IMMOBILIZATION OF DIFFERENT CHROMOPHORES ON CELLULOSE ACETATE BUTYRATE FLAT-SHEET TO DETECT HEAVY METAL IONS

by

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PROSES IMMOBILISASI KROMOFOR BERBEZA DI KEPINGAN RATA SELULOSA ASETAT BUTYRATE UNTUK MENGESAN ION LOGAM BERAT

ABSTRAK

Keracunan logam berat telah menjadi masalah besar kepada alam sekitar dan kesihatan manusia. Pengesan optik yang mampu milik dan mudah digunakan sedang dikaji agar pencemaran logam berat dapat dikawal dan dikurangkan. Kajian ini memfokuskan jenis-jenis kromofor sebagai reagen optik yang boleh mengesan logam berat di kepekatan yang berbeza. Kromofor yang dipilih untuk kajian ini adalah dithizone (DTZ), 1-(2-pyridylazo)-2-naphthol (PAN) and 4-(2-pyridylazo)-resorcinol (PAR) dan logam berat yang digunakan ialah Pb^{2+}. Ketika proses mengesan logam berat di kepekatan 5ppm, perubahan warna DTZ ialah yang paling ketara. Perubahan warna PAR dan PAN pula, tidak begitu ketara, menjadikan DTZ sebagai reagen optik yang paling sesuai antara ketiga-tiga kromofor yang dicadangkan. Kromofor-kromofor tersebut juga digunakan untuk mengesan logam berat di kepekatan yang berbeza. Di kepekatan 5ppm dan 10ppm, kromofor-kromofor tersebut mampu menghasilkan perubahan warna yang ketara dengan DTZ menghasilkan perubahan warna yang paling ketara. DTZ juga mampu menghasilkan perubahan warna di kepekatan 3ppm walaupun tidak ketara seperti di kepekatan 5ppm dan 10ppm. DTZ menunjukkan sensitivity yang paling baik antara ketiga-tiga kromofor yang diuji.

IMMOBILIZATION OF DIFFERENT CHROMOPHORES ON CELLULOSE ACETATE BUTYRATE FLAT-SHEET TO DETECT HEAVY METAL IONS

ABSTRACT

Heavy metals poisoning has been a serious problem to the environment and human health. Affordable and easy to use optical sensors are being developed in effort to monitor and reduce the heavy metal contaminations. This study focuses on the types of chromophores as the optical reagent that can detect the heavy metals at different heavy metal concentrations. The chromophore selected for this research are dithizone (DTZ), 1-(2-pyridylazo)-2-naphthol (PAN) and 4-(2-pyridylazo)-resorcinol (PAR) and the heavy metal used is Pb^{2+}. The color changes of DTZ are the most significant during heavy metal detection at 5ppm heavy metal concentration. The color changes of PAR and PAN on the other hand, are not so noticeable making DTZ as the most suited to be used as the optical reagent among the three chromophores suggested. The chromophores are also used to detect the heavy metals at different concentrations. At 5ppm a.nd 10ppm, the chromophores are able to produce color changes with the most significant changes belong to DTZ. DTZ is also able to produce color changes at 3ppm concentration although it is not as significant as the detection at 5ppm and 10ppm concentrations. DTZ showcases the best sensitivity between the three chromophores tested.

CHAPTER 1

INTRODUCTION

Chapter 1 introduces the overview of this research and significance of optical sensors in detecting the heavy metal pollutants. Overall, this chapter consists of the research background for the heavy metal detection and application of chromophore as the heavy metal optical sensors indicators, the problem statement, and the objectives of this final year project.

1.1 Research Background

In the recent decades, the presence of pollutant, such as lead and other heavy metals in drinking water has been a grave concern due to its harmful effect on the human health. Heavy metals are inherently non-biodegradable, highly toxic and easy to accumulate which can cause severe harm to human health if left untreated (Zhang et al., 2019). Elevated heavy metal lead levels in human blood, for instance, can cause serious mental and physical health problems especially for children (René et al., 2022). Thus, it is important to monitor and detect these heavy metal pollutants to prevent contamination.

To detect these heavy metals, there are several types of sensors been explored and produced. One of which is optical sensors field that being widely explored to detect these heavy metals. They are defined as small devices capable of reacting selectively and sensitively to the presence of a heavy metal, resulting in optical information (Oehme and Wolfbeis, 1997). Optical-based sensors, particularly fluorescent ones, are very sensitive and adapted to miniaturization. Furthermore, the analysis of excitation and emission spectra as well as fluorescence intensity, lifetime, and anisotropy from the fluorescence signal analysis can provide a lot of information (René et al., 2022).

In optical-based sensors, chromophore is being used as the transmitter reagent. Most of the indicator dyes and reagents utilized in sensor applications, including the chromophore, are mostly used in immobilized form. The indicator dyes and reagents are immobilized on polymers that serve as the solid supports. There are several types of polymeric materials and blends in sensor applications, and they are mostly employed in the form of thin membranes or films. (Oehme and Wolfbeis, 1997). Polymeric materials have always been utilized for coating or surface functionalization due to their flexibility, diversity, and high energy density. (Kush et al., 2021).

In removing the heavy metals, chitosan and chitosan composites have been reported as eco-friendly and efficient methods for the sorption of many pollutants, particularly potentially toxic metal ions (Ambaye et al., 2022). In recent years, blend polymer was developed to improve the polymeric membrane's adsorption capacity as chitosan has a promising adsorptive material (Aquino et al., 2018). To study the color responsive sensors during detection and removal of lead, the cellulose acetate butyrate/chitosan blend polymers will be used to form a flat sheet as the matrix platform for the immobilization of transmitter reagent, chromophores.

