

**CAPABILITY OF CHROMOPHORES-CELLULOSE
ACETATE/CHITOSAN MEMBRANE BASED
COLORIMETRIC SENSOR FOR HEAVY METAL
DETECTION**

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COLORIMETRIC SENSOR FOR HEAVY METAL
DETECTION**

by

CHAN ZI YING

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LIST OF ABBREVIATIONS

AAS	Atomic absorption spectroscopy
AB	Azobenzene
APC	Amorphous polycarbonate
CA/CS	Cellulose acetate/chitosan
CE	Counter electrode
DHA	Docosahexaenoic acid
DMF	N,N-Dimethylformamide
DTZ	Dithizone
EO	Electro-optic
EPA	Eicosapentaenoic acid
FAO	Food and Agriculture Organization
FTIR	Fourier transform infrared spectroscopy
GC	Glass carbon
HMI	Heavy metal ions
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission
MeCN	Acetonitrile
PAR	4-(2-pyridylazo)-resorcinol
PAN	1-(2-pyridylazo)-2-naphthol
PSI	o-phenolsalicylimine
PV	Pyrocatechol violet
PVDF	Polyvinylidene difluoride

RBG	Red blue green
RE	Reference electrode
SEM	Scanning electron microscopy
SWASV	Square wave anodic stripping voltammetry
TIPS	Thermally induced phase separation
WE	Working electrode
WHO	World Health Organization
XRF	X-ray fluorescence spectrometry

**KEUPAYAAN SENSOR KOLORIMETRI YANG BERDASARKAN
KROMOFOR-MEMBRAN SELULOSA ASETAT/KITOSAN DALAM
PENGESANAN LOGAM BERAT**

ABSTRAK

Pengesanan logam berat di lokasi sumber air dengan cepat dan ringkas menggunakan kolorimetri sensor memainkan peranan yang penting dalam bidang industri, alam sekitar dan biologi sains. Namun, keupayaan kolorimetri sensor untuk menghasilkan pertukaran warna yang ketara amat bergantung kepada reagen optik seperti kromofor dan keadaan pengesananannya. Dalam pengajian ini, reagen optik seperti *dithizone (DTZ)*, *pyridylazo naphthol (PAN)* and *pyridylazo resorcinol (PAR)* yang berfungsi untuk menghasilkan pertukaran warna telah digunakan untuk pengesanan Pb^{2+} . Larutan *DTZ* pada pH4.11 didapati berupaya untuk menunjukkan transisi warna yang jelas dalam beberapa saat daripada hijau gelap kepada ungu semasa didedahkan kepada $0.25ppm Pb^{2+}$ ($1ppm Pb(NO_3)_2$) disebabkan pembentukan *DTZ-Pb* kompleks manakala pengesanan logam berat dengan menggunakan *PAN* dan *PAR* tidak dapat dikesan dengan mata kasar. Oleh itu, *DTZ* telah digunakan untuk mencipta sensor kolorimetri yang stabil dan canggih dengan memerangkap *DTZ* atas membran selulosa asetat/kitosan (*CA/CS*). Membran yang diterangkan dengan *DTZ* pada pH 4.11 menunjukkan respons yang paling bagus dalam keadaan pengesanan yang kering dan tanpa basuh dengan etanol semasa mengesan Pb^{2+} . Selain itu, reaksi pembentukan *DTZ-Pb* kompleks juga telah diselidik dengan *FTIR*, *SEM* dan *UV-vis* spektroskopi. Keupayaan sensor kolorimetri yang berdasarkan kromofor-membran *CA/CS* untuk mengesan $0.25ppm Pb^{2+}$ ($1ppm Pb(NO_3)_2$) telah membuktikan aplikasinya dalam pengawasan pembuangan timah hitam menurut had yang ditetapkan

dalam Environmental Quality (Sewage and Industrial Effluents) Regulations, 1979:
Standard B (0.5ppm Pb).

**CAPABILITY OF CHROMOPHORES-CELLULOSE ACETATE/CHITOSAN
MEMBRANE-BASED COLORIMETRIC SENSOR FOR HEAVY METAL
DETECTION.**

ABSTRACT

Simple and fast on-site detection of heavy metals using colorimetric sensor plays an important role in various fields of industry, environment and biological sciences. However, the ability of a colorimetric sensor to induce a distinct color transition highly depends on the optical reagent, for example, type of the chromophore and its detecting conditions such as concentration and pH. In this study, optical reagents such as dithizone (DTZ), pyridylazo naphthol (PAN) and pyridylazo resorcinol (PAR) that responsible to induce color changes were used for Pb^{2+} detection. DTZ solution at pH4.11 was found to be capable of displaying distinct color transition from dark green to purplish within seconds upon exposed to 0.25ppm Pb^{2+} (1ppm $Pb(NO_3)_2$) due to formation of DTZ-Pb color complex whereas PAN and PAR didn't provide naked eyes recognition of Pb^{2+} detection. Therefore, DTZ was used to further construct a stable and sophisticated colorimetric sensor by covalently immobilize DTZ on CA/CS membrane. DTZ at pH 4.11 immobilized on membrane strip had showed the most attracting result in response to Pb^{2+} in dry sensing condition and without ethanol washing. Besides, the complexation reaction of lead with DTZ has also been explored by FTIR, SEM and UV-vis spectroscopy. Capability of DTZ-CA/CS membrane based colorimetric sensor to detect 0.25ppm Pb^{2+} (1ppm $Pb(NO_3)_2$) had proved its practical application in lead emission monitoring comply with the limit of Environmental Quality (Sewage and Industrial Effluents) Regulations, 1979: Standard B (0.5ppm Pb).

