DEVELOPMENT OF COLORIMETRIC CHEMOSENSORS FOR METAL IONS RECOGNITION IN AQUEOUS ENVIRONMENT USING STATISTICAL APPROACH

BY

HAKIMAH BINTI ISMAIL

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Kulliyyah of Science
International Islamic University Malaysia

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ABSTRACT

Three thiosemicarbazone derivatives named 2-acetylpyrazine thiosemicarbazone (P1), *N*(1)-(2-acetylpyrazine)-*N*(4)-(2-hydroxyphenyl)-thiosemicarbazone (P2) and the novel *N*(1)-(4-acetylpyidine)-*N*(4)-(2-hydroxyphenyl)-thiosemicarbazone (P3) were synthesized through the proposed methods. The characterization through melting point analysis, Fourier transform infrared (FTIR) spectroscopy, CHNS elemental analysis and nuclear magnetic resonance (NMR) spectroscopy showed high purity of the products. The synthesized thiosemicarbazones were optimized as selective and sensitive chemosensors for cobalt (Co^{2+}) and mercury (Hg^{2+}) ions through spectroscopic study using ultraviolet-visible (UV-Vis) spectroscopy. Colorimetric response was observed where P1, P2 and P3 changed their color from colorless to orange, orange and yellow respectively after being added with their respective metal ions. Response surface methodology (RSM) was used to study the effects of interactive variables and identify the most optimum condition for each chemosensor. The absorbance values of P1-Co^{2+}, P2-Co^{2+} and P3-Hg^{2+} at 450, 423 and 414 nm respectively were collected as responses. Results showed that metal ion concentration, pH and time were significant to the model. The optimum condition for 100 μM P1 was found with 70 μM Co^{2+} in 8:2 v/v DMSO/Tris-HCl at pH 5.3 after 15 minutes reaction, P2 at 100 μM was optimum with 80 μM Co^{2+} in 8:2 v/v DMSO/Tris-HCl at pH 7.5 after 10 minutes reaction whereas P3 at 20 μM was ideal with 60 μM Hg^{2+} in 8:2 v/v DMSO/citrate-phosphate at pH 7.8 after 18 minutes reaction. These chemosensors were highly sensitive as the limit of detection (LOD) of P1, P2 and P3 towards selected metal ions was found to be low at 1.64, 1.52 and 3.56 μM respectively. The binding stoichiometry of P1-Co^{2+}, P2-Co^{2+} and P3-Hg^{2+} were 2:1, 2:1 and 1:1 molar ratio respectively. The interference analysis showed that the presence of 1 molar equivalent of other metal ions did not interfere the interactions of P1-Co^{2+}, P2-Co^{2+} and P3-Hg^{2+}. Computational study through COSMO-RS showed the compatibility of chemosensors to selected solvent DMSO. Calculation of molecular electron potential (MEP) and Fukui function in density functional theory (DFT) suggested that nitrogen, sulphur and oxygen atoms of the chemosensors involved in the interaction with metal ions. Calculation of highest occupied molecular orbital-lowest unoccupied molecular orbital (HOMO-LUMO) energy gap and formation energy showed that the formation of P1-Co^{2+}, P2-Co^{2+} and P3-Hg^{2+} were preferred over non-interacted chemosensors in the presence of the metal ions. The evaluation on several water samples showed that they were efficient to be used for environmental water samples. The optimized P1, P2 and P3 in this study promised three new selective and sensitive chemosensors from thiosemicarbazone derivatives for Co^{2+} and Hg^{2+} detection.

