified with nanomaterials to improve the limit of detection, sensitivity, and selectivity (Dai et al., 2018).

There are many electrochemical methods that can be used for the detection of heavy metal ions which includes voltammetry, potentiometry, and conductometry. Voltammetry which includes cyclic voltammetry (CV), anodic stripping voltammetry (ASV), and square wave anodic stripping voltammetry (SWASV) is utilized by analyzing the current-potential or potential-time curve (Dai et al., 2018). ASV has become a mature and widely used electrochemical detection method for a wide variety of heavy metal ions detection because high sensitivity in heavy metal ions detection and broad application range. The concept of ASV in the detection of heavy metal ions is based on the heavy metal ions deposited at the negative electrode under negative voltage and stripping off the heavy metal ions at the reversed electrode potential (Dai et al., 2018).

1.2 Problem statement

Heavy metal detection is important for environmental monitoring because heavy metal pollution significantly endangers human health and the surrounding environment. In the past, hanging mercury drop and related electrodes were used for heavy metal ions sensing applications. However, the toxicity of mercury has raised a concern to the public because mercury can cause serious harm. Thus, this issue limits the potential use of mercury related electrode. As an alternative for mercury related electrode, tin is known as an environmentally green material with low toxicity and good conductivity. Theoretically, nanosize Tin provides a high surface area, enhances catalytic properties of electrodes, and has the potential to improve the sensitivity and specificity of sensors. Tin sheets have been reported in the application of heavy metal sensors. However, tin sheets are not suitable for disposable sensors because of low sensitivity and lack of specificity due to the small surface area for reaction to take place. Although tin-based nanoparticles have been widely studied for gas sensing applications, tin-based nanoparticles have not been widely studied as heavy metal sensors as compared to other metal-based nanoparticles. From the literature review, not much research has been conducted on the tin nanoparticles (SnNPs) modified electrode for the detection of heavy metal ions. Also, lack of study has been conducted on SnNPs modified indium tin oxide (ITO) electrode as working electrode for heavy metal detection. Thus, in this study, SnNPs modified indium tin oxide (ITO) electrodes were synthesized and used to detect heavy metal ions, in particular Cd^{2+} and Pb^{2+} ions. This sensor is beneficial for environmental monitoring that will benefit society.

1.3   Objectives

The general objective of the proposed research is to investigate the feasibility of using SnNPs as heavy metal ions sensors. The specific objectives are as follows:

i.   To synthesize and characterize SnNPs using chemical reduction method.

ii.   To fabricate SnNPs/ITO modified electrodes using drop casting method.

iii.   To determine the electrochemical performance of SnNPs/ITO modified electrode as heavy metal ions sensor.

1.4   Research scope

In this work, SnNPs were synthesized via the chemical reduction method, and the properties of the as-synthesized SnNPs were characterized by X-ray diffraction (XRD), Transmission electron microscopy (TEM), and zeta potential. The SnNPs were synthesized with and without the addition of PVP to analyze the effect of PVP on the properties of SnNPs. Then, the as-synthesized SnNPs were drop casted on ITO electrodes for modification of ITO electrodes, followed by dried and heat-treated in an oven. Then, the Nafion layer was deposited on SnNPs/ITO to improve the sensitivity and selectivity of the modified electrode. The electrochemical properties of the modified electrodes were studied via cyclic voltammetry (CV). Next, square wave anodic stripping voltammetry (SWASV) was used to analyze the selectivity and sensitivity of the modified electrodes for the detection of Cd^{2+} and Pb^{2+} ions. Then, the interference study of other heavy metal ions on Nafion/SnNPs WPVP/ITO modified electrode was studied in ICP multi-element standard solution. The feasibility of Nafion/SnNPs WPVP/ITO modified electrode was studied in real seawater sample application. Finally, the reproducibility of the Nafion/SnNPs WPVP/ITO modified electrode was studied towards simultaneous detection of Cd^{2+} and Pb^{2+} ions.

1.5   Thesis outline

This thesis contains five chapters. The first chapter discusses the background of this research, problem statement, objectives, and research scope. The second chapter covers the literature review on the effect of heavy metals, conventional detection techniques, sensors, electrochemical detection techniques for heavy metal ions, modification of working electrodes, and synthesis methods for SnNPs. The third chapter explains the project's methodology, materials and chemicals used in this project, parameters for the setup, and characterization methods. The fourth chapter focuses on the result analysis and discussion of this research. Finally, the fifth chapter contains the conclusion of this research and suggestions for future research work.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Heavy metal ions have high toxicity which can cause significant consequences to human beings and the environment. Heavy metal ions that are present in water may enter small organisms and bio-accumulate within the food chain where humans have the risk of being exposed to heavy metal ions or ingestion of heavy metal ions through various processes causing adverse effects. In this chapter, the sources and health effects of heavy metal ions on human beings and the environment are discussed. The heavy metal ions detection via conventional techniques and electrochemical techniques are explained. Finally, the nanomaterials that have been used to modify the working electrode for heavy metal ions detection are also discussed in this chapter as well.