CHAPTER ONE

INTRODUCTION

1.1 Heavy Metal in Wastewater

In recent years, the rapid economic development causes the increasingly discharge of wastewaters containing heavy metals to the environment. Major sources of these heavy metals are cosmetics and their by-products, fertilizers and other chemicals generated from industrial or household waste (Bansod et al., 2017, Shazili et al., 2006). These heavy metals are non-biodegradable and will accumulate in the bodies of humans and other living organisms through the consumption of heavy metal contaminated fishes and seafoods, causing serious damage to the human central nervous system, endocrine system, kidneys, bones and livers (Bansod et al., 2017).

Contamination of heavy metal ions, particularly Pb^{2+} , causes serious negative effect to human health such as renal malfunction and retardation of brain development. Various neurotoxic effects in children are also caused by the contamination of Pb^{2+} in the environment. In some cases, children are suffered from seizures and coma (Lidsky and Schneider, 2003). To resolve or reduce such scenarios, laws and regulations are enforced by establishing permissible limits for heavy metals discharge in marine organisms, rivers and drinking water. For example, the chemical standard of 0.05ppm of lead (Pb) for natural mineral water is stated in Food Regulations 1985 and Environmental Quality (Sewage and Industrial Effluents) Regulations, 1979 established a limit of 0.1ppm and 0.5ppm of lead (Pb) for the sewage and industrial effluent according to two standards of

effluent discharge: Standard A and Standard B respectively (Department of Environment, 2010, Kementerian Kesihatan Malaysia, 2014).

1.2 Sensor in The Detection of Heavy Metal

Traditional methods that are employed for heavy metals detection are mostly spectroscopy-based techniques such as atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectrometry (ICP-MS) and X-ray fluorescence spectrometry (XRF) (Azmi and Low, 2017, Gumpu et al., 2015). Atomic absorption spectrometric (AAS) method is able to quantitatively detect the heavy metals by measuring the absorbed radiation by the heavy metals (Báez, 2012). ICP-MS method detect heavy metal by first converting the atoms of the heavy metal in the sample to ions by the ICP source and the ions produced are separated and detected by the mass spectrometer (Sneddon and Vincent, 2008). These techniques have the benefits of high selectivity and sensitivity to the analytes species. However, they are associated with shortcomings of time consuming, expensive and complicated in their operating procedures where trained personnel are required to carry out the analytical procedures that involve complex instrument (Bansod et al., 2017, Cui et al., 2015, Rasheed et al., 2018, Gumpu et al., 2015). Efficient and low-cost sensors are preferred to detect and measure the concentration of heavy metals in the environment.

Recently, chemical sensor has been widely used to detect heavy metal on-site due to its simple analytical procedures and short detection time. Chemical sensor is further classified as optical sensor and electrochemical sensor (Azmi et al., 2018). Optical sensor detects heavy metal by inducing color changes which can be observed by naked eyes

within short detection time. Meanwhile, electrochemical sensor measure the generated electrical signal in correspond to the electrochemical reactions within the solution containing metal ions by passing current to the aqueous solution with biosensing electrodes (Bansod et al., 2017). Recent focus on heavy metal detection techniques has been shifted to chemical sensors which provide more advantages than the spectroscopy-based techniques.

1.3 Problem statement

Among the heavy metal detection techniques, colorimetric sensor or optical sensor is found to be the most suitable technique to detect heavy metal in the environment due to its rapid on-site detection ability and user-friendly (Azmi et al., 2018). However, lack of sophisticated facilities in countries such as Africa, Kenya and Cambodia emphasis the importance of rapid and on-spot color detection by colorimetric sensor to determine the presence of heavy metal in the sample.

The simple procedure of colorimetric sensor is able to provide quick detection of heavy metals in the environment as compared to the complicated procedure of spectroscopy-based techniques that require trained personnel to carry out the analysis. However, the ability of a colorimetric sensor to induce a distinct color changes highly depends on the detecting agent, for example, type of chromophore and its detecting conditions. The variables particularly pH of the chromophore and sensing condition will affect effectiveness of the sensor. The spectra absorbance of chromophore will alter with pH due to the change in electron density under acidic or alkaline conditions. In fact, the chromophores will then be protonated under acidic condition and causes the deterioration of their coordinated ability to metal ions. Meanwhile under alkaline condition, the metal

ions will precipitate which make the metal ions content in the sample solution decreases (Yan et al., 2017). These will weaken the interaction of chromophore with the heavy metal ions, thus, reduce detecting sensitivity of a sensor. Besides, electrostatic interactions between the polymer surface (detection platform of a sensor) and the immobilized chromophore may also influenced by pH (Goddard and Hotchkiss, 2007). When the polymer surface is modified to acidic or alkaline, the electrostatic interactions between the polymer surface and the immobilized chromophore will be weaken or strengthen depending on the interacting charged of a chromophore. Therefore, this research is conducted to explore the effect of pH to immobilize chromophores on polymer detecting platform and also study the performance of colorimetric sensor at different pH conditions.

1.4 Research objectives

This research is carried out:

- 1) To select appropriate chromophores to interact with heavy metal.
- 2) To explore the effects of pH to immobilize the chromophores on the membrane.
- 3) To determine the interaction between heavy metal and sensor in different pH condition of the chromophore.

1.5 Scope of study

In this research, chromophores which serve as optical indicators in the colorimetric sensor are dithizone (DTZ), pyridylazo naphthol (PAN) and pyridylazo resorcinol (PAR). Selection of appropriate chromophore is done based on the performance of Pb^{2+} detection which can be determined through the distinctness of color transition and

changes in uv-vis absorption spectra. Chromophore is immobilized on cellulose acetate/chitosan membrane that serve as sensing platform for direct detection of Pb^{2+} .

