RESORCINOL BASED POLYMERIC MATRICES FOR APPLICATIONS IN Cu(II) DETERMINATION

Kotchakriangkrai Sanai

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เมทริกซ์พอลิเมอร์ที่มีเรซอร์ซินอลสำหรับประยุกต์ในการหาปริมาณทองแดง(II)

นายกชเกรียงไกร สนัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาเคมี
มหาวิทยาลัยเทคโนโลยีสุรนารี
ปีการศึกษา 2557

RESORCINOL BASED POLYMERIC MATRICES FOR APPLICATIONS IN Cu(II) DETERMINATION

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the degree of doctor of philosophy.

Thesis Examining Committee

(Assoc. Prof. Dr. Jatuporn Wittayakun)
Chairperson

(Asst. Prof. Dr. Sanchai Prayoonpokarach)
Member (Thesis Advisor)

(Asst. Prof. Dr. Kunwadee Rangsriwatananon)
Member

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(Asst. Prof. Dr. Anchalee Samphao)
Member

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กชเกรียงไกร สนัย : เมทริกซ์พอลิเมอร์ที่มีเรซอร์ซินอลสำหรับประยุกต์ในการหาปริมาณทองแดง(II) (RESORCINOL BASED POLYMERIC MATRICES FOR APPLICATIONS IN Cu(II) DETERMINATION) อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.สัญชัย ประยูรโภคราช, 177 หน้า

งานวิจัยนี้นำเสนอการพัฒนาเซ็นเซอร์เคมีเชิงแสงสำหรับหาปริมาณทองแดง(II) โดยรีเอเจนต์ที่ทำหน้าที่เป็นสารรับรู้ คือ 5-เมทิล-4-(2-ไทเอโซลิลเอโซ)เรซอร์ซินอล (5เอ็มทาร์) มีการศึกษาวัสดุที่ใช้เป็นเมทริกซ์รองรับชนิดต่าง ๆ คือไคโตซาน ไคโตซานที่มีอะมิโนซิลิกาผสมอยู่ 5 %โดยน้ำหนักต่อปริมาตร และแนฟิออน 5เอ็มทาร์ถูกตรึงแบบกายภาพบนไคโตซานและไคโตซานที่มีอะมิโนซิลิกาผสมอยู่ 5 % โดยน้ำหนักต่อปริมาตร และถูกตรึงบนแนฟิออนโดยแรงทางไฟฟ้า มีการศึกษาสภาวะที่เหมาะสมในการตรึงในแต่ละเมทริกซ์รองรับ มีการประเมินพารามิเตอร์ที่มีอิทธิพลต่อการตอบสนองของฟิล์มรับรู้ต่อทองแดง(II) ได้แก่ ความเข้มข้นของ5เอ็มทาร์ เวลาที่ใช้ในการตรึง พีเอชของสารละลาย และชนิดของตัวรบกวน ด้วยวิธีการที่พัฒนาขึ้นมา ขีดจำกัดการตรวจวัดสำหรับทองแดง(II) มีค่า 32, 20 และ 13 พีพีบี จากฟิล์มรับรู้ที่ทำจากไคโตซาน ไคโตซานที่มีอะมิโนซิลิกาผสมอยู่ และแนฟิออนตามลำดับ การคืนสภาพของฟิล์มรับรู้หลังจากที่ผ่านการสัมผัสกับทองแดง(II) มาแล้ว ทำได้โดยแช่ฟิล์มในสารละลายกรดไฮโดรคลอริกความเข้มข้น 1.0โมลาร์ ฟิล์มรับรู้ที่ทำจากไคโตซานและแนฟิออนนำไปใช้หาปริมาณ ทองแดง(II) ในสารตัวอย่างน้ำและเหล้าขาวได้ผลเป็นที่น่าพอใจ

มีการศึกษาการดูดซับ 5เอ็มทาร์บนเอ็มซีเอ็ม-41 ที่ผ่านและไม่ผ่านการเผา และตัวดูดซับที่ได้นำไปใช้ในการดูดซับทองแดง(II) ในสารตัวอย่างน้ำ 5เอ็มทาร์ชอบที่จะดูดซับบนเอ็มซีเอ็ม-41 ที่ไม่ผ่านการเผามากกว่า เมื่อเปรียบเทียบกับเอ็มซีเอ็ม-41 ที่ผ่านการเผา โดยความจุการดูดซับมีค่า 131.6 มิลลิกรัมต่อกรัม สำหรับเอ็มซีเอ็ม-41 ที่ไม่ผ่านการเผา และ 7.0 มิลลิกรัมต่อกรัม สำหรับเอ็มซีเอ็ม-41 ที่ผ่านการเผา การดูดซับทองแดง(II) บนเอ็มซีเอ็ม-41 ที่ไม่ผ่านการเผาที่ตรึงด้วย 5เอ็มทาร์จะเป็นไปตามการดูดซับแบบแลงเมียร์และมีอันดับปฏิกิริยาเป็นอันดับสองเทียม ค่าความจุการดูดซับสำหรับทองแดง(II) คือ 24.2 มิลลิกรัมต่อกรัม ในสารละลายพีเอช 5.0 และเวลาการดูดซับ 15

นาที ตัวดูดซับมีความเลือกสรรที่สูงสำหรับทองแดง(II) และสามารถคืนสภาพได้อย่างน้อยที่สุด 5 ครั้งโดยแช่ในสารละลายอีดีทีเอ ความเข้มข้น 0.2 โมลาร์ ด้วยเวลา 30 นาที

สาขาวิชาเคมี ลายมือชื่อนักศึกษา__________________________

ปีการศึกษา 2557 ลายมือชื่ออาจารย์ที่ปรึกษา____________________

KOTCHAKRIANGKRAI SANAI : RESORCINOL BASED POLYMERIC MATRICES FOR APPLICATIONS IN Cu(II) DETERMINATION. THESIS ADVISOR : ASST. PROF. SANCHAI PRAYOONPOKARACH, Ph.D. 117 PP.

5-METHYL-4-(2-THIAZOLYAZO)RESORCINOL/ COPPER(II) OPTICAL CHEMICAL SENSING FILM/ CHITOSAN/ SILICA/ NAFION/ MCM-41

The development of optical chemical sensors for the determination of Cu(II) by using a chromogenic sensing reagent, 5-methyl-4-(2-thiazolylazo)resorcinol (5MTAR) as a sensing reagent was presented. Different supporting matrix materials including chitosan, 5 % w/v amino-silica particles suspended in chitosan and Nafion were studied. 5MTAR was immobilized in chitosan and the amino-silica particle chitosan mixture via physical interaction and in Nafion via electrostatic force. The condition of the immobilization of each supporting matrixes was optimized. The influence of the set of parameters on the response of the sensing films to Cu(II), normally the concentration of 5MTAR, immobilization time, pH of the solutions and interference species were evaluated. With the developed methods, limit of detection for Cu(II) were 32, 20 and 13 ppb with sensing films fabricated from chitosan, amino-silica chitosan and Nafion, respectively. Regeneration of the sensing films after exposure to Cu(II) was possible by soaking the matrixes in 1.0 M HCl. The chitosan and Nafion sensing films were used with satisfactory results for the determination of Cu(II) in water and Thai whisky samples.

Furthermore, the adsorption of 5MTAR on calcined and non-calcined MCM-41 (MCM-41-NC) was studied and the derived adsorbents were used in the adsorption of Cu(II) in water samples. 5MTAR was preferably adsorbed on MCM-41-NC compared to calcined MCM-41 with the adsorption capacities of 131.6 mg/g for MCM-41-NC and 7.0 mg/g for calcined MCM-41. Adsorption of Cu(II) on 5MTAR-immobilized MCM-41-NC followed Langmuir adsorption and pseudo-second-order kinetic model. The adsorption capacity for Cu(II) was 24.2 mg/g in a pH 5.0 solution and the adsorption time of 15 min. The adsorbent showed high selectivity to Cu(II) and could be regenerated at least 5 times by soaking in 0.2 M EDTA for 30 min.

School of Chemistry Student’s Signature______________________

Academic Year 2015 Advisor’s Signature______________________

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I would like to acknowledge the scholarship under strategic scholarships for Frontier Research Network Ph.D. Program from the Commission on Higher Education of Thailand. Thanks also to all good friends in the Material Chemistry Research Group and other friends for their friendship and all their helps. Finally, a note of thanks goes to my family, for their encouragement and supporting me spiritually throughout my life.