2.2 Effect of heavy metals to the human beings and the environment

Heavy metal pollution is a critical issue due to its high toxicity and non-biodegradable. Heavy metal contaminants can be present in the environment through natural processes and anthropogenic activities (Engwa et al., 2019). Anthropogenic activities such as mining, smelting, foundries, leaching of metals, etc. are the primary causes of heavy metal pollution. Heavy metal pollution also occurs via natural processes such as volcanic activity, soil erosion, metal corrosion, and geological weathering (Briffa et al., 2020). These heavy metal contaminants would gradually enrich the environment and bio-accumulate living organisms, entering the food chain and slowly jeopardizing human health because humans are usually the last of the food chain. Heavy metal toxicity has several consequences to human body as it may affect the central nervous function that results in mental disorder, and damage the vital organs

such as the lungs, liver, or kidney leading to other disease conditions. Long-term exposure to heavy metal elements may slowly cause degeneration processes of physical, muscular, and neurological that cause diseases such as multiple sclerosis, Parkinson's disease, Alzheimer's disease, and muscular dystrophy. Moreover, repeatedly long-term exposure will damage nucleic acids, causing mutation and eventually leading to cancer (Engwa et al., 2019).

Arsenic is a heavy metal that can cause adverse effect to both ecological and human health due to its prominent toxic and carcinogenic. Arsenic is found and distributed in soil, water, air, and rocks in the form of oxides, sulfides or as a salt of iron, sodium, calcium, copper, etc. (Jaishankar et al., 2014). Workers may be exposed to arsenic through work environment via oral, respiratory, or dermal routes; but arsenic is likely to be orally absorbed by ordinary people from contaminated water source, agricultural and fish products. Inorganic arsenic compounds in the forms of trivalent (As^{3+}) and pentavalent (As^{5+}) are commonly found in contaminated water, air, or soil, whereas organoarsenicals are commonly exposed through ingestion of contaminated agricultural and fish products (Hong et al., 2014). When humans are exposed to As^{5+}, arsenate will reduce or partially reduce to As^{3+}, arsenite in the blood, then As^{3+} is spread to tissues and commonly absorbed hepatocytes. As^{3+} is being absorbed by the cells at faster rate than As^{5+} and is considered more toxic as compared to As^{5+}. Besides, during reduction of As^{5+} in the body produces intermediates such as methylarsinic acid (MMA^{3+}) and dimethylarsinic acid (DMA^{3+}) which are relatively more toxic than inorganic arsenic, and both intermediates are able to damage deoxyribonucleic acid (DNA). Thus, the toxicity and carcinogenicity of arsenic are closely related to the metabolic processes (Briffa et al., 2020).

Cadmium is toxic heavy metal usually a byproduct of zinc production where humans or animals may be exposed at work or in the environment. Cadmium mostly is used in alkaline rechargeable batteries as an electrode component. Cadmium also is used for special alloy production, coatings, pigments, plating and as a plastic stabilizer (Engwa et al., 2019; Jaishankar et al., 2014). Cadmium waste that spread into the environment may remain for several decades and eventually accumulate within the food chain and presence in human body. Exposure to cadmium for human mostly via inhalation and ingestion which possibly causing serious intoxication. Cadmium metal is known as potent carcinogen which affect the kidneys, lungs, pancreas, and prostate, the high toxicity of cadmium caused the vital DNA repair activity to be deactivated and increasing the instability of genome which leads to various human cancer (Briffa et al., 2020).

Chromium is heavy metal that can be in solid, liquid or in gas forms, which presence in soils, plants, and animals. Chromium can presence in many different oxidation states such as divalent, trivalent, four-valent, five-valent, and hexavalent state. Cr^{3+} and Cr^{6+} are the most stable oxidation states among others. Cr^{3+} is an essential nutritional compound for humans and animals which plays an important role in glucose metabolism, but Cr^{6+} on the other hand is a highly toxic and carcinogenic compound which possess risks to human health (Jaishankar et al., 2014). In digestive tract and airways, Cr^{6+} is absorbed at faster rate as compared to Cr^{3+}. If the chromium excretes from lungs via mucociliary clearance, then chromium may enter the gastrointestinal tract which usually absorbed in the jejunum (Briffa et al., 2020). The intracellular reduction of Cr^{6+} and Cr^{3+} along with the oxidative reduction causes toxicity mechanism and carcinogenicity of chromium. Besides, the formation of Cr^{3+} from the reduction of Cr^{6+} will form toxic complexes and peptides that can cause DNA

lesions. Cr^{6+} is more toxic than Cr^{3+} because Cr^{6+} has higher redox potential and able to penetrate more into cells (Briffa et al., 2020).

Copper is an essential nutrient for humans, animals, and plants which is also required by many enzymes to function normally. However significant exposure of copper may affect human health (Taylor et al., 2019). Copper can change oxidation states from Cu^{2+} to Cu^+ via redox reaction by cuproenzymes. Changing oxidation states of copper causes copper to become toxic as superoxide and hydroxyl radicals will be formed. Copper often used in production of copper pipes, cables, wires, or copper utensils, etc. Besides, copper also can be used for intrauterine device (IUD) or presence in composition of birth control pills (Engwa et al., 2019). There is high concentration of copper heavy metal that is distributed in the soil and water due to anthropogenic activities and taken up by plants causes copper presence in our human's food sources such as nuts and seeds, avocado, oyster, lobster, dark chocolate, etc. (Briffa et al., 2020).