خلاصة البحث

في هذه البحث تم استخدام الإجراءات المقترحة لعمل ثلاث مشتقات ثيوسيميكاربازون: 2-أسيتيل بيرازين ثيوسيميكاربازون (P1)، ن(1)-(2-أسيتيل بيرازين)-ن(4)-(2-هيدروكسي فينيل)-ثيوسيميكاربازون (P2) و ن(1)-(4-أسيتيل بيريدين)-ن(4)-(2-هيدروكسي فينيل)-ثيوسيميكاربازون (P3). تم تحديد نقاء المنتجات من خلال تحليل نقطة الانصهار، والتحليل الطيفي للأشعة تحت الحمراء (FTIR)، وتحليل عناصر CHNS، والرنين المغناطيسي النووي (NMR) من خلال التحليل الطيفي باستخدام التحليل الطيفي للأشعة فوق البنفسجية المرئية (UV-Vis)، تم تحسين ثيوسيميكاربازونات المنتجة كمستشعرات كيميائية انتقائية وحساسة لأيونات الكوبالت (Co^{2+}) والزئبق (Hg^{2+}). بعد خلطها بأيونات المعادن الخاصة بها، تغير لون P1 و P2 و P3 من عديم اللون إلى البرتقالي والبرتقالي والأصفر على التوالي. تمت دراسة تأثيرات المتغيرات المتفاعلة باستخدام منهجية سطح الاستجابة (RSM) لتحديد الحالة الأكثر مثالية لكل جهاز استشعار كيميائي. تم الحصول على قراءات امتصاص P1-Co^{2+} و P2-Co^{2+} و P3-Hg^{2+} عند 450 و 423 و 414 نانومتر على التوالي كاستجابات. تم العثور على تركيز أيون المعدن، ودرجة الحموضة، والوقت لتكون مهمة للنموذج. تم العثور على أفضل الظروف لـ 100 مايكرومتر من P1 لتكون 70 مايكرومتر من ثاني أكسيد الكربون في 2:8 v/v Tris-HCl/DMSO عند الرقم الهيدروجيني 5.3 بعد تفاعل 15 دقيقة، P2 عند 100 مايكرومتر كان الأمثل لتكون 80 مايكرومتر من ثاني أكسيد الكربون في 2:8 v/v Tris-HCl/DMSO عند الرقم الهيدروجيني 7.5 بعد 10 دقائق من التفاعل و بينما كان P3 عند 20 مايكرومتر مثاليًا مع 60 مايكرومتر Hg^{2+} في DMSO v/v 2:8/سترات-فوسفات عند الرقم الهيدروجيني 7.8 بعد تفاعل 18 دقيقة. تم العثور على حد الكشف (LOD) لـ P1 و P2 و P3 تجاه أيونات المعادن المختارة منخفضًا عند 1.64 و 1.52 و 3.56 مايكرومتر على التوالي، مما يشير إلى أن هذه المستشعرات الكيميائية كانت شديدة الحساسية. كانت مقاييس العناصر الكيميائية الملزمة P1-Co^{2+} و P2-Co^{2+} و P3-Hg^{2+} هي النسب المولية 2:1 و 2:1 و 1:1 على التوالي. لم يتداخل وجود مكافئ مولاري واحد من أيونات المعادن الأخرى مع تفاعلات P1-Co^{2+} و P2-Co^{2+} و P3-Hg^{2+} وفقًا لتحليل التداخل. كشفت دراسة حسابية باستخدام COSMO-RS أن المستشعرات الكيميائية كانت متوافقة مع المذيب المحدد DMSO. اقترح حساب إمكانات الإلكترون الجزيئي (MEP) ووظيفة Fukui في نظرية الكثافة الوظيفية (DFT) أن ذرات النيتروجين والكبريت والأكسجين في المستشعرات الكيميائية كانت متورطة في التفاعل مع أيونات المعادن. كشف حساب أعلى مداري جزيئي مشغول-أدنى فجوة طاقة مدارية جزيئية غير مشغولة (HOMO-LUMO) وطاقة التكوين أنه في وجود أيونات معدنية، كان تكوين P1-Co^{2+} و P2-Co^{2+} و P3-Hg^{2+} مفضلًا على المستشعرات الكيميائية غير المتفاعلة. أظهر تقييم العديد من عينات المياه أنها كانت مناسبة للاستخدام مع عينات المياه البيئية. تم تحسين مشتقات ثيوسيميكاربازون P1 و P2 و P3 في هذه الدراسة لإنتاج ثلاثة مستشعرات كيميائية انتقائية وحساسة جديدة لاكتشاف Co^{2+} و Hg^{2+}.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science in Chemistry.