Kotchakriangkrai Sanai

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CHAPTER I

INTRODUCTION AND BACKGROUND

1.1 Introduction

Copper is one of the heavy metals used in many applications such as electrical devices, construction and industrial machinery as shown in Figure 1.1 (http://www.kme.com/en/a_wide_range_of_uses). Consequently, copper waste could be generated from its applications. At low concentration, it is an essential nutrient for plants, animals and human, important to carbohydrate and lipid metabolism (Yari and Afsha, 2006). However, exposure to high amount of copper ion can be harmful to human health and cause, for example nose and throat irritation, vomiting, diarrhea, and liver and kidneys damage. Drinking water and food can be potential sources for an intense copper exposure. The World Health Organization (WHO) suggested that the concentration of copper(II) ions (Cu(II)) in drinking water should not exceed 2 mg/L (WHO, 2003). Therefore, periodically monitoring of Cu(II) level in the environment and drinking water is required. In the tap water, contamination of Cu(II) could be from corrosion of copper pipes and fitting. Cu dissolves into water as Cu(II) when contacts with water where pH < 6.5. Carbon dioxide dissolved in tap water is major contributor for the pH value of the water which is less than 6.5. Together with oxygen (O_2) in the acidified water, corrosion of copper in the the plumbing system could be resulted (Oliphaut, 2010).

Cu(II) could be determined by many techniques for example, flame atomic absorption spectrometry (Duran, Gundogdu, Bulut, Soylak, Elci, Senturk and Tufekci, 2007), graphite furnace atomic absorption spectroscopy (Cabon, 2002), inductively coupled plasma optical emission spectrometry (ICP-OES) (He, Hu, Jiang, Chang, Tu and Zhang, 2010) and voltammetry (Lua, Longb, Li, Zhana, Zhaoa and Liua, 2004). These methods provided detection limits for Cu(II) in part per billion levels. However, these methods required relatively expensive equipment, a skilled operator and sample pretreatment.

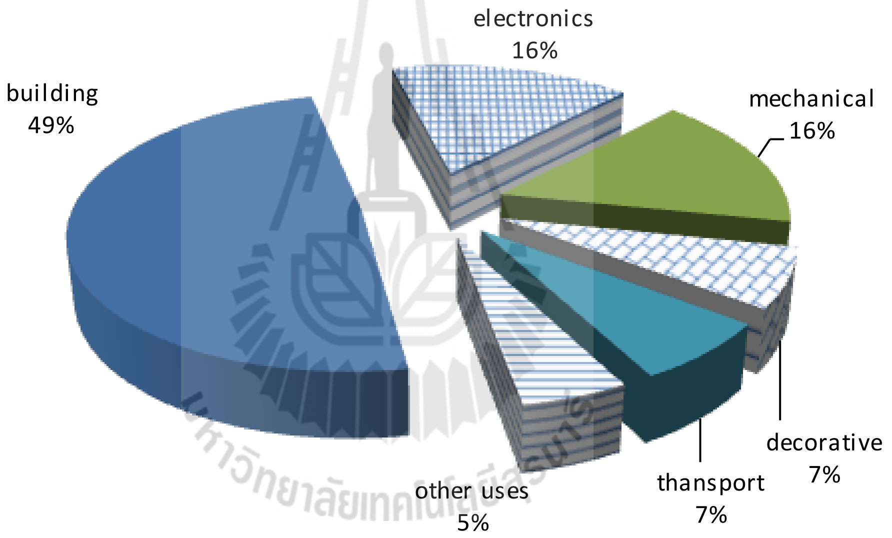


Figure 1.1 Applications of copper (http://www.kme.com/en/a_wide_range_of_uses).

Optical chemical sensors offer an alternative for determination of metal ions in aqueous samples. The sensors have some advantages such as high sensitively, electrical passiveness (nonelectrical contact with the sensor) and multiplexing capabilities. Optical chemical sensors based on an organic dye immobilized into a suitable polymer matrix for the determination of Cu(II) have been developed in recent years and some of these are summarized in Table 1.1. Some of the developed sensors might be less useful for practical applications due to complication of sensor fabrication procedures (Hashemi et al., 2008), poor limit of detection (Mahendra et al., 2004), unavailable commercial sensing reagent (Chamjangali et al., 2009; Sands et al., 2002) and serious influence by cationic interferents (Chamjangali et al., 2009). Therefore, in this research, we have attempted to construct optical chemical sensor for the determination of Cu(II) in aqueous solutions based on 5-methyl-4-(2-thiazolylazo)resorcinol (5MTAR) which are simple to fabricate and highly selective to Cu(II).

Wada and Nakagawa (1975) synthesized a chromogenic dye, 5MTAR and found that the dye could form complex with Cu(II) and Hg(II). Chung and Chung (2003) used 5MTAR as a chelating agent for the determination of Co(II) at pH 7.2 by a reversed-phase capillary high-performance liquid chromatography. There have been no reports of using 5MTAR as a sensing reagent for the determination of Cu(II) in aqueous solutions. Therefore, in this work, 5MTAR was incorporated in various polymer matrices and evaluated for the performance in the detection of Cu(II).

Table 1.1 Some optical chemical sensors for Cu(II) determination.

Sensing reagent	Supporting matrix	LOD (ppb)	Main interferent	Signal mode	References
Cupron	XAD-2[1]	5016.5	N/A[2]	Reflectance	Mahendra, Gangaiya, Sotheeswaran and Narayanaswamy, 2003
FSBF	XAD-7	10160.0	N/A	Reflectance	Mahendra, Gangaiya, Sotheeswaran and Narayanaswamy, 2002
Lucifer yellow	CUD[3]	N/A	Hg(II)	Fluorescence	Mayr and Werner, 2002
PAN[4]	Nafion	127.0	N/A	Absorbance	Coo and Belmonte, 2002
DPAN[5]	Nafion	N/A	Ni(II)	Absorbance	Sands, Cardwell, Cattrall, Farrell, Iles and Kolev, 2002
Pyrocatechol violet	PVC	N/A	N/A	Absorbance	Steinberg, Lobnik and Wolfbeis, 2003
Salophen	PVC	3.0	Ag(I), Na(I)	Absorbance	Gholivand, Niroomandi, Yari and Joshagani, 2005
Dithizone	TAC[6]	76.2	Hg(II)	Absorbance	Safavi and Bagheri 2005
Calmagite	Agarose	0.1	No	Absorbance	Hashemi, Glab and Ingman, 2008

Table 1.1 Some optical chemical sensors for Cu(II) determination. (Continued).

Sensing reagent	Supporting matrix	LOD (ppb)	Main interferent	Signal mode	References
PPDOT[7]	TAC	50.8	Co(II), Fe(II), Ag(I), Ni(II), EDTA	Absorbance	Chamjangali, Soltanpanah and Goudarzi, 2009
1,1′-(4-nitro-1,2-phenylene)bis(azan-1-yl-1-ylidene)bis(methan-1-yl-1-lidene)dinaphthalen-2-ol	PVC	25.4	No	Absorbance	Ghaedi, Tashkhourian, Montazerozohori, Biyareh and Sadeghian, 2014
6-bromo-3-(2-methyl-2,3-dihydrobenzo[d]thiazol-2-yl)-2H-chromen-2	TAC	15.9	No	Absorbance	Pourbasheer, Morsali, Banaei, Aghabalazadeh, Ganjali and Norouzi, 2014
(N′1E,N′2E)-N′1,N′2-bis(pyridine-2-ylmethylene)oxalohydrazide	PVC	51.4	No	Absorbance	Ghaedi, Shahamiria, Mirtamizdoust, Hajati and Taghizadeh, 2015

[1] Polystylene-diphenylbenzene co-polymer

[2] Not reported

[3] Cellulose beads/polyurethane hydrogel D4

[4] 1-(2-pyridylazo)-2-naphthol

[5] 4-decyloxy-2-(2-pyridylazo)-1-naphthol

[6] Triacetyl cellulose

[7] 1-phenyl-1,2-propanedione-2-oximethiosemicarbazone

1.2 Research objectives

The aims of this work are as follows,

1. To develop optical chemical sensing films based on 5MTAR as a sensing reagent for the determination of Cu(II)

2. To investigate various supporting matrices that would be appropriate for immobilization of 5MTAR

3. To study parameters that affect response of sensing films to Cu(II) and

4. To evaluate the performance characteristics of the developed sensing films in the determination of Cu(II) in aqueous samples.

1.3 Scope and limitations of the study

Scope and limitations of this work are as follows,

1. 5MTAR will be used as a sensing reagent for Cu(II).

2. The dye and dye-metal complexes in aqueous solutions, the dye and dye-metal complexes in supporting matrices (chitosan, Nafion, silica particles/chitosan and MCM-41) will be characterized by a fiber optics uv-vis spectrometer.

3. Response of the developed sensing films with and without metal ions will be investigated with the fiber optics UV-Vis spectrometer.