Lead is one of the most critical environmental pollutants that brought major threat for human health because lead can induce inflammatory cascades in various tissues. Lead is natural component in earth's crust, and primary source came from lead-acid batteries in car, lead piping, hair dye, soldering, insecticides (Briffa et al., 2020). Lead exposure in human is commonly via digestive system, respiratory system, and skin, lead will bind with sulfhydryl and nucleophilic functional groups that causes oxidative stress. The free radicals produced by lead may damage the cell membrane via lipid peroxidation causing inflammatory cascades (Boskabady et al., 2018). The toxicity of lead is mainly due to the ability of lead metal ions to replace other bivalent or monovalent cations and interfere the biological metabolism of the cell. Long-term exposure to lead increases the blood pressure because lead affect the crucial hormonal

and neuronal systems that regulate the heart rate. Moreover, high lead concentration in plant causes the reactive oxygen species (ROS) to be produced faster than usual and damaging the chlorophyll. Thus, this suppressing the photosynthesis and overall growth of the plant (Jaishankar et al., 2014).

Mercury is non-essential heavy metal that is in shiny silvery color and becoming odorless and colorless gas when being heated. Mercury is a very toxic element that exist in three forms in nature which are metallic, organic, and inorganic (Jaishankar et al., 2014). Thus, mercury may cause adverse effect to the environment. Mercury is widely used in thermometers, barometers, dental amalgams, mercury arc lamp, catalyst, etc. The primary source of mercury pollution is anthropogenic activities and widely distributed in the environment and contaminated water resources. Thus, human can hardly escape from the exposure to mercury. Human exposure to mercury commonly via inhalation and ingestion. As mercury enters human body, mercury adheres to red blood cells due to high affinity for the sulfhydryl group. Thus, mercury can pass through the blood-brain barrier easily and deposited on many tissues and various organs in the human body (Briffa et al., 2020). Moreover, mercury able to alter tertiary and quaternary structure of proteins and membrane permeability which poisons the cellular function by attaching sulfhydryl and selenohydryl groups (Jaishankar et al., 2014).

In short, heavy metals had brought adverse effect to the living organism in the environment and possibly lead to greater risk of human health by accumulating in human body via food chain, occupational exposure, etc. Exposure of heavy metal ions such as lead (Pb), mercury (Hg), cadmium (Cd) at low concentration or some essential nutrient metal ions such as iron (Fe), zinc (Zn), cobalt (Co), etc., at high concentration

are toxic to human beings. The sources and health effects of heavy metals are shown in Table 2.1.

Table 2.1: Sources and health effects of heavy metals (Briffa et al., 2020)

Heavy metal	Sources	Health effects
Arsenic (Ar)	Atmospheric deposition Mining Insecticides Bronze production	Highly effects dermal region (Cancer) Brain & Cardiac problems DNA damage Infertility and miscarriages
Cadmium (Cd)	Plastic stabilizer Phosphate fertilizers Pesticides Batteries	Osteoporosis Prostate cancer Lung diseases Renal issues DNA impairment
Chromium (Cr)	Steel fabrication Electroplating Textile Dye paints Alloys	Lung disorder (bronchitis, cancer), Renal and reproductive system Liver and kidney diseases Coma Death
Copper (Cu)	Copper polishing Plating Printing Alloys Fertilizers	Abdominal disorders, Metabolic activity abnormalities Wilson's disease
Lead (Pb)	Batteries Coal combustion Paint industry	Serious effect on mental health (Alzheimer's disease), Nervous system
Mercury (Hg)	Coal combustion Fish Mining Paint industry Paper industry Volcanic eruption	Sclerosis Blindness Minamata disease Deafness Gastric problems Renal disorders

2.3 Conventional detection techniques for heavy metal ions

Nowadays, environmental pollution due to heavy metal ions has increased aggressively due to rapid development of industrialization, urbanization, and anthropogenic activities. As a result, high concentration of heavy metals is released into the environment in a short period of time, endangering living species and bio-accumulating within the food chain, posing major health risks to humans, who are often the last consumers in the food chain (Jin et al., 2020). The monitoring of the

heavy metal ions is necessary to control and prevent heavy metal pollution to the environment. In conventional detection techniques, advanced analytical instruments have been built to detect heavy metal ions at trace quantities from parts per million (ppm) to even parts per quadrillion (ppq) range (Kumar et al., 2017). The analytical instruments such as atomic absorption spectroscopy (AAS), mass spectroscopy (MS), inductively coupled plasma mass spectrometry (ICP-MS), and X-ray fluorescence (XRF) can offer comprehensive selectivity and sensitivity information to nanogram level of heavy metal ions, however these analytical instruments are relatively expensive, lack of field portability, skilled operator, complicated operating procedures, and relatively slow to obtain result (Kumar et al., 2017).