…………………………………….…...
Erna Normaya Abdullah
Supervisor

…………………………………….…...
Mohammad Norazmi Ahmad
Co-Supervisor

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science in Chemistry.

…………………………………….…...
Ahmad Fakhrurrazi Ahmad Noorden
Internal Examiner

…………………………………….…...
Musa Ahmad
External Examiner

This thesis was submitted to the Department of Chemistry and is accepted as a fulfilment of the requirement for the degree of Master of Science in Chemistry.

…………………………………….…...
Wan Hazman Danial
Head, Department of Chemistry

This thesis was submitted to the Kulliyyah of Science and is accepted as a fulfilment of the requirement for the degree of Master of Science in Chemistry

…………………………………….…...
Jesni Shamsul Shaari
Dean, Kulliyyah of Science

DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Toxicity of metal ions has become a consideration for a very long time as various cases on this issue have been reported. Metals can be classified as essential and non-essential metals, based on their importance to human body. Cobalt is an essential metal which produces cobalamin complex (Vitamin B_{12}) by binding with a corrin ring (Nordberg et. al, 2007). In human body, this complex is important for the activity of some enzymes such as isomerases and methyltransferases. As an essential metal, cobalt is required in a moderate amount and can be consumed from diets such as vegetables, agricultural products, meat, dairy products and supplements. An excessive intake of cobalt may affect organs and tissues and lead to many health problems such as cardiovascular, nervous, endocrine and respiratory systems, as well as fatality.

Mercury, on the other hand, is a non-essential metal and among the most toxic material for human. The accumulation of mercury in human body mainly affects the central nervous system which leads to memory loss, Alzheimer and Autism in children. It also affects other organs and biological systems such as reproductive, motor and cardiovascular. The main concern of mercury toxicity is the possibility of this hazardous element to be transferred from one organism to another in food chain. The detection of mercury content in human body is also difficult due to its short half-life. Severe and lethal cases on mercury toxicity have been reported including the famous Minamata disease that happened in 1960s which affected the health of more than two thousand people with death of more than a thousand people (Crichton et al., 2017).

The detection of harmful metal ions is important so that the toxicity especially to human can be avoided. The common techniques to detect the presence and concentration of metal ions are through laboratory analyses such as atomic absorption spectroscopy (AAS), atomic emission spectroscopy (AES), inductively-coupled plasma mass spectroscopy (ICP-MS), neutron activation analysis, X-ray fluorescence spectroscopy and voltammetry (Koduru & Lee, 2014). Although these techniques offer high sensitivity to metal ions, they are costly, time-consuming and require trained personnel to conduct the laboratory analyses. Therefore, chemosensors made from organic compounds have been studied extensively to replace the traditional methods. Chemosensors usually involve spectroscopic analyses such as ultraviolet-visible (UV-Vis) and fluorescence. These techniques are lower in cost, have comparable sensitivity to the traditional methods while promising easier sample preparation. The presence of selected metal ions can easily be identified through colorimetric changes and spectral shifts. These offer quick detection which is very useful for on-site analysis.

Chemosensors are optimized in the laboratory to identify their best condition for metal ions detection. The common optimization technique involves study on the effect of one factor at a time while fixing the values of other factors. Factors affecting the sensitivity of chemosensors include solvent system, pH, time, chemosensor concentration, metal ion concentration and temperature. The optimization of one factor at a time does not represent the effects of overall factors on the sensitivity. Therefore, response surface methodology (RSM) was introduced for optimization of chemosensors as it allows the study on the effects of multiple variables to the responses. RSM involves mathematical and statistical calculation to identify the best condition for the chemosensors in detecting metal ions (Bezerra et al., 2008).