4. Performance characteristics of the developed sensing films such as a response time, lifetime, a working range, and a limit of detection will be evaluated.

1.4 Background

1.4.1 Chemical sensors

Sensors can be classified into two groups that are physical sensors and chemical sensors. Physical sensors are sensitive to physical parameters such as temperature, pressure, magnetic field and force. A chemical sensor is a device which responds selectively to an analyte upon binding with the analyte (Cattrall, 1997). Chemical sensors can be categorized as optical, electrochemical, heat sensitive, and mass sensitive chemical sensors based on a type of a transducer employed for signal generation.

Figure 1.2 shows a simplified diagram of components and function of a chemical sensor. When an analyte reacts with a receptor part of the chemical sensor, a chemical signal is produced and converted into an analytical signal by a transducer. An optical chemical sensor is of interest in this work because it is easy to prepare, simple to use, low cost, applicable as a test kit, and high selectivity and sensitivity. The main focus of this work is to develop the receptor part which composes mainly of a sensing reagent and a suitable supporting matrix.

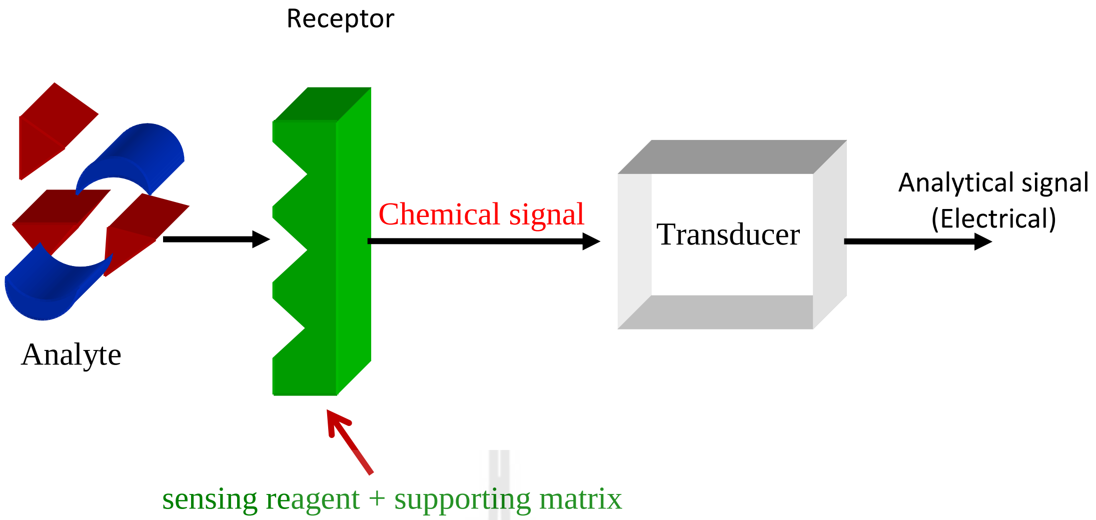


Figure 1.2 Components and function of a chemical sensor.

1.4.2 Sensing reagents

Organic dyes or indicator dyes are commonly used as sensing reagents for optical chemical sensors and can be classified according to their functional groups as azo, carbonyl, nitro, cyanide and so on. Table 1.1 shows organic dyes that were used as reagents in chemical sensors. In this work, 5MTAR is used as a sensing reagent and its chemical structure is shown in Figure 1.3. It is a derivative of 4-(2-thiazolylazo)resorcinol (TAR) and can selectively form complex with Cu(II) ion in 20% dioxane with a formation constant of 1.78 x 10^4 (Wada and Nakagawa, 1975).

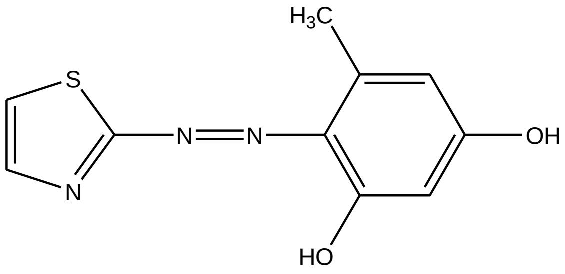


Figure 1.3 A structure of 5MTAR.

1.4.3 Supporting matrix

Supporting matrix is a material that is used to immobilize a sensing reagent. Polymers are widely used as supporting matrices and in some cases they can act as a selective element or a transducer. Requirements of polymers to be used as a supporting matrix are homogenous, compatible with a sensing reagent, low intrinsic absorbance or fluorescence (for optical chemical sensors), long operation lifetime and physically and chemically stable (Mohr, 2006). Some polymers for example, chitosan, polyvinyl chloride (PVC) and Nafion have been used in optical chemical sensors and are discussed here.

Chitosan is a cationic natural polysaccharide. It has hydroxyl and amine functional groups on the polymer chain as shown in Figure 1.4. These functional groups can be modified to couple with organic dyes under a mild reaction condition. In addition, the active groups on the polymer chain can form hydrogen bonds with water. Consequently, a chitosan film swells in an aqueous solution. Swelling can result in film instability. Several methods have been used to chemically modify natural chitosan. The hydroxyl and amine groups on a glucosamine unit of chitosan provide the reactive sites for crosslinking reaction which improves mechanical strength, chemical stability, and hydrophilicity of the polymer.

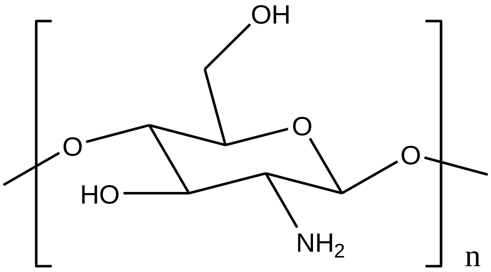


Figure 1.4 A structure of chitosan.

PVC is a hydrophobic polymer which is useful in sensor applications because it is insoluble in aqueous solutions and optically transparent. A structure of PVC is shown in Figure 1.4. A film made from PVC alone is relatively rigid which is resistant to ion diffusion. Therefore, a plasticizer is added to reduce the rigidity and enhance the permeability the film. Tributylphosphate (TBP) (Fouladgar and Ensafi, 2010), dioctyl sebacate (DOS) (Oehme et al., 1998) and dioctyl phthalate (DOP) (Gholivand et al., 2005) are examples of plasticizers used in chemical sensors.

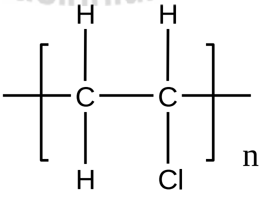


Figure 1.5 A structure of PVC.

Nafion is an ionic polymer that consists of a polytertrafluoro ethylene backbone and perfluorovinyl ether pendent side chain with sulfonic acid group built into its structure as shown in Figure 1.6. Cationic dyes can be simply immobilized onto Nafion by ion exchange process (Mohr, 2006).

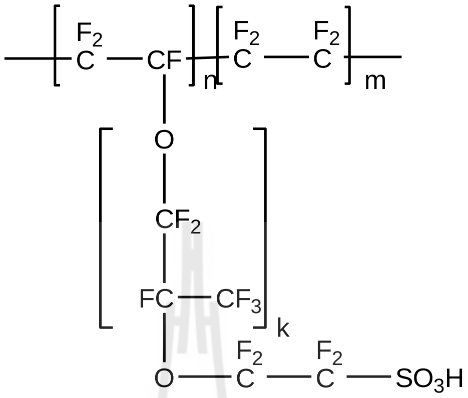


Figure 1.6 A structure of Nafion.

To fabricate sensing films, a sensing reagent can be physically or chemically immobilized onto a supporting matrix (Oehme et al., 1998; Mohr, 2006). Physical immobilization relies on electrostatic interaction between the sensing reagent and the supporting matrix. A physical immobilization method is simple; however, leaching of the sensing reagent from the support could affect sensitivity and stability of the chemical sensor. In chemical immobilization, a chemical bond is formed between the sensing reagent and the supporting matrix. Processes for chemical immobilization are rather more complicated because chemical modification of the sensing reagent and the supporting matrix might be required before chemically bonded the two together. In addition, the modification should not alter the sensing characteristics of the chemical sensor.

The amount of immobilized sensing reagent is one of the parameters that influences the sensor sensitivity as the maximum amount immobilized will result in optimum signal response from an analyte. For optical chemical sensors in the form of sensing films, the amount of immobilized sensing reagent in a film is limited to the amount of functional groups of the polymer support that can bind with the sensing reagent. Therefore, in this work we will also investigate the effect of silica microparticles or silica nanoparticles incorporated into the polymer film on the performance characteristics of the sensing film. The silica particles will also be modified with appropriate functional groups on their surface.