1.2 Problem Statement

The heavy metals in water are a serious matter because of the toxicity on humans and ecosystems. The usage of lead in the old plumbing system is one of the sources of lead in water. Even though the usage of lead in the plumbing system is now declining, there are still concerns regarding this matter as there are still other sources of lead pollution such as lead-based paints, drinking water in low and middle-income developing countries and more (René et al., 2022).

While there are several types of heavy metals sensors being developed, optical sensors are particularly friendly as they can provide easy detection due to the visible response, affordable, and easy to be used. In this paper, the optical sensors are used as lead detector, with chromophore immobilize on polymeric materials as the signal transmitter. However, the sensitivity and ability of each type of chromophores to produce color changes during the heavy metal detection differ from one another. The effectiveness of the sensor depends on the detecting sensitivity of the chromophores at different heavy metal concentration.

The flat sheet polymeric materials are used as the solid support for the chromophore and adsorbent of the heavy metals. The solid platform used for the immobilization of the chromophore is important to ensure the chromophores are efficiently immobilized. The types and composition of the polymeric materials used in the membrane film can affect the mechanical properties of the membrane film (Guillermo et al., 2005). The strength of the membrane film can also change for different polymer concentration. This study will evaluate the sensitivity and effectiveness of the colorimetric sensor for different types of chromophores at different heavy metal concentration and the effect of different polymer concentration on the membrane film properties.

1.3 Research Objectives

The objective of this research:

i. To evaluate the effect of different concentration of the cellulose acetate butyrate on the solid platform.

ii. To immobilize chromophore on the solid platform membrane to produce sensor.

iii. To select the most sensitive chromophore to be used in the heavy metal sensor.

1.4 Scope of study

In this research, the transmitter reagent used to indicate the color changes during the heavy metal detection is chromophore. The chromophores Dithizone (DTZ), 1-(2-pyridylazo)-2-naphthol (PAN) and 4-(2-pyridylazo)-resorcinol (PAR) are used to determine the sensitivity of each chromophore as the optical sensors of heavy metal. The color changes of each chromophore during heavy metal detection are used as the indicator to select the best chromophore to be used in the optical sensors. The chromophores are used by immobilizing them on a solid platform, cellulose acetate butyrate flat sheet matrix platform.

The solid platform used could affect the immobilization of chromophores, thus, the effect of different cellulose acetate butyrate concentration from 3 to 10 wt.% on the formation of the membrane film is also studied to ensure the solid platform used can be used to effectively immobilized the chromophore. The characterization of the colorimetric sensors is done by RBG indication tool to evaluate the color changes of the sensors using RBG values of the colorimetric sensor before and after heavy metal detection.

1.5 Sustainability

The sustainability of this research can be based on the Sustainable Development Goals (SDGs) which consists of a set of 17 global goals. These goals are intended to create a better and sustainable future. This research closely related to Goal 6 which is *clean water and sanitation*. This research promotes clean water and aquatic life as it is used to detect heavy metal contamination. Through this heavy metal detector project, heavy metal pollution and contamination in the water source are hopefully can be controlled and reduced to ensure a safer and cleaner water. The optical sensors are also tested in different concentrations to ensure the heavy metal can be detected at low concentration and not exceeding the safe limits in the water.

CHAPTER 2

LITERATURE REVIEW

This chapter will present the previous studies and reviews from credible scientific records related to this final year project topic. The overview on the heavy metal sensors, optical sensors transmitter reagent and the immobilization platform for the reagent are also covered in this chapter.

2.1 Heavy metal pollutants in the environment and human health

Heavy metals contamination in the environment has been an increasing concern due to the toxicity that can be a threat to the ecosystem and the human health. Due to the used of these heavy metals in the industries and technological applications, the risk of exposure to the human are increasing (Tchounwou et al., 2012). Sources of the heavy metals in the environment include agricultural, industrial, and atmospheric sources. The mining and foundries areas are well known sources of the heavy metal (Tchounwou et al., 2012). There are some heavy metals which can be beneficial to the human such as zinc, iron, and cobalt and some are harmful and can induce toxicity such as cadmium, arsenic, mercury, and lead (Tchounwou et al., 2012). Table 2.1 illustrates the list for different global regulatory authorities that regulates these standards for safe consumption of drinking water.

Table 2.1 shows the maximum permissible limits of various heavy metals according to different regulatory authorities [Mukherjee et al., 2021]

Heavy metal	FDA (mg/L)	WHO (mg/L)	CODEX (mg/L)	EU (mg/L)
Cadmium	0.005	0.003	0.003	0.005
Arsenic	0.01	0.01	0.01	0.01
Lead	0.005	0.01	0.01	0.01
Chromium	0.1	0.05	-	0.05

In this study, the heavy metal lead is used as the target analyte to be detected by the color sensor. Lead is a naturally occurring metal in the environment, however, the mining, fossil fuels burning activities etc. have increased the concentration of lead in the environment. The applications of lead in the industries are also high such as the lead-acid batteries and metal products industries (Tchounwou et al., 2012). In the United States alone, the usage of lead in 2004 was estimated to be 1.52 million metric tons in various industries with the highest percentage which is 83% of the lead usage is used in the lead-acid batteries production (Tchounwou et al., 2012).

Though the usage of lead has been greatly reduced in the industries by eliminating lead in gasoline, reducing usage of lead in the plumbing systems and residential paints, etc. the lead contamination is still happening due to the exposures from the soil, dust, or lead-contaminated deteriorated paint (Tchounwou et al., 2012). In Malaysia, the release of heavy metal sludge from the industrial activities is one of the causes for lead exposure to the environment. The heavy metal releases then can contaminate and toxicate the ground water which can cause damage to the marine ecosystem. Lead poisoning in human can cause headache, irritability, and loss of memory, which are some of the early symptoms for the lead exposure on the central nervous system. Lead exposure to human remains a serious health problem which can affect the human internal organs (Tchounwou et al., 2012). Table 2.2 shows several uses of lead and effects of lead on human.