Various organic compounds have been optimized as chemosensors for metal ions recognition including thiosemicarbazone derivatives. The presence of many electron donor atoms such as sulphur and nitrogen in thiosemicarbazone derivatives increases their potential to interact with metal ions thus, making it suitable to be used as chemosensors (Sarkar et al., 2015). The interaction of thiosemicarbazone derivatives with metal ions are through monodentate, bidentate or tridentate interactions, depending on the substituents on the thiosemicarbazide moiety. There are numerous studies that proved the high sensitivity and selectivity of thiosemicarbazone derivatives as metal ion chemosensors.

Therefore, in this study, three thiosemicarbazone derivatives named 2-acetylpyrazine thiosemicarbazone (P1), *N*(1)-(2-acetylpyrazine)-*N*(4)-(2-hydroxyphenyl)-thiosemicarbazone (P2) and the novel *N*(1)-(4-acetylpyidine)-*N*(4)-(2-hydroxyphenyl)-thiosemicarbazone (P3) were synthesized. The products were characterized by melting point analysis, CHNS elemental analysis, Fourier-transform infrared (FTIR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy to evaluate their purity. The thiosemicarbazone derivatives were optimized as sensitive and selective cobalt and mercury chemosensors through spectroscopic study using UV-Vis spectroscopy. Response surface methodology (RSM) was used to study the interactive effects of metal ion concentration, pH and time on their sensitivity as chemosensors. The chemical properties of the title compounds and their interactions with metal ions were clarified through computational study using conductor-like screening model for realistic solvents (COSMO-RS) and density function theory (DFT). The optimized chemosensors were tested on several environmental water samples to evaluate their practicality in real water samples. This research is aimed to produce highly sensitive and selective chemosensors for cobalt and mercury ions.

1.2 PROBLEM STATEMENT

The exposure to hazardous metal ions such as cobalt and mercury can lead to various health problems. The early detection of the presence of metal ions in samples is important to avoid the toxicity effects. However, metal ions are not easily detected unless using common laboratory techniques which are expensive, time consuming and require specific training. Chemosensors which offer low-cost, user-friendly and fast detection of metal ions however, require a massive number of experiments for optimization procedure through spectroscopic study. The common optimization technique only studies the effect of one factor at a time thus, does not imply the complete effects of all factors to the chemosensor sensitivity. Most chemosensor studies concentrate only on experimental study which limits the knowledge on the chemosensor-metal ion interactions at molecular level. The practicality of these chemosensors in environmental water samples is also unclear as the test was not included in some of the previous studies.

1.3 RESEARCH OBJECTIVES

The study is aimed to achieve the following objectives:

1. To synthesis three thiosemicarbazone derivatives including one novel compound and characterize them through melting point analysis, FTIR spectroscopy, CHNS elemental analysis and NMR spectroscopy.
2. To optimize the synthesized thiosemicarbazone derivatives as chemosensors for cobalt and mercury ions through spectroscopic study using RSM.
3. To identify the mechanism of interaction between chemosensor and metal ions through computational study involving COSMO-RS and DFT.

4. To evaluate the practicality of the optimized chemosensors for cobalt and mercury detection in environmental water samples.

1.4 RESEARCH QUESTIONS

1. How do the target thiosemicarbazone derivatives can be synthesized and what are their physical and chemical properties?
2. What are the optimum conditions for the title compounds to be highly sensitive and selective chemosensors for cobalt and mercury ions?
3. How do the interactions between the chemosensors and metal ions occur?
4. How efficient the optimized thiosemicarbazones as cobalt and mercury ions chemosensors in environmental water samples?

