UNIVERSITI PUTRA MALAYSIA

GOLD ELETRODE MODIFIED BY DICARBOXYL-CALIX[4]ARENE AS ELECTROCHEMICAL SENSING PLATFORM FOR DETECTION OF LEAD(II), NICKEL(II) AND ZINC(II) IONS

SITI FATIMAH NUR BINTI ABDUL AZIZ

FS 2018 13

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By

SITI FATIMAH NUR BINTI ABDUL AZIZ

Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia, in Fulfilment of the
Requirements for the Degree of Master of Science

December 2017

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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December 2017

Chairman : Shahrul Ainliah Alang Ahmad, PhD
Faculty : Science

Persistent of heavy metal pollution in water samples is the most concerned issues in worldwide because of the toxicity effect that poses a major threat to all life of environments. Thus, this leads to development of a wide range of tools and techniques of sensing including conventional methods like atomic absorption spectroscopy (AAS) and inductively-coupled plasma mass spectroscopy (ICP-MS) which more tedious to handle. As an alternative way, analytical approach which able to offer simplicity and faster response was explored by development of electrochemical sensor with various type of modifier as the sensing interface to detect the heavy metal ions. In this project, a simple and highly sensitive of electrochemical technique was developed for the determination of heavy metal ions such as lead(II), nickel(II) and zinc(II) ions in water samples using calix[4]arene derivative. The calixarene compound was chosen to develop a sensor based on dicarboxyl-calix[4]arene modified on gold surface (COOH-C4 electrode) as it has been known as one of great metallic cation sensing receptor due to its ability to chelate with various ions. Initially, the gold surface was modified with 3-mercaptopropionic acid (MPA) using self-assembly technique (MPA/Au electrode). The subsequent modification of MPA/Au with dicarboxyl-calix[4]arene compound was carried out by crosslinking the carboxyl groups from MPA and dicarboxyl-calix[4]arene through linkers, namely ethylenediamine (EDA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxy succinimide (NHS) via one-pot reaction. The modified electrodes were characterized using X-ray photoelectron spectroscopy (XPS) and fourier transform infrared spectroscopy (FTIR). The results indicated the confirmation of each stage of electrode modification. The electrochemical analyses of COOH-C4 electrode showed an enhanced of electrocatalytic activity and higher current towards analytes (Ni^{2+}, Pb^{2+} and Zn^{2+}), compared to bare gold surface and MPA/Au electrode. Under optimum conditions, the differential pulse voltammetry response of COOH-C4 displayed a wide linear response range from 0.28 - 2.5 mg/l for Pb^{2+} ion, 2.85 – 6.65 mg/l for Zn^{2+} ion and 0.13 -1.68 mg/l for Ni^{2+} ion with the detection of limit of 0.0062 mg/l, 1.5 mg/l, 0.34 mg/l, respectively. A simultaneous study using similar concentration of analytes was also done and as a result, the COOH-C4 electrode was found to be sensitive towards Pb(II) ions compared to the other metal ions. Due to poor peak separation and low R^2 value (0.7646) of Zn(II) and Ni(II)ions, respectively, the LOD reading was only obtained for Pb(II) ion which at 0.01 mg/l. Furthermore, the fabricated electrode shows good selectivity towards the Pb^{2+} ion in presence of possible interfering species. The developed COOH-C4 electrode then was successfully applied to determine Pb^{2+} ion in river water. A satisfactory precision of relative standard deviation at 3.12% and acceptable recoveries of 92 % were obtained which demonstrated the potential of dicarboxyl-calix[4]arene modified on electrodes (COOH-C4 electrode) as heavy metals sensing.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

PERMUKAAN EMAS DIUBAH DIKARBOSIL-KALIKS[4]ARENA SEBAGAI PELANTARAN PENGESAN ELEKTROKIMIA UNTUK MENGESAN ION PLUMBUM(II), NIKEL(II) DAN ZINK(II)

