วิธีตรวจวัดปรอท(II) อย่างง่ายโดยใช้อนุภาคระดับนาโนเมตรของทองคำร่วมกับ 2-เมอร์แคปโทเบนโซไทอาโซล

นางสาวดุษฎี ร่วมสนิท

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเคมี ภาควิชาเคมี
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2555

SIMPLE METHOD FOR MERCURY(II) DETERMINATION USING GOLD NANOPARTICLES WITH 2-MERCAPTOBENZOTHIAZOLE

Miss Dusadee Ruamsanith

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry
Department of Chemistry
Faculty of Science
Chulalongkorn University
Academic Year 2012

Thesis Title SIMPLE METHOD FOR MERCURY(II) DETERMINATION USING GOLD NANOPARTICLES WITH 2-MERCAPTOBENZOTHIAZOLE
By Miss Dusadee Ruamsanith
Field of Study Chemistry
Thesis Advisor Assistant Professor Fuangfa Unob, Ph.D.
Thesis Co-advisor Luxsana Dubas, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

……..……………………………………Dean of the Faculty of Science
(Professor Supot Hannongbua, Dr.rer.nat)

THESIS COMMITTEE

…………………………………………..Chairman
(Assistant Professor Warinthorn Chavasiri, Ph.D.)

…………………………………………..Thesis Advisor
(Assistant Professor Fuangfa Unob, Ph.D.)

…………………………………………..Thesis Co-advisor
(Luxsana Dubas, Ph.D.)

…………………………………………..Examiner
(Puttaruksa Varanusupakul, Ph.D.)

…………………………….…….………External Examiner
(Waraporn Threeprom, Ph.D.)

ดุษฎี ร่วมสนิท : วิธีตรวจวัดปรอท(II) อย่างง่ายโดยใช้อนุภาคระดับนาโนเมตรของทองคำร่วมกับ 2-เมอร์แคปโทเบนโซไทอาโซล (SIMPLE METHOD FOR MERCURY(II) DETERMINATION USING GOLD NANOPARTICLES WITH 2-MERCAPTOBENZOTHIAZOLE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ดร.เฟื่องฟ้า อุ่นอบ, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: อ.ดร.ลักษณา ดูบาส, 45 หน้า.

ทำการพัฒนาวิธีตรวจวัดไอออนปรอท(II) โดยใช้อนุภาคระดับนาโนเมตรของทองคำร่วมกับ 2-เมอร์แคปโทเบนโซไทอาโซล อนุภาคทองคำระดับนาโนเมตรที่ดัดแปรด้วย 2-เมอร์แคปโทเบนโซไทอาโซลสามารถถูกเหนี่ยวนำให้เกิดการรวมกลุ่มเมื่อเติมไอออนปรอท(II) ทำให้สีของสารละลายเปลี่ยนจากสีชมพูเป็นม่วง และวิเคราะห์สารละลายด้วยเครื่องสเปกโทโฟโตมิเตอร์ที่ความยาวคลื่น 680 และ 530 นาโนเมตร ทำการหาค่าที่ดีที่สุดของตัวแปรที่ส่งผลต่อการวิเคราะห์ได้แก่ ความเข้มข้นของอนุภาคระดับนาโนเมตรของทองคำ ความเข้มข้นของสารละลาย 2-เมอร์แคปโทเบนโซไทอาโซล ขนาดของอนุภาคระดับนาโนเมตรของทองคำ และเวลาของการทำปฏิกิริยา โดยสามารถทำการตรวจวัดไอออนปรอทได้ด้วยสารละลายอนุภาคระดับนาโนเมตรของทองคำเข้มข้น 0.25 mM ดัดแปรด้วยสารละลาย 2-เมอร์แคปโทเบนโซไทอาโซลเข้มข้น 5.0 μM ระยะเวลาในการวิเคราะห์ 3 นาที ค่าพีเอชที่เหมาะสมต่อการวิเคราะห์ปรอทคือ พีเอช 4-5 ช่วงความเป็นเส้นตรงในการวิเคราะห์ปริมาณไอออนปรอทคือช่วงความเข้มข้น 20-100 μg/L (R^2=0.99) และมีขีดจำกัดต่ำสุดของการตรวจวัดคือ 5 μg/L การตรวจวัดสามารถทำได้โดยใช้เทคนิคทางสเปกโตรเมตรีและการมองสี

ภาควิชา เคมี
สาขาวิชา เคมี
ปีการศึกษา 2555

ลายมือชื่อนิสิต
ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก
ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม

5272315123 : MAJOR CHEMISTRY
KEYWORDS : MERCURY / GOLD NANOPARTICLES / ADSORPTION

DUSADEE RUAMSANITH : SIMPLE METHOD FOR MERCURY(II) DETERMINATION USING GOLD NANOPARTICLES WITH 2-MERCAPTOBENTOBENZOTHIAZOLE. ADVISOR: ASST.PROF. FUANGFA UNOB, Ph.D. CO-ADVISER: LUXANA DUBAS, Ph.D., 45 pp.

A method for mercury (II) ions detection was developed by using 2-mercaptobenzothiazole (MBT) with gold nanoparticles (MBT-AuNPs). The MBT-AuNPs can be induced to aggregate in the presence of Hg(II) ions resulting in the change in solution color from pink to purple. The solution was then analyzed by UV-VIS spectrophotometer at 680 and 530 nm. Parameters that affected the Hg(II) determination including gold nanoparticles concentration, MBT concentration, particles sizes of gold nanoparticles, reaction time were optimized. The detection of Hg(II) could be performed by using 0.25 mM AuNPs solution modified with 5.0 µM MBT solution with the total analysis time of 3 min. The suitable pH for Hg(II) determination was pH 4-5. A good linearity (R^2 = 0.99) was obtained for the determination of Hg(II) in the concentration range of 20-100 µg/L with the detection limit of 5 µg/L. The detection can be performed spectrophotometrically and visually.

Department: Chemistry	Student's Signature
Field of Study : Chemistry	Advisor's Signature
Academic Year : 2012	Co-advisor's Signature

ACKNOWLEDGEMENTS

My research can be successfully completed with helpfulness and support from my respectful thesis advisor, Assistant Professor Dr. Fuangfa Unob, for constructive criticism, inspiration, and encouragement during my research and having been giving advisements for solving many problems through my experiments. Even more, I would like to acknowledge the advice and guidance of Dr. Luxsana Dubas, with her informal relationship, insightful discussions and suggestions make me pleasure and be joyous to my progress laboratory skill. I would like to extend my appreciation to Assistant Professor Dr. Wanlapa Aeungmitrepirom and Assistant Professor Dr. Apichat Imyim for their precious suggestions. I would like to express my sincere appreciation for them with my great thankfulness. Additionally, I gratefully acknowledge my thesis committees, videlicet, Assistant Professor Dr. Warinthorn Chavasiri, Dr. Puttaruksa Varanusupakul, and Dr. Waraporn Threeprom, who give me opinions and valuable suggestions for my work and devote their valuable time for my thesis defense examination. Furthermore, sincere thanks to all members of EARU (Environmental Analysis Research Unit) for their kindness, encouragement and lovely friendship, and the good supports and suggestion concerning experimental techniques particularly from Miss Kanchalar Keeratirawee, Miss Wanwisa Janrungroatsakul and Miss Warangkana Kaodee. I would like to thank my all classmates in Master degree of Analytical chemistry, who gave me spirituality and helpfulness for doing my work until my research was completely finished. This thesis was financially supported by CU Graduated School Thesis Grant and Center for petroleum, Petrochemicals and Advanced Materials.

And not forgotten to utter mention, I would like to thank my beloved family, especially my lovely parents who have been giving me many supports and great willingness for further my lab until success comes. The usefulness of this work, I dedicate to my parents and all the teachers who have taught me since my childhood.

CONTENTS

Page

Page

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF SCHEMES

Scheme **Page**

LIST OF ABBREVIATIONS

M	Molar
µM	Micromolar
nM	Nanomolar
nm	Nanometer
ng/L	Nanogram per liter
µg/L	Microgram per liter
ng/g	Nanogram per liter
µmole	Micromole
µL	Microliter
˚C	Degree Celsius
TEM	Transmission electron microscopy
MBT	2-Mercaptobenzothiazole
AuNPs	Gold nanoparticles

CHAPTER I

INTRODUCTION

1.1 Statement of purpose

Mercury is a heavy metal that is widely used in many applications such as in thermometer, tooth amalgam fillings, electrical switches, electronic and petrochemicalindustry. The release of mercury from its products and inappropriate treatment of industrial waste would cause environmental pollution problem due to mercury contamination. Human could be exposed to mercury by touching, eating and inhaling, which the mercury can be accumulated inside the body and has adverse effects on human health in both acute and chronic poisoning. The symptoms of mercury poisoning include brain damage and it could be the cause of death [1]. Because of the extreme toxicity of mercury thus the monitoring of Hg(II) in the environment is important.

Generally, high efficiency techniques such as the cold vapor atomic absorption spectroscopy and inductively coupled plasma mass spectroscopy are used for Hg(II) determination [2,3]. However, these instruments are rather expensive and complicated. Thus, the development of a simple and inexpensive method is still a challenge. Colorimetric method have attached favourable attention for environmental monitoring, biological and industrial samples recently due to its simplicity, rapidity, good precision and the use of basic equipment [4-6].

Recently, gold nanoparticles are a great deal for using as colorimetric Hg(II) sensor owing to its optical properties and its surface plasmon resonance (SPR) that changes depending on the size, shape, the surrounding media and the interaction with neighbouring particles [7,8]. The SPR spectra are related with colour of AuNPs solution. For example, AuNPs in the size range of 5-20 nm would appear ruby red [9] and the color of larger particles or aggregates of smaller particles are purple or blue. AuNPs modified with chelating ligands can be induced to the aggregation in the presence of metal ions, resulting in the change in solution colour. Thus, it can be used as the colorimetric sensor of metal ions. Many researchers reported the use of AuNPs functionalized with biomolecules for mercury detection such as papain [10], L-cysteine [11], peptide [12] and DNA [13]. Their colorimetric sensors were successfully used to detect and quantify mercury in nanomolar level. In this study, 2-mercaptobenzothiazole (MBT) is chosen to be a chelating ligand for gold nanoparticles modification due to the presence of thiol group in its molecule that is capable of forming complex with mercury [14,15]. In addition, it seems to be more stable than biomolecules which was previously evaluated in the literature.

In this research, a simple and sensitive method for mercury determination is developed using gold nanoparticles modified with 2-mercaptobenzothiazole and UV-Vis spectrophotometer detection.

1.2 Objectives

1. To develop a method for Hg(II) determination and optimize the parameters that affect the Hg(II) determination.
2. To apply the devloped method to determine Hg(II) in real water samples.

1.3 Scopes of this research

Gold nanoparticles were firstly prepared by citrate reduction of $HAuCl_4$. Then, the obtained gold nanoparticles and 2-mercaptobenzothiazole was used as a media to study the suitable condition for Hg(II) determination. AuNPs aggregation is followed by UV-Visible spectrophotometry and transmission electron microscopy (TEM). Various parameters such as concentration of gold nanoparticles, size of nanoparticles, concentration of 2-mercaptobenzothiazole, reaction time, pH and co-existing ions were investigated. Finally, the optimized condition would be evaluated the performance of this method.

1.4 The benefit of this research

A simple and high precision method to quantify Hg(II) content in water sample at sub micromolar level was developed.

CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 Mercury measurement

According to the high toxicity even through low concentration of mercury, the monitoring and measurement of mercury is important. There are several sensitive methods for mercury measurement. Generally, the spectrometry method has been used to measure mercury ions concentration in many sources [2, 3]. In some cases, the concentration of mercury in samples is lower than the detection limit of instruments. Therefore, sample preparation or preconcentration techniques are required to improve the sensitivity of mercury determination. Herein, the methods for determination of mercury are reviewed.

Wu *et al*. [16] proposed a method to improve the sensitivity of mercury determination by chemical vapor generation techniques coupled to inductively coupled plasma mass spectrometry (CVG-ICP-MS). They reported the use of stannous chloride ($SnCl_2$), potassium tetrahydroborate (KBH_4) and formic acid (HCOOH) as reducing agent for reduction of Hg(II) to Hg(0) in chemical vapor generation. They found that the sensitivity and detection limit of CVG-ICP-MS was as low as 0.001 µg/L.