1.5 RESEARCH HYPOTHESIS

The title compounds would be successfully synthesized according to the proposed methods and confirmed through characterization procedure including melting point analysis, FTIR spectroscopy, CHNS elemental analysis and NMR spectroscopy. The optimization through RSM offers the study on the effects of independent factors and their interactions to the sensitivity of chemosensors. The mechanism of interactions between chemosensors and metal ions would be clarified through computational chemistry. This study would produce three chemosensors for cobalt and mercury ions from thiosemicarbazone derivatives which are efficient in environmental water samples.

1.6 RESEARCH SIGNIFICANCE

This study will contribute to the society by producing three new chemosensors for cobalt and mercury detection. The toxicity can be avoided as these chemosensors are promising high sensitivity and selectivity to the metal ions. The optimization data through spectroscopic study using RSM is beneficial to engineers in producing mechanical probes using the same mechanism from this study. The computational study is beneficial to scientists in understanding the chemical behavior and interaction mechanism of chemosensor-metal ion interactions.

CHAPTER TWO

LITERATURE REVIEW

2.1 TOXICITY OF METAL IONS

Metals can be classified as essential or non-essential based on their importance and functionality to human. Essential metals are important elements to living organisms for maintaining biological structures, mechanisms and other body systems (Nordberg et al., 2007). Essential metals should be consumed properly because both insufficient and excessive intake can affect biological functions and consequently lead to various health problems. On the other hand, non-essential metals are elements that are not required by human body and can lead to many serious health problems even in a trace amount. Metals can be found from ecological water resources such as rivers, lakes and sea, natural food resources such as vegetables and other agricultural products, as well as various industries such as cosmetics, pharmaceuticals, and chemicals (Koduru & Lee, 2014).

2.1.1 Cobalt

Cobalt is one of ten essential metals for human, which is required in trace amount. It binds with a corrin ring to form cobalamin complex (Vitamin B_{12}) which is an important cofactor for several B_{12}-dependent enzymes such as isomerases and methyltransferases (Crichton et al., 2017; Nordberg et. al, 2007). In the industry, cobalt is used in production of steel and alloys, rechargeable batteries, carbon nanotubes, fertilizers and some medicines. Human exposure to cobalt is through dietary intakes from meat, dairy products and supplements, treatment of several diseases, environmental pollution and industrial activities (Leyssens et. al, 2017).

The exposure towards cobalt is normally at considerate level, sufficient enough to regulate human biological system. However, at high consumption, cobalt toxicity was often recognized when the effect had become worse. The prolong exposure to Co^{2+} can affect various organs and tissues, and biological systems such as cardiovascular, nervous, endocrine and respiratory (Leyssens et. al, 2017; Mao et al., 2011; Peters et. al, 2017). In 1960s, a severe and lethal cardiomyopathy occurred among heavy beer drinkers when Co^{2+} was used in the beer as foam stabilizer. Cobalt toxicity was also reported from anemia treatment, occupational exposure, dietary supplements, blood doping agent and orthopaedic joint replacements (Crichton et al., 2017).

2.1.2 Mercury

Mercury is among the most toxic non-essential metals, which usually being exposed to human from environmental resources (Crichton et al., 2017). It can be found in various industries such as chemical catalysts, dental fillings, paints, batteries, lamps and fungicides. Mercury exists in three oxidation states as metallic or ionic mercury. Industrial releases usually contain inorganic mercury which easily converted to more toxic organic mercury by bioorganisms. It has become a major concern as mercury can be bioaccumulated and transferred from one organism to another through food chain. Even in a trace amount, mercury can affect nervous, renal, reproduction, immune and cardiovascular systems. It affects fertility and blood pressure regulation, can cause insomnia, memory loss, headaches and many other health problems (Gupta et al., 2017; Zahir et al., 2005).

The toxicity effect of mercury is often referred to Minamata disease that happened in 1956 (Crichton et al., 2017). In this case, toxic mercury was found in