1.5 References

Cabon, J. Y. (2002). Determination of Cu and Mn in seawater by graphite furnace atomic absorption spectrometry with the use of hydrofluoric acid as a chemical modifier. **Spectrochimica Acta Part B**. 57: 939-950.

Cattrall, R. W. (1997). Chemical Sensors, Oxford University Press, New York.

Chamjangali, M. A., Soltanpanah, S., Goudarzi, N. (2009). Development and characterization of a copper optical sensor based on immobilization of synthesized 1-phenyl-1,2-propanedione-2-oxime thiosemicarbazone on a triacetylcellulose membrane. **Sensors and Actuators B: Chemical**. 138: 251-256.

Coo, L. D., Belmonte, C. J. (2002). Nafion-PAN optical chemical sensor: optimization by FIA. **Talanta**. 58: 1063-1069.

Duran, C., Gundogdu, A., Bulut, V. N., Soylak, M., Elci, L., Senturk, H. B., Tufekci M. (2007). Solid-phase extraction of Mn(II), Co(II), Ni(II), Cu(II), Cd(II) and Pb(II) ions from environmental samples by flame atomic absorption spectrometry (FAAS). **Journal of Hazardous Materials**. 146: 347-355.

Dybko, A., Wroblewski, W. (2002). Analyte recognition and signal convertion in potentiometric and optical chemical sensors. **Journal of Environmental Studies**. 11: 5-10.

Faridbod, F., Ganjali, M. R., Dinarvand, R., Norouzi P. (2007). The fabication of potentiometric membrane sensors and their appications. **American Journal of Biotechnology**. 25: 2960-2987.

Fouladgar, M., Ensafi, A. A. (2010). A novel optical chemical sensor for thallium(III) determination using 4-(5-bromo-2-pyridylazo)-5-(diethylamion)-phenol. **Sensors and Actuators B: Chemical**. 143: 590-594.

Ghaedi, M., Shahamiri, A., Mirtamizdoust, B., Hajati, S., Taghizadeh, F. (2015). A novel polyvinyl chloride-membrane optical sensor for the determination of Cu^{2+} ion based on synthesized (N$'^{1}$E,N$'^{2}$E)-N$'^{1}$,N$'^{2}$-bis(pyridine-2-ylmethylene)oxalohydrazide: Experimental design and optimization. **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy**. 138: 878–884.

Ghaedi, M., Tashkhourian, J,, Montazerozohori, M., Biyareh, M. N., Sadeghian, B. (2013). Highly selective and sensitive determination of copper ion by two novel optical sensors. **Arabian Journal of Chemistry**. [online]. Available: http://dx.doi.org/10.1016/j.arabjc.2013.08.008.

Gholivand, M. B., Niroomandi, P., Yari, A., Joshagani, M. (2005). Characterization of an optical copper sensor based on N,N-bis(salycilidene)-1,2-phenylenediamine. **Analytica Chimica Acta**. 538: 225-231.

Hashemi, P., Abolghasemi, M. M., Alizadeh, K., Zarijani, R. A. (2008). A calmagite immobilized agarose membrane optical sensor for selective monitoring of Cu^{2+}. **Sensors and Actuators B: Chemical**. 129: 332-338.

He, Q., Hu, Z., Jiang, Y., Chang, X., Tu, Z., Zhang L. (2010). Preconcentration of Cu(II), Fe(III) and Pb(II) with 2-((2-aminoethylamino)methyl)phenol-functionalized activated carbon followed by ICP-OES determination. **Journal of Hazardous Materials**. 175: 710-714.

http://www.kme.com/en/a_wide_range_of_uses, accessed March 2014.

Hulanicki, A., Glab, S., Ingman, F. (1991). Chemical sensor definition and classification. **Pure and Applied Chemistry**. 63: 1247-1250.

Leelasattarathkul, T., Liawruangrath, S., Rayanakorn, M., Liawruangrath, B., Oungpipat, W., Youngvises, N. (2007). Greener analytical method for the determination of copper(II) in wastewater by micro flow system with optical sensor. **Talanta**. 72: 126-131.

Lua, G., Longb, D., Li D., Zhana, T., Zhaoa, H., Liua, C. (2004). Determination of copper in food by stripping voltammetry using Cu(II)-[2-(2,3,5-triazolylazo)-5-dimethylaninobenzonic] complex. **Food Chemistry**. 84: 319-322.

Mahendra, N., Gangaiya, P., Sotheeswaran, S., Narayanaswamy, R. (2002). Investigation of a Cu(II) fibre optic chemical sensor using fast sulphon black F (FSBF) immobilised onto XAD-7. **Sensors and Actuators B: Chemical**. 81: 196-201.

Mahendra, N., Gangaiya, P., Sotheeswaran, S., Narayanaswamy, R. (2003). Investigation of a fibre optic copper sensor based on immobilized α-benzoinoxime (cupron). **Sensors and Actuators B: Chemical**. 90: 118-123.

Mayr, T., Werner, T. (2002). Highly selective optical sensing of copper(II) ions based on fluorescence quenching of immobilized Lucifer yellow. **Analyst**. 127: 248-252.

Mohr, J. G. (2006). Optical Chemical Sensors, **NATO Science Series**. 224: 297-321.

Oehme, I., Prattes, S., Wolfbeis, S. O., Mohr J. G. (1998). The effect of polymeric supports and methods for immobilization on the colorimetric reagent zincon. **Talanta**. 47: 126-131.

Oliphaut, R. A. (2010). Causes of copper corrosion in pumping system, Foundation of water research. UK.

Pourbasheer, E., Morsali, S., Banaei, A., Aghabalazadeh, S., Ganjali, M. R., Norouzi, P. (2014). Design of a novel optical sensor for determination of trace amounts of copper by UV/vis spectrophotometry in the real samples. **Journal of Industrial and Engineering Chemistry**. [online]. Available: http://dx.doi.org/10.1016/j. jiec.2014.12.011.

Safavi, A., Bagheri, M. (2005). Design of a copper(II) optode based on immobilization of dithizone on a triacetylcellulose membrane. **Sensors and Actuators B: Chemical**. 107: 53-58.

Sands, T. J., Cardwell, T. J., Cattrall, R. W., Farrell, J. R., Iles, P. J., Kolev S. D. (2002). A highly versatile stable optical sensor based on 4-decyloxy-2-(2-pyridylazo)-1-naphthol in Nafion for the determination of copper. **Sensors and Actuators B: Chemical**. 85: 33-41.

Steinberg, I. M., Lobnik, A., Wolfbeis, O. S. (2003). Characterisation of an optical sensor membrane based on the metal ion indicator pyrocatechol violet. **Sensors and Actuators B: Chemical**. 90: 230-235.

Wada, H., Nakagawa, G. (1975). Some 4-(2-thaiazolyazo)resocinol derivatives as analytical reagents. **Bunseki Kagaku**. 24: 758-761.

WHO (2003). Copper in drinking-water. Background document for preparation of WHO Guidelines for drinking water quality. Geneva. World Health Organization.

Yari, A., Afshari, N. (2006). An optical copper(II)-selective sensor based on a newly synthesized thioxanthone derivative, 1-hydroxy-3,4-dimethylthioxanthone. **Sensors and Actuators B: Chemical**. 119: 531-537.

Youngchan, S., Deokkyu, L., Kangtaek, L., Kyung, H. A., Bumsang, K. (2008). Surface properties of silica nanoparticles modified with polymers for polymer nanocomposite applications. **Journal of Industrial and Engineering Chemistry**. 14: 515-519.

CHAPTER II

AN OPTICAL CHEMICAL SENSOR FOR THE DETERMINATION OF COPPER(II) BY USING 5-METHYL-4-(2-THIAZOLYAZO)RESORCINOL IMMOBILZED ON CHITOSAN FILM

2.1 Abstract

A chromogenic sensing reagent, 5-methyl-4-(2-thiazolylazo)resorcinol (5MTAR), was immobilized on a chitosan film and the film was used for the determination of Cu(II). A chitosan film was made from a solution of 1 %w/v of chitosan in acetic acid by a casting technique. The obtained film with the size of 0.8 x 4.0 cm^2 was immobilized with 5MTAR. The optimum condition for the immobilization was achieved using 5MTAR concentration of 0.05 %w/v and a contact time of 120 min. The developed sensing film when immersed in a solution of Cu(II) at pH 4.0 had adsorption maximum at 555 nm. The sensing film responded linearly to Cu(II) in the concentration range of 0.1 to 8.0 ppm with a response time of 10 min and the limit of detection was 32 ppb. Percent relative standard deviations (%RSD) were 4.40% and 1.74% when 6 sensing films were tested in the solutions with 5.0 and 8.0 ppm Cu(II), respectively. The sensing film could be regenerated by soaking in 0.1 M HCl for 1 min. The major interferences were Hg(II) and Zn(II). The sensing film was used for the determination of Cu(II) in water samples with satisfactory results.