The factor that affect the immobilization of chromophore such as pH is also studied in this research. pH study that carried out is the pH of chromophores during immobilization. Moreover, the sensing condition of the colorimetric sensor is studied and detection limit of the colorimetric sensor is determined.

The prepared colorimetric sensor before and after Pb^{2+} detection was characterized by scanning Fourier transform infrared spectroscopy (FTIR) for the determination of membranes' and chromophores' functional group; electron microscopy (SEM) for the analysis of physical morphology of membrane before and after immobilization of chromophores as well as upon detected Pb^{2+} ; UV-Vis spectroscopy to evaluate the color changes of aqueous chromophores when detected Pb^{2+} ; RGB indication tool to evaluate the RGB values of the membrane-based colorimetric sensor before and after Pb^{2+} detection.

CHAPTER TWO

LITERATURE REVIEW

2.1 Discharge of Heavy Metal in Environment

Heavy metals pollution has been a major concern and issue of the rapid growth of industrial and population expansion in Malaysia. The most heavily discharged metals are from manufacturing sectors especially electronic and semiconductor industries with discharged wastes includes Cadmium (Cd), Copper (Cu), Zinc (Zn), Lead (Pb), Nickel (Ni), Iron (Fe), Aluminum (Al), Manganese (Mn), Chromium (Cr) and Tin (Sn) (Shazili et al., 2006, Alina et al., 2012). In fact, heavy metal wastes are produced from industrial activities such as electroplating, etching, and preparation of various metal components in industries, that generated waste of heavy metals directly as by-products and also as a result of from waste treatment process. Table 2.1 summarized the processes that are categorized as metal finishing operations that has the potential to generate the heavy metal wastes (Sundaravadivel et al., 2006).

Table 2.1: Categories of metal finishing operations. Information are adapted from Sundaravadivel et al. (2006).

Chemical/ electrochemical conversions	Diffusion coating operations	Case-hardening operations
<ul style="list-style-type: none">• Phosphating• Chromating• Anodising• Passivation• Metal coloring• Electroplating	<ul style="list-style-type: none">• Lattice diffusion• Spraying• Cladding• Vapour deposition• Vacuum coating• Hot dipping	<ul style="list-style-type: none">• Carburising• Carbo-nitriding• Nitriding• Micro-casing• Thermal hardening

According to the Malaysia Environmental Quality Report (MEQR) in year 2011, heavy metal sludge was one of the main wastes produced in Malaysia with the amount of 173,837.06 metric tonnes per year, which has contributed to 10.72% of the total waste (Department of Environment, 2011). The amount of heavy metal sludge produced is expected to increase every year due to the rapid growth of industrial activities in Malaysia.

2.1.1 Health and Environmental Effect

The discharge of heavy metals from the industries will accumulate in the sediments of river, rivers and marine environments due to their property of non-biodegradable. When heavy metals reach the sediments of rivers, heavy metals are strongly bounded to colloidal components on the soil surface. Decomposition of soil organic matter or soil acidification induced through soil internal process results in the increased of mobilized of metal concentrations and thus, cause hazards either to the growing organisms in the soil or to the ground water (Sundaravadivel et al., 2006, Baath, 1989).

The entry of heavy metals to the rivers and marine environments are both in particulate and dissolved forms (Sundaravadivel et al., 2006, Agusa et al., 2005). Heavy metals discharge to the marine environments can damage the marine organisms as well as ecosystem due to the toxicity and its accumulative behavior. According to the work of Rahman et al. (2012), fishes have the ability to concentrate heavy metals in their muscles and these heavy metals may be accumulated in the food chain and eventually consumed by organisms (Devagi et al., 2008, Rahman et al., 2012). Indeed, fishes and shell fishes are common main dishes taken by Malaysian to provide nutritional value in the form of protein, and minerals such as calcium, phosphorus, iron and copper as well as

eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in our daily diets (Alina et al., 2012). These metals are essential for the metabolic activities in human, however, the excessive amount will jeopardize the human health (Beldi et al., 2006). Moreover, the nonessential metals such as Cd, Pb, Ni, and Cr that present in marine organisms are toxic to human even in relatively low concentration. These heavy metals will enter human body through various sources especially seafood and fishes and disrupt cellular functions in human body (Gumpu et al., 2015). Although these heavy metals will be excreted through liver, kidney or spleen, however, the excretion process is very slow. A long storage of these heavy metals may leads to organ damage (Gumpu et al., 2015). For example, Cd cause injures to the kidney, including impaired kidney function, poor reproductive capacity, hypertension, tumors and hepatic dysfunction; Pb can cause renal failure and liver damage and excessive intake of Cr, Zn and Cu cause nephritis, anuria and extensive lesions in the kidney (Rahman et al., 2012).

99% of water supply for domestic use in Malaysia is from surface water and another 1% of water supply is from groundwater (Azrina et al., 2011). Water supply from surface water and groundwater that are widely used as drinking water in Malaysia are susceptible to heavy metal pollutions due to the industrial discharge of sludge that containing high concentrations of heavy metal. Thus, proper treatment process is required to remove the hazardous heavy metals content in the drinking water before reaching to the consumer.

2.1.2 Permissible Limit of Heavy Metal in Environment

In Malaysia, there are laws and regulations established to control the permissible limit of heavy metal in the drinking water, river and fishes to ensure safety for public consumption. According to Food Regulations 1985, the chemical standard for natural mineral water is shown in Table 2.2 (Kementerian Kesihatan Malaysia, 2014).