Mousavi *et al*. [17] developed a sensitive and efficient method for determination of trace mercury in water sample. Mercury was preconcentrated by solid phase extraction (SPE). It was trapped with silver by amalgamation inside SPE column. Then, mercury was released by resistance heating before determination with cold vapor atomic absorption spectrometry. The detection limit of this method was 3 ng/L.

In addition, the combination of chromatographic and spectrometric method gives the researchers the ability to perform speciation and determination of trace mercury ions.

Souza *et al*. [18] developed a simple method for mercury speciation in hair sample using high-performance liquid chromatography coupled to inductively coupled plasma mass spectrometry. The detection limit of this method was 15 ng/g, 10ng/g and 38 ng/g for inorganic mercury, methyl mercury and methyl mercury, respectively.

Silva *et al*. [19] developed a sensitive method for mercury determination at low concentration levels in rice sample. The sample was digested by microwave assisted digestion and analyzed by cold vapor atomic fluorescence spectrometry. The detection limit of this method was 0.9 ng/g.

Krupp *et al*. [20] developed a technique for separation and molecular identification of mercury and their compounds in plants using electrospray mass spectrometry equipped with ion trap. This technique included mercury separation by high-performance liquid chromatography and simultaneous detection by inductively coupled plasma mass spectrometry (LC-ICP/MS) and electrospray mass spectrometry (LC/MS).

From previous research, there are several techniques to determine mercury consisting of direct measurement and combination of measurement techniques. Mostly, they used sample preparation techniques for trace level of mercury sample before measurement by analytical instrument. The use of their techniques was complicated, time-consuming and high cost. Therefore, other techniques were developed to overcome these limitations. The spectrophotometric and colorimetric methods have been proposed. These methods follow the appearance or change of absorption/emission intensities and also the solution color change when analytes interact with ligand molecules. Ligand molecules that cause the solution color change after the complexation with the target analytes are so-called colorimetric sensors. For example, Yuan *et al*. [4] developed a colorimetric and fluorometric sensor probe for mercury ion detection. The sensor molecule was highly selective to Hg(II) ions, resulting in the solution color change from purple to red-pink and enhancement in fluorescence signal. Their probe can be used for naked-eye detection and determination of Hg(II) by fluorescent spectrometry.

Furthermore, the chelating molecules could be attached onto solid support to fabricate colorimetric sensors. Lee *et al*. [21] developed the colorimetric and spectrophotometric sensor for determination of Hg(II) in water sample using azo-coupled macro-cyclic immobilized on silica nanotube (SNT). Hg(II) ions selectively reacted with SNT resulting in color change from yellow to violet. This colorimetric sensor could show the color change at the mercury concentration as low as 0.1 mM. Moreover, this chemosensor can be used as portable kit for on-site analysis by changing the support to glass substrate instead of silica nanotube and the color change remained the same. The test-kit sensor also showed response as violet color at mercury concentration of 0.1 mM.

Recently, nanoparticles were popular as colorimetric sensor such as silver nanoparticles and gold nanoparticles [22].

2.2 Gold nanoparticles

Gold nanoparticles are in form of colloidal gold of particles size in the range of 1-100 nanometers. The physical properties of gold in nanoscale are different form bulk gold. For example, the melting point of bulk gold is 1064 ºC but the melting point of nanoscale gold particles is about 300-500 ºC depending on their size [23]. Regarding the optical properties of gold nanoparticles, the small scale of gold has red color instead of the observed yellow color as in the bulk one. The observed color of gold nanoparticles depends on their size, for example the color of 13 nm, 32 nm and 55 nm nanoparticles are red, pinkish red and pink, respectively [24]. The change of optical properties can be explained by the phenomena of surface plasmon resonance.

2.2.1 Surface plasmon resonance

The surface plasmon resonance is a phenomenon describing the optical properties of nanoparticles resulted from a resonance of free electron on the metal surface. When gold nanoparticles are exposed with incident light, the electromagnetic field of light induces corrective oscillation of free electrons on the surface of metal nanoparticles. The electron oscillation around the surface causes the charge separation with respect to ionic core of nanoparticles, resulting to the resonance along the electric field direction (Fig 2.1). The coherent oscillation of metal free electron with electromagnetic field is called surface plasmon resonance (SPR) [8, 25-26]. SPR adsorption band of metal nanoparticles with the size larger than 2 nm is strong and broad in UV-Vis range. The wavelength and intensities of SPR band of nanoparticles can be different owing to size, shape, composition and the dielectric constant of surrounding media [7, 8]. In case of gold nanospheres, the resonance occurs in visible region at approximately 520 nm wavelength and the particles appear in red color. For the gold nanorods, the oscillation of free electrons occurs both long and short nanorod axis, resulting the strong SPR band in the near infrared region and weak band in visible region [27]. The increase in the particle size induces red shift of SPR wavelength and increase of the absorption intensity.

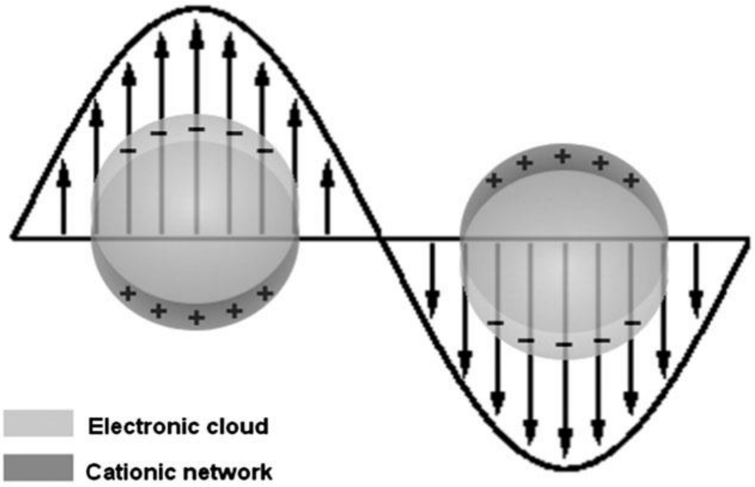


Figure 2.1 Scheme of oscillation of free electrons of nanoparticles along with electromagnetic field of incident light [8].

In addition, when nanoparticles are exposed to the light, the energy loss of incident light (extinction energy) can occur via absorption and scattering process of the particles. These optical properties including the light absorption, fluorescence, Mie scattering and surface enhance raman scattering are useful in many applications [26].

2.2.2 Aggregation of gold nanoparticles

Gold nanoparticles in diameter of 5-20 nm display a red color with the surface plasmon resonance band around 520 nm. The aggregation of gold nanoparticles leads to the shift of absorption spectra to longer wavelength and change the color of solution to purple. These principles have been applied to detect various analytes such as DNA, protein and metal ions [28]. The aggregation of gold nanoparticles is probably occurs when the target analyte is adsorbed on the gold nanoparticles leading to the decrease the inter-particle distance. In addition, the use of a cross-linking agent can induce the aggregation. The suitable cross-linking agent can bind with gold nanoparticles and target analyte. The interparticles bond such as hydrogen bonding, electrostatic attraction and metal-ligand coordination can overcome the electrostatic or steric repulsion.

2.2.3 Application

From the unique properties of gold nanoparticles, they have attracted interest in many applications including biological, biomedical and environmental application. In biomedical application, gold nanoparticles are useful in disease therapy because the gold nanoparticles are stable, non-toxic, non-degradable. The transport of gold nanoparticles can be followed by monitoring its optical property [29].The example of biomedical application includes drug delivery [30] and cancer therapy [31, 32].

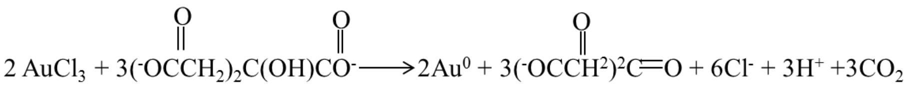
Furthermore, the gold nanoparticles chemosensor is recently gaining interested in biological molecules detection and analysis of target analytes in environmental samples because it is simple and uncomplicated method. Many researchers reported the use of gold nanoparticle colorimetric sensor for target analyte detection by following the aggregation of gold nanoparticles as the change of solution color such as use for detection of acetamiprid in vegetables sample [33] and as colorimetric probe for cysteine determination [34]. Besides the biological analyte, there are also the applications as colorimetric sensors for metal ions detection in environmental samples. The examples include colorimetric detection of heavy metal using gold nanoparticles with alkanethiols [35] and determination of Ca(II) in serum by colorimetric detection of gold nanoparticles immobilized with triethanolamine [36].

2.3 Chemical preparation of gold nanoparticles

Gold nanoparticles can be simply prepared by chemical reduction. The redox reaction between gold salts and reducing agent changes the oxidation state of gold from 3 to 0. The reducing agents commonly used for gold nanoparticles preparation include sodium citrate [37], sodium borohydride [38], ascorbic acid [39], glucose [40] and mixture of tannic acid and sodium citrate [41].

2.3.1 Citrate reduction method

The simple and commonly used method for preparation of nanoparticles is chemical reduction of chloroaurate ions solution by sodium citrate [37]. The citrate reduction method produces spherical shaped gold nanoparticles. Citrate ions are adsorbed onto the gold nanoparticles surface and prevent the particles from aggregation, thus citrate ions act as both reducing agent and stabilizer. The gold nanoparticles with various particle sizes can be prepared by controlling the ratio of gold and citrate amount. The size of gold nanoparticles increased with increasing the content of citrate [24]. The overall mechanism of citrate reaction can be presented as



$$2\,AuCl_3 + 3(^{-}O\overset{\overset{O}{\|}}{C}CH_2)_2C(OH)\overset{\overset{O}{\|}}{C}O^{-} \longrightarrow 2Au^0 + 3(^{-}O\overset{\overset{O}{\|}}{C}CH^2)^2C{=}O + 6Cl^- + 3H^+ + 3CO_2$$

Figure 2.2 The overall mechanism of citrate reduction [42].

2.4 Literature review

The gold nanoparticles have been utilized extensively in many applications due to its unique properties. Many researchers demonstrated the use of gold nanoparticles as colorimetric sensor to determine mercury ion concentration. They would follow the aggregation of gold nanoparticles by observing the change of color solution color change and analysis by UV-Vis spectrometer. Thus, the results and the detection are easily monitored by both naked eye detection and spectrophotometry. There are many reports on gold nanoparticles sensor development including the improvement of detection limit by using the selective ligands toward the target metals.

Guo *et al*. [10] developed the detection system using gold nanoparticles modified with papain (P-AuNPs) for Hg(II), Pb(II), Cu(II) ion detection. Papain is a protein that contains cystein residues selective with these metals ions. When metals ions solutions were added into P-AuNPs system, the solution color turned from red to blue due to metal ions induced gold nanoparticles-papain aggregation. The detection system could detect these metal ions at concentration as low as 200 nM.

Chai *et al*. [11] proposed the use of L-cysteine functionalized gold nanoparticles (Cys-GNPs) as colorimetric sensor for Hg(II) detection. This research reported that Cys-GNPs aggregation was induced by Hg(II). The detection of Hg(II) at low concentration (0.1-10 µM) could be achieved by ultraviolet radiation. The sensor could be used to determine Hg(II) concentration in a linear range of 100 nM-2 µM (r^2=0.9747). This sensor could detect Hg(II) with naked eye at minimum detectable concentration of 100 nM.

Huang *et al.* [43] developed a highly selective sensor for Hg(II) ion determination using 3-mercaptopropionic functionalized gold nanoparticles (MPA-AuNPs) in 2,6-pyridinedicarboxylic acid (PDCA). In the presence of Hg(II) ions, the solution color changed from red to purple and UV-Vis absorption wavelength displayed a red shift. This sensor is selective with Hg(II) at solution pH 9 controlled by Tris-borate buffer and the method provided a linear range between 250-500 nM with correlation coefficient of (r^2) 0.95 and a detection limit of 100 nM.

Wu *et al.* [13] designed a colorimetric probe for Hg(II) by using mercury-specific-DNA functionalized AuNPs (Au-MSD). The Au-MSD could interact with Hg(II) ions and formed thymine-Hg(II)-thymine complex. The Au-MSD aggregated rapidly in solution containing $MgCl_2$ salt. On the other hand, Hg(II) could stabilize Au-MSD and their aggregation was inhibited. Thus, the solution color of Au-MSD-Hg(II) are still red. This probe was used to determine Hg(II) in the concentration range of 0.1-10 µM Hg(II). The detection limit of 60 nM was obtained.