2.2 Introduction

Chitosan is a poly[β-(1→4)-2-amino-2-deoxy-D-glucopyranose]. The polymer has reactive functional groups which are hydroxyl and amine that can be useful for chemical modification. These functional groups can also interact with water via hydrogen bonding that results in swelling of chitosan. Modification of chitosan by crosslinking with epichlorohydrin or glutaraldehyde reduces the swelling effect (Rinaudo, 2006; Ngah, Endud and Mayanar, 2002).

Chitosan has some interesting features such as optical transparency, biodegradability, non-toxicity, high mechanical strength, low cost, ease of modification and high amount of active groups (Loo-Teck, Yong and Huijun, 1998). A thin and optically transparent chitosan film can be formed simply from an acidic aqueous solution of chitosan. Therefore, from these advantages it is used in many applications such as metal and organic dye adsorption, solid phase extraction, electrochemical and optical chemical sensors.

Some applications of chitosan as a supporting matrix for a sensing reagent in optical chemical sensors have been reported. Sombatsri, Wittayakun, Sanai, Kajsanthia and Prayoonpokarach (2012) embedded disodium-1-nitroso-2-naphthol-3,6-disulfonate (nitroso-R) on a chitosan film crosslinked with epichlorohydrin via electrostatic interaction. The sensor was used for the determination of Co(II) in water samples. Yusof and Ahmad (2002) developed an optical Pb(II) sensor based on gallocynin immobilized in chitosan membrane. The sensing reagent interacted with the membrane via hydrogen bonding. Yusof and Ahmad (2002) reported an optical chemical sensor for the determination of Co(II) in water based on 2-(4-pyridylazo)resorcinol (PAR) hydrogen bonded with a chitosan membrane. Abdullah, Ahmad, Karuppiah, Heng and Sidek (2006) used hydrogen bonded tyrosinase on chitosan film as a biosensor for the determination of phenol in industrial wastewaters.

In this work, chitosan was investigated as a supporting matrix for 5MTAR to develop a Cu(II) sensor. The conditions for the immobilization were investigated. The performance of the developed sensor was evaluated and the sensor was applied for determination of Cu(II) in tap water samples.

2.3 Experimental

2.3.1 Preparation of solutions

Solutions of 5MTAR (95% Aldrich) with a concentration range of 0.01-1.00% w/v were prepared by dissolving appropriate amount of 5MTAR in 40% v/v ethanol in DI water to produce 100 mL solutions.

A stock solution of chitosan was prepared by dissolving 1.0 g chitosan powder (MW > 600,000, Fluka) into 100 mL of 1% v/v acetic acid solution with stirring provided for 3 h. The obtained solution was filtered, sonicated for 30 min to remove air bubbles and kept at 4 ^{o}C for further used.

A stock solution of 500 ppm Cu(II) was prepared by dissolving 0.1901 g of $Cu(NO_3)_2 \cdot 3H_2O$ (99.99%, Merck) in DI water and the volume of the solution was adjusted in a 100-mL volumetric flask.

Phosphate buffer solutions with pH in the range of 3-9 were prepared by mixing 0.1 M phosphoric acid (85%, UNIVAR) and 0.1 M dibasic sodium phosphate (99.5%, Riedel-de Haën). The amount of the phosphate salt and the volume of phosphoric acid solution were varied to obtain the desired pH and a pH-meter was used to monitor the pH of the solution.

A 0.25% v/v glutaraldehyde solution was prepared by dilution of 10 mL 25% w/w glutaraldehyde (Unilab) in a 1000-mL volumetric flask using DI water as a solvent.

2.3.2 Preparation of chitosan sensing film

A 4 mL of 1 % w/v chitosan stock solution was cast on an area 8 x 19 cm^2 of a transparent film and dried at 60 ^oC in a hot air oven for 15 h. The dried film was cut into a size of 4.0 x 9.5 cm^2 and soaked in 0.25% v/v glutaraldehyde for 7.5 min to crosslink chitosan. After the film was removed from the glutaraldehyde solution, it was washed with 20 mL DI water for 3 times and dried at room temperature. The dried film was cut into a size of 0.8 x 4.0 cm^2.

Parameters that affected the amount of 5MTAR immobilized in chitosan films were studied. These parameters are solvents, dye concentration and contact time. Solutions prepared from a mixture of ethanol and water were used to dissolve the dye and the percent of ethanol was varied in the range of 30-100%.

To immobilize 5MTAR, a chitosan film with the size of 0.8 x 4.0 cm^2 was immersed into 7.0 mL solution of the dye contained in 15-mL vial for a given time. After that, the film was washed 2 times with 10 mL of 1 M NaCl and then with DI water. The film was dried at room temperature and kept in desiccator. The concentration of the dye was varied in the range of 0.001-0.1 % w/v.

Immobilization time was investigated by varying the time that the film was soaked in 0.05 %w/v 5MTAR solution from 10 to 240 min. After immobilization, the film was washed with 1.0 M HCl for 10 min and DI water. The absorbance of the film was measured at 467 nm which was the absorption maximum of the dye.

Effect of the film thickness on the response of the sensing film was investigated. It is assumed that the thickness increased with the volume of the chitosan solution used for making the film. The thickness of the films was varied by controlling the volume of the chitosan solution (20-160 µL) cast onto an area of 1 x 1 cm^2 transparent film. After drying, the films were soaked in 0.25% v/v glutaraldehyde solution for 7.5 min with stirring provided. After crosslinking, the films were washed with DI water. To immobilized the sensing reagent, the films were soaked in 0.05% w/v 5MTAR solution with shaking provided for 2 h and then washed with 1 M NaCl until the absorbance of 5MTAR in the washed solution could not be detected.

2.3.3 Absorbance measurement with the sensing films

Absorbance of a sensing film with the size of 0.8 x 4.0 cm^2 was measured with a fiber optic spectrometer (CHEMUSB4-VIS/NIR, Ocean Optics). The film was dipped in 2 mL of a studied solution that was contained in a 10-mm cuvette as shown in Figure 2.1. A signal obtained from the sensing film dipped in 2 mL of phosphate buffer was used as a blank signal. The wavelength range in this study was 350-800 nm.

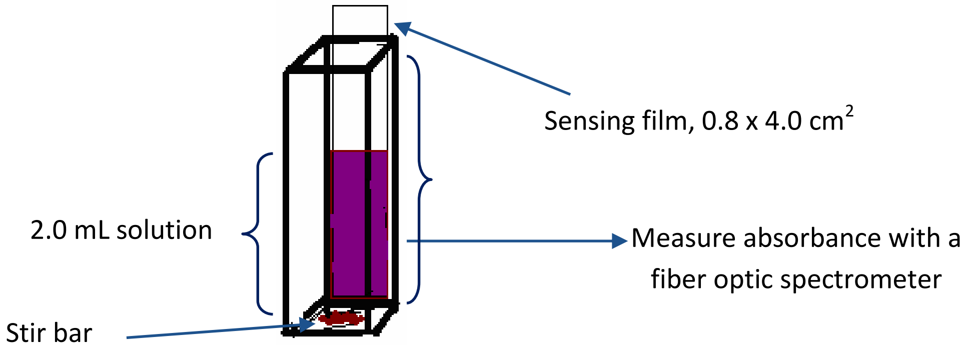


Figure 2.1 A setup for absorbance measurement with the sensing film.

2.3.4 Effect of the pH on the response of the sensing film to Cu(II)

The effect of the pH on the response of the sensing films to Cu(II) was studied in phosphate, acetate and citrate buffer. The pH of the solutions was varied in the range of 2-7. The concentration of Cu(II) was fixed at 5.0 ppm and the absorbance at 555 was measured at 10 min after the film was exposed to the Cu(II) solution.

2.3.5 Regeneration and reversibility

To reuse the sensing films, Cu(II) has to be removed from the film and this could be achieved only when the reaction between Cu(II) and 5MTAR is reversible. Solutions of HCl, EDTA and NaCl were investigated as regenerating reagents. The sensing film after exposed to 5.0 ppm Cu(II) was used in the regeneration process. The film was dipped into a beaker containing 20 mL of the studied reagent with stirring provided. After the film was removed from the reagent, it was washed with DI water and phosphate buffer at pH 4.5. The absorbance at 555 nm of the sensing film in phosphate buffer at pH 4.5 was then recorded and compared to the signal of the film before exposed to the Cu(II) solution.