AAS is the most frequently used tool for determination of most metals and metalloids because AAS offers high sensitivity for many applications with relatively low interference. The sample was first atomized to free gaseous atoms by atomizer and absorb electromagnetic radiation at a specific wavelength which is proportional to concentration of the analyte atoms then produce other measurable wavelength as signals (Fernández et al., 2018; Hill and Fisher, 2017). AAS consists of four main components which are lamp to provide narrow spectral line, atomizer to convert sample into free gaseous atoms, wavelength selector to ensure specific light to be absorbed, and a detector to measure the intensity of light being emitted through the wavelength selector (Fernández et al., 2018). These four major components of AAS are arranged in a straight line as shown in Figure 2.1.

13
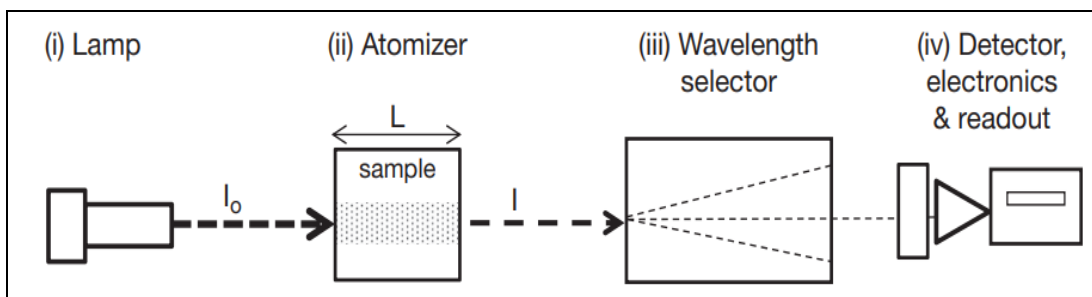
Figure 2.1: Schematic diagram of atomic absorption spectroscopy (Fernández et al., 2018)

MS is usually used with other spectral data to determine the structures of organic compounds. MS able to obtain mass spectrum with very little amount of sample material about nanogram only from small and valuable samples (Thompson, 2018). The mass spectrometry analysis is first started by producing gas phase ions from the sample via electron ionization in the ion generation chamber and accelerated into the mass analyzer tube. Then, these generated ions will be separated via mass analyzer tube in accordance with their mass-to-charge ratio (m/e) and are then collected and detected in ions collector chamber. Lastly, the mass spectrum of the sample can be produced when the signal is transferred to data collection system. In MS, low pressure is maintained in ions generation chamber, mass analyzer tube, and ions collector chamber to minimize any events of reaction, scattering, and neutralization of the ions (Baghel et al., 2017). Figure 2.2 shows the schematic diagram of MS.
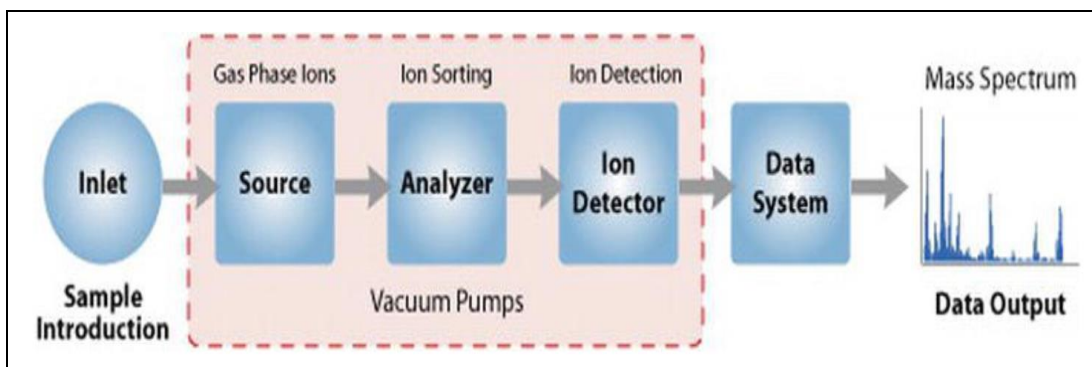
Figure 2.2: Schematic diagram of mass spectrometry (Baghel et al., 2017)