Oleh

SITI FATIMAH NUR BINTI ABDUL AZIZ

Disember 2017

Pengerusi : Shahrul Ainliah Alang Ahmad, PhD
Fakulti : Sains

Pencemaran logam berat yang berterusan dalam sampel air telah menjadi isu pada tahap membimbangkan di seluruh di dunia di sebabkan kesan toksik yang menjadi ancaman utama kepada semua kehidupan dan alam sekitar. Oleh yang demikian, ini menggalakan pembangunan pelbagai alat dan teknik pengesan termasuklah kaedah konvensional seperti spektrosfotometri serapan atom (AAS) dan spektrometer jisim plasma gandingan teraruh (ICP-MS) yang lebih sukar dikendalikan. Sebagai cara alternatif, pendekatan analitikal yang berkebolehan menawarkan kesederhanaan dan tindak balas yang lebih cepat telah diterokai melalui perkembangan pengesan elektrokimia dengan pelbagai jenis pengubah sebagai pengantaramuka untuk mengesan ion logam berat. Dalam kajian ini, teknik elektrokimia yang mudah dan lebih sensitif telah direka untuk mengesan ion logam berat seperti ion plumbum, nikel dan zink di dalam sampel air dengan menggunakan dikarboksil-kaliks[4]arena. Sebatian kaliks[4]arena dipilih untuk membangunkan sensor berdasarkan dikarboksil-kaliks[4]arena diubahsuai pada permukaan emas (COOH-C4 elektrod) kerana kehebatannya sebagai reseptor pengesan kation logam yang mampu untuk mengkelat pelbagai jenis ion. Sebagai permulaan, permukaan emas telah diubahsuai dengan 3-merkaptopropionik asid (MPA) menggunakan teknik swa-susunan untuk memperoleh permukaan elektrod MPA/Au. Seterusnya, elektrod MPA/Au diubahsuai dengan sebatian dikarboksil-kaliks[4]arena yang dilakukan melalui taut-silang kumpulan karboksil dari MPA dan dikarbosil-kaliks[4]arena melalui penghubung etilenediamina (EDA), 1-etil-3- (3-dimetilaminopropil) karbodiimida (EDC) dan N-hidroksi suksinimida (NHS) menerusi tindak balas satu langkah. Elektrod yang telah diubahsuai dicirikan menggunakan spektroskopi fotoelektron sinar-X (XPS) dan fourier spektroskopi inframerah (FTIR). Keputusan menunjukkan pengesahan setiap peringkat pengubahan elektrod. Analisis elektrokimia elektrod COOH-C4 menunjukkan peningkatan aktiviti elektrokatalitik dan arus yang lebih tinggi terhadap analit (Ni^{2+}, Pb^{2+} dan Zn^{2+}), berbanding permukaan emas dan elektrod MPA/Au.

Pada keadaan optimum, tindakbalas voltammetri denyut pembeza pada COOH-C4 menunjukkan julat tindak balas linear yang luas dari 0.28 - 2.5 mg/l untuk ion Pb^{2+}, 2.85 - 6.65 mg/l untuk ion Zn^{2+} dan 0.13 -1.68 mg/l untuk ion Ni^{2+} dan masing-masing menunjukkan had pengesanan 0.0062 mg/l, 1.5 mg/l dan 0.34 mg/l. Satu kajian serentak menggunakan kepekatan analit yang sama juga dilakukan dan sebagai hasilnya, elektrod COOH-C4 didapati lebih sensitif terhadap ion Pb(II) berbanding ion logam lain. Disebabkan pemisahan puncak yang tidak jelas dan nilai R^2 yang rendah (0.7646) untuk ion Zn(II) dan Ni(II), bacaan LOD hanya diperoleh untuk ion Pb(II) iaitu pada 0.01 mg/l. Tambahan pula, elektrod yang diubah menunjukkan selektiviti dan kestabilan yang baik terhadap ion Pb^{2+} dengan kehadiran spesis bendasing. Elektrod COOH-C4 kemudiannya digunakan untuk mengesan ion Pb^{2+} dalam air sungai. Sisihan piawai relatif yang memuaskan pada 3.12% dan perolehan semula yang boleh diterima sebanyak 92% menunjukkan potensi dikarboksil-kaliks[4]arena yang diubah suai pada elektrod (elektrod COOH-C4) berfungsi sebagai pengesan logam berat.

ACKNOWLEDGEMENTS

In the name of Allah, The Most Gracious and Merciful, first and the foremost, I would like to thank God for His blessing in giving me the chance to finish my master research. With His guidance and help, this project runs smoothly according to the plan. Special appreciation and deepest gratitude goes to my Supervisor, Dr. Shahrul Ainliah Alang Ahmad for her continuous support and supervision during conduction of this research. I acknowledge her support in helping me patiently and strengthen my spirit to get through the whole challenging journey. Her commitment and guidance have contributed to the completion of this thesis.