Table 2.2 shows the uses of lead and its effects on human [Briffa et al., 2020]

Uses of Lead	Effects on Human
• Lead piping, • Cable sheeting, • Lead acid batteries in cars,	• Hypertension, • Miscarriages, • Stillbirths,

• Lead crystal glass, • Sports equipment, • Weight belts for divers, • Canister for corrosive liquids, • In buildings for roofing, • Stained glass windows, • Ammunition and projectiles	• Brain injury, • Renal impairment, • Cognitive impairment, • In children: - Brain and central nervous system development altered - Reduced intelligence, - A decline in educational achievement, - A reduction in the attention span

2.2 Heavy metal detection techniques

To detect and control the lead level in the environment, analytical techniques and sensing-based techniques are the methods that have been used in producing the heavy metal detectors. Analytical techniques such as atomic absorption spectroscopy (AAS), atomic fluorescence spectroscopy (AFS), inductively coupled plasma-mass spectroscopy (ICP-MS), high-performance liquid chromatography etc. are the effective and recognized traditional methods used to detect the heavy metal (Alberti et al., 2020). These techniques, however, are costly, time-consuming, and labor-intensive despite being highly sensitive and selective as they require a well-trained analyst and professional, time-consuming sample preparation, several hours of detection and costly instruments (Sharma et al., 2006). Furthermore, due to the bigger size of the equipment, they cannot be employed as portable devices for on-site detection (Ullah et al., 2018). Table 2.3 shows several examples on the use of conventional techniques for heavy metal detection.

Table 2.3 shows the summary of conventional techniques for heavy metal detection [Mukherjee et al., 2021]

Heavy metal	Sample pretreatment	Methods of detection	Detection limit
Chromium	Solid-phase extraction	ICP-MS	4.43 ng/L
Zinc	Chelating with 5,7-dichlorooxine	AAS	0.5 μg/L
	CPE pre-concentration	FAAS	0.2 μg/L
Copper	Solid-phase extraction	FAAS	0.2 μg/L

There are also sensing-based techniques been applied to the heavy metal detectors production. The sensing-based techniques can be classified into two main types, optical and electrochemical sensors (Azmi et al., 2018). A chemical sensor detects a particular analyte and convert the chemical stimuli into a signal. Generally, chemical sensors have three parts which are the receptor, signal transducer and a read-out mechanism (Sharma et al., 2006). The receptor is a moiety that directly interacts with the analyte and can convert the changes in the molecular environment's chemical composition into changes in physical or chemical characteristics such as changes in electron distribution energy of frontier orbitals, redox potential, and so on. The transducer then converts and amplifies the chemical changes into an observable analytical signal output, such as changes in optical signal and electrochemical activity. Lastly, the read-out moiety will be reporting the recognition event and processing the transducer signal (Sharma et al., 2006).

2.1.2 Optical sensor

An ideal sensor should have high binding constant, low detection limit and less response time (Sharma et al., 2006). Optical sensor is preferable among other types of sensors and considered as a better approach for detecting the heavy metals since it provides low detection limit, high selectivity and sensitivity, and an inexpensive method, as it does not require expensive equipment or complex detection method (Alberti et al., 2020, Piriya V.S et al., 2017). It also provides small response time and in-situ and real-time monitoring (Sharma et al., 2006). Optical sensors have great potential and wide range of application as on-site heavy metal detection (Ullah et al., 2018).

Piriya V.S et al. (2017) reviewed the usage of colorimetric sensors for the detection of chemical compounds such as heavy metal ions, toxic gases, and organic compounds. For the heavy metal detection, gold nanoparticles is used which help provided the optical property for the color changes. Heavy metal detection in water, however, used gold nanoparticles capped with chitosan as the optical sensors. The colorimetric sensors are proved to be simple and rapid as the detection is done in a single step with no complex instrumentation (Piriya V.S et al., 2017).

2.1.3 Signal transmitters of optical sensor

Optical-based sensors usually use signal transmitter or reagent as indicators to detect the heavy metals. The optical indicator can generate color changes that can be seen with the naked eye. Without the need of any spectroscopic equipment, the sensor output signal can produce measurable color changes as the result of the reaction between the analyte with the optical indicator ligand that is immobilized on the membrane substrate (Azmi et al., 2018). The operation of optical sensors is based on changes in optical properties such as absorption, transmission, emission, etc. caused by the binding of an immobilized indicator with the analyte (Ullah et al., 2018).

Fluorescent chemical compound has been widely used as a type of indicators for the optical sensors. Fluorescent sensors have been developed based on changes in the physicochemical properties of a fluorophore caused by the analytes. It is one of the most used signals in the optical sensors application as it can provide a lot of information through the analysis of excitation and emission spectra as well as fluorescence intensity, lifetime, and anisotropy (René et al., 2022). Generally, the fluorescent sensor is made up of a fluorescent component that signals the binding event and a receptor element for specific binding to analyte (Ullah et al., 2018). The figure 2.1 shows the example of simplified schematic for the Pb-based fluorescent sensor namely Pb-1 to Pb-19.

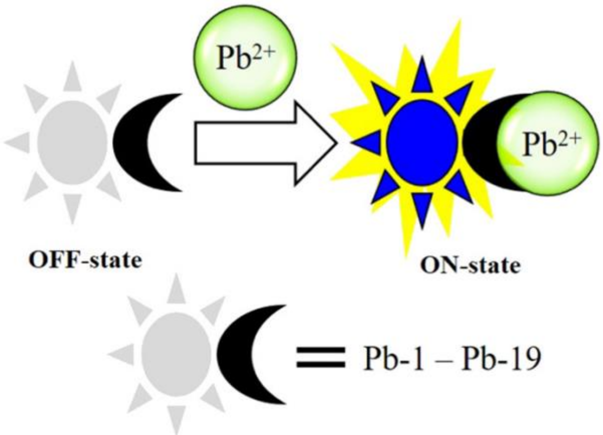


Fig. 2.1 A simplified schematic of the Pb-based fluorescent sensor for the detection of Pb^{2+} (Rasheed et al., 2018).