ICP-MS is an analytical technique which can detect heavy metal ions at trace levels. There are six basic components in ICP-MS which are sample chamber, inductively coupled plasma (ICP), interface, ion optics, mass analyzer, and detector as shown in Figure 2.3. Firstly, the liquid sample will be nebulized to form fine aerosol in sample chamber. Then, the fine aerosol will be transferred towards the argon plasma and ionized by high temperature plasma which producing the ions. The generated ions will be introduced through the interface region and passes through electrostatic lenses known as ion optics that focuses and guides the ion beam to the quadrupole mass analyzer and the ions can then be separated by the mass analyzer in accordance with their mass-to-charge ratio (m/e). Finally, these ions are measured at the detector (Wilschefski and Baxter, 2019).
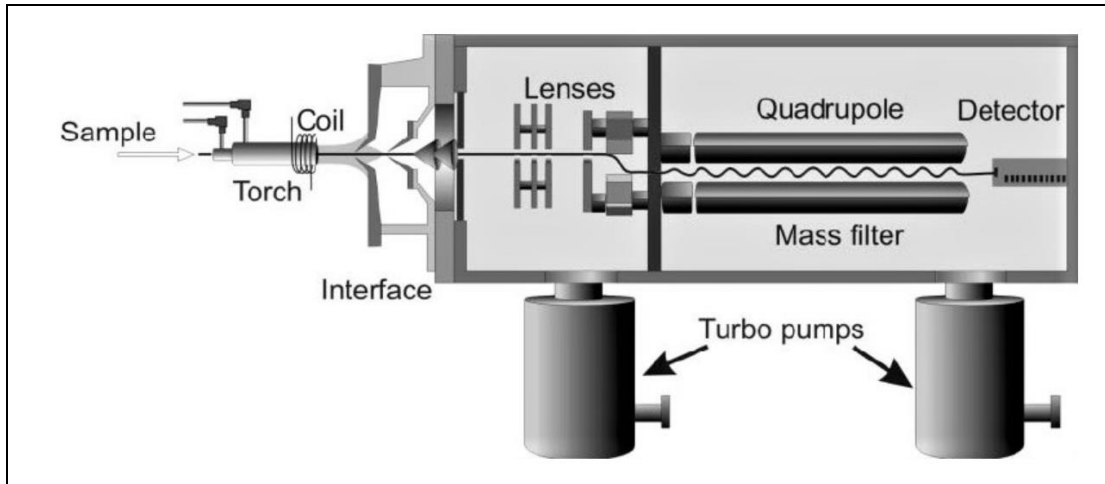
Figure 2.3: Schematic diagram of inductively coupled plasma mass spectrometry (Wilschefski and Baxter, 2019)

XRF is a non-destructive elemental analysis which can be used to quantify heavy metal ions concentration. Firstly, a focused X-ray beam will be utilized to irradiate the sample, which will release distinctive secondary X-rays. A core shell electron will be ejected as a photoelectron from a specific atom upon excitation by X-ray photon. Then, this core shell electron leaving a core hole is being filled by a neighboring orbital electron with higher energy and this resulting the X-ray fluorescence photon is being emitted. This X-ray fluorescence photon carries energy that is equivalent to the difference of binding energies of the two shells involved in the electron transition. Because binding energies vary with nuclear charges, every element has its own photon energy. As a result, XRF can quantitatively detect heavy metal ions based on their unique photon energy, allowing for multi-element analysis (Zhang et al., 2018). Figure 2.4 shows the schematic diagram of XRF.
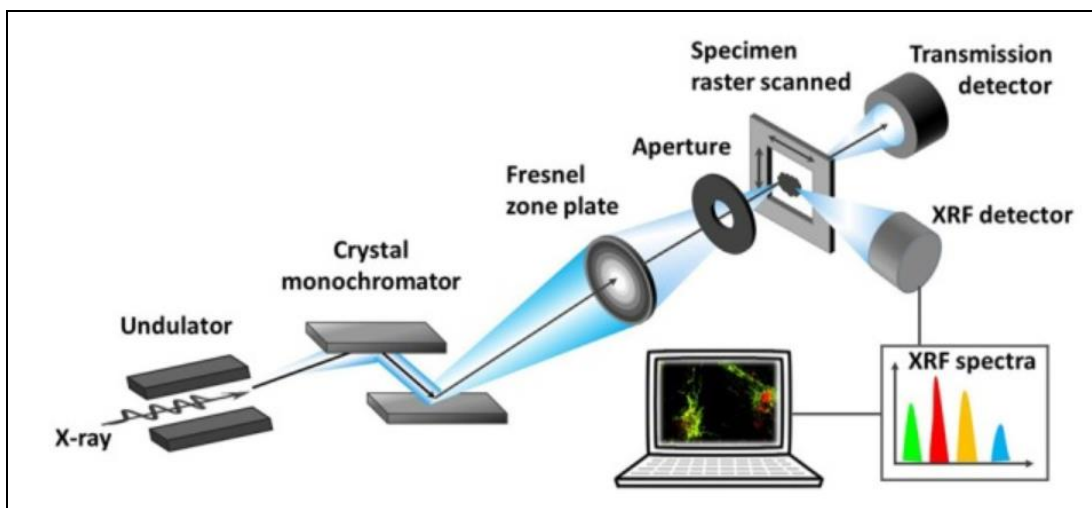
Figure 2.4: Schematic diagram of X-ray fluorescence (Zhang et al., 2018)

2.4   Sensors for heavy metal ions detection

Apart from the conventional detection techniques for heavy metal ions via advanced analytical instruments, there are many sensors can be used to detect heavy metal ions at trace level such as biosensor, optical sensor, fluorescent sensor, and electronic tongue. Generally, sensor is a device that composes of a transducer and a processor which able to provide selective quantitative or semiquantitative analytical information by recognizing the element biologically, chemically, or electronically (Xiang et al., 2020).