Table 2.2: Standard for natural mineral water. Information are adapted from Kementerian Kesihatan Malaysia (2014).

Chemicals	Maximum permitted proportion in milligram per litre (mg/l)
Arsenic	0.05
Barium	1
Borate (calculated as H ₃ BO ₃)	30
Cadmium	0.01
Copper	1
Chromium (IV)	0.05
Cyanide (calculated as CN ⁻)	0.01
Fluoride (calculated as F ⁻)	2
Lead	0.05
Manganese	2
Mercury	0.001
Nitrate (calculated as NO ₃ ⁻)	45
Nitrites (calculated as NO ₂ ⁻)	0.005
Organic matter (calculated as O ₂)	3
Selenium	0.01
Sulphide (calculated as H ₂ S)	0.05
Zinc	5

Environmental Quality (Sewage and Industrial Effluents) Regulations, 1979 also established a limit for the sewage and industrial effluent according to two standard of effluent discharge: Standard A for discharge upstream of any raw water intake, and Standard B for discharge downstream of any raw water intake (Department of Environment,

2010). The permissible limit for heavy metal discharge in sewage and industrial effluent is shown in Table 2.3.

Table 2.3: Parameter limits of effluent of standards A and B. Information are adapted from Department of Environment (2010).

Parameter (1)	Unit (2)	Standard	
		A (3)	B (4)
(i) Temperature	°C	40	40
(ii) pH Value	-	6.0-9.0	5.5-9.0
(iii) BOD ₅ at 20°C	mg/l	20	50
(iv) COD	mg/l	50	100
(v) Suspended Solids	mg/l	50	100
(vi) Mercury	mg/l	0.005	0.05
(vii) Cadmium	mg/l	0.01	0.02
(viii) Chromium, Hexavalent	mg/l	0.05	0.05
(ix) Arsenic	mg/l	0.05	0.10
(x) Cyanide	mg/l	0.05	0.10
(xi) Lead	mg/l	0.10	0.5
(xii) Chromium, Trivalent	mg/l	0.20	1.0
(xiii) Copper	mg/l	0.20	1.0
(xiv) Manganese	mg/l	0.20	1.0
(xv) Nickel	mg/l	0.20	1.0
(xvi) Tin	mg/l	0.20	1.0
(xvii) Zinc	mg/l	1.0	1.0
(xviii) Boron	mg/l	1.0	4.0
(xix) Iron (Fe)	mg/l	1.0	5.0
(xx) Phenol	mg/l	0.001	1.0
(xxi) Free Chlorine	mg/l	1.0	2.0
(xxii) Sulphide	mg/l	0.50	0.50
(xxiii) Oil and Grease	mg/l	Not	10.0
		Detectable	

A permitted level of heavy metal toxicity in fish and shellfish is also stated by the Food and Agriculture Organization and World Health Organization (WHO) to compare the contamination of heavy metals in fish and shellfish. Malaysia permitted limit is stated

in Food Act 1983 and Food Regulations 1985 (Alina et al., 2012, Kementerian Kesihatan Malaysia, 2014). Table 2.4 shows the permitted level of heavy metals.

Table 2.4: Limits of various heavy metal contaminations. Information are adapted from Kementerian Kesihatan Malaysia (2014) and Alina et al.(2012).

Metal	Malaysia Limits (mg/g)	FAO/WHO, 2004 Limits (µg/g)
Mercury (Hg)	0.5	0.5
Arsenic (As)	1	0.1-5.0
Cadmium (Cd)	1	0.05
Lead (Pb)	2	1.5

2.2 Heavy Metal Detection Techniques

In order to avoid these harmful heavy metals release to the environment, monitoring and detection of heavy metals in the environment is indispensably important. Various approaches have been developed and continuously improved to be employed for on-site detection of heavy metal. Methods that have been used to detect heavy can be classified as spectroscopy-based techniques and sensing-based techniques.

2.2.1 Spectroscopy Based Techniques

Traditional methods that are employed for heavy metals detection are mostly spectroscopy-based techniques such as atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectroscopy (ICP-MS), inductively coupled plasma optical emission (ICP-OES) and X-ray fluorescence spectrometry (XRF) (Bansod et al., 2017, Gumpu et al., 2015, Sneddon and Vincent, 2008, Rasheed et al., 2018, Hong et al., 2011). Atomic absorption spectrometric (AAS) method is able to quantitatively detect

heavy metals by measuring the absorbed radiation by the heavy metals (Báez, 2012). The concentration of the heavy metals is calculated based on Beer-Lambert law where the absorbance is directly proportional to the concentration of analyte absorbed for the existing set of conditions (Báez, 2012). This technique is left behind after introduce the more scientific advances of inductively coupled plasma optical emission (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS).

Inductively coupled plasma mass spectroscopy (ICP-MS) is an analytical technique that combine the ICP which is the ion source and mass spectrometer in the system (Sneddon and Vincent, 2008). It quantifies the heavy metal by first producing ions from ICP and then pass to the MS through a small orifice for the mass spectrum scanning (Sneddon and Vincent, 2008). An inductively coupled plasma optical emission (ICP-OES), on the other hand, is use to quantify heavy metals by measuring the electrical signal that converted from the light emitted by the atoms of a metal in the plasma (Sneddon and Vincent, 2008).

XRF is an analytical tool that measure the radiation emitted by the sample to determine the elements present. The elements present in the sample will emit fluorescent X-ray radiation with discrete energies (equivalent to colors in optical light) that are characteristic of the elements and a different energy will indicate different color (Brouwer, 2003).