A work done by Ju and Chen (2014) shows the development of fluorescent sensor in detecting the Fe^{3+} ion. They have developed a simple synthetic strategy to prepare highly fluorescent nitrogen-doped GQDs (N-GQDs) for the detection of Fe^{3+} in which the N-GQDs is prepared by a simple hydrothermal method with hydrazine. The N-GQDs is a possible type of promising fluorescence probe for sensing metal ions. To study the sensitivity of N-GQDs for Fe^{3+} detection, the photoluminescence (PL) properties of the N-GQDs and the un-doped GQDs in the presence of Fe^{3+} were studied. The results show that the N-GQDs exhibit an exceptional improvement in fluorescence intensity and higher sensitivity towards Fe^{3+} quenching in a wide concentration range (Ju and Chen, 2014).

2.2 Chromophore

Other type of signal transmitter used in optical sensors is chromophore, which is part of a molecule or chemical group responsible for its color. Chromophore is an extended delocalized system of electrons in a compound which will gives its color (Shukla et al., 2012). Molecule has color when it absorbs certain wavelengths of visible light while transmitting or reflecting others. A chromophore is a region in a molecule where the energy difference between 2 separate molecular orbitals falls inside the range of the visible spectrum (Shukla et al., 2012).

2.2.1 Chromophore usage as the transmitter reagent

The chromophores are the groups of atoms responsible for the color, while auxochromes are electron withdrawing or donating substituents that cause or heighten the color of the chromophores by changing the adsorption towards longer wavelengths as well as increasing the absorption intensity (Coulibaly, 2021). These four are some of the well-known chromophore's group, azo (–N=N–), carbonyl (–C=O), methine (–CH=), nitro (–NO2), and quinoid groups. The auxochromes are acids or bases where the most prominent are amine (–NH3), carboxyl (–COOH), sulfonate (–SO3H), and hydroxyl (–OH) (Coulibaly, 2021). The formation of complexes between the heavy metal ions and the chromophore ligands have been developed and tested. The complex is formed when an electron-donating ligand or ion interacts with a metal ion. The formation of complexes allows them to be detected using spectrophotometric methods or chromatography (Coulibaly, 2021). Azmi et al. (2018) formed colored complexes by combining dithizone ligand with metal ions such as Hg2+, Cu2+, and Zn2+. The reaction of the color changes varies depending on the type of metal ions interacting with the ligand.

The chromophore reagents are able to be used as a selective and optical transducer for optical sensors in the aqueous medium, as the binding of the metal ions to the receptors are proven to cause visible color changes. However, the data collection for the use of the heavy metal ions detectors in aqueous medium relies on UV-visible spectroscopic titration experiments and Benesi–Hildebrand (B–H) plots, which require skilled professional and infrastructure (Azmi et al., 2018). If there was no need for specialized technologies to detect the color changes, the optical sensors would be much ideal.

The common types of chromophores used as the transmitter reagent in the colorimetric sensor are Dithizone (DTZ), 1-(2-pyridylazo)-2-naphthol (PAN) and 4-(2-pyridylazo)-resorcinol (PAR). Gupta et al. (2015) studied the interaction of azo compound, PAN, with aluminum ions. The PAN synthesized by Gupta et al. (2015) interacts strongly and selectively with aluminum ions and thus, been used in preparing the colorimetric sensor for aluminum ions. From the study, PAN is shown to be able to detect aluminum ions both qualitatively and quantitatively (Gupta et al., 2015).

2.3 Solid phase detection

Chromophores are mostly used as a transmitter reagent of optical sensors by immobilizing it onto polymeric materials and blends. Dithizone-colorimetric sensor was bind to the cellulose acetate (CA) membrane, which act as the immobilization and detection platform in the study by Azmi et al. (2018). The work demonstrates that the practicability of using a porous CA membrane as the solid detection platform, and its sensitive binding interactions with dithizone demonstrated its practical uses for on-site quick detection of heavy metals. The performance and sensitivity of the optical sensor, however, can be affected by the change in the physical and chemical characteristic of the membrane used (Azmi et al., 2018). This study focuses on the effect of the membranes structural on the chromophore immobilization which was done by preparing the polymer blend membrane at different polymer concentration. The membrane-based sensor produced using CA and 25% glutaraldehyde is shown to have a good binding ability with DTZ and good membrane stability against 90 aging days. The sensor produced from this work can be used as a stable optical sensor, capable of showing different color changes in response to the presence of Hg^{2+}, Cu^{2+} and Zn^{2+} ions (Azmi et al., 2018).

Jayawardane et al. (2013) research shows the detection of Cu (II) in natural and wastewater using novel polymer inclusion membranes application to produce paper-based sensor with enhanced selectivity and sensitivity. The reagent used is 1-(2-pyridylazo)-2-naphthol (PAN) which react with Cu (II) to form Cu (II)-PAN complex. The polymer inclusion membrane technology can incorporate a wide range of extractants and color reagents thus, it is expected that it will help the development of numerous highly selective and sensitive paper-based devices for a variety of analytes of interest (Jayawardane et al., 2013). The solid phase detectors are a much preferable platform to be used in the optical sensors' application as they offer an attractive alternative to conventional solvent extraction by drastically reducing or eliminating the use of solvents (Jayawardane et al., 2013).