I would like to include special note of thanks to Dr. Ruzniza Mohd Zawawi, my co-supervisor, for her advice and assistance in conducting the experimental work. My special thanks are extended to Dr. Faridah Lisa Supian, the lecturer from UPSI, for sharing knowledge and contribution in the project progress. Her comments and suggestions on the research continuity helped me to improve my research work.

I would also like to express my deepest appreciation and indebtedness to my parent, Abdul Aziz Ma’arof and Leha Abdullah who always give me support, morally, physically and financially to go through this so called blood and sweat journey. I am also grateful to my siblings for their caring, love and courage which strenghen my spirit to get to the end.

Moreover, the completion of this thesis could not be possible without the assistance and support of so many people whose names may not all be enumerated especially my friends (Ila, Miza, Zurmira, Ain, Feesa, Husna, Nisa) and my other cheerful labmates.

I also place on record, my sense of gratitude to one and all who, directly or indirectly, have lent their helping hand in this research including staffs from Faculty of Science and ITMA. Their contribution are gratefully acknowledge.

Thank You.

SITI FATIMAH NUR ABDUL AZIZ

I certify that a Thesis Examination Committee has met on 5 December 2017 to conduct the final examination of Siti Fatimah Nur binti Abdul Aziz on her thesis entitled "Gold Electrode Modified by Dicarboxyl-Calix[4] Arene as Electrochemical Sensing Platform for Detection of Lead(II), Nickel(II) and Zinc (II) Ions" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Tan Yen Ping, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Jaafar bin Abdullah, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Sharifah Mohamad, PhD
Associate Professor
University of Malaya
Malaysia
(External Examiner)

NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 29 January 2018

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Shahrul Ainliah Alang Ahmad, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Ruzniza Mohd Zawawi, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Member)

Faridah Lisa Supian, PhD
Associate Professor
Faculty of Science and Mathematics
Universiti Pendidikan Sultan Idris
(Member)

ROBIAH BINTI YUNUS, PhD
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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
CPE	Carbon Paste Electrode
CME	Chemically Modified Electrode
CV	Cyclic Voltammetry
DPV	Differential Pulse Voltammetry
EIS	Electrochemical Impedence Spectroscopy
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
EDA	Ethylene Diamine
RoHS	European Union Restriction of Hazardous Substances
FS-FAAS	Fast Sequential flame atomic absorption spectrometry
FTIR	Fourier Transform Infrared Spectroscopy
GCE	Glassy Carbon Electrode
ICP-MS	Inductively Coupled Plasma – Mass Spectroscopy
ICP-OES	Inductively Plasma Optical Emission Spectrometry
LOD	Limit of Detection
MPA	Mercaptopropionic Acid
NHS	N-Hydroxysuccinimide
ΔEp	Peak-to-peak separation
SPE	Screen Printed Electrode
SPGE	Screen Printed Gold Electrode
SAM	Self-assembled Monolayer
SI-LOV	Sequential Injection Lab-On-Valve

ATSDR	United States Agency for Toxic Substances and Disease Registry
US EPA	United States Environmental Agency
WHO	World Health Organization
XPS	X-ray Photoelectron Spectroscopy

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Contamination of water sources by heavy metals has become one of severe environmental pollutions and public issues. The pollutions can cause to disruption of human metabolic function through accumulation in vital organs such as kidney, bones and liver by displacing some vital nutritional minerals, thus hindering their biological function. Moreover, the contamination of heavy metal can cause the plant to experience oxidative stress which later leads to cellular damage, disturbing its ionic homeostasis (Singh, Gautam, Mishra, & Gupta, 2011). Various comparable studies and techniques have been developed to achieve the best performance of heavy metals detection. To serve the similar aim, in this study, an electrochemical approach using differential pulse voltammetry (DPV) was implemented. The DPV measurement was chosen as mode of detection for three analytes; lead(II), zinc(II) and nickel(II) ions. Generally, the current response produced through the technique which proportional to amount of detected target analyte was extrapolated to determine the sensitivity of the fabricated sensor. Furthermore, the electrochemical approach able to offer greater sensitivity and selectivity of heavy metals ions detection due to presence of modifier incorporated with active binding site which having high affinity towards heavy metal ions the employment of modified electrodes through the electrochemical technique. In this regard, macrocyclic molecules; calixarene carboxylic acid derivative was chosen as a modifier for the development of modified gold electrodes in electrochemical detection of heavy metals. Mercaptopropionic acid (MPA) was modified priorly on the gold surface through self-assembled monolayer (SAM) due to facile and simplicity of the method.