These spectroscopic methods are highly sensitive, selective and they able to simultaneous determine different type of heavy metal ions concentration accurately (Cui et al., 2015, Bansod et al., 2017). However, these techniques have the disadvantages of

high instrument cost and only trained personnel is entitled to carry out the analysis because of its complicated operating procedure (Bansod et al., 2017, Cui et al., 2015, Rasheed et al., 2018). Besides, multi-steps sample preparation which involves difficult analytical procedures is also appeared as one of the major drawback because the aim of rapid heavy metal detection is not satisfy (Rasheed et al., 2018, Bansod et al., 2017).

2.2.2 Sensing Based Techniques

Attention has been shifted to the sensing-based techniques in the heavy metals detection to overcome the shortcomings of the spectroscopy-based techniques. Sensing-based techniques are able to detect the presence of heavy metals through the changes of the properties in the system such as color, fluorescent or redox potential when the heavy metals bind selectively and reversibly with the detection reagent (Navneet and Subodh, 2011). The sensors are categorized based on the properties change in the system: color (colorimetric chemosensors) or fluorescence (fluorescent chemosensors) or redox potentials (electrochemical sensors) as shown in Figure 2.1 (Navneet and Subodh, 2011).

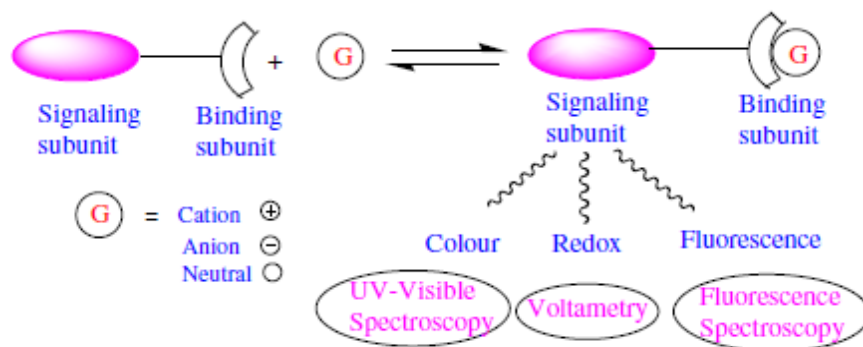


Figure 2.1: Schematic presentation of interaction of chemosensor with guest analyte (Navneet and Subodh, 2011).

Guest analyte that bind to the detection reagent can be charged (cation, anion) or neutral. The dynamic forces such as dipole-dipole, H-bonding, charge dipole, cation- π etc. are responsible for the reversible binding of substrate analyte. Additionally, many of the sensors utilize the dynamic exchange of covalent bonds (Navneet and Subodh, 2011). Determination of heavy metals with color and fluorescence transition are analyzed using UV-vis spectroscopy and fluorescence spectroscopy respectively. They also provide naked eyes recognition of the heavy metal detection. However, redox reaction occurred in electrochemical sensor upon exposure to heavy metal is determined via voltammetry. Researchers such as Gupta et al. (2015) had synthesized a new azo-compound, 1-(2-pyridylazo)-2-naphthol to detect aluminum with a detection limit of 1.81×10^{-8} M in fluorescence sensors. Azmi and Low (2017), Zargoosh and Babadi (2015) had suggested membrane-based colorimetric sensor using dithizone as detection reagent. In the research of Xuan et al. (2016), fully integrated electrochemical micro-sensor was evaluated for the detection of cadmium and lead with detection limits of $0.4 \mu\text{g L}^{-1}$ and $1.0 \mu\text{g L}^{-1}$ respectively in acetic-acid buffered solution using the square wave anodic stripping voltammetry (SWASV) technique.

2.2.2(a) Electrochemical Sensor

Electrochemical sensor detects heavy metal ions by using sensing electrodes to allow the current to flow through the aqueous solution and generate electrical signal (Bansod et al., 2017, Cui et al., 2015). Change of current, potential, electrochemical impedance, capacitance or electrochemiluminescence due to the presence of heavy metal ions is measured for heavy metal ions detection (Cui et al., 2015). The electrochemical sensor usually consists of three-electrode system containing working electrode (WE),

counter electrode (CE) and reference electrode (RE) as shown in Figure 2.2 (Cui et al., 2015, Bansod et al., 2017).

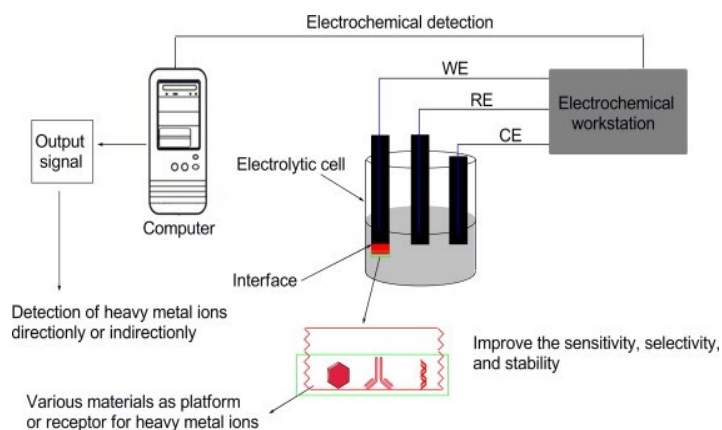


Figure 2.2: Schematic illustration of general principle of electrochemical sensing of heavy metal ions (Cui et al., 2015).