2.3.1 Polymeric materials as the solid supports

Optical sensors are used by immobilizing the transmitter reagent onto polymeric materials and blends. The immobilization of the sensors on the polymer matrices are usually via adsorption, entrapment, ion exchange and covalent binding procedures. Polymers act as the solid supports for the reagent and the choice of the polymer materials can affect the sensor's performance. The choice of the polymer materials could have significant effects on the optical sensors' selectivity, sensitivity, response time and stability (Guillermo et al., 2005, Oehme and Wolfbeis, 1997).

Both organic and inorganic polymer materials are used as the solid supports for the transmitter reagent of the optical sensors. However, most of the optical sensors developed used organic polymers as the supports for the reagent such as polyvinyl chloride, polystyrene, polydimethyl siloxanes, cellulose derivatives, ion exchange resins, etc (Reglero Ruiz et al., 2018). Organic polymers are commercialized at low prices in a variety of molecular sizes, possess homogenous structures, sufficient chemical, thermal and photochemical stability, can be easily manufactured as thin transparent films or (micro)beads with high reproducibility, allow for high indicator loading and analyte diffusion due to their (micro)porous structure, and are compatible with a wide range of organic/inorganic molecules that may be used as necessary additives in the sensing phase are few of the many reasons for its popularity (Reglero Ruiz et al., 2018).

Cellulose acetate (CA) membrane, a derivative of cellulose, is a common and one of the primary polymer membranes for aqueous based separation of water treatments (Moradihamedani & Abdullah, 2017). Because of their strong mechanical strength and good thermal and chemical stability, cellulose acetate is commonly used in colorimetric sensors (Azmi et al., 2018). However, CA has problems such as intolerance to harsh pH environments that can lead to hydrolyzation and vulnerability towards biodegradation (Ong et al., 2013). Cellulose acetate butyrate (CAB) polymer, which is a derivative of CA and a cellulose mixed ester of acetic and butyric acids, has been studied (Fordyce & Meyer, 1940). CAB membranes has better stability, better thermal and chemical stability compared with cellulose acetate materials and great film forming and mechanical properties (Ong et al., 2013, Ng et al., 2020, Xing et al., 2013). CAB polymer membrane can be applied as the polymer membrane for the solid support of the optical sensor.

Polymer blending method is a famous method that has been used to enhance the polymer material. It is a proven method of obtaining new materials/blends with vastly different and unique qualities that are intermediate between those of pure components, as well as economic benefits (Moradihamedani & Abdullah, 2017). Chitosan is a natural polymer and promising adsorptive material that has been applied as a matrix in the sensor development, making great contribution to the adsorption of metal ions due to its abundant amino and hydroxyl groups (Safitri et al., 2021, Zhang et al., 2019). It also possesses outstanding properties including biocompatibility, biodegradability, bioactivity, and non-toxicity (Zhang et al., 2019) as well as good solubility in organic media and water. However, pure chitosan membranes lack mechanical strength and chemical stability. Polymer blending of chitosan with CAB thus, can improve the adsorption capacity of the CAB membrane as well the mechanical strength of chitosan membranes in which CAB will act as the mechanical support while chitosan will contribute amino group to the membrane matrix through the blending process (Azmi et al., 2018, Aquino et al., 2018).

2.4 Production of color responsive optical sensor

The cellulose acetate butyrate/chitosan flat sheet matrix platform from CAB/chitosan blend polymer is proposed as the platform for the heavy metals' optical sensor due to the excellent adsorption properties of the blend polymers (Aquino et al., 2018). The signal transmitter for the color responsive optical sensor of heavy metal will be immobilized onto the solid platform to generate color changes that can be seen with the naked eye during the heavy metal detection. The sensor output signal will produce measurable color changes without the need of any spectroscopic equipment at different heavy metal concentration (Azmi et al., 2018).

CHAPTER 3

MATERIALS AND METHODS

This chapter explains the methodologies applied in this final year project, which includes the general research flow, immobilization of chromophores and characterization of the colorimetric sensor.

3.1 Materials and equipment

Cellulose acetate butyrate powder and chitosan (low molecular weight) were purchased from Sigma-Aldrich (St. Louis, MO). Formic acid (90-100%) which is used as the solvent in the membrane preparation was purchased from Fisher Scientific (UK). Dithizone (DTZ), 1-(2-pyridylazo)-2-naphthol (PAN) and 4-(2-pyridylazo)-resorcinol (PAR) were supplied by Merck (Darmstadt, Germany). Lead (II) nitrate is obtained from Sigma-Aldrich (St. Louis, MO). Ethanol for the preparation of the chromophore solutions. All chemicals used were of analytical grade and used without further purification. The equipment used in this research is the color indication tools based on the RBG values. The color changes of the sensors after detecting the heavy metals will be evaluated by this tool.

3.2 General research methodology

Generally, this final year project focused on the immobilization of different chromophores on the solid platform to detect heavy metals. The experiment will be utilizing the color indication tools to study the color changes of the heavy metal detector sensors using different types of chromophores. Figure 3.1 shows the general research flow of this experiment.