Yang *et al.* [44] developed a simple, cheap and sensitive sensor for colorimetric detection of Hg(II). The 4-mercaptopyrridine molecule bound to AuNPs through nitrogen atom and induced the aggregation of the AuNPs. The aggregation of 4-mercaptopyrridine-AuNPs was inhibited by Hg(II) and the degree of non-aggregation was represented by the color change from blue to purple or red depending on Hg(II) content. The response of the sensor was linearly dependent on Hg(II) concentration in the range of 0.15–3.00 µM. The detection limit was 55 nM.

Regarding 2-mercaptobenzothiazole (MBT) ligand, it is mostly used as coagulant for metal ions in flotation collector. It is a heterocyclic thiamide compound containing nitrogen and sulfur atom which can form complex with metal ions. MBT and its derivatives have been used as chemosensor in metal ions detection. Freg *et al.* [45] developed a highly sensitive fluorescence chemosensor which was 2-mercaptobenzothiazole benzoate for Cd(II), Hg(II) and Zn(II). This sensor was highly sensitive and selective fluorescence enhancement for Zn(II) ions.

Song.*et al.* [46] studied the preparation of novel raman probe for SERs-based immunoassay by using 2-mercaptobenzothiazole (MBT) modified AuNPs. MBT solution was dropped into colloidal gold solution under vigorous stirring. The aggregation of AuNPs-MBT occurred when the negatively charged citrate ions was replaced by MBT molecules. The formation of aggregates was monitored by UV-visible spectrophotometer. The SER-based immunoassay was successfully performed in real sample.

As shown in the previous researches, 2-mercaptobenzothiazole can bind with Hg(II) ions and also can be modified on gold nanoparticles and used as colorimetric sensor. In this work, we are interested in producing a colorimetric method by using gold nanoparticles with 2-mercaptobenzothiazole for determination of Hg(II).

CHAPTER III

EXPERIMENTAL

3.1 Instruments

3.1.1 UV-Visible spectrophotometer

The absorption spectra of gold nanoparticles solution were recorded by UV-Visible spectrophotometer model HP 8435 using quartz cuvette in the wavelength range of 400 to 800 nm.

3.1.2 Cold vapor atomic absorption spectrometer (CVAAS)

The determination of Hg(II) concentration was performed by cold vapor atomic absorption spectrometer from Perkin Elmer (FIAS 100 coupled to AA Analyst 100). For Hg(0) vapor generation, 1% $SnCl_2$ in 3% HCl solution and 3% HCl solution were used as a reducing agent and carrier solution, respectively.

3.1.3 Transmission electron microscopy (TEM)

Transmission electron microscope model JEM-2100 was used to observe the particles size of gold nanoparticles. A drop of sample solution was placed onto carbon grids and dried before insertion to TEM operation system.

3.1.4 pH meter

A pH of sample solution was measured by pH meter model S220 (Mettler Toledo).

3.2 Chemicals

All chemicals used in this research are shown in table 3.1.

Table 3.1 Chemicals list

Chemicals	Supplier
Gold (III) chloride trihydrate (99.9%)	Sigma-Aldrich
Trisodium citrate dihydrate	Fisher chemicals
Mercaptobenzothiazole (99.0%)	Fluka
Dimethylsulfoxide (99.9%)	Sigma-Aldrich
Metal standard solutions (1000 ppm)	Merck
Nitric acid (65% w/w)	Merck
Sodium hydroxide	Merck
Ethylenediaminetetraacetic acid disodium salt	Merck

3.3 Preparation of solutions

Gold (III) solutions

Gold (III) chloride trihydrate solution (1.0 mM) was prepared by dissolving an appropriate amount of gold (III) chloride trihydrate in de-ionized water and used for gold nanoparticles preparation.

Citrate solution

Citrate solution (0.5% w/v) was prepared by dissolving an appropriate amount of trisodium citrate dihydrate in de-ionized water and used for gold nanoparticles preparation.

2-Mercaptobenzothiazole solution

2-Mercaptobenzothiazole solution was prepared by dissolving MBT in dimethyl sulfoxide and used to prepare the MBT solution of desired concentrations in the range of 0.1–10 µM for investigating the suitable MBT concentration in gold nanoparticles modification.

Nitric acid solutions

Nitric acid solutions (1% v/v) was prepared by diluting the concentrated nitric solution (65% w/w) with de-ionized water and used for pH adjustment.

Sodium hydroxide solutions

Sodium hydroxide solutions (1% w/v) was prepared by dissolving an appropriate amount of sodium hydroxide in de-ionized water and used for pH adjustment.

3.4 Preparation and characterization of gold nanoparticles

The gold nanoparticles (AuNPs) solutions were prepared by using the citrate reduction of $HAuCl_4$ solution. The 10 mL of $HAuCl_4$ solution (1.0 mM) was mixed with 10 mL of DI water in an Erlenmeyer flask. The mixture was stirred continuously and heated in a water bath until the temperature reached 85˚C. The 2 mL of citrate solution (0.5% w/v) was subsequently added rapidly into the solution of $HAuCl_4$ and the yellow color of gold solution finally turned to red within 15 minutes. The colloidal solution was allowed to cool to room temperature and kept in a glass bottle until use. The absorption spectra and the particles size of the obtained AuNPs were measured by UV-Visible spectrophotometer and transmission electron microscopy (TEM), respectively.

3.5 Optimization of Hg(II) determination method

3.5.1 Procedure of Hg(II) determination

The starting AuNPs solution used in the experiment was obtained by the dilution of 0.5 mM AuNPs (0.5 mL) with de-ionized water (0.5 mL). For determination of Hg(II) concentration, 1.0 mL of 0.25 mM AuNPs solution and 1.0 mL of MBT in dimethylsufoxide (DMSO) were mixed in a test tube under vigorous stirring for a desired period of time (reaction time I). Then, 1.0 mL of Hg(II) aqueous solution was added to the AuNPs-MBT solution and the mixture was stirred for a desired time interval (reaction time II) before the analysis. The absorption spectra of AuNPs-MBT and AuNPs-MBT-Hg(II) were recorded by UV-Visible spectrophotometer in the wavelength range of 400 to 800 nm and their aggregation profiles were observed by TEM. The parameters including AuNPs concentration, MBT concentration, reaction time I and II were optimized.

3.5.2 Effect of the AuNPs concentration

Gold nanoparticles solution in the concentration range of 0.25, 0.30, 0.40 and 0.50 mM were prepared by diluting 0.50 mM AuNPs solution with the appropriate amount of deionized water. Meanwhile, the concentration of MBT was maintained at 1 µM. The reaction time I and II was 1 and 2 minutes, respectively.

3.5.3 Effect of MBT concentration

In order to evaluate the influence of MBT concentration on Hg(II) determination, the MBT solution of different concentrations ranging from 0.1 to 10 µM was prepared and used. The concentration of AuNPs was maintained at 0.25 mM. The reaction time I and II was 1 and 2 minutes, respectively.

3.5.4 Effect of the AuNPs size

To investigate the effect of AuNPs size, solutions containing AuNPs of 13 and 20 nm in diameter were prepared by the reduction method with citrate solution (0.5%) of varied volume. Meanwhile, the concentration of AuNPs and MBT were maintained at 0.25 mM and 5.0 µM, respectively. The reaction time I and II was 1 and 2 minutes, respectively.

3.5.5 Effect of reaction time

The reaction time for AuNPs-MBT to aggregate in the presence of Hg(II) ions was studied using 20 and 100 µg/L Hg(II) as sample solution. The reaction time was divided into 2 ranges in this experiment. First, the reaction time between AuNPs and MBT (reaction time I) were varied from 1 to 10 minutes. The aggregation of AuNPs with MBT was monitored by UV-Vis spectrometer. Then, using suitable reaction time I, the reaction time between AuNPs-MBT and Hg(II) ions were studied in the range of 1 to 30 minutes. The concentration of AuNPs and MBT were maintained at 0.25 mM and 5.0 µM, respectively.

3.5.6 Effect of pH

A solution containing 60 µg/L Hg(II) was used as a sample to study the effect of pH. The solution pH was adjusted by 1% HNO_3 and/or 1% NaOH to a value in the range of 1 to 7. In addition, blank solutions containing no Hg(II) ions, adjusted the pH to the studied values with 1% HNO_3 and/or 1% NaOH were used as samples to compare the effect of pH on AuNPs-MBT particles aggregation. The concentration of AuNPs and MBT were maintained at 0.25 mM and 5.0 µM, respectively. The reaction time I and II was 1 and 2 minutes, respectively.

3.5.7 Effect of co-existing ions

The effect of co-existing ions was individually investigated by adding 0.12 µmole of Ag(I), Cu(II), Pb(II), Cd(II), Co(II), Mn(II), Ni(II), Cr(VI), As(III), Zn(II), Fe(III) or Mg(II) to AuNPs-MBT solution, compared to the solution containing 0.12 µmole of Hg(II) ion. The solution color was observed after adding each individual metal ion. MBT and AuNPs solution concentration was fixed to 5 µM and 0.25 mM, respectively. The reaction time I and II was 1 and 2 min.

3.6 Evaluation of method performance

In this section, the method performance was evaluated by using standard Hg(II) ions solution at various concentration levels. The analysis was repeated 3 times for each concentration under optimal conditions. The calibration curve was constructed between absorbance ratio at 680 and 530 nm against the concentration in the range of 20 to 100 µg/L Hg(II). The signal of the concentration that is the limit of detection ($\mathrm{S_{LOD}}$) was calculated by Eq. 3.1 [48];

$$\mathrm{S_{LOD}} = \mathrm{S}_{Bl} + 3\mathrm{SD}_{Bl} \qquad (3.1)$$

where S_{Bl} and SD_{Bl} represent the blank solution signal and the standard deviation of the blank solution signal, respectively. In this study, SD_{Bl} was substituted by the term of $\mathrm{S}_{y/x}$, which is the statistic value estimating the random errors in the y-direction or in this case, the errors in absorbance ratio for the analysis of each point of calibration (Eq. 3.2). Then, the limit of detection (LOD) was calculated by comparing $\mathrm{S_{LOD}}$ to the calibration curve.

$$S_{y/x} = \sqrt{\frac{\sum_i (y_i - \hat{y})^2}{n-2}} \qquad (3.2)$$

The accuracy and precision of this method were presented as the percentage of recovery (Eq.3.3) and the relative standard deviation, respectively. For evaluating the method accuracy, 60 µg/L of Hg(II) ions solution were spiked into two brands of drinking water samples and one brand of mineral drinking water sample. The Hg(II) detection procedure were done according to section 3.5.1 under optimum condition. The amount of Hg(II) ions in spiked and non-spiked samples were calculated from the linear equation of the linear curve constructed between the absorbance ratio of 680/530 and the concentration of Hg(II) in the range of 20 to 100 µg/L.

$$\% \text{ recovery} = \frac{(\text{Xs} - \text{Xb})}{\text{S}} \times 100 \qquad (3.3)$$

where Xs = the mass of Hg(II) found in the spiked sample (µg)

Xb = the mass of Hg(II) found in the non-spiked sample (µg)

S = the mass of Hg(II) spiked in the sample (µg)

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Characterization of gold nanoparticles (AuNPs) and 2-mercaptobenzothiazole modified gold nanoparticles

In this research, gold nanoparticles were prepared by citrate reduction method. The observed color of gold nanoparticles solution was wine red and the UV-Vis spectrum of this solution showed the absorption maxima at approximately 520 nm, corresponding to the UV-Vis absorption of the particles of 13 nm in diameter according to the previous research [24]. The colloidal AuNPs solution was stable over 14 days due to the colloids stabilization by citrate anions covered on AuNPs. After the addition of 2-mercaptobenzothiazole into AuNPs solution, the solution color changed from red to pink. In the presence of Hg(II) ions, the color of solution turned from pink to purple depending on Hg(II) ions concentration. This phenomena allow the determination of Hg(II) concentration.

The change in AuNPs solution was monitored by UV-visible spectrophotometry and transmission electron microscopy (TEM). Figure 4.1 shows the UV-Vis spectrum of the red color colloidal gold solution that has absorption maxima in visible region at about 520 nm. After adding MBT, the absorption maxima shifted to longer wavelength (526-530 nm). In the presence of Hg(II), the intensity of absorption at 530 nm decreased while a band around 600-800 nm appeared.