Biosensors are small size devices that allow simple and fast determination for heavy metal ions in water sample which allow for onsite application. Besides, biosensor may be used to determine not only the presence and concentration of heavy metal ions in water, but also their biological consequences, such as toxicity and cytotoxicity. Biosensors are analytical sensory devices that combine physical and chemical sensing techniques through direct interaction with biological and physicochemical elements. Basically, the biological element serves as a receptor for detecting heavy metal ions in the sample via reaction between the heavy metal ions

with the bioreceptor. The physicochemical element serves as a transducer which converts the reaction response from the bioreceptor into a measurable signal (Odobašić et al., 2019). Immobilization of biological element or bioreceptor in proximity of the transducer can be accomplished by physical entrapment or chemical attachment.

There are many types of biosensors such as enzyme-based biosensors, antibody-based biosensors, DNA-based biosensors, etc. In enzyme-based biosensors, enzyme is employed to immobilize on the transducer surface, which serves as a connection between heavy metal ions and a transducer (Mukherjee et al., 2021). Generally, the detection of enzyme-based biosensors is based on heavy metal induced activation or inhibition of their activities which convert the metal ions into quantifiable product (Osorio-González et al., 2019). The class of enzymes such as phosphatases, oxidases, dehydrogenases, peroxidases can be employed as bioreceptors for heavy metal ions detection in the environment. There is one main drawback of enzyme-biosensor which is lack of selectivity because there are some enzymes that can simultaneously detect several types of heavy metal ions causes lack of information for the heavy metal ions presence in the sample (Odobašić et al., 2019). As for antibody-based biosensors, antibodies are immobilized onto the sensor surface via several approaches which are covalent bonding, non-covalent immobilization, and coupling by affinity interactions. Antibody also is known as immunoglobulins (Ig) that can be categorized into five groups based on the structure and functions of the antibody, which includes IgA, IgD, IgE, IgG, and IgM. IgG has a better affinity and specificity than other classes, thus it is the most extensively used class for heavy metal ions detection in antibody-based biosensors. Monoclonal and polyclonal are antibodies that are commonly employed for biosensors (Odobašić et al., 2019). In DNA-based biosensors, single stranded DNA (ss-DNA) molecules or probe ss-DNA are employed

as heavy metal ions recognition element. The detection mechanisms rely on the probe ss-DNA hybridization with complementary target ss-DNA or changes in structural integrity of probe ss-DNA caused by heavy metal ions. Both mechanisms will cause changes in the mass transport, light absorption, emissions, or proton concentration and producing signal. Thus, transducer element converts the signals to measurable data (Saidur et al., 2017).

Optical detection techniques for heavy metal ions often used optical fibers, integrated optics, capillary-type devices, specific indicator dyes, ionophores, etc. The heavy metal ions usually detected by conventional methods such as reflection, absorption, or luminescence spectrometry (Malik et al., 2019). Fluorescent-based optical sensors consist of fluorophore, a fluorescent chemical compound that can emit light after being excited at lower wavelengths, and concentration of the heavy metal ions presence will affect the intensity of the light emitted by the fluorophore. However, many fluorescent indicators can bind with several metal ions that reduce the selectivity of the optical sensors. As for ionophore-based optical sensors, ionophores that are ion complexing organic molecules have been employed for heavy metal ions detection because they are able to bind with selective ions. Besides, chromogenic material can be introduced into ionophores in detecting heavy metal ions. Thus, during detection of heavy metal ions, protons that equal to the charges of the heavy metal ions will be released from the chromo-ionophore, which is responsible for the changes of color of the chromo-ionophore.

Electronic tongue (e-tongue) is a multisensory array which made of low selective, non-specific, and cross sensitive chemical sensors incorporated with chemometrics device to process multivariate data (Kirsanov et al., 2021). Normally, huge amount of data is obtained from the e-tongue, so multivariate statistical

approaches for quantitative and/or qualitative analysis is required to visualize information from both simple and complicated liquid media (Shimizu et al., 2019). The concept of e-tongue is based on several electrodes with no or low-selectivity in a sensor array to detect the heavy metal ions in complex liquid efficiently. A sensor arrays or electronic tongues made of potentiometric chemical sensors was developed by Rudnitskaya et al., 2008 for fast determination of ultra-low activities of copper, zinc, lead, and cadmium ions in seawater.

2.5　Electrochemical techniques for heavy metal ions detection

Electrochemical techniques are commonly used for heavy metal ions detection because the instrumentation setup is usually small and compact, easy to modify the electrode, simple and portable that are suitable for onsite application (Bansod et al., 2017). Besides, electrochemical techniques are usually more economic, user friendly, simpler procedure for heavy metal ions detection in samples and short analytical time. However, electrochemical techniques have a lower sensitivity and higher limit of detection (LOD) compared to conventional techniques (Malik et al., 2019). In electrochemical sensing of heavy metal ions, the incorporation of biosensing electrode is required to improve the sensitivity and LOD by flowing current through the aqueous solution to produce a useful and quantifiable electrical signal due to the presence of heavy metal ions. The electrical parameters such as current, voltage, capacitance, electrochemical impedance, and electrochemiluminescence will change due to the presence of heavy metal ions which allows detection. The electrochemical sensing techniques can be classified into potentiometry, amperometry, voltammetry, coulometric, electrochemical impedance, and electrochemiluminescent techniques (Cui et al., 2015; Malik et al., 2019). Generally, electrochemical techniques use three