As shown in Figure 2.2, reaction is occurred on the working electrode (WE) that commonly made up of inert materials such as Au, Ag, Pt, glassy carbon (GC) and film electrodes (Metrohm Autolab, 2011). Reference electrode (RE) acts as a reference point in the electrochemical sensor system for the control and measurement of potential. In order to achieve the high stability of the reference electrode potential, a redox system is employed with constant concentrations of each components of the redox reaction (Metrohm Autolab, 2011). Counter electrode (CE) which made up of inert material such as Pt, Au, graphite and GC has the function of closing the current circuit in the electrochemical sensor system (Metrohm Autolab, 2011). There are a few classifications of electrochemical techniques for detection of heavy metal ions: static techniques, potentiostatic, galvanostatic, impedance measurement and electrochemiluminescence as shown in Figure 2.3 (Bansod et al., 2017).

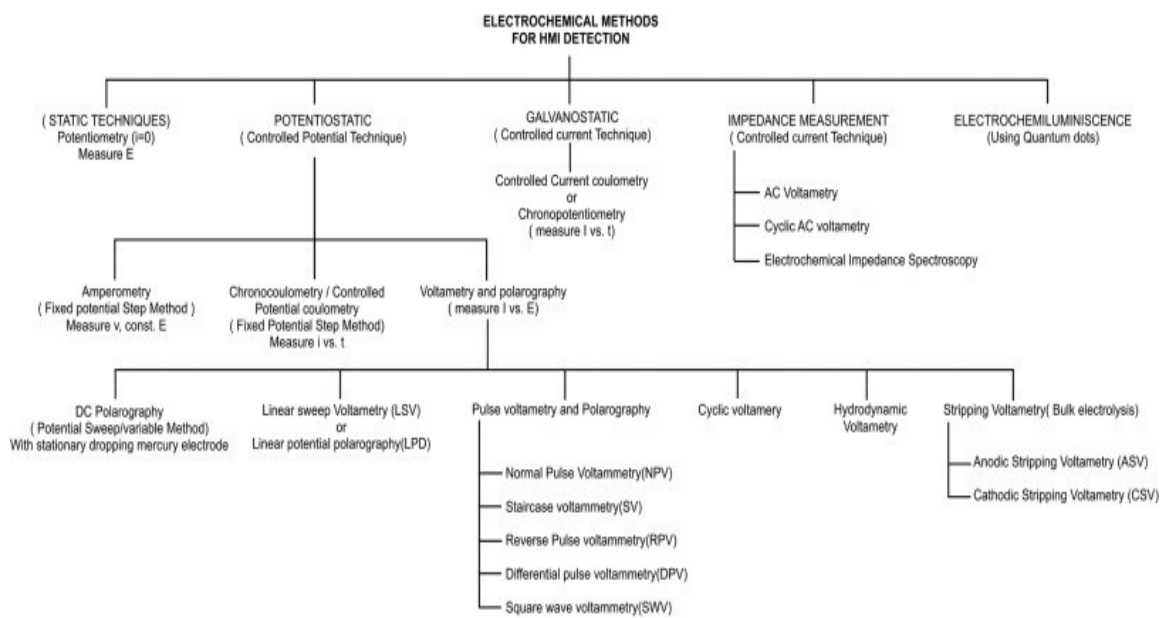


Figure 2.3: Classification tree for various electrochemical methods for HMI detection (Bansod et al., 2017).

Electrochemical sensor has the advantages of lower instrumentation and operating cost, shorter analytical time and user-friendly which does not involve complicated analytical procedures. However, detection of heavy metal ions using electrochemical sensor has the limitation of low sensitivity and Limits of detection (LOD) (Bansod et al., 2017). The benefits of electrochemical sensor in heavy metal detection have attracted the interest of researchers to modify and study on this sensor. For example, Zhang et al. (2017) designed a PtAu/CNF membrane which can be directly used as a sensor electrode for simultaneous detection of trace Cd^{2+} , Pb^{2+} , and Cu^{2+} with a sensitivity of $0.10 \mu\text{M}$ and correlation coefficients of 0.976, 0.993, and 0.976 respectively by square wave anodic stripping voltammetry (SWASV). Gumpu et al. (2017) also proposed the modification of gold (Au) electrode by $[\text{Ru}(\text{bpy})_3]^{2+}$ -GO to improve the sensitivity towards Cd(II), Pb(II), As(III) and Hg(II) metal ions with a lowest detection limit of 2.8, 1.41, 2.3 and 1.6 nM

respectively. Therefore, from above discussion, it can be deduced that different electrodes are coupled to meet the suitability of detection of specific elements in order to increase the sensitivity and limits of detection.

2.2.2(b) Optical/Colorimetric Sensor

Optical sensor also known as colorimetric sensor is a quantitative heavy metal detection probe that employed dye or gold nanoparticles as optical indicator (Hong et al., 2011, Azmi and Low, 2017). The detection mechanism is based on reaction occurs between heavy metal and optical indicator that induce a color change with naked eyes detection. Heavy metal ion is attracted to the optical reagents and chemically bind to them to form complex. This causes the bathochromic or hypsochromic shift of absorption spectra or visual color change in the colorimetric sensor which is influenced by the transition of electron densities on the chromophore moiety due to the charged heavy metal ions present (Navneet and Subodh, 2011, Suksai and Tuntulani, 2005, Yan et al., 2017). Therefore, colorimetric sensor has the common application in detecting heavy metal ions on-site. Figure 2.4 below shows the hypsochromic and bathochromic shifts in the colorimetric sensor.