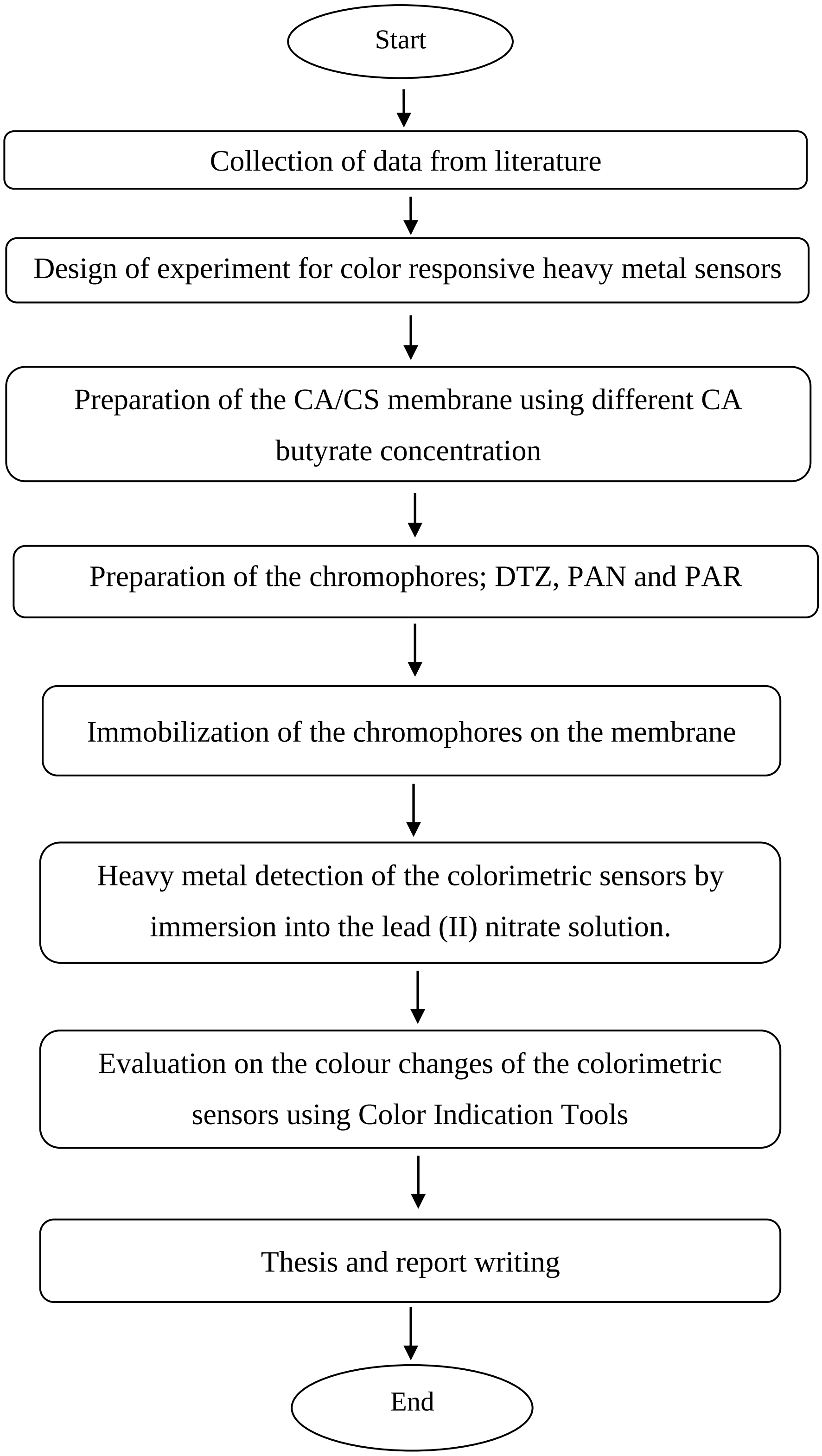


Figure 3.1 Research flow for the detection of heavy metal using colorimetric sensors experiment

3.3 Preparation of solid platform at different cellulose acetate butyrate concentration

The solid platform is prepared by dissolving 10, 8, 5 and 3 wt% of cellulose acetate butyrate and 1 wt% of chitosan in the formic acid. The cellulose acetate butyrate and chitosan powders are measured using analytical balance in the 50ml beaker. The solvent, formic acid is measured using the analytical balance in the 100ml beaker. The formic acid is then placed on the stirrer at 40°C while the CA/CS polymer mixtures is added slowly into the solvent to obtain the polymer solution. The polymer solution is then poured onto a glass plate to be cast with a 500 µm casting gap. The cast-film membrane is immersed into a mixed bath of ethanol and distilled water for 24 hours and air-dried for another 24 hours.

3.3.1 Effect of cellulose acetate butyrate concentration

The polymer solutions are prepared with different cellulose acetate butyrate concentration by adjusting the weight percentage of the CA polymer to 10, 8, 5 and 3 wt% before mixing with formic acid. The solutions then cast onto the glass plate to form the membrane films. The membrane films are then characterized by evaluating the brittleness of the membrane films formed.

3.4 Preparations of chromophores

Dithizone (DTZ), 1-(2-pyridylazo)-2-naphthol (PAN) and 4-(2-pyridylazo)-resorcinol (PAR) solutions are prepared by dissolving the solid form of chromophores in ethanol solution. 5 mg of chromophore is measured using analytical balance before being dissolved into ethanol in the 50 ml volumetric flask to produce 100ppm chromophore solution. The chromophore is stirred into the ethanol for 5 minutes to ensure the solution is homogenous.

3.5 Immobilization of chromophores on the solid platform

The DTZ, PAR and PAN chromophores are immobilized on the prepared solid platform of CA/CS membrane. The membrane film is first cut into strips of 4cm x 50 mm. Then, 10 ml of the chromophore solutions prepared is poured into a petri dish before immersing the membrane strips into the solutions. The membrane strips are immersed for 2 hours and washed with distilled water three times to remove the excess chromophores. The strips are air-dried at room temperature for 12 hours. After drying, photos of each strip are taken to be evaluated using the RBG online indication tool (IMAGECOLORPICKER.com).