electrodes setup, which consists of working electrode (WE), counter electrode (CE), and reference electrode (RE) as shown in Figure 2.5.
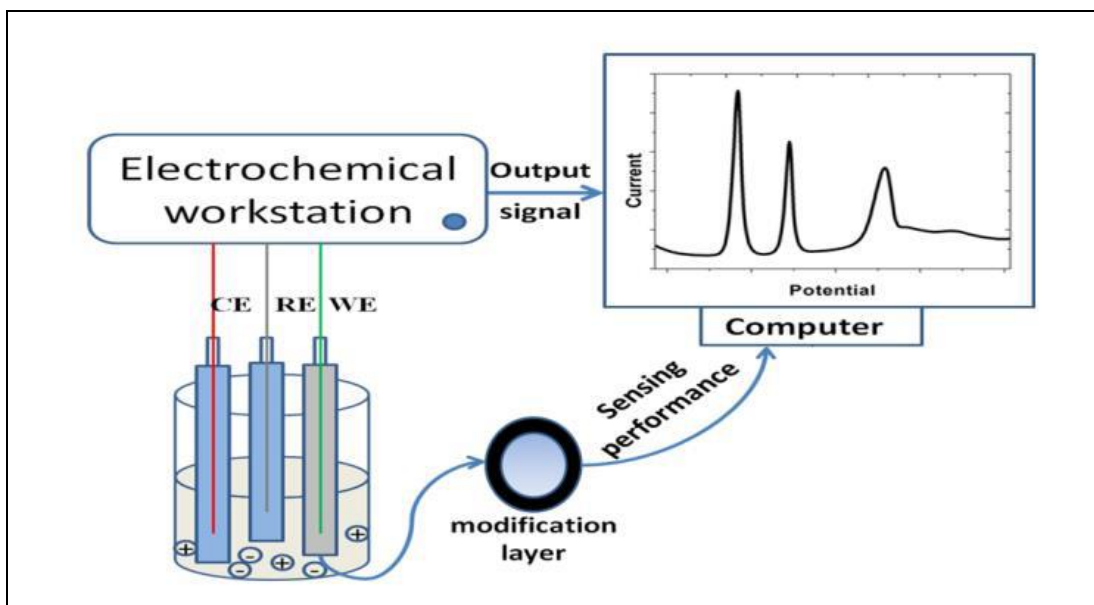

Figure 2.5: Schematic diagram of general principle of electrochemical sensing of heavy metal ions (Lu et al., 2018)

Potentiometry analysis measures the electromotive force (EMF) without applying any electric current and is commonly used to analyze the heavy metal ions quantitatively in the solution (Malik et al., 2019). This technique is considered low cost, short response time, high selectivity and broad range of analyte can be determined. However, the electrode is hard to be miniaturized, has high detection limit, and low sensitivity (Bansod et al., 2017). For example, Aglan et al. (2018) performed detection of Hg (II) via potentiometric technique in different aqueous media such as water, dental amalgam, and fish tissue with screen-printed electrode (SPE) modified with zirconium antimonate ionophore with detection limit of 5×10^{-8} mol/L and the modified electrode can be used in pH range of 2.5-8.5.

Amperometry analysis involves measurement of current due to the reduction of heavy metal ions by a fixed potential difference that is applied in between a working

electrode and a reference electrode in the sample solution. Because of the fixed potential difference of the working electrode, heavy metal ions are detected and undergo a faradic reaction. However, because the working electrode has a small surface area, only a small quantity of heavy metal ions will react, and the faradic reaction is incomplete. Thus, the electrode surface must be modified to increase the surface area and improve their sensitivity towards heavy metal ions (Malik et al., 2019).

Next, voltammetry analysis is the most extensively utilized for heavy metal ions detection as compared to other electrochemical techniques because voltammetry analysis has high accuracy, low LODs and high sensitivity. In voltammetric analysis, the current is measured by varying the applied voltage to acquire the current versus voltage curve. There are many types of voltammetry such as linear sweep voltammetry (LSV), cyclic voltammetry (CV), square wave voltammetry (SWV), differential pulse voltammetry (DPV), and stripping voltammetry (SV) (Lu et al., 2018).