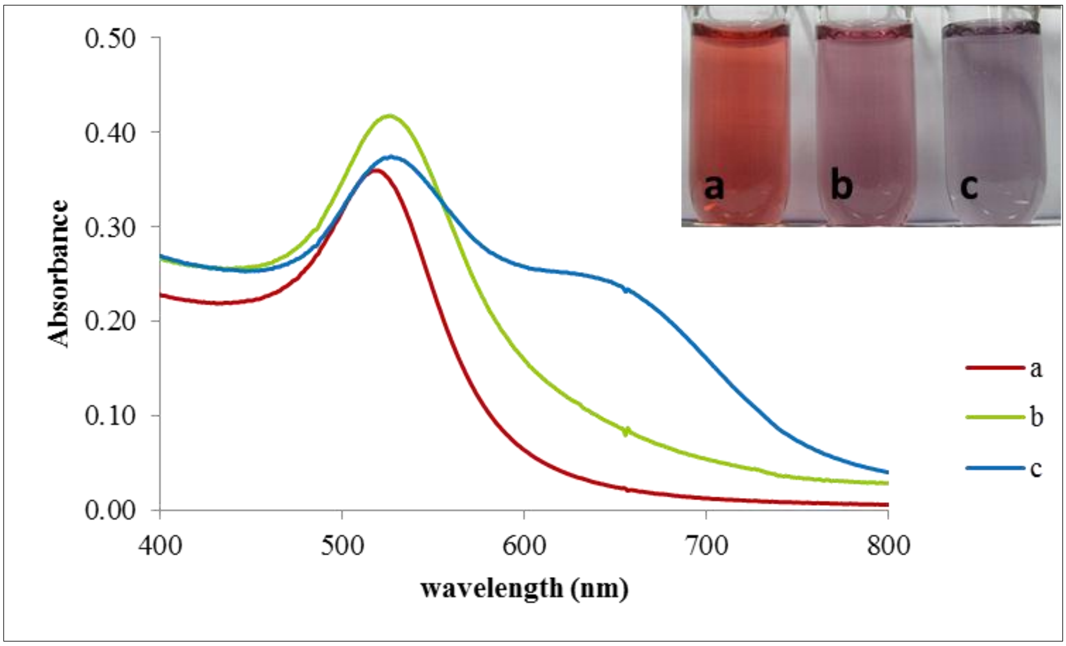


Figure 4.1 The color and UV-Vis absorption spectra of (a) AuNPs, (b) AuNPs-MBT and (c) AuNPs-MBT with 60 µg/L Hg(II); AuNPs 0.25mM, MBT 5.0 µM, total analysis time 3 min.

The particles dispersion and aggregation of AuNPs, AuNPs-MBT before and after the addition of Hg(II) ions were observed by TEM (Fig. 4.2). TEM image of AuNPs solution shows spherical AuNPs with mean diameter of 13 nm. In the presence of MBT, the inter-particles distance between AuNPs decreased, leading to particles aggregation. Finally, the degree of aggregation of AuNPs-MBT remarkably increased after adding Hg(II) ions, resulting in large cluster of particles.

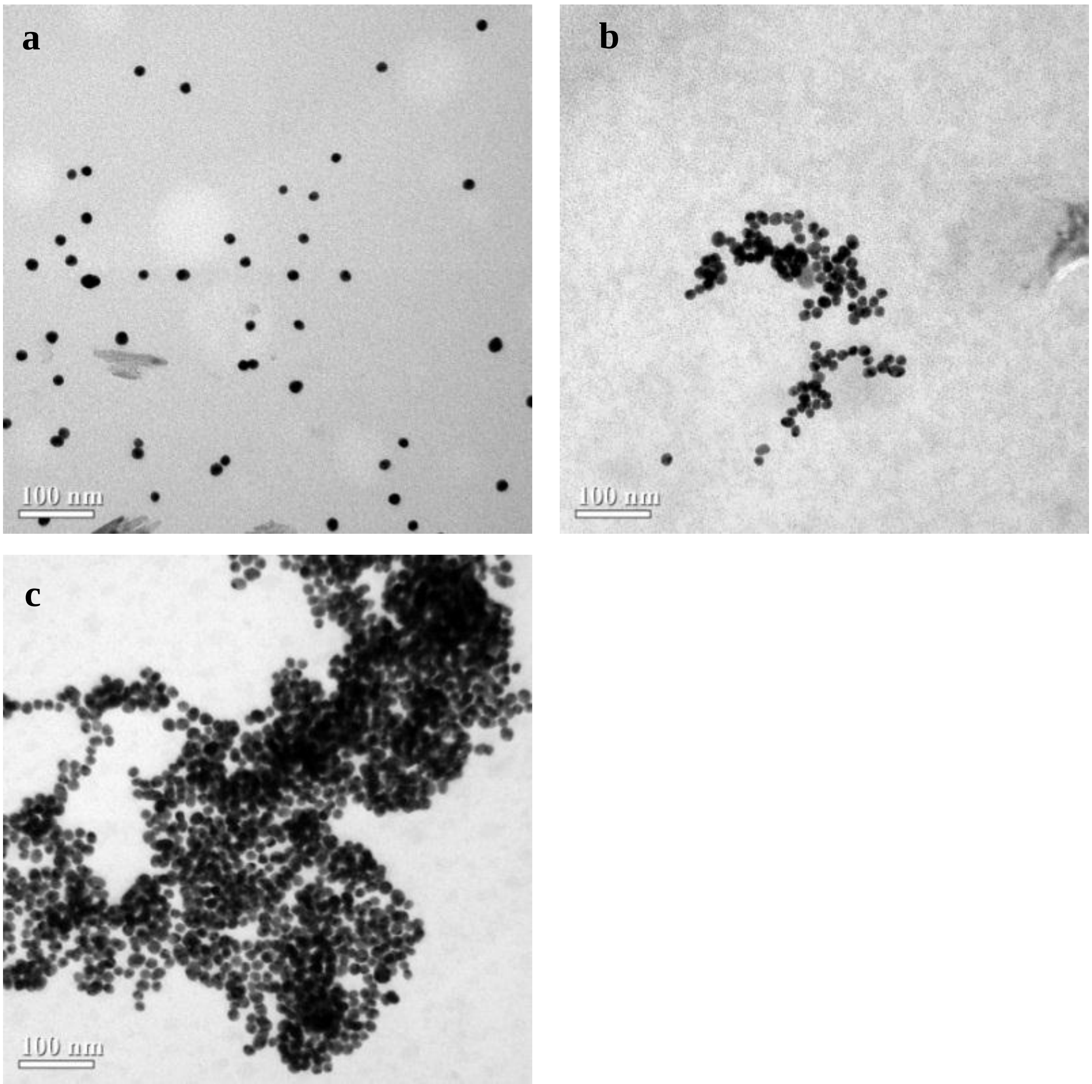


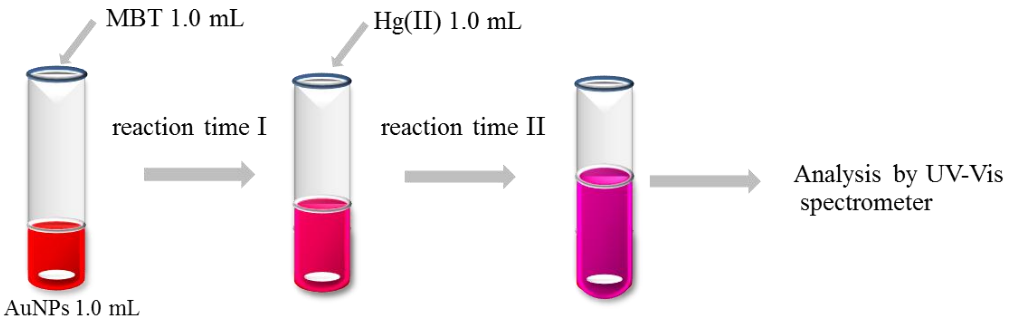
Figure 4.2 TEM images of (a) AuNPs, (b) AuNPs-MBT and (c) AuNPs-MBT with 60 μg/L Hg(II).

4.1.1. Mechanism of nanoparticles aggregation

By the results obtained, a mechanism was proposed as described in the following part. In pure AuNPs solution, AuNPs dispersed well in solution due to the presence of citrate anions on particles surface resulting in electrostatic repulsion of negatively charged nanoparticles. After the addition of MBT, MBT molecules that contain sulfur and nitrogen atom would bind with the metallic nanoparticles through covalent Au-S bond [46, 49, 50] and the aggregation of AuNPs occurred. Finally, higher degree of aggregation observed in the presence of Hg(II) is believed to be due to the binding of Hg(II) ions with MBT ligand that induced the AuNPs-MBT particles to get close to each other and form larger cluster.

4.2 Optimization of Hg(II) determination

Herein, a procedure in Hg(II) detection by following the solution color change and UV-Vis absorption spectra is propose (scheme 4.1). A solution of MBT was mixed with AuNPs solution under stirring for a desired time (reaction time I), then Hg(II) was added into AuNPs-MBT solution. The color was observed and the UV-Vis spectrum was recorded at few minutes after Hg(II) addition (reaction time II). The effect of AuNPs solution concentration, MBT concentration, reaction time I and II was investigated. The solutions of 10-150 µg/L Hg(II) were used. The amount of Hg(II) was quantified by cold-vapor atomic absorption spectrometer before use. As shown in Fig. 4.1, the absorption spectra of AuNPs-MBT and AuNPs-MBT-Hg displayed a red shift with absorption maxima at approximately 530 and 680 nm, respectively. Hence, the determination of Hg(II) ion can be evaluated from calibration curve using absorbance ratio at 680 and 530 nm (Absorbance 680/530).



Scheme 4.1 The steps of Hg (II) determination.

4.2.1 Effect of gold nanoparticles concentration

The concentration of gold nanoparticles would affect the sensitivity, linear range of developed colorimetric method, and in particular the color change of solutions. Thus, the effect of starting AuNPs concentration was studied using fixed concentration of MBT (1 µM). The concentration of Hg(II) was in the range of 20 to 100 µg/L and reaction time I and II was kept to 1 and 2 min, respectively. Figure 4.3 shows the calibration curves of Hg(II) obtained by using 0.25, 0.30, 0.40 and 0.50 mM AuNPs.

It was found that the method with AuNPs concentration of higher than 0.30 mM had low sensitivity as there was no significant change in absorbance ratio and solution color in the presence of different Hg(II) concentration, except at 100 µg/L Hg(II). It could be explained that in solution containing high concentration of AuNPs, the amount of AuNPs was much higher than the amount of MBT and Hg(II) and some part of AuNPs remained individually dispersed in solution. Hg(II) ions could not induce AuNPs-MBT to aggregate by the time that the solution was analyzed. Although the method with 0.30 mM AuNPs showed the highest sensitivity of all but the linear range was narrower than the method with 0.25 mM AuNPs. Therefore, the AuNPs concentration of 0.25 mM was chosen as starting AuNPs solution and the solution color change observed was in agreement with the change in Hg(II) concentration. It turned from pink to purple when increased Hg(II) concentration.

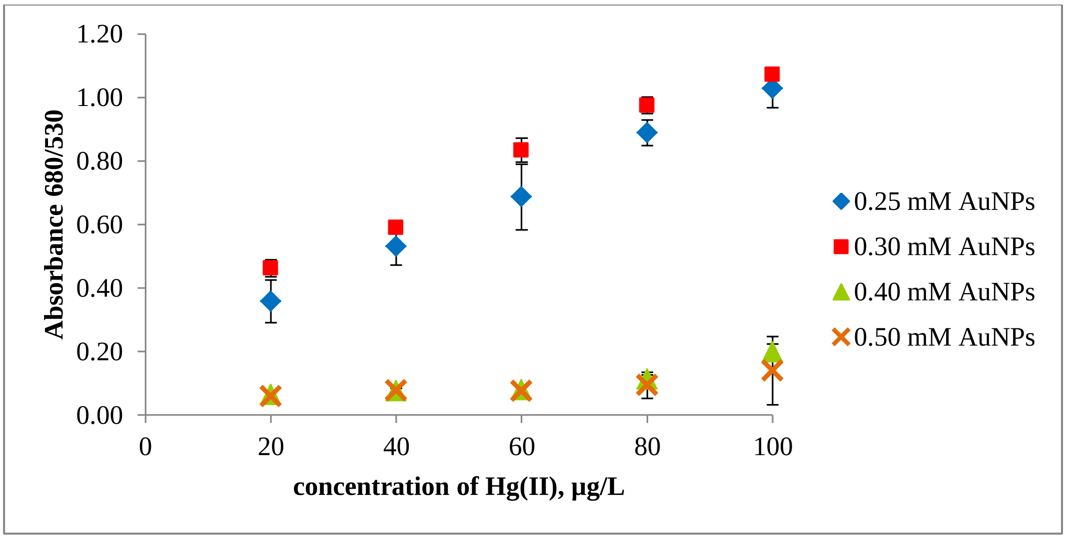


Figure 4.3 The calibration curves of Hg(II) determination (20-100 µg/L) using different concentration of starting AuNPs solution.