3.6 Performance of colorimetric sensors on different heavy metal concentrations

Lead (II) nitrate solution is first prepared at 100 ppm using 50 ml volumetric flask. The lead (II) nitrate solutions are prepared by dissolving lead (II) nitrate into distilled water. The solutions are then diluted to 1, 3, 5, and 10ppm. The heavy metal is stirred into the distilled water for 5 minutes to ensure the solution is homogenous. For each heavy metal concentration, 9 membrane strips are used in the detection process to study the sensitivity of the three different chromophores. Each chromophore will have 3 membrane strips tested with each heavy metal concentrations for more accurate results. The strips immobilized with chromophores are immersed in a small container containing 3 ml of heavy metal solutions for 20 minutes. Then, the membrane strips are air-dried for 12 hours and taken photos to be evaluated using the RBG online indication tool (IMAGECOLORPICKER.com).

3.6.1 Evaluation using color indication tool

Photos of the color changes of membrane immobilized chromophore during heavy metals detection is captured by using camera. The position of the sample and the light intensity of the surrounding need to be constant. Thus, the photos are taken in a fixed position to ensure the surrounding are constant. The color changes are evaluated based on RBG value by comparing the before and after photos of the membrane immobilized with chromophore during heavy metal detection.

CHAPTER 4

RESULTS AND DISCUSSION

In this chapter, the results of the experiment will be presented and thoroughly discussed to achieve the research objectives.

4.1 Effect of cellulose acetate butyrate concentration

In this experiment, the solid platform for the immobilization of chromophore used is cellulose acetate flat sheet matrix platform. The flat sheet matrix platform is prepared from polymer solutions of cellulose acetate butyrate and chitosan dissolved in formic acid as solvent. The polymer solutions are prepared with different cellulose acetate butyrate concentration to study the state of the membrane form to ensure the chromophore could immobilize on the solid platform better. In the work by Azmi et al. (2017), the blending of chitosan with cellulose acetate were being prepared at different polymer compositions. The work has shown that the different polymer concentration effects the membrane binding ability. For this research, the effects of different cellulose acetate butyrate concentration on the mechanical properties, brittleness, of the membrane films are being evaluated. The concentration of the cellulose acetate butyrate used varies from 3, 5, 8 and 10 wt%.

10 wt% of cellulose acetate butyrate is first dissolved into the formic acid together with chitosan powder. The polymer mixture is stirred into the formic acid a little at a time and around 4 hours is taken for the polymer to be dissolved into the formic acid before left to be stirred overnight to ensure homogenous solution. The polymer solution formed, however, is too viscous and the membrane film cannot be cast. The process is repeated using 8 wt% of cellulose acetate butyrate and the time taken for the polymer to be dissolved into formic acid also reduced to 3 hours. Even though the taken for the polymer solution to form is less than the 10 wt%, the solution formed is still quite viscous and the membrane film formed is fragile. Figure 4.1 shows a cut of membrane film from polymer solution of 8 wt% of

cellulose acetate butyrate.

Figure 4.1 Membrane condition for 8 wt% of cellulose acetate butyrate after drying process

For the 5 wt% of cellulose acetate butyrate, only 2 hours is taken for the polymer to be dissolved into formic acid. The membrane produced is less fragile than 8 and 10 wt% making it the most suitable membrane for the immobilization process. Polymer solution with 3 wt% of cellulose acetate butyrate is also prepared, and while the time taken for the polymer mixture to be dissolved into the formic acid is the shortest, the polymer solution cannot be produced into a membrane as it dissolved being placed in the mixed bath. This suggested that the membrane conditions are thus affected by the concentration of the cellulose acetate butyrate. Figure 4.2 (a) shows the condition of the polymeric membrane for 3 wt% of cellulose acetate butyrate after being immerse in the mixed bath and figure 4.2 (b) shows a cut of membrane film from polymer solution of 5 wt% of cellulose acetate butyrate.

(a) (b)

Figure 4.2 Membrane condition for: (a) 3 wt% of cellulose acetate butyrate after mixed bath immersion and (b) 5 wt% of cellulose acetate butyrate after drying process

The membrane film formed by using 5 wt% of cellulose acetate butyrate has the best mechanical properties in term of the membrane film brittleness than the other membrane films of different cellulose acetate butyrate concentration. From the membrane films formed, the membrane for 5 wt% of cellulose acetate butyrate is the most flexible membrane and thus, suitable to be used as the solid support for the transmitter reagent.

4.2 Performance of the different chromophores as the heavy metal detector

The three types of chromophores used as the potential transmitter reagent of this optical sensor project are dithizone (DTZ), 1-(2-pyridylazo)-2-naphthol (PAN) and 4-(2-pyridylazo)-resorcinol (PAR). The three chromophores are used to detect the heavy metal in the heavy metal solutions by immersing them in the heavy metal solutions, after being immobilized on the selected membrane of 5wt% of cellulose acetate butyrate. The color changes of the membrane strips before and after chromophore immobilization are captured using the camera. Figure 4.3 (a) shows the color of the membrane strip before chromophore immobilization, figure 4.3 (b) shows the color of the membrane strip after chromophore PAN immobilization and figure 4.3 (c) shows the color of the membrane strip after chromophore DTZ immobilization.