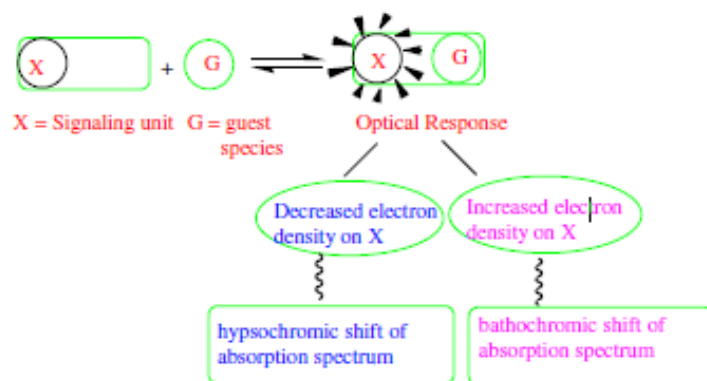


Figure 2.4: Schematic presentation of hypsochromic and bathochromic shifts in chemosensors (Navneet and Subodh, 2011).

The hypsochromic shift is defined as the shift of a spectral band to higher frequency or shorter wavelength upon substitution or change in medium and it is informally referred to as blue shift; bathochromic shift is defined as shift of a spectral band to lower frequencies or longer wavelengths upon substitution or change in environment and it is informally referred as a red shift (Nic et al., 2006, Pahari and Chauhan, 2006). Bathochromic shifts can be occurred in two ways: i) by conjugation of chromophoric groups or ii) by attaching auxochromes to the system (Pahari and Chauhan, 2006).

The quantitative detection of heavy metal can be determined by naked eyes without the use of expensive equipment and complex operation (Azmi and Low, 2017, Piriya et al., 2017). The configuration of colorimetric sensor is comparatively simple therefore, it provides real time analysis with little time consuming (Rasheed et al., 2018, Piriya et al., 2017). The high sensitivity, selectivity and accuracy of colorimetric sensor also overcome the problem of low detection limit of heavy metal ions that faced by the electrochemical sensor (Rasheed et al., 2018). Thus, in present work, colorimetric sensor is developed for heavy metal ions (HMI) detection.

2.3 Chromophores

Chromophore is any isolated covalently bonded group that shows a characteristics absorption in the UV/Visible region (Pahari and Chauhan, 2006). Chromophore with specific unsaturated groups will appear color when the molecule absorbs certain wavelength of visible light and transmit to others. Attachment of other component such as HMI will cause the shift of the position and intensity of the absorption band of a chromophore which is known as auxochromes (Pahari and Chauhan, 2006). Auxochromes produce the hypsochromic and bathochromic shifts and causes color changes of the chromophore formed with heavy metal ions (Pahari and Chauhan, 2006).

2.3.1 Type of Chromophore

Dithizone (DTZ), rhodamine, 4-(2-pyridylazo)-resorcinol (PAR), 1-(2-pyridylazo)-2-naphthol (PAN), metalloporphyrin, o-phenolsalicylimine (PSI), bromocresol green, Nile red, pyrocatechol violet (PV) and bromophenol red are among the common chromophores that have been used and researched as the detecting agent in the colorimetric sensor (Azmi et al., 2018, Gupta et al., 2015, LaGasse et al., 2014, Steinberg et al., 2003). Rhodamine, PSI and PAN have turn-on fluorescence behaviour of aluminium interaction with their ligands while DTZ, PAR and metalloporphyrin don't exhibit this characteristic (Rasheed et al., 2018, Gupta et al., 2015). Researchers such as Azmi et al. (2018) have utilized DTZ as chemoresponsive indicator to detect mercury ion. In their work, dithizone are successfully immobilized on cellulose acetate membrane and the membrane-based sensor was able to detect 3-10ppm of Hg solution. LaGasse et al. (2014) has also proposed a colorimetric sensor array using bromocresol green, Nile red and

bromophenol red to detect SO₂ and NH₃ gas. Arrays printed on porous polymer (polypropylene and polyvinylidene difluoride) membrane showed the best spot quality, quick response, reproducibility and with lowest noise.


2.3.2 Physical and Chemical Properties of Chromophore

Chromophore is organic molecule that able to impart color depending on its electronic properties (Ahmet et al., 2016, Pahari and Chauhan, 2006). It is generally found in organic dye molecule with properties of excellent brilliance and contrast color strength (Ahmet et al., 2016). However, they are weak in term of durability and thermal and solvent stability (Ahmet et al., 2016). In order to overcome these shortcomings, the structure of the chromophore is modified before it was immobilized on a polymer support. For example, Liu et al (2015) has designed a new NLO chromophore DIMER1 dual D-Pi-A nunchaku-like structure to improve stability of chromophore in solvent. In the study, chromophores are physically attached on the amorphous polycarbonate (APC) and its temporal stability of the electro-optic (EO) activity was significantly improved by the winding effect among the chromophores and polymers which could make this doping system more likely to the EO polymers with chromophore as the side chain.

Chromophores are mainly categorized into : (1) chromophores contains of π -electrons or (2) chromophores comprises of both π -electrons and n -electrons (Pahari and Chauhan, 2006). Π -electrons are usually found in unsaturated compounds such as dienes, trienes, aromatic compounds, etc while n -electrons are electrons that are not involved in bonding between atoms in the molecules such as lone pair of electrons in nitrogen, oxygen, sulphur or halogens (Pahari and Chauhan, 2006, S, 2011). Chromophore that contain π -

electrons only can undergo $\pi \rightarrow \pi^*$ transition while chromophore contain π -electrons and n -electrons is able to undergo $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transition. Table 2.5 summarized the examples of electronic transition occurred at different wavelength.

Table 2.5: Examples of electronics transition. Information are adapted from Pahari and Chauhan (2006).