4.2.2 Effect of the concentration of 2-mercaptobenzothiazole

Because MBT was used as a ligand that can bind both AuNPs and Hg(II) ion strongly, the concentration of MBT would affect the aggregation of AuNPs when AuNPs-MBT form and hence further affect the aggregation of AuNPs-MBT after adding Hg(II) ions. The solution color would also change accordingly. Therefore, it is necessary to find a suitable MBT concentration for the determination of Hg(II).

The concentration of MBT was varied in the range of 0.1 to 10.0 µM for the modification of 0.25 mM AuNPs and the detection of 20 to 100 µg/L Hg(II). Figure 4.4 shows the calibration curve of Hg(II) using each of different MBT concentration to prepare AuNPs-MBT. When MBT concentration higher than 5.0 µM was used, AuNPs-MBT solution aggregated quickly and the solution color turned from red to purple. When Hg(II) ion was further added into this AuNPs-MBT solution, the color of solution turned to deep blue or gray. The precipitation of AuNPs-MBT-Hg particles were observed when solution of high Hg(II) concentration was analyzed (5.0 µM). The absorption spectrum shifted to longer wavelength (600-700) and linear relation between signal and mercury concentration was narrow (20-60 µg/L). In addition, the MBT concentration is lower than 5.0 µM did not provide a good sensitivity. Under the condition of using low MBT concentration, the color of AuNPs solution after adding MBT changed from red to pink and the spectra maxima shifted to 530 nm. However, after the adding of Hg(II) ions, the color of solution was not visibly change and the change in absorbance ratio was not observed, indicating that the AuNPs-MBT aggregation was not induced by the presence of Hg(II) ions in the studied concentration range. Therefore, the concentration of 5.0 µM MBT was chosen because it provided a good sensitivity and linear range.

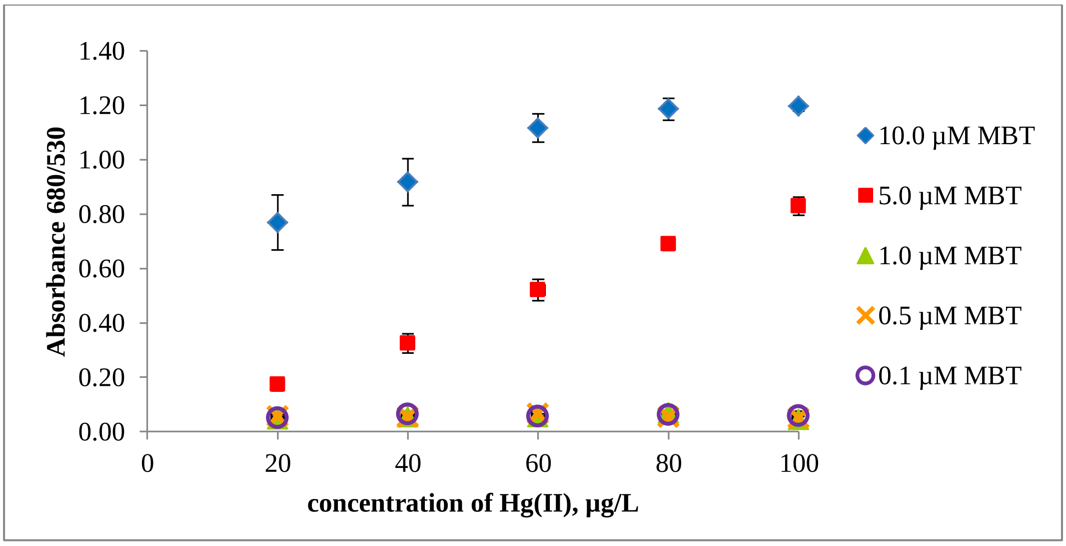


Figure 4.4 The calibration curves of Hg(II) determination (20-100 µg/L) using AuNPs (0.25 mM) modified with different MBT concentrations.

4.2.3 Effect of gold nanoparticles size

AuNPs of different sizes were employed to investigate the effect on the aggregation behavior and performance in Hg(II) determination. The particles size can be controlled by varying the volume of 0.5% citrate solution used in AuNPs preparation while the concentration of starting Au(III) ions is fixed. Figure 4.5 shows that the synthesized gold nanoparticles solutions have absorption maxima at 520 and 526 nm, corresponding to the absorption of the particles with diameter of 13 nm and 20 nm, respectively [24]. TEM image of 13 nm and 20 nm AuNPs are shown in Fig. 4.6. These nanoparticles (0.25 mM AuNPs) were modified with 5.0 µM MBT and used for Hg(II) determination. The reaction time between AuNPs and MBT was 1 min before adding the Hg(II) ions. The mixture was analyzed by UV-Vis spectrophotometer at 2 min after the Hg(II) addition (Fig. 4.7).

The 20 nm AuNPs aggregated rapidly with MBT and the solution color turned from red to deep purple. There was no difference in color of both solutions and absorption spectra when solutions of various Hg(II) concentration were added. On the other hand, the degree of aggregation of 13 nm AuNPs increased in increasing Hg(II) concentration. Therfore, the 13 nm AuNPs was chosen for Hg(II) determination.

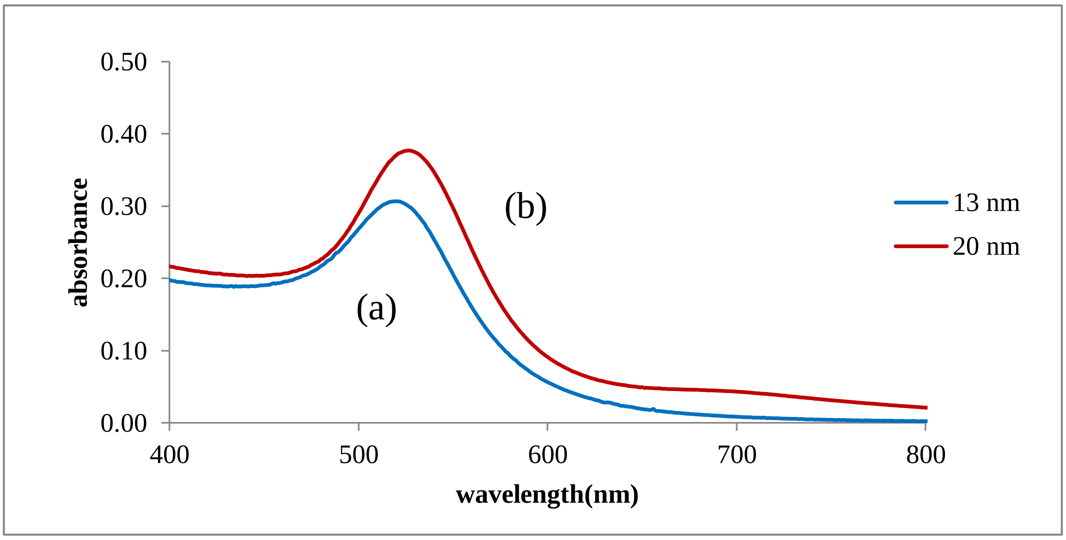


Figure 4.5 UV-vis absorption spectra of solution of gold nanoparticles with diameter of (a) 13 nm (b) and 20 nm.

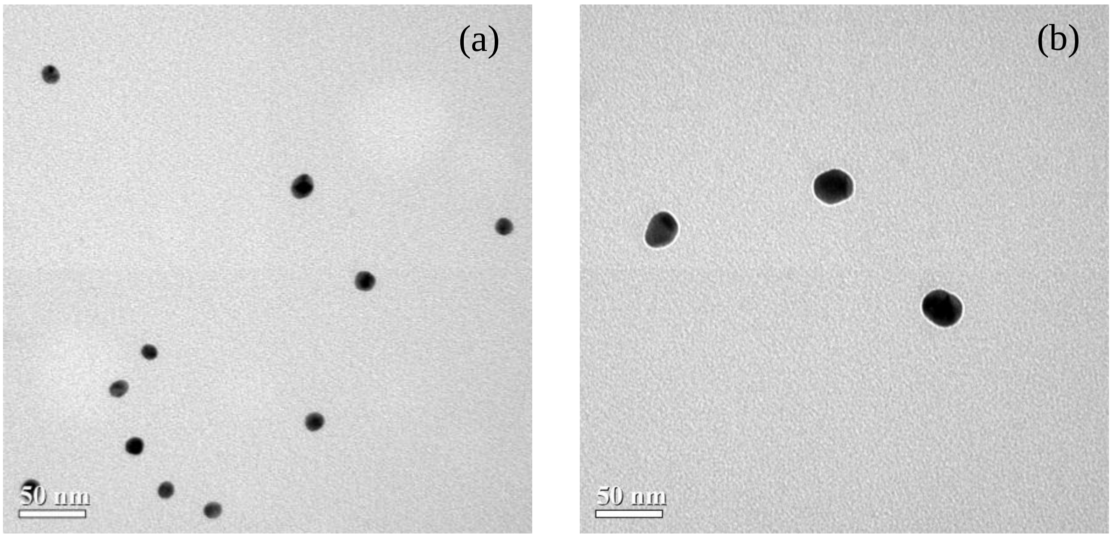


Figure 4.6 TEM images of gold nanoparticles of average diameter of (a) 13 nm and (b) 20 nm.

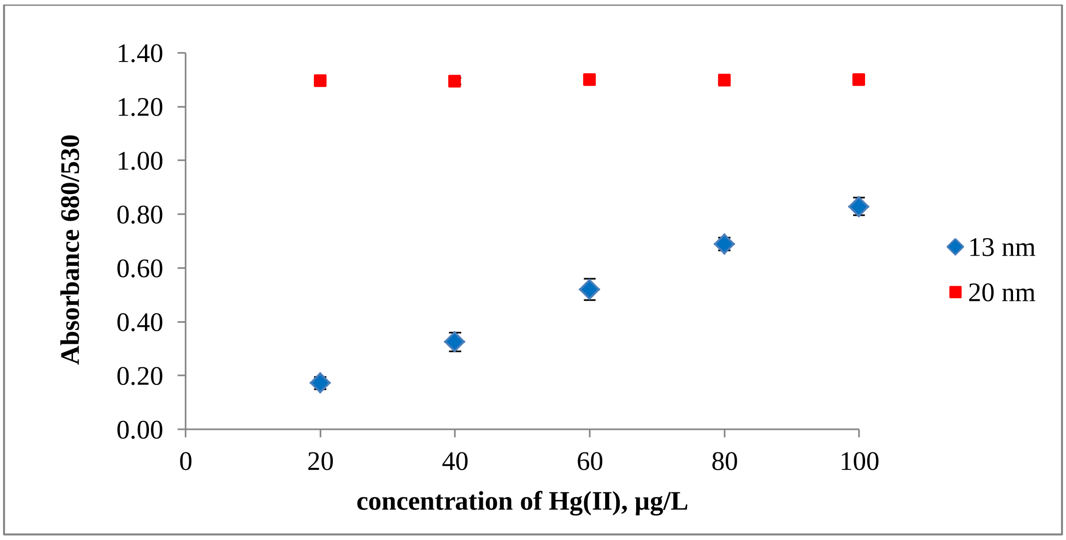


Figure 4.7 The calibration curve of Hg(II) using AuNPs (0.25 mM) with diameter of 13 and 20 nm modified with MBT (5.0 µM).

4.2.4 Effect of reaction time

In the preliminary study, it was observed that the degree of particles aggregation was likely to depend on certain parameters including AuNPs concentration, MBT concentration, Hg(II) concentration and reaction time. Therefore, the reaction time between AuNPs and MBT (reaction time I, scheme 4.1) and between AuNPs-MBT and Hg(II) ions (reaction time II, scheme 4.1) are important factors that may improve the linear range and limit of detection. In this study, we divided the experiments into two parts: the studies of the effect of reaction time I and the reaction time II on the aggregation of AuNPs-MBT before and after adding of Hg(II) .