1.2 Heavy Metals

Naturally found as sub-element in minerals and ores, heavy metals are type of elements which have been classified as hazardous metallic compounds that can impose adverse effects towards living organisms due to its persistency, toxicity and could not be degraded or destroyed, thus remain stable on-site that located nearby industrial area (Ding, Zhu, Xu, & Qian, 2015; Järup, 2003; Saha, Mollah, Alam, & Rahman, 2016). In general, heavy metals with atomic number greater than 20 are distinguishable from other existing metals as they are heavier with density more than 5 g/cm^3. Heavy metals can be classified into several groups such as metalloids, transition metals, basic metals, lanthanides, actinides, and metals of groups III to V (Kamunda, Mathuthu, & Madhuku, 2016). Among them, mercury, lead, and cadmium are the three most dangerous elements while zinc, manganese, copper, and selenium metalloid still can be portrayed as essential elements when present at their permissible level (Hurdebise, Tarayre, Fischer, Colinet, Hiligsmann, & Delvigne, 2015; March, Nguyen, & Piro, 2015). Rapid industrialization and urbanization has lead to accumulation of the heavy metals in plants and river. For instance, these type of metals mostly can be found in production of textile, glass etching, agricultural and pharmaceutical industry.

Some are used as components in production of pigment, paint, cooking utensil and components for hardware, automobile and electronic industries. (He, Yang, & Stoffella, 2005; Tchounwou, Yedjou, Patlolla, & Sutton, 2012). Nevertheless, an increase in anthropogenic activities including uncontrolled domestic discharged, industrial and agricultural wastes, poor effluent management and removals of metals from water pipe system, these hazardous substances can be found in relatively low quantity which tend to accumulate abundantly (March et al., 2015; Saha et al., 2016). These pollutants may lead to several health problems for example enzyme impairment as they have high affinity towards sulphur group which present as one of element in enzyme composition. Furthermore, the bio-accumulation process (organism absorb foreign substances faster than which it lost by catabolism or excretion) over a time causes an increase of chemical concentration in a biological organism which effects on human health (Kampa & Castanas, 2008). The heavy metal ions are able to bind with the sulfhydryl groups of protein in our body as they can mimic the metabolic of non-toxic elements, causing bad consequences like mutagenesis of the cells (Kudr, Nguyen, Gumulec, Nejdl, Blazkova, Ruttkay-Nedecky, Hynek, Kynicky, Adam, & Kizek, 2014). Some of the metals are also able to act as catalysts and involve in the oxidative reactions of the biological macromolecules, leading to the oxidative tissue damage due to intoxication. Through the inhibition of various mitochondrial enzymes and uncoupling of oxidative phosphorylation, the impairment of cellular respiration will occur due to metal toxicity (Godwill, Jane, Scholastica, Marcellus, Eugene, & Gloria, 2015). One of the severe cases related to metal poisoning reported from mass media that highlighted from abc news in 2016 (Jocobo, 2016) . The report reveals serious symptoms such as skin rashes, dizziness, headache and stunted growth were diagnosed on 4 year-old twins due to water crisis effect which caused by lead intoxication (Ding et al., 2015; Järup, 2003).

By all mean, an ample clean source of water that free from toxic contaminants heavy metals has become a necessity to many people. To minimize the effect, prevention step has been taken by United States Agency for Toxic Substances and Disease Registry (ATSDR) by set up a list to categorize heavy metals like arsenic, lead, and mercury as the most dangerous heavy metal pollutants (Kamunda et al., 2016). The European Union Restriction of Hazardous Substances (RoHS) also has banned the consumption of lead, cadmium, and mercury in the electrical and electronic equipments because of their toxicity effect which develop rapidly (H. N. Kim, Ren, Kim, & Yoon, 2012). World Health Organization (WHO) and United States Environmental Agency (US EPA) who are concerned with the international public health have defined the permissible level for each of these hazardous metals in the drinking water system to monitor the water quality as shown in Table 1.1 (Bhavtosh Sharma1, 2012). Reviews on occurrence and risk upon exposure of heavy metal specifically lead, zinc and nickel which became targets of interest in this presence work were further discussed in the literature.

Table 1.1 : The permissible level of heavy metal ions in drinking system by several organizations.