LSV is the simplest technique where the applied potential is scanned linearly with time (Sawan et al., 2020). LSV starts to scan at a potential where there is no electrode reaction and continues to potential where the analyte is reduced to a more negative values or oxidized to a more positive value. The resulting current will be recorded throughout the scanning to acquire voltammogram as shown in Figure 2.6. Peak potential (E_p) in the voltammogram reveals the information of the analyte involved, whereas peak current (i_p) gives information on the concentration of the analyte, and number of electrons involved in the electrochemical reaction (Bontempelli et al., 2018).
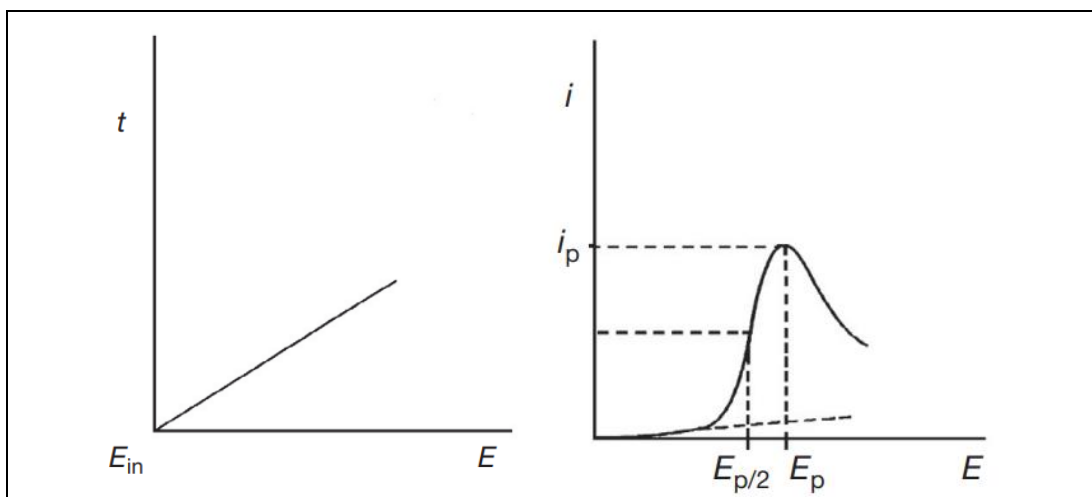
Figure 2.6: Potential waveforms and voltammogram from linear sweep voltammetry
(Bontempelli et al., 2018)

CV analysis involves applied potential swept in one direction then only swept in reverse direction between two extreme values with a steady rate in the order of few mV/s to few V/s. In CV analysis, current will flow through the electrode which either oxidize or reduce the analyte during the applied potential is swept in forward and reverse directions, and the forward and reverse scan currents between WE and CE are measured and plotted versus applied potential to acquire cyclic voltammogram. CV analysis is an electrochemical technique that is extensively used to determine the electrochemical properties relevant to electroactive surfaces. Besides, CV analysis allows to understand the redox mechanisms and the electron transfer reactions of electroactive ions in solution (Nnamchi and Obayi, 2018). A common cyclic voltammogram representing a reversible single electrode transfer reaction is shown in Figure 2.7.
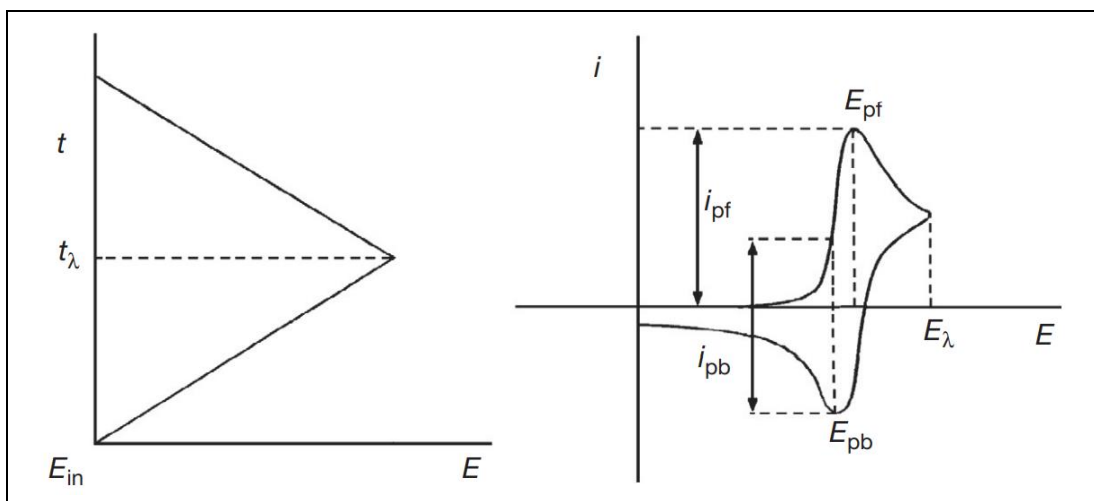
Figure 2.7: Potential waveforms and voltammogram from cyclic voltammetry analysis (Bontempelli et al., 2018)

Stripping voltammetry (SV) can be categorized into three types which are anodic, cathodic, or adsorptive stripping voltammetry (ASV, CSV, or AdSV, respectively). SV consists of two steps which are pre-concentration and stripping steps. In pre-concentration step, heavy metal ions from the sample solution will undergo reduction to zero valent metal and deposit onto the WE. In stripping step, the zero valent metal that accumulated will be re-oxidized to cations. Stripping the metal electrochemically allows for low LOD within ppb range, multielement determination and possibility for onsite application of heavy metal ions detection (Ferrari et al., 2020). During fast oxidation step, a high stripping current peak will be reached, and the stripping peak along with the peak current will be varied depending on the types of heavy metal ions (Gao and Huang, 2013). Moreover, stripping technique can be employed in combination with different pulse voltammetry techniques and resulting new detection techniques such as linear sweep anodic stripping voltammetry (LSASV), differential pulse anodic stripping voltammetry (DPASV), and square-wave anodic stripping voltammetry (SWASV). These techniques are relatively effective to detect

24