The reaction time between AuNPs and MBT was varied between 1 to 10 minutes using 0.25 mM AuNPs and 5.0 µM MBT. Figure 4.8 shows the evolution of absorbance ratio as reaction time increased. The absorbance ratio did not clearly change during the first two minutes, but gradually increased from 2 to 10 minutes, revealing that the aggregation of AuNPs induced by MBT continuously occurred. This result is in agreement with the report of the previous research [46]. To shorten analysis time, we fixed the reaction time between AuNPs-MBT at 1 min.

In order to investigate the effect of reaction time II, 20 and 100 µg/L Hg(II) solution were used. Reaction time II was varied from 1 to 30 minutes and the results are shown in Fig. 4.9. Obviously, the degree of AuNPs-MBT-Hg(II) aggregation was related with time and the rate of aggregation depended strongly on Hg(II) concentration. Absorbance of AuNPs-MBT-Hg(II) at 680 nm increased with time and the color turned from pink to blue. In solution containing high Hg(II) concentration, AuNPs-MBT aggregation induced by Hg(II) ions occurred rapidly and reached a constant degree of aggregation in a short time. The absorbance ratio remained constant after 15 minute, revealing that the highest degree of aggregation under such condition was attained.

According to this observation, the change of solution color and absorbance could be detected immediately after the adding Hg(II) ions to AuNPs-MBT solution. To shorten the total analysis time, the reaction time of 2 minutes was selected for all subsequent experiments and the total analysis time was 3 minute. Nevertheless, when increased the reaction time II to 4 minute, the linear range covered 10 µg/L Hg(II) ions as shown in Fig. 4.10 with the total analysis time of 5 minute. Therefore, longer reaction time II could also be used depending on purpose of the use.

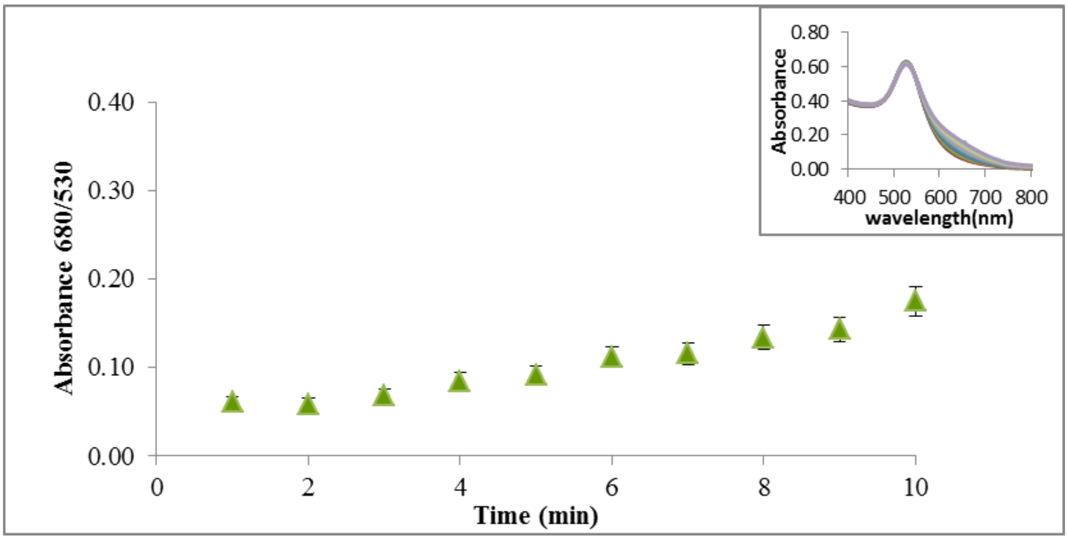


Figure 4.8 The degree of AuNPs-MBT aggregation in the absence of Hg(II) ion, inset: UV-Vis spectrum of AuNPs-MBT solution at different reaction time.

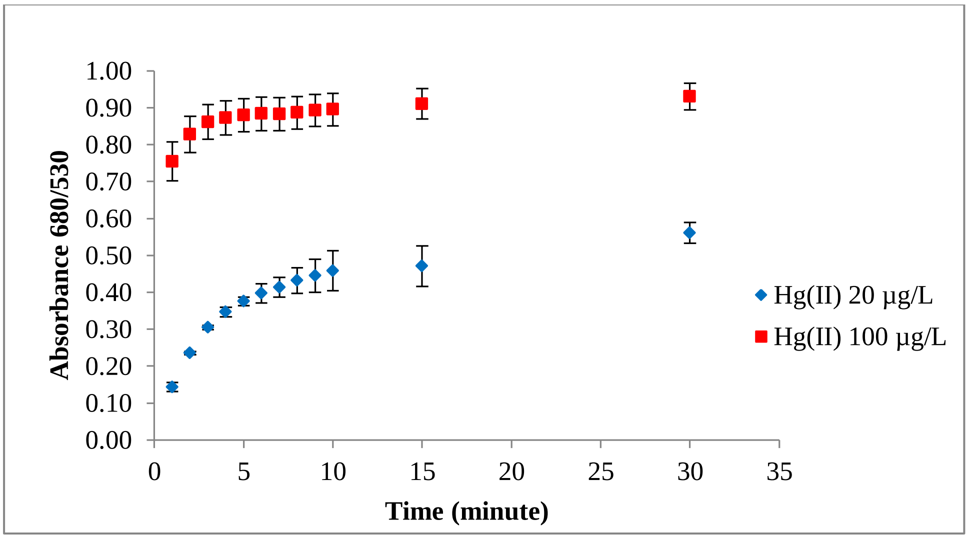


Figure 4.9 The degree of AuNPs-MBT aggregation in the presence of Hg(II) 20 and 100µg/L in function of reaction time.

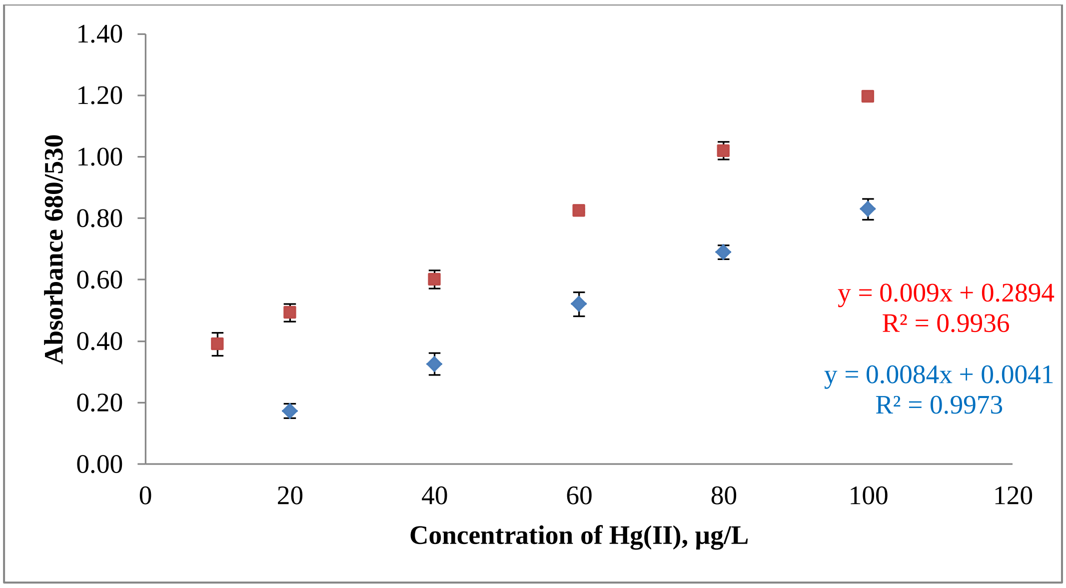


Figure 4.10 The calibration curves of Hg(II) (10-100 µg/L) using (■) 4 min of reaction time II and (◆) 2 min of reaction time II.

4.2.5 Effect of pH

The sample solution pH may affect the aggregation of AuNPs and the complex formation between Hg(II) and ligand. In this study, the effect of pH was studied. A solution of 60 µg/L Hg(II) was used as sample solution. The pH of Hg(II) solution was adjusted to a range of 1-7 using 1% HNO_3 and/or 1% NaOH solution. The results are shown in Fig 4.11. At pH 1-3 of Hg(II) solutions, AuNPs-MBT aggregated immediately after adding sample solution. The solution color turned to blue or purple and the spectra shifted to longer wavelength. At pH 4-5, the color of solution was purple. On the other hand, the aggregation was not observed at pH 6-7 and the solution color remained pink. To compare the effect of pH solution, the blank solution containing no Hg(II) ions at pH 1-7 was used. It was found that the absorption spectra shifted to a longer wavelength at pH 1-3 and the solution color changed from pink to blue or purple. Meanwhile, the AuNPs-MBT absorption spectra and color of solution did not change when blank solution of pH 4 to 7 was added.

In strong acid solution without Hg(II) ions, the aggregation of AuNPs-MBT observed could be explained by the protonation of citrate anions covered on AuNPs-

MBT particles surface (pK_a citrate = 6.39, [43]). As a result, the net charge on AuNPs-MBT particles would decrease and these colloidal particles became less stable and aggregated with each other. When Hg(II) solutions having pH 1-3 were analyzed, the aggregation occurred would probably the result from the effect of citrate protonation and/or the effect of Hg(II) ions. The change in absorbance ratio that was the result from Hg(II) ions was not clearly seen, compared to the change observed in blank solution of the same pH (Fig. 4.11).

On the other hand, AuNPs-MBT aggregated more slowly when Hg(II) solution having pH value of 4 or 5 was added. The aggregation of nanoparticles solution was not observed in the presence of Hg(II) solution at pH 6-7 during the chosen reaction time. It is possible that the complexation between MBT and Hg(II) species did not occur at pH 6-7. The Hg(II) ions are present in different species in solution depending on the solution pH. Hg(II) in solution of pH higher than 6 exist as $Hg(OH)_2$ species [51, 52]. These neutral molecules may not interact with the MBT molecule at pH 6-7 and hence did not induce AuNPs-MBT aggregation. Therefore, the suitable pH in the Hg(II) determination was 4-5.

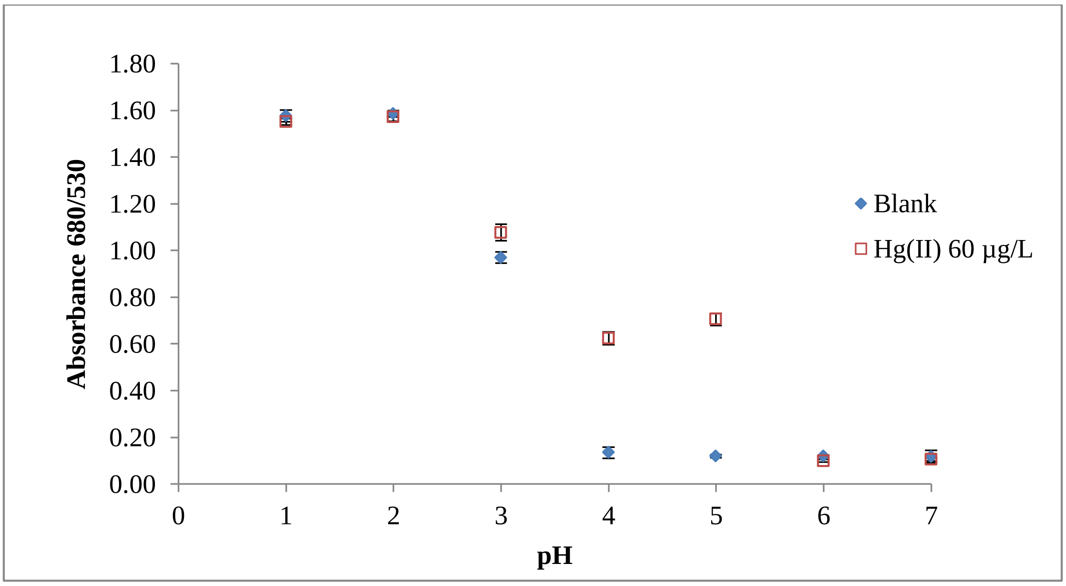


Figure 4.11 Effect of solution pH on the aggregation of AuNPs-MBT with Hg(II) ions (60 µg/L) and without Hg(II) ions (Blank).