2.3.6 Reproducibility and Repeatability

Reproducibility in the process of the film preparation was evaluated using 16 sensing films prepared from 4 batches which 4 films was randomly selected from each batch. Each sensing film was soaked in 5.0 ppm Cu(II) solution and the absorbance was measured at the optimum condition. %RSD of the response signals was calculated.

The repeatability of the response signal was assessed using 12 sensing films prepared from the same batch. The films were tested in both 1.0 and 5.0 ppm Cu(II) solutions (6 films/solution) and the measurements were made at the optimum condition. %RSDs of the response signals according to the study in each Cu(II) solution were calculated.

2.3.7 Stability and lifetime of the sensing film

To evaluate the stability of the sensing films in aqueous solutions, the sensing films were soaked into 2.0 mL of 0.1 M HCl or 0.1 M phosphate buffer at pH 4.5 for 4 h. The absorbance signals of the sensing films were recorded every 5 min.

Lifetime of the sensing film was studied by immersing the sensing film in 10.0 mL phosphate buffer for 3 weeks. The absorbance of the sensing films was measured at the wavelength at 467 nm for every 3 days.

2.3.8 Selectivity

The effect of foreign species on the determination of Cu(II) was studied at the optimum measurement condition. A sensing film was immersed in 3.15 x 10^{-5} M Cu(II) solution and the absorbance denoted as A was rescored. Similar absorbance measurement was done with a solution of Cu(II) mixed with a studied ion. The absorbance obtained from this measurement step is denoted as A_i. The concentration of Cu(II) and the studied ion was fixed at 3.15 x 10^{-5} M. To evaluate the interference effect, %error was calculated using equation (2.1) as follows,

$$\%\text{error} = \frac{A - A_i}{A} \times 100 \qquad (2.1)$$

An ion that contributes the %error within ±5.0% is considered as not a significant interference.

2.3.9 Analysis of tap water samples

To test the analytical validity of the developed sensing films, determination of Cu(II) in water samples was performed. Tap water samples were collected from two locations at F1 building, Suranaree University of technology, Thailand. Water samples were collected and stored in 500-mL polypropylene bottles after a faucet was turned on for 3 min. The water samples were used without any treatment. For comparison purpose, the concentration of Cu(II) in the samples was also measured by a flame atomic absorption spectrometer (FAAS) using AOAC method no. 974.24 (AOAC International, 1995).

2.4 Results and discussion

2.4.1 Solution study of Cu(II) and 5MTAR reaction

A solution of 2.72 x 10^{-5} M 5MTAR which was prepared by dissolving 5MTAR in a solution with pH 3.5 of 0.1 M phosphate buffer mixed with acetonitrile with the volume ratio of 70:30, reacted with Cu(II) causing a change in the absorption spectrum as shown in Figure 2.2. A new absorption peak appeared at 550 nm, which was attributed to Cu(II)-5MTAR complex. In addition, the color of the solution changed from orange to dark brown. The stoichiometric ratio of Cu(II):5MTAR was 1:2 evaluated by a method of the continuous variation. The result also agreed with that obtained using a mole ratio method as shown in Appendix A. Absorption spectra of 5MTAR in solutions at different pHs are shown in Appendix B.

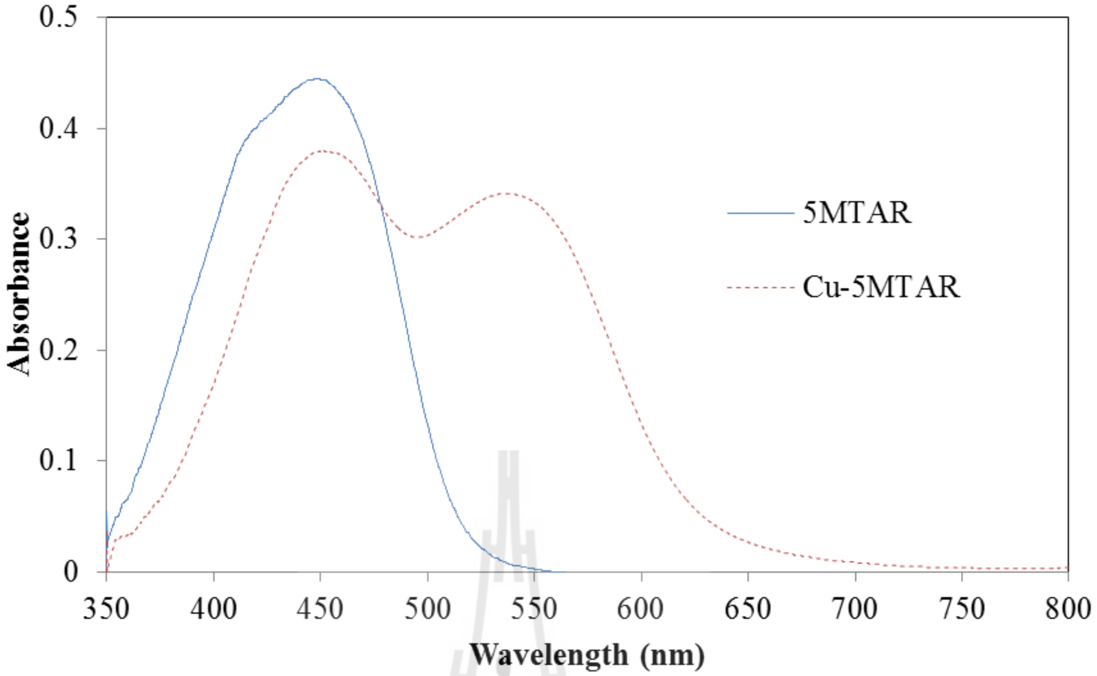


Figure 2.2 Absorption spectra of 5MTAR solution and 5MTAR mixed with Cu(II). The concentration of 5MTAR in the solution was 2.72 x 10^{-5} M and that of Cu(II) was 1.5 x 10^{-5} M.

A possible structure of the complex is shown in Figure 2.3. The structure is similar to that of Mn(II), Co(II) and Ni(II) complexes of 4-(2-thiazolyazo)resorcinol suggested by Karipcin, Dede, Percin-Ozkorucuklu and Kabalcilar (2010). When Cu(II) formed complex with 5MTAR, the hydrogen of one of the hydroxyl groups on the dye molecule was liberated. Therefore, this reaction is pH dependent. The formation constant of the complex was 2.44 x 10^{10} which was calculated using a method reported by Likussar and Boltz (1971). The proton dissociation equilibria of 5MTAR is shown in Appendix C.

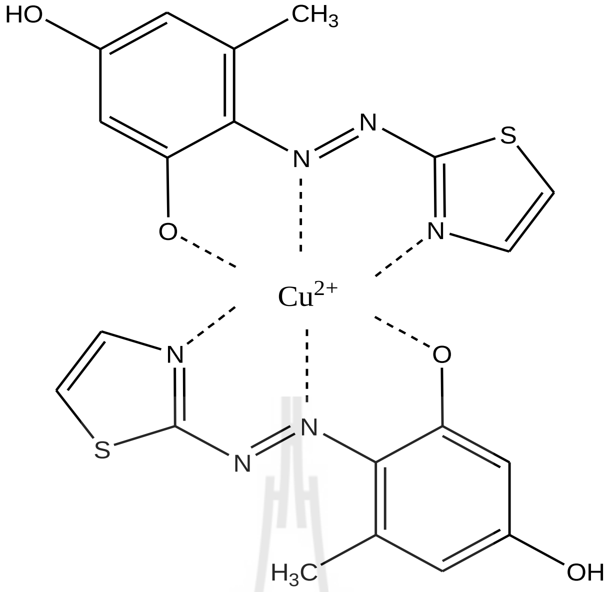


Figure 2.3 Suggested structure of 5-MTAR-Cu(II) complex.

2.4.2 Response of the sensing films to Cu(II)

The absorption spectra of the sensing film soaked in phosphate buffer at pH 4.5 had the absorption maximum at 467 nm as shown in Figure 2.4. When the sensing film was dipped in 12.5 ppm Cu(II) solution at pH 4.5, there is a change in the absorption spectrum due to Cu(II)-5MTAR complex. The blank corrected Cu-5MTAR/chitosan spectrum shows an absorption maximum at 555 nm. Therefore, the absorbance measurement at 555 nm was chosen for further work.

The films with different thicknesses were tested for their responses to 12.5 ppm Cu(II) in phosphate buffer pH 4.5 and the results are shown in Table 2.1. It was assumed that the thicker film was obtained with the larger volume of chitosan solution. Sensing film no.1 has the shortest response time, but the lowest response signal. A longer response time and a larger signal were observed when the film thickness was increased. The larger signal was obtained with a thicker film because of more amount of 5MTAR immobilized onto the film and the longer response time was due to a longer diffusion path for Cu(II). Although higher signals were obtained with the thicker films, the higher sensing reagent concentration could result in an incoherent response because of an inhomogeneous distribution of the reagent in the film (Ahmad and Narayanasswamy, 1995). Therefore, in this work, the film fabricated with 26 26 µL/cm^2 chitosan solution was selected for further study. In addition, when preparing the film with the volume of chitosan solution < 26 µL/cm^2, the dispersion of chitosan on the transparent film was poor.