Metals	USEPA (mg/l)	WHO (mg/l)	National Drinking Water Quality Standard (Malaysia) (mg/l)
Arsenic	0.01	0.01	0.01
Aluminium	0.05 – 0.2	NM	0.2
Zinc	5.0	NM	3.0
Cadmium	0.005	0.003	0.003
Chromium	0. 1	0.05	0.05
Copper	1.3	2.0	1
Iron	0.3	NM	0.3
Lead	0.015	0.01	0.01
Mercury	0.002	0.006	0.001
Manganese	0.05	NM	0. 1
Nickel	--	0.07	0.02

NM : not mentioned

1.3 Structural Properties of Self-assembled Monolayers (SAMs)

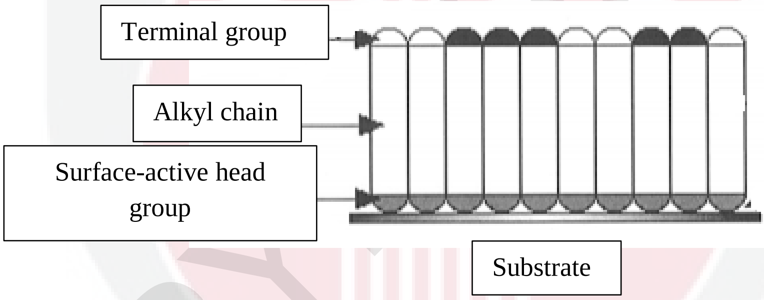

Figure 1.1 : Description of general monolayer (Ulman, 1996)

Self-assembled monolayers (SAMs) is one of well-known and versatile molecules used in surface modification. Driven by spontaneous reaction, the surface is able to offer better chemical and mechanical stabilities of film compared to Langmuir-Blodgett (LB) technique (Smith, Lewis, & Weiss, 2004). Generally, LB technique involve spontaneous process of self-organization of amphiphilic molecules at liquid-solid interface. The LB monolayer is formed by passing through the interface several times and result in addition of new layers on the substrate (Zasadzinski, Viswanathan, Madsen, Garnaes, & Schwartz, 1994) (Netzer, Iscovici, & Sagiv, 1983). Figure 1.1 shows the formation of self-assembled monolayer on a substrate. The monolayer can be divided into three parts, firstly is surface active head group which attached strongly by chemisorption on the surface of substrate, second is alkyl chain which connected with different functionalities and lastly is the terminal group is free to be tailored and interact to other functional group for further functionalization (El-Desawy, 2007).

Uniformity, well-ordered structure and high stability are among the crucial properties for a monolayer formation. Several factors that affect the rate of formation are chain length and concentration of adsorbate, type of solvents, temperature and cleanliness of substrate (Bain, Troughton, Tao, Evall, Whitesides, & Nuzzo, 1989). In early process formation of monolayer, a rapid adsorption of incomplete monolayer occurs and then slowly continues with displacement of any contaminant, elimination of attached solvent within monolayer, followed by lateral diffusion to minimize defect and lastly process of monolayer packing. Although SAMs can be developed in short time, a prolonged immersion is required to form a less-defect monolayer (Bain et al., 1989). Previous studies have reported that within an hour, a packed alkanethiol was able to be formed, however, to form an ordered and uniform monolayer, it took days (Wink, Van Zuilen, Bult, & Van Bennekom, 1997). Thus, in this research, SAM was chosen by introducing mercaptopropionic acid (MPA) onto the gold surface.

The choice of suitable solvent is an important factor to be considered, particularly, ethanol is used as preferable solvent due to its low toxicity, less tendency embedding into monolayer, economical and available in its high purity form (Bain et al., 1989) (Wink et al., 1997). The versatility of SAMs can be proved based on its applicability on various type of solid support. Self-assembly of thiol, disulphides, and sulphides on gold substrate were found to be the most favourable approach (Wink et al., 1997). Besides, work on silicon and indium-tin-oxide (ITO) substrates by attachment of silane derivatives are other common approach done through self-assembly` method (Vuillaume, Boulas, Collet, Allan, & Delerue, 1998) (Hillebrandt & Tanaka, 2001).