4.2.6 The effect of co-existing ions

To investigate the effect of co-existing ions on Hg(II) detection, the reaction of AuNPs-MBT with potential interfering heavy metal ions was studied. An individual metal ions solution (0.12 µmole) of Ag(I), Cu(II), Pb(II), Cd(II), Co(II), Mn(II), Ni(II), Cr(VI), As(III), Zn(II), Fe(III) or Mg(II) was used as sample solution. The solution of AuNPs (0.25 mM) modified with MBT (5 µM) was used for this study. Figure 4.12 shows the solution color in the presence of each metal ions. It was found that the presence of Ag(I), Pb(II) and Cu(II) ions strongly induce with the aggregation of AuNPs-MBT particles. This result could be explained by hard- soft acids and base theory, the MBT molecule contains sulfur and nitrogen atom that can strongly bind with soft and borderline acid metal ions [45]. The use of masking agent to form complexes with interfering ions such as Ag(I), Pb(II) and Cu(II) in sample prior to Hg(II) detection is recommended. Masking agents should bind strongly with these interfering ions.

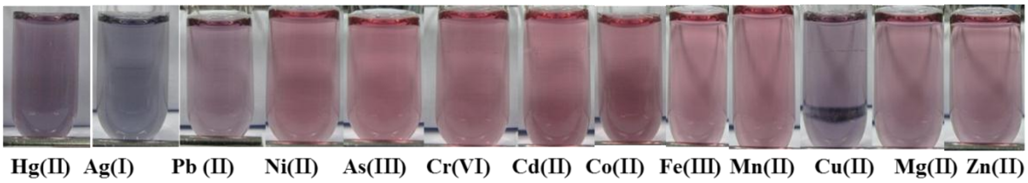


Figure 4.12 Solution of AuNPs-MBT in the presence of various heavy metal ions.

4.3 Evaluation of method performance

After the optimum condition of the developed method was obtained, the method was validated as described in the following part.

Calibration curve and linearity

Under the optimum conditions, the calibration curve was constructed using the concentration of Hg(II) ion in the range of 20 to 100 µg/L. As shown in Fig. 4.13, the linear regression equation was y = 0.0084x + 0.1435. The absorption spectra of Hg(II) were shown in Fig. 4.14. The change in absorption spectra related with the solution color that changed from pink to purple in the presence of different concentration of Hg(II) ions (Fig. 4.15).

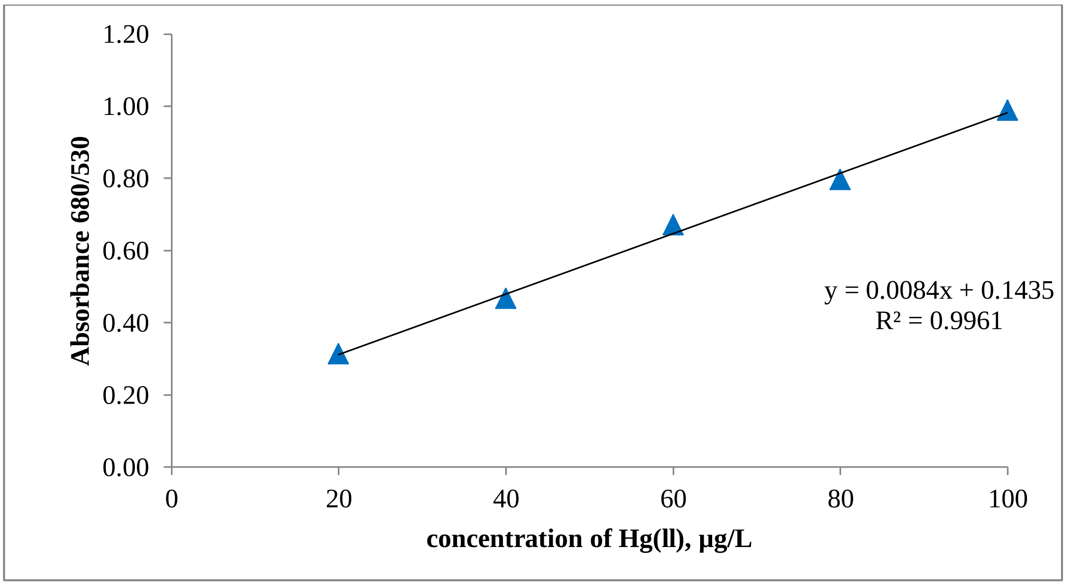


Figure 4.13 The calibration curve of Hg(II) ions obtained under optimum condition.

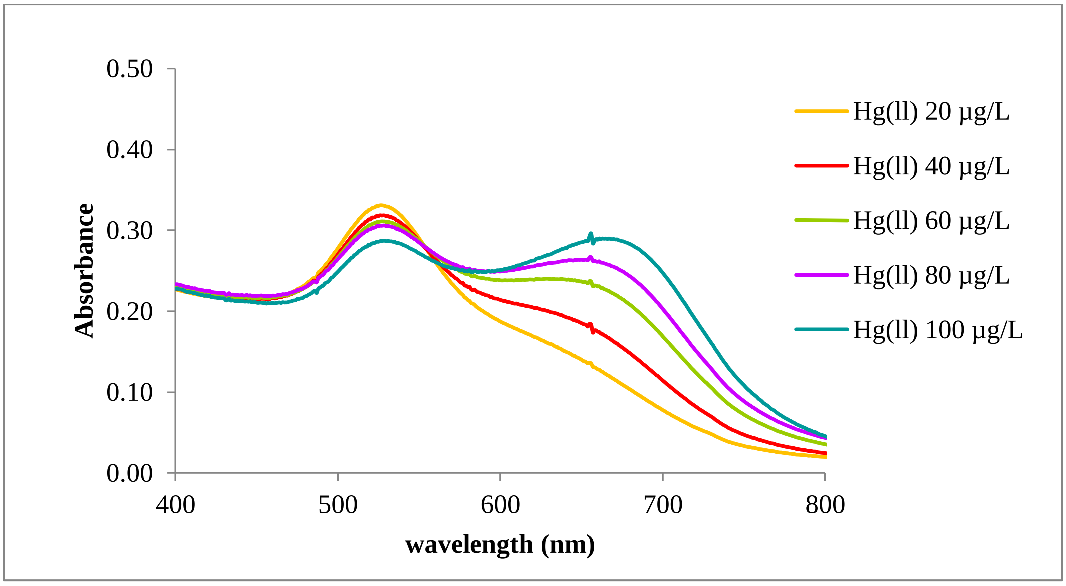


Figure 4.14 UV-Vis absorption spectra of AuNPs-MBT solution containing different Hg(II) ions concentration.

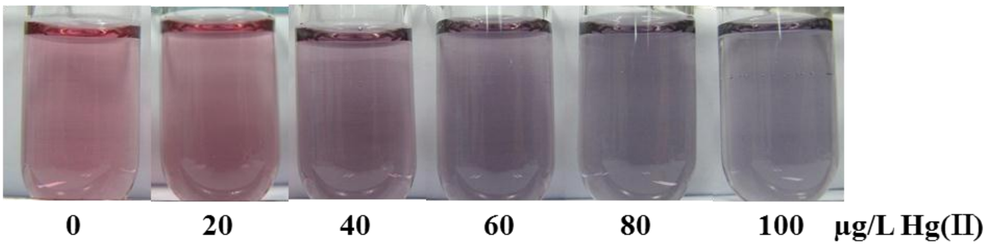


Figure 4.15 AuNPs-MBT solution color after the adding 20 to 100 µg/L Hg(II) ions.

Limit of detection

The limit of detection of this method was calculated using the Eq. 3.1 according to Section 3.6. It was found that the limit of Hg(II) ion detection by spectrophotometry was as low as 5 µg/L (25 nM) and the change of solution color was observed at 40 µg/L (0.2 µM) Hg(II).

Table 4.1 shows the performance of methods using different ligands to modify AuNPs for Hg(II) ions detection. Comparing between the LOD obtained in this study and the LOD values reported in the literatures, it was found that this developed method has the detection limit lower than the other previously proposed methods.

Table 4.1 Comparison of the limit of detection

References	**Ligand**	**Linear range (µM)**	**Limit of detection (nM)**
Guo *et al.*[10]	Papain	1.00-40.00	200
Chai *et al.* [11]	L-cysteine	0.10-2.00	100
Wu *et al.* [13]	DNA	0.10-10.00	60
Yang *et al.* [44]	4-mercaptopyridine	0.15-3.00	55
This method	2-mercaptobenzothiazole	0.10-5.00	25

Reproducibility

The reproducibility of this method were investigated by performing the analysis of 20 and 100 µg/L Hg(II) solutions. It was found that the Intra-day percentage of relative standard derivation of signal ratio of 20 µg/L and 100 µg/L Hg(II) solution was 3-13 % and 3-4% (n=3), respectively. The Inter-day percentage of relative standard derivation of signal ratio of 20 µg/L and 100 µg/L Hg(II) solution was 20 % and 10 % (n=9), respectively. These results reveal an acceptable precision for the analysis at this concentration level [53]. The relative standard deviation of the calibration slope was 5 % (n = 3) over 3 days.

Accuracy

The accuracy of the method was evaluated by spiking known amount of Hg(II) ions into drinking water samples. The percentage of recovery (%recovery) was then determined. A solution of 60 µg/L Hg(II) was spiked into two brands of drinking water and one brand of mineral drinking water sample. Unfortunately, the aggregation of AuNPs-MBT was observed during un-spiked samples analysis, in particular mineral drinking water sample caused a high degree of aggregation. The results reveal that the other ions in samples also induced the aggregation of AuNPs-

MBT. Nevertheless, higher degree of aggregation was achieved when samples spiked with Hg(II) ions were analyzed, compared to un-spiked samples. The accurate recovery could not be obtained in this case and the accuracy of this method has not yet evaluated. More study is required.

CHAPTER V

CONCLUSION

5.1 Conclusion

A method for mercury (II) ions detection was developed by using 2-mercaptobenzothiazole (MBT) with gold nanoparticles (MBT-AuNPs) as media. The aggregation induced by MBT caused the change in solution color from red to pink after adding the ligand. In the presence of Hg(II) ions, AuNPs-MBT aggregates was induced to form larger clusters and the color of solution turned from pink to purple depending on Hg(II) ions concentration. This phenomena allow the determination of Hg(II) concentration. The solution was then analyzed by UV-VIS spectrophotometer. The particles aggregation was confirmed by TEM.

The effects of gold nanoparticles concentration, MBT concentration, size of gold nanoparticles and reaction time were investigated. The concentration of gold nanoparticles and MBT solution was studied in the range of 0.25 to 0.50 mM and 0.1 to 10 µM, respectively. The concentration of 0.25 mM of gold nanoparticles and 5 µM of MBT solution was chosen to prepare AuNPs-MBT solution. The particles sizes of gold nanoparticles affected the determination of Hg(II). Gold nanoparticles with the size of 20 nm did not produce linear response with respect to Hg(II) concentration. Thus, the gold nanoparticles size of 13 nm in diameter was chosen for the determination. The effects of reaction times were studied and the reaction times of 1 and 2 minutes were chosen for the reaction between AuNPs and MBT and between AuNPs-MBT and Hg(II), respectively.

The suitable pH for Hg(II) determination was pH 4 to 5. A good linearity ($R^2 = 0.99$) was observed for the determination of Hg(II) in the concentration range of 20 to 100 µg/L. The limit of Hg(II) ion detection by spectrophotometry was as low as 5 µg/L and the change in color solution was observed at 40 µg/L Hg(II). The results from the study of co-existing ions effect indicating that Ag(I), Cu(II) and Pb(II) ions were the potential interfering ions in Hg(II) determination. The use of masking agents was suggested.

5.2 Suggestion of future work

- The method sensitivity should be improved in order to be able to determine trace Hg(II) in the real sample.
- The effect of co-existing ion should be minimized to improve the accuracy of the method in Hg(II) determination.

REFERENCES

[1] Holmes, P.; James, K. A. F.; Levy, L. S. Is low-level environmental mercury exposure of concern to human health? Science of the Total Environment. 408 (2009) 171–182.

[2] Krata, A.; Bulska, E. Critical evaluation of analytical performance of atomic absorption spectrometry and inductively coupled plasma mass spectrometry for mercury determination. Spectrochimica Acta Part B. 60 (2005) 345–350.