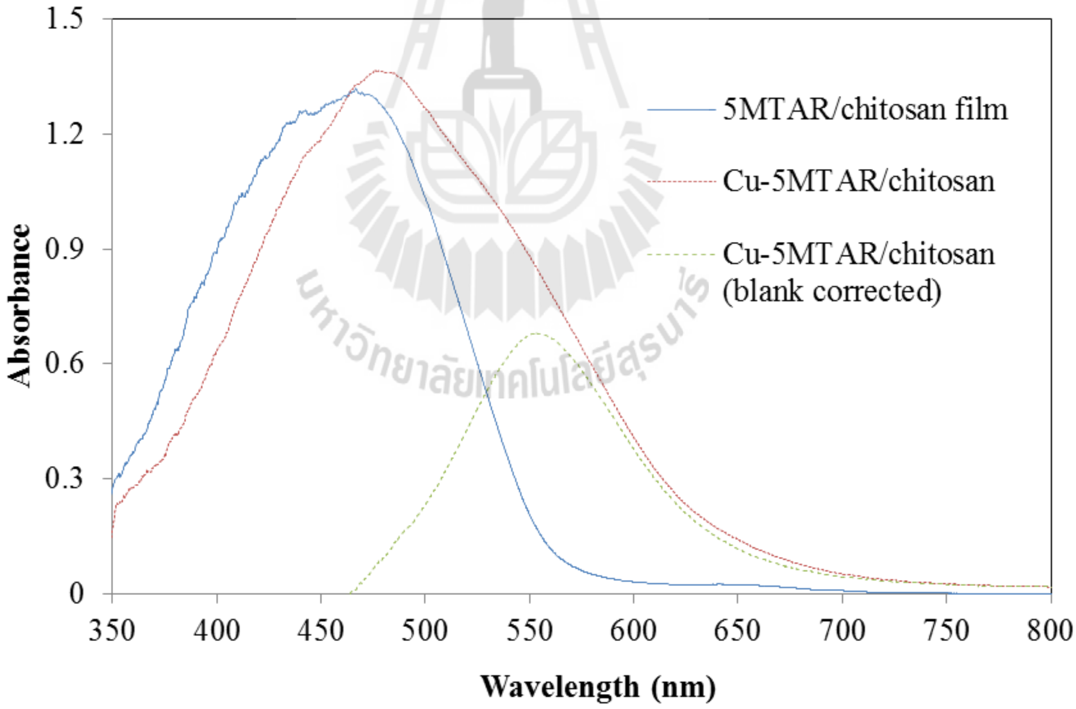


Figure 2.4 Absorption spectra of the sensing film and the sensing film in 12.5 ppm Cu(II) solution. The pH of the solution was controlled at 4.5 with phosphate buffer.

Table 2.1 Effect of the film thickness on the response time and the response signal of the sensing films.

Sensing film no.	**Chitosan solution (μL/cm^2)**	**Response time[a] (min)**	**A (555 nm)**
1	26.3	17	0.681
2	41.1	30	1.240
3	82.2	50	1.547
4	164.5	>80	>1.401

[a] A response time is defined as the time that the signal reaches ~95% of the signal at the equilibrium state.

2.4.3 Study of immobilization conditions

The effect of a solvent on the dye immobilization was studied using 0.05% w/v 5MTAR. Solutions of ethanol mixed with water were used to dissolve 5MTAR and the percent of ethanol was varied in the range of 30-100%. Figure 2.5 shows the absorbance of 5MTAR in the films after the immobilization process. The maximum absorbance was obtained with 40% ethanol solution. At the ethanol concentration lower than 40%, 5MTAR poorly dissolved in the solution; therefore, the observed signal was lower. The absorbance of 5MTAR in the film decreased when increasing the ethanol content because 5MTAR interacted stronger with the solvent than with the chitosan film. Therefore, 40% ethanol solution was selected for further study.

The optimum concentration of 5MTAR was evaluated using the dye concentrations in the range of 0.001-0.1 %w/v. Figure 2.6 shows the optimum concentration at 0.05 %w/v 5MTAR which was used in further study.

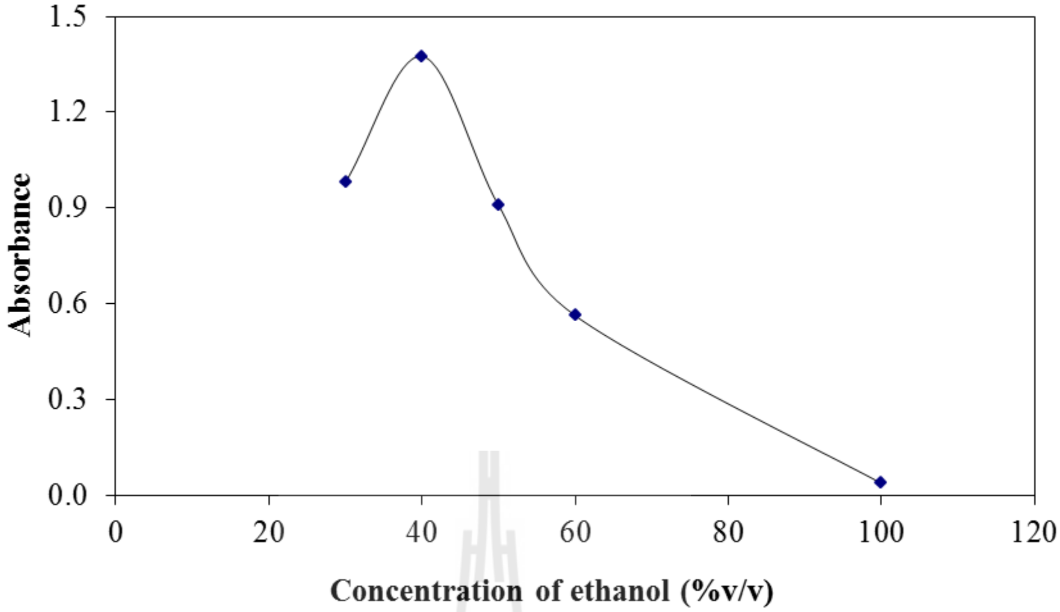


Figure 2.5 Effect of ethanol on the immobilization of 5MTAR on the chitosan film.

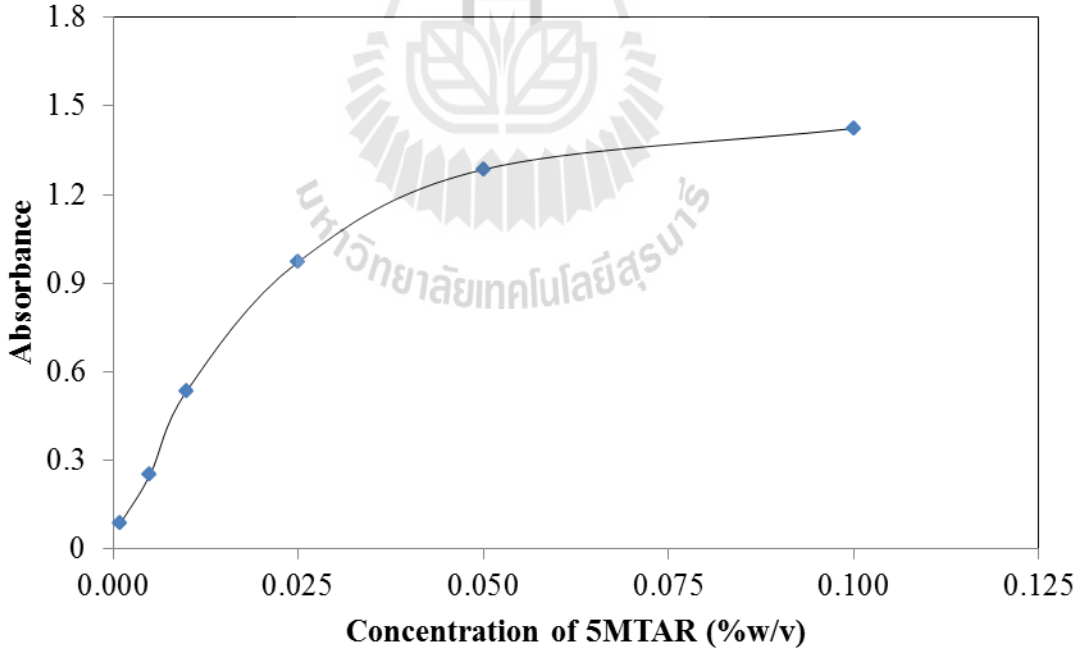


Figure 2.6 Optimization of the 5MTAR concentration for the immobilization on the chitosan film.