1.4 Gold Substrate

1.4.1 Gold as Self-Assembly Platform

Gold is the most convenient metal surface used in SAMs due to various incorporation of functional group which commonly results in well-defined formation of monolayer. In 1983, Nuzzo and Allara were among the early researchers came up with tremendous work in performing well-oriented self-assembled of derivative specifically dialkyl sulfides on gold (Bain et al., 1989; Wink et al., 1997). In addition, the beneficial of gold as a platform for adsorption of thiol monolayer prevents formation of any stable oxide on the surface. Moreover, gold has a great affinity towards sulphur atom, thus the prominent Au-S bonding will lead to formation of monolayer despite of other functionalities(Bain et al., 1989; J. Kim; Wink et al., 1997). Due to that, most of the works reported are on self-assembled of organothiol molecule on gold surface. Briefly, adsorption of monolayer is likely to happen when spontaneous formation of S-Au bond simutaneously after the elimination of thiol group (Chira, Covaci, & Radu, 2012). Due to its robustness in surface modification, gold substrate looks impressive to be used as a platform for wide range applications including in development of electrochemical sensor. Fabrication of thiol-supported gold has become well-known approach in developing electrochemical sensor and immunosensor.

Research published by Tlili and co-workers showed the facile assembled of 11-mercaptoundecanoic acid onto gold surface which was attached with antibody through EDC/NHS coupling. This method was used as the strong interaction between thiol and gold that assisted in maintaining the stability of biological receptor (Tlili, Abdelghani, Hleli, & Maaref, 2004). Based on the previous reported work, the similar strategy was implemented in this study by using different types of thiol derivatives.

1.5 Calixarene

Evolves as uncharacterized by-product from synthesis of baeklite in 1872 by Baeyer, macrocyclic compound is also known as calixarene started to draw attention due to its versatility in binding various type of guest ions. In 1940, Zinke and Ziegler initiated step which focused on producing a cyclic tetramer calixarene while the first work on its conformation was published by Comforth in 1955 (Agrawal, Pancholi, & Vyas, 2009; Düker, Kutter, Dülcks, & Azov, 2014; Jacques Vicens, 2012; Ward, Li, Regan, Deasy, & Kelleher, 2015)**.** Zinke and group was successfully produced the cyclic compound from condensation process of p-substituted phenols with formaldehyde. Subsequently, a few years later, formation of calixarene from base and acid induced process was discovered by Gutche and Högberg. By varying the ratio of alkali with phenol derivatives, calixarene cavity can be tuned to different size (Jacques Vicens, 2012). Figure 1.2 shows the general structure of calix[n]arene. Generally, calix[n]arene composed of lower rim with attached oxygen group, ranging from numerous functionalities and upper rim of alkyl group which later can be modified with desired functional group. Its scaffold is made of manifold phenolic unit that links with methylene bridges which offers π- π interactions. Due to its rigid cavity or called central annulus, these host molecules are fascinating to be used in molecular recognition study. The versatility of calixarene also can be seen in a numerous fields such as an anti-oxidant in polymer synthesis, ion exchange resin to coat material in electronic application, composed as films for chemical sensor, ionophore in boosting hydrolysis reaction and play exceptional role in extracting and detecting various type of metal ions and biomolecules like amino acids (Bozkurt, Durmaz, Yilmaz, & Sirit, 2008; Dartiguelongue, Leybros, & Grandjean, 2017; Hassen, Martelet, Davis, Higson, Abdelghani, Helali, & Jaffrezic-Renault, 2007)**.** Generally, the basket-shaped of this supramolecular compound has calix[n]arene as a common form where value of n is ranging from 4 to 8 which eventually imply the cavity size and shape (Bayrakcı, Ertul, & Yilmaz, 2009; Hassen et al., 2007; C. Liu, Lin, Wang, Jiang, & Lin, 2005). Furthermore, apart from having preorganized scaffold structure, this supramolecular compound possesses fascinate physical and chemical properties like high melting point, high chemical and thermal stability, low toxicity and solubility (Zaghbani, Tayeb, Fontas, Hidalgo, Vocanson, Dhahbi, & Antico, 2011).