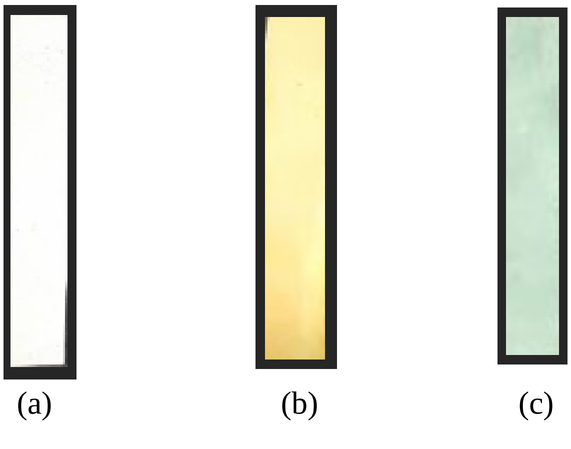
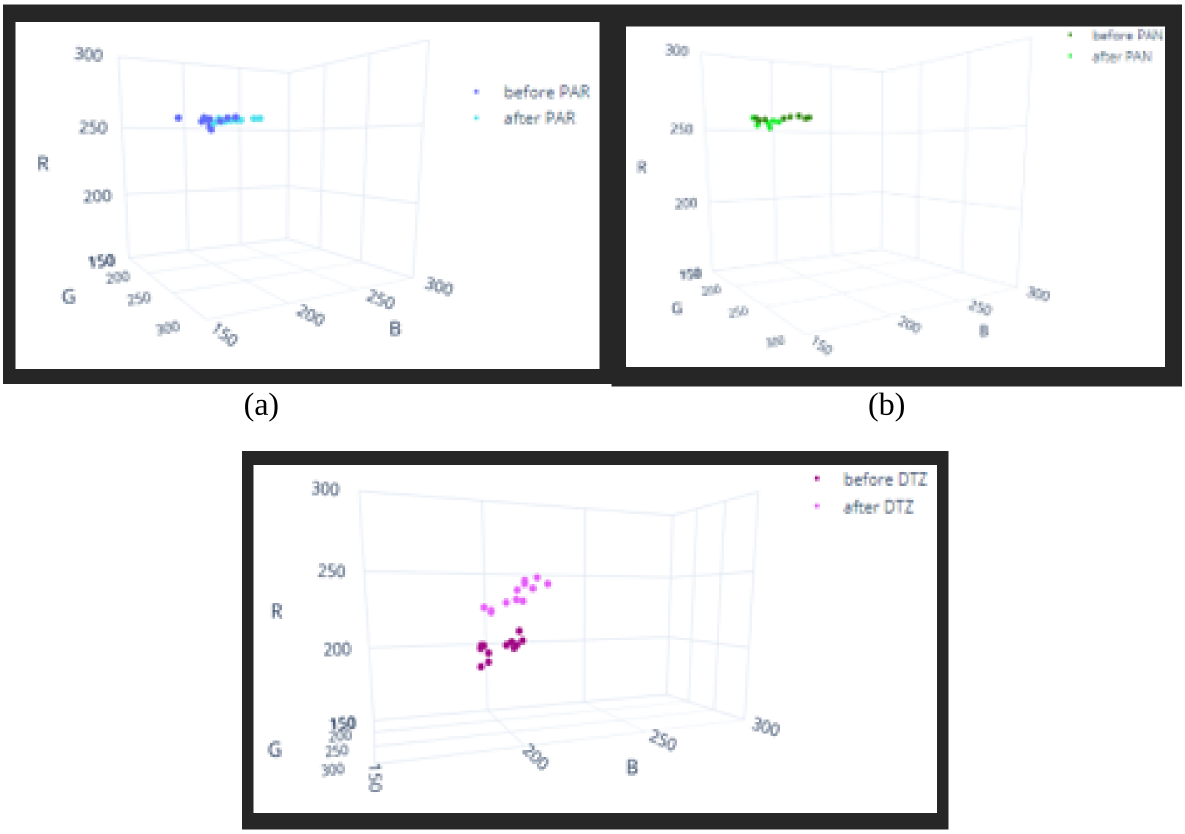


Figure 4.3 Membrane strip: (a) before chromophore PAN immobilization (b) after chromophore immobilization and (c) after chromophore DTZ immobilization

The color changes of the membrane strips before and after the chromophore immobilization on the membrane strips are due to the chromophore. PAN and PAR chromophore solutions both exhibit yellowish color while DTZ chromophore solution exhibits greenish color in nature, thus, the membrane strips will exhibit the greenish and yellowish colors due to the chromophores immobilized on them.

The color changes of each of the chromophore immobilized membrane strips before and after detection with 5 ppm of Pb^{2+} ions are also captured using the camera and evaluated using the RBG online indication tool (IMAGECOLORPICKER.com). The RBG value of each strip and type of chromophore are recorded and displayed in the 3d scattered graph in Fig 4.4 (plotly.com).



(a) (b)

(c)

Figure 4.4 Performances of the colorimetric sensors for: (a) PAR chromophore (b) PAN chromophore and (c) DTZ chromophore.

From the figure, the RBG value for PAR immobilized membrane strips before the heavy metal detection are not too different with the RBG value after the detection. The RBG values of PAR chromophore are almost similar due to the membrane strips insignificant changes of color before and after the heavy metal detection. Thus, PAR immobilized membrane strips are shown to be not quite efficient as optical sensor for heavy metal detection. The PAN immobilized membrane strips also demonstrated similar results as the PAN immobilized membrane strips. The RBG values for PAN observed from the graph shows small differences before and after the heavy metal detection as the color changes are not too different. The PAN immobilized membrane strips thus, is also not the best optical sensor for heavy metal detection.

The RBG values of the DTZ immobilized membrane strips, however, are quite different before and after the detection of heavy metal. This indicates that the color changes of DTZ chromophore during heavy metal detection is quite significant unlike the other two chromophores. DTZ immobilized membrane strips are shown to be the best among the three suggested chromophores in producing color changes during the heavy metal detection and thus, the most suited to be used in the colorimetric sensor as transmitter reagent. Table 4.1 shows the average RBG value for the chromophores before and after the heavy metal detection.

Table 4.1 Average RBG value for the chromophores before and after the heavy metal detection.

Chromophore	Before			After		
	Red	Green	Blue	Red	Green	Blue
PAR	253	245	190	253.5	240	202
PAN	253	242	165	251	225	159
DTZ	200	210	190	230	215	200