[3] Gao, Y.; Shi, Z.; Long, Z.; Wu, P.; Zheng, C.; Hou. X. Determination and speciation of mercury in environmental and biological samples by analytical atomic spectrometry. Microchemical Journal. 103 (2012) 1–14.

[4] Yuan, M.; Li, Y.; Li, J.; Li, C.; Liu, X.; Lv, J.; Xu, J.; Liu, H.; Wang, S.; Zhu, D. A colorimetric and fluorometric dual-modal assay for mercury ion by a molecule. Organic Letters. 12 (2007) 2313-2316.

[5] Li, H.; Zhaia, J.; Tiana, J.; Luoa, Y.; Sun, X. Carbon nanoparticle for highly sensitive and selective fluorescent detection of mercury(II) ion in aqueous solution. Biosensors and Bioelectronics. 26 (2011) 4656–4660.

[6] Vasimalai, N.; John, S. A. Ultrasensitive and selective spectrofluorimetric determination of Hg(II) using a dimercaptothiadiazole fluorophore. Journal of Luminescence. 131 (2011) 2636–2641.

[7] Kelly, K. L.; Coronado, E.; Zhao, L. L.; Schatz, G. C. The Optical properties of metal nanoparticles: the influence of size, shape, and dielectric environment. Journal of Physical Chemistry B. 107 (2003) 668-677.

[8] Moores, A.; Goettmann, F. The plasmon band in noble metal nanoparticles: an introduction to theory and applications. New Journal of Chemistry**.** 30 (2006) 1121–1132.

[9] Rosi, N. L.; Mirkin, C. A. Nanostructures in biodiagnostics. Chemical Reviews.105 (2005) 1547-1562.

[10] Guo, Y.; Wangb, Z.; Qub, W.; Shaoa, H.; Jiang, X. Colorimetric detection of mercury, lead and copper ions simultaneously using protein-functionalized gold nanoparticles. Biosensors and Bioelectronics. 26 (2011) 4064–4068.

[11] Chai, F.; Wang, C.; Wang, T.; Ma, Z.; Su, Z. L-cysteine functionalized gold nanoparticles for the colorimetric detection of Hg^{2+} induced by ultraviolet light. Nanotechnology. 21(2010) 1-6.

[12] Si, S.; Kotal, A.; Mandal, T. K. One-dimensional assembly of peptide-functionalized gold nanoparticles: an approach toward mercury ion sensing. Journal of Physical Chemistry C. 111 (2007) 1248-1255.

[13] Wu, J.; Li, L.; Zhua, D.; Hea,P.; Fanga, Y.; Cheng, G. Colorimetric assay for mercury (II) based on mercury-specific deoxyribonucleic acid-functionalized gold nanoparticles. Analytica Chimica Acta. 694 (2011) 115–119.

[14] Jeoung, M. S.; Choi, H. S. Spectrophotometric determination of trace Hg(II) in cetyltrimethylammonium bromide media. Bulletin of the Korean Chemical Society. 25 (2004) 1877-1880

[15] Filho, N. L. D.; Gushikem, Y.; Polito, W. L. 2-Mercaptobenzothiazole clay as matrix for sorption and preconcentration of some heavy metals from aqueous solution. Analytica Chimica Acta. 306 (1995) 167-172.

[16] Wu, L.; Zheng, C.; Ma, Q.; Hu, C.; Hou, X. Chemical vapor generation for determination of mercury by inductively coupled plasma mass spectrometry. Applied Spectroscopy Reviews. 42 (2007) 79–102.

[17] Mousavi, H. Z.; Asghari, A.; Shirkhanloo, H. Determination of Hg in water and wastewater samples by CV-AAS following on-line preconcentration with silver trap. Journal of Analytical Chemistry. 65 (2010) 935–939.

[18] Souza, S. S.; Rodrigues, J. L.; Souza, V. C. O.; Barbosa, Jr. F. A fast sample preparation procedure for mercury speciation in hair samples by high-performance liquid chromatography coupled to ICP-MS. Journal of Analytical Atomic Spectrometry. 25 (2010) 79–83.

[19] Silva, M. J.; Paimb, A. P. S.; Pimentelc, M. F.; Cervera, M. L.; Guardia, M. Determination of mercury in rice by cold vapor atomic fluorescence spectrometry after microwave-assisted digestion pollution. Analytica Chimica Acta. 667 (2010) 43–48.

[20] Krupp, E. M.; Milne, B. F.; Mestrot, A.; Meharg, A. A.; Feldmann, J. Investigation into mercury bound to biothiols: structural identification using ESI–ion-trap MS and introduction of a method for their HPLC separation with simultaneous detection by ICP-MS and ESI-MS. Analytical and Bioanalytical Chemistry. 390 (2008)1753–1764.

[21] Lee, S. J.; Lee, J. E.; Seo, J.; Jeong, I. Y.; Lee, S. S.; Jung, J. H. Optical sensor based on nanomaterial for the selective detection of toxic metal Ions. Advanced Functional Materials. 17 (2007) 3441–3446.

[22] Sumesh, E.; Bootharaju, M. S.; Pradeep, A. T. A practical silver nanoparticle-based adsorbent for the removal of $Hg^{2+}$from water. Journal of Hazardous Materials. 189 (2011) 450–457.

[23] Liu, H. B.; Ascencio, J. A.; Perez-Alvarez, M.; Yacaman, M. J. Melting behavior of nanometer sized gold isomers. Surface Science. 491 (2001) 88-98.

[24] Basu, S.; Ghosh, S. K.; Kunda, S.; Panigrahi, S.; Praharaj, S. P.; Jana, S.; Pal, T. Biomolecule induced nanoparticle aggregation: effect of particle size on interparticle coupling. Journal of Colloid and Interface Science. 313 (2007) 724-734.

[25] Huang, X.; El-Sayed, M. A. Gold nanoparticles: optical properties and implementations in cancer diagnosis and photothermal therapy. Journal of Advanced Research. 1 (2010) 13-28.

[26] Huang, X.; Jain, P. K.; El-Sayed, I. H.; El-Sayed, M. A. Gold nanoparticles: interesting optical properties and recent application in cancer diagnostics and therapy. Nanomedicine. 2 (2007) 681-693.

[27] Link, S.; El-Sayed, M. A. Spectral properties and relaxation dynamics of Surface plasmon electronic oscillations in gold and silver nanodots and nanorods. Journal of Physical Chemistry B. 103 (1999) 8410-8426.

[28] Shipway, A. N.; Lahav, M.; Gabai, R.; Willner, I. Investigations into the electrostatically induced aggregation of Au nanoparticles. Langmuir. 16 (2000) 8789-8795.

[29] Yeh, Y. C.; Creran, B.; Rotello, V. M. Gold nanoparticles: preparation, properties, and applications in bionanotechnology. Nanoscale. 4 (2012) 1871-1880.

[30] Chomoucka, J.; Drbohlavova, J.; Huskab, D.; Adam, V.; Kizekb, R.; Hubaleka, J. Magnetic nanoparticles and targeted drug delivery. Pharmalogical Research. 62 (2010) 144–149.

[31] Jr, B. J. R. Dendrimer-based nanoparticles for cancer therapy. American Society of Hematology. 2009 (2009) 708-719.

[32] Nam, J.; Won, N.; Jin, H.; Chung, H.; Kim, S. pH-induced aggregation of gold nanoparticles for photothermal cancer therapy. Journal of American Chemical Society. 131 (2009) 13639-13645.

[33] Xu, Q.; Du, S.; Jin, G. D.; Li, H.; Hu, X. Y. Determination of acetamiprid by a colorimetric method based on the aggregation of gold nanoparticles. Microchimica Acta. 173 (2011) 323-329.

[34] Zhang, Y. P.; Chen, J.; Bai, L. Y.; Zhou, X. M.; Wang, L. M. Gold nanoparticle-based optical probe for quick colorimetric visualization of cysteine. Journal of the Chinese Chemical Society. 57 (2010) 972-975.

[35] Hung, Y. L.; Hsiung, T. M.; Chen, Y. Y.; Huanh, Y. F.; Huang, C. C. Colorimetric detection of heavy metal ions using label-free gold nanoparticles and alkanethiols. Journal of Physical Chemical C.114 (2010) 16329-16334.

[36] Ma, M.; Wang, J.; Zheng, X. Enhancement of the colorimetric sensitivity of gold nanoparticles with triethanolamine to minimize interparticle repulsion. Microchim Acta. 172 (2011) 155-162.

[37] Turkevich, J. Colloidal gold. Part I. Gold Bulletin. 18 (1985) 86-91.

[38] Shan, C.; Li, F.; Yuan, F.; Yang, G.; Niu, L.; Zhang, Q. Size-controlled synthesis of monodispersed gold nanoparticles stabilized by polyelectrolyte-functionalized ionic liquid. Nanotechnology. 19 (2008) 1-6.

[39] Sun, K.; Qiu, J.; Liu, J.; Miao, Y. Preparation and characterization of gold nanoparticles using ascorbic acid as reducing agent in reverse micelles. Journal of Materials Science. 44 (2009) 754–758.

[40] Pacławski, K.; Streszewski, B.; Jaworski, W.; Luty-Błocho, M.; Fitzner, K. Gold nanoparticles formation via gold(III) chloride complex ions reduction with glucose in the batch and in the flow microreactor systems. Colloids and Surfaces A. (2012) 1-8.

[41] Muhlpfordt, J. A. The preparation of colloidal gold particles using tannic acid as an additional reducing agent. Experientia. 38 (1982) 1127-1128.

[42] Kumar, S.; Gandhi, K. S.; Kumar, R. Modeling of formation of gold nanoparticles by citrate method. Industrial & Engineering Chemistry Research. 46 (2007) 3128-3136.

[43] Huang, C. C.; Chang, H. T. Parameters for selective colorimetric sensing of mercury(II) in aqueous solutions using mercaptopropionic acid-modified gold nanoparticles. Chemical Communication. (2007) 1215-1217.

[44] Yang, X.; Liu, H.; Xu, J.; Tang, X.; Huang, H.; Tian, D. A simple and cost-effective sensing strategy of mercury(II) based on analyte-inhibited aggregation of gold nanoparticles. Nanotechnology. 22 (2011) 1-6.

[45] Feng, L.; Chen, Z. 2-Mercaptobenzotiazole benzoates as highly sensitive fluorescent enhancement chemosensor for transition metal ions. Sensor and Actuators B. 120 (2007) 665-668.

[46] Song, C.; Wang, Z.; Yang, J.; Zhang, R.; Cui, Y. Preparation of 2-mercaptobenzothiazole-labled immune-Au aggregates for SERS-based immunoassay. Colloidal and Surface B: Biointerfaces. 81 (2010) 285-288.

[47] Pearson, R. G.; Hard and soft acids and bases. Journal of the American Chemical Society. 85 (1963) 3533-3539.

[48] Miller, N. J.; Miller, C. J. Statics and chemometrics for analyticle chemistry. 4th ed. Eillis Horwood, 2000.

[49] Woods, R.; Hope, G. A.; Watling, K. A SERS spectroelectrochemical investigation of the interaction of 2-mercaptobenzothiazole with copper, silver and gold surfaces. Journal of Applied Electrochemistry. 30 (2000) 1209-1222.

[50] Wang, G.; Harrison, A.; Li, X.; Whittaker, G.; Shi, J. Wang, X.; Yang, H.; Cao, P.; Zhang, Z. Study of the adsorption of benzimidazole and 2-mercaptobenzothiazole on an iron surface by confocal micro-Raman spectroscopy. Journal of Raman Spectroscopy. 35 (2004) 1016–1022.

[51] Barriada, J. L.; Herrero, R.; Prada-Rodríguez, D.; Sastre de Vicente, M. E. Interaction of mercury with chitin: A physicochemical study of metal binding by a natural biopolymer. Reactive & Functional Polymers. 68 (2008) 1609–1618.

[52] Knocke, W. R.; Hemphill, L. H. Mercury(II) sorption by waste rubber. Water Research. 15 (1981) 275-282.

[53] Huber, L. Validation and qualification in analytical laboratories. 2nd ed. Interpharm Press, 1999.

VITA

Miss Dusadee Ruamsanith was born on November 15, 1986 in Nakhon Si Thammarat, Thailand. She graduated with a Bachelor of Science degree from Srinakarinwirot University in 2008. After that, she has been a graduate student at the Department of Chemistry Chulalongkorn University and become a member of Environmental Analysis Research Unit. She finished her Master’s degree of Science in 2012.