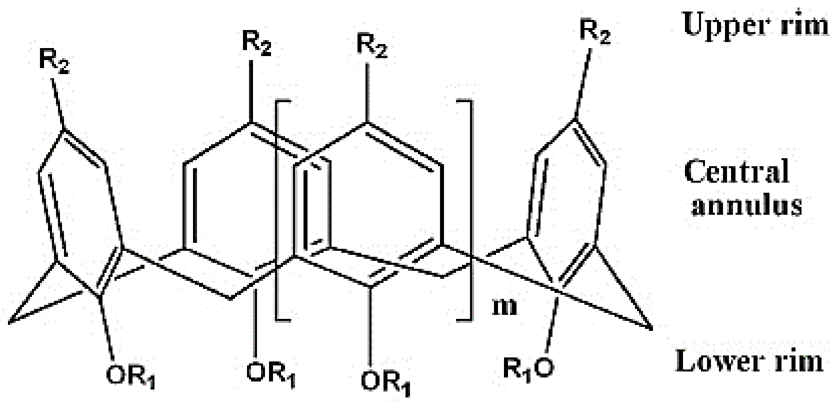

Figure 1.2 : General structure of calixarene

The uniqueness of calixarene can also be observed from it's conformation and orientation where its tetramer and pentamer structures are available either in cone, partial cone, 1,2-alternate and 1,3-alternate. Utmost work was found on cone conformation as the cavity size is suitable to fit ionic and neutral species distinctly. Figure 1.3 illustrates the structure of calixarene in different conformation (Arnaud-Neu & Schwing-Weill, 1997; Bayrakcı et al., 2009; Mokhtari, Pourabdollah, & Dalali, 2011). Besides that, calixarene can be classified based on functionalization of the rims which are heterocalixarene where the benzene cavity is built from heterocyclic moieties, heteracalixarene with nitrogen, oxygen and sulphur heteroatom embedded between the phenol units such as thia, aza and oxa-calixarenes which mostly linked to carbonyl group to offer more binding sites (Agrawal et al., 2009).

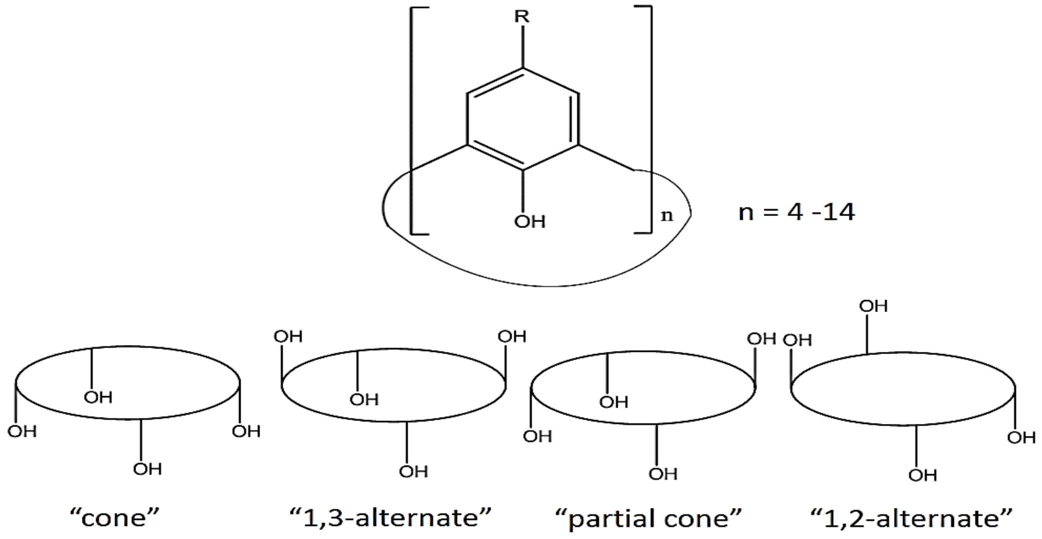

Figure 1.3 : Conformational shapes of calixarene

1.6 Problem Statement

The developmental effect and health problems like retardation, skin dermatitis, abdominal pain, fever and headache are among the side effects of non-decayed heavy metals pollutant exposure. Hence, monitoring of heavy metals present in water sources is very much needed. A conventional methods such as inductively coupled plasma mass spectrometry (ICP-MS) or atomic absorption spectrometry (AAS) have been well-known to detect the presence of these contaminants. Despite of high sensitivity measurement, they are tedious to handle in term of sample preparation and require well-trained personnel. In addition, the AAS method facing some limitation in term of severe matrix effect and difficulty to deliver a response because ionization process is distracted as hotter flame was used for sample dissociation. Meanwhile, for detection of metal ion using ICP-MS, the method is likely to face spectral interference due to line-rich spectra produced by the hot plasma source. Besides, existence of other methods like fast sequential flame atomic absorption spectrometry (FS-FAAS), sequential injection lab-on-valve (SI-LOV) and fluorescence spectrometry are also facing limitation in term of simplicity and portability. As an alternative way, electroanalytical methods have attracted wide attention with better sensitivity, selectivity and delivering fast response with on-site mode through a simple sample preparation and method of detection. In present study, a modified thiolated gold electrode was used as a surface platform to enhance the sensitivity of electrochemical sensor by attachment of receptor called carboxyl-calix[4]arene derivative onto it. The main attractive properties of the scaffold compound calixarene to be chosen as a heavy metal detector is due to the outstanding ability in binding wide range of guest ion through its open structure and functionalized rims. Its specialty in forming strong interaction with ions that fit into its rims, eventually resolve any selectivity issue, thus make it promising to be one of great heavy metal sensor. Prior to the modification with the calixarene cerivatives, the thiolated gold surface (MPA/Au) was prepared through self-assembled of 3-mercaptopropionic acid (MPA). The thiolation process was chosen as it able to resist polymerization of thiol derivative on the electrode surface at room condition which commonly occurred on modification by using silane derivative. In addition, the thiol monolayer is more resistive towards contaminant comes from surrounding during the sample preparation, thus inert condition is optional. The MPA/Au electrode was then fabricated with calixarene derivative namely dicarboxyl-calix[4]arene by one pot reaction using EDA and EDC/NHS as linkers. The EDC/NHS linkers were used to attach the carboxyl group of MPA/Au electrode surface with carboxyl group of the calixarene compound by diamine derivative (EDA) in the simplest way as no vigorous chemical reaction is required.