Compound	$\lambda_{\max}(\text{nm})$
$\sigma \rightarrow \sigma^*$	
CH ₄	122
CH ₃ – CH ₃	135
$\pi \rightarrow \pi^*$	
CH ₂ = CH ₂	162
CH = CH	173
(CH ₃) ₂ C = O	190
CH ₃ – N 	201
$\eta \rightarrow \sigma^*$	
H ₂ Ö:	167
CH ₃ Ĉl:	172
(CH ₃)N:	227
$\eta \rightarrow \pi^*$	
(CH ₃) ₂ Ĉ:	280

2.4 Detection Platform of Colorimetric Sensor

Optical reagent in the colorimetric sensor is immobilized or entrapped in both organic and inorganic polymer materials (Guillermo et al., 2005, Azmi and Low, 2017). The polymer material which act as the sensing platform in the colorimetric sensor allows

the optical reagent to easily access to the heavy metal ions and also improve thermal stability of the chromophore (Liu et al., 2012). Poly(styrene), poly(methyl methacrylate), poly(vinyl chloride), poly(tetrafluoroethylene), poly(dimethyl siloxanes) as well as cellulose derivatives are among the widely used organic polymers (Guillermo et al., 2005). Inorganic polymer materials such as silica gel, glass or fused silica are also widely applied due to their dimensional stability which able to resist swelling (LaGasse et al., 2014). Polymeric support is preferable to inorganic support. Moreover, the polymer materials also maintain or improve the appropriate photophysical features of the optical reagent (chromophore) (Guillermo et al., 2005). In a research carried out by Tung and Guan (1996), the photophysical and photochemical behavior of azobenzene (AB) incorporated into solvent-swollen acid form Nafion (Nafion-H⁺) membranes was improved which exhibited strong fluorescence at room temperature due to the protons of the Nafion-H⁺ participate in the photochemical and photophysical processes of AB.

2.4.1 Characteristic of the detection platform

Selection of polymer material for colorimetric sensor as solid support and detection platform should consider a lot of features such as chemical inertness, high thermal stability, optical transparency, affordability, compatibility with the indicator dye, good mechanical properties, permeability to analyte, low intrinsic fluorescence or absorbance and the flexibility or porous structure to allow quick analyte diffusion (Guillermo et al., 2005, LaGasse et al., 2014, Goddard and Hotchkiss, 2007). Organic polymer has the properties of homogeneous structures, thermally and photochemically stabilized (Guillermo et al., 2005). For example, in a work carried out by LaGasse et al. (2014), responsive dyes immobilized on organic PVDF membrane showed significantly

higher total response to NH₃ and SO₂ gas which attributed by faster diffusion of the analyte caused by increased hierarchical porosity.

Moreover, the polymeric supports also have the property of controllable film thickness which enable them to be manufactured as thin films with high reproducibility and the microporous structure of the polymers allows the high optical reagent loading and analyte diffusion (Guillermo et al., 2005). Azmi and Low (2017) carried out the development of colorimetric sensor by manipulating the proportion of chitosan and cellulose acetate in membrane preparation in order to obtain the highest binding capacity and high binding stability for the chromophore. In their work, it was found that membrane developed by using 1 wt.% chitosan and 17 wt.% cellulose acetate have the highest binding capacity and high binding stability up to 90 days of measurement.

Detection platform that employing inorganic polymers is usually when dimensional stability, temperature tolerance and chemical inertia is a must (Guillermo et al., 2005). In contrary with organic polymers, inorganic materials are not easily manufactured as thin films, and have less porous structure to the analyte and light transmission once they are in non-particulate form (Guillermo et al., 2005).

2.4.2 Cellulose Acetate Membrane

Cellulose acetate membrane is one of the most common organic material used as solid support and sensing platform in colorimetric sensor. Cellulose is a naturally abundant syndiotactic homopolymer that contains D-glucopyranose units linked to one another by β -(1-4)-glycosidic bonds (Voicu et al., 2016). Owing to the low solubility of pure cellulose in common solvents, cellulose derivatives such as cellulose acetates,

carboxymethyl cellulose, nitrocellulose, hydroxypropyl cellulose, and hydroxyethyl cellulose which are soluble in a wide range of common solvents are preferably used as the alternatives to pure cellulose (Voicu et al., 2016). Among the cellulose derivatives, cellulose acetate is commonly used to synthesis membrane because it is soluble in a wide range of aprotic polar solvents such as acetone, N,N-Dimethylformamide (DMF), Acetonitrile (MeCN) and etc. (Voicu et al., 2016, Chen et al., 2004). Cellulose acetate membrane is an adsorptive membrane that has hydrophilic surface and low non-specific protein binding (Chen et al., 2004). This membrane is required to be deacetylated to obtain reactive hydroxyl groups with pore size changed and rigidity loss (Chen et al., 2004). For example, Voicu et al. (2016) has synthesized cellulose acetate membrane for covalent immobilization of sericinon to it using glutaraldehyde as linker between amino-propyl-triethoxysilane and sericin.

Indeed, synthesis of cellulose acetate membrane can be done by controlled phase separation of polymer solutions into two phases: one with a high polymer concentration and other with a low polymer concentration (Pandey and Chauhan, 2001). After the phase separation, the phase of high polymer concentration solidifies shortly and forms membrane. Phase separation of polymer solutions for porous polymeric membranes can be carried out in several ways. The techniques include immersion precipitation, thermally induced phase separation (TIPS), air casting of a polymer solution and precipitation from vapor phase (Pandey and Chauhan, 2001). In regards of a membrane structure, the porous polymeric membrane can be classified as symmetric or asymmetric. A symmetric porous film has uniform porosity. However, an asymmetric porous film has pore structure