1.7 Novelty of Research

To date, in this study, detection of heavy metal ions using calixarene derivative called dicarboxyl-calix[4]arene on a solid state electrochemical sensor was developed by several modification steps on a gold surface (gold electrode and screen printed gold electrode (SPGE). Mercaptopropionic acid (MPA) was initially self-assembled on the gold surface. Binding affinity of electrode surface towards detection lead(II), zinc(II) and nickel(II) ions was enhanced by functionalizing dicarboxyl-calix[4]arene compound on the modified gold electrode. The strong interaction between the host and guest ion is existed due to its exceptional structure, open cavity with functionalized upper and lower rim for better trapping.The outstanding properties of calixarene as electrochemical sensor in capturing metal ions was reviewed in research done by Honeychurch in 2001. However, different calixarene moities, thiolated derivatives were used as a receptor on screen printed carbon electrode to trace metals which then showed excellent sensitivity (Honeychurch, Hart, Cowell, & Arrigan, 2001). This project which involved modification of electrochemical sensor based calixarene modified on thiolated gold surfaces has become one good strategy that offer excellent performance of heavy metal detector.

1.8 Objectives of the Study

The aim of this research is to develop an electrochemical detection of heavy metal ions (lead(II), zinc(II) and nickel(II) ions) by dicarboxyl-calix[4]arene receptor modified on gold surface. The gold surface was priorly self-assembled with thiol derivative called 3-mercaptopropionic acid (MPA). The functionalization of dicarboxyl-calix[4]arene tetramer type was then performed onto the thiolated gold surface through one-pot reaction with presence of EDA EDC/NHS as linkers. Below are the sub-objectives targeted for this study.

i. To prepare and characterize self-assembled monolayer of 3-mercaptopropionic acid (MPA) on Au electrode followed by functionalization of dicarboxyl-calix[4]arene on electrode via EDC/NHS activations

ii. To study analytical performance of modified electrode for determination of Pb^{2+}, Zn^{2+} and Ni^{2+} ions using voltammetric approaches such as Cyclic Voltammetry (CV) and Differential Pulse Voltammetry (DPV).

1.9 Scope and Limitation

In this presence study, the recognition of calixarene based-sensor, COOH-C4 is specific towards lead ion due to high affinity of the metal ion with the active binding site (hydroxyl group at lower rim of calix[4]arene), thus promised a better sensitivity. There is a limitation in this study when employing the fabricated sensor to detect wide range of heavy metal ions like zinc(II) and nickel(II) ions. Based on the analytical performance study of the developed COOH-C4 electrode towards Ni(II) detection, the obtained LOD value has exceeded the permissible level of Ni(II) ion. The poor sensitivity of Ni(II) ion was due to comparable difference in the metal ions radii with Pb(II) and Zn(II) ions. In addition, from the simultaneous study using same concentration of metal ions solution (Pb(II), Zn(II) and Ni(II) ions), the COOH-C4 electrode was found to be less selective towards Zn(II) and Ni(II) ions in presence of Pb(II) ion. This portrayed by poor peak separation of Zn(II) and Ni(II) ions and low R^2 value of Ni(II) ion from the extrapolation of plotted calibration curve. In term of stability, the COOH-C4 electrode could give the best detection performance within two weeks as it start to degrade longer than the given time.

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