With the fixed concentration of 5MTAR at 0.05 %w/v, the immobilization time was varied. When the immobilization time was increased, the absorbance of the film increased as shown in Figure 2.7. A time of 120 min was chosen for further study because it provided the signal >93.3% of the steady state signal.

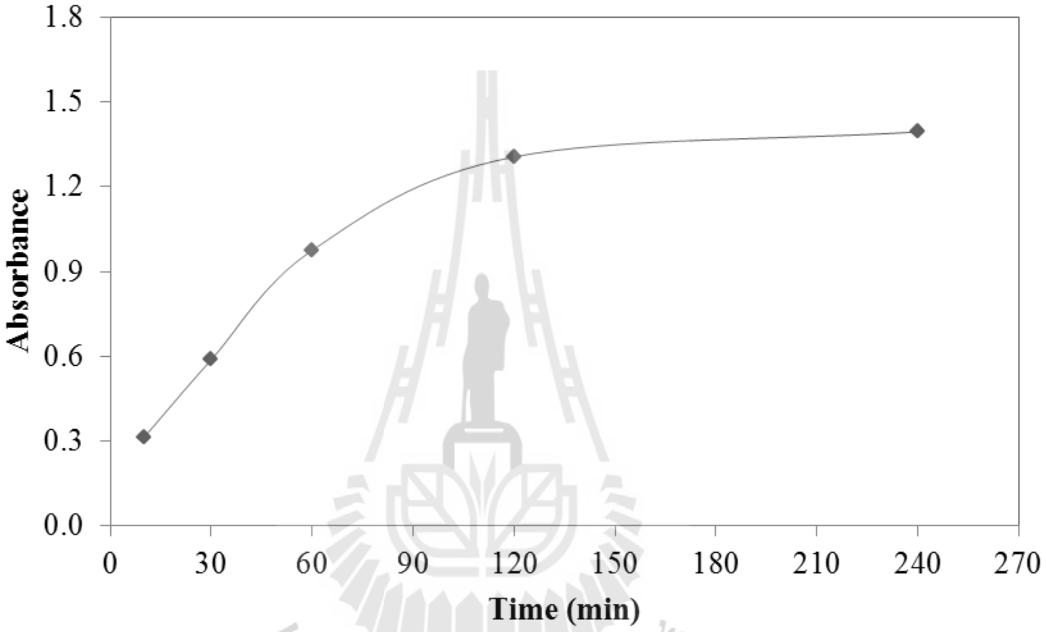


Figure 2.7 Influence of time on the immobilization of 5MTAR. The concentration of 5MTAR was 0.05 %w/v.

2.4.4 Effect of pH on the response of sensing films to Cu(II)

Response signals from the sensing films in the solutions of 12.5 ppm Cu(II) at different pHs over the range of 2.0-9.0 were measured. The pH of the solutions was controlled with phosphate buffer. Figure 2.8 shows that the response signals were comparatively high at pH 4-5. When the pH of the solution was lower than 4.0, the signal decreased because protonation of 5MTAR might compete with the formation of

Cu(II)-5MTAR complex. At the pH higher than 5.0, the response signal decreased because Cu(II) could react with hydroxide ions (Chamjangali, Soltanpanah and Goudarzi, 2009). In this work, we selected pH at 4.0 for further study, although the response signal was not the optimum value, the interference from other ions was lower.

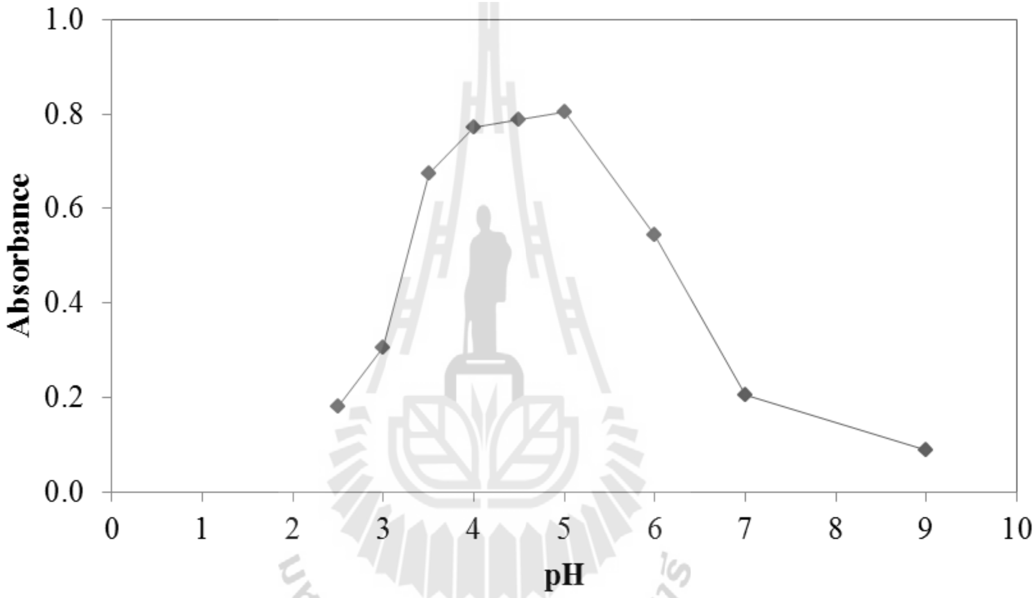


Figure 2.8 Effect of pH on the response signals of the sensing films studied in 12.5 ppm Cu(II) solutions.

2.4.5 Calibration curves and detection limits

Time profile response curves of the sensing films with different concentrations of Cu(II) are shown in Figure 2.9 The signal approaches equilibrium faster when the sensing film was in the solution with higher concentration of Cu(II). Signals at different times on the time profile response curves of the sensing film were used to construct calibration curves for Cu(II) and the linear equations are shown in

Table 2.2. The graphs with $R^2 > 0.99$ were obtained with the response times 5, 10 and 15 min and among these the lowest limit of determination calculated based on 3 times a standard deviation of the blank signals was obtained at the response time of 15 min. However, the response time of 10 min was selected for further study to compromise for a shorter analysis time.

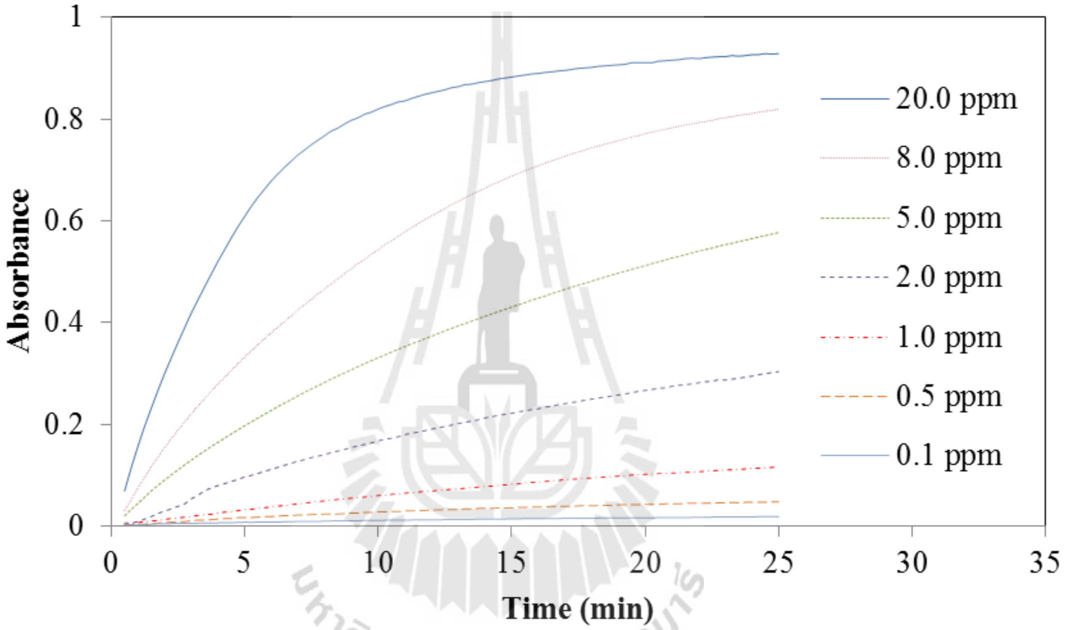


Figure 2.9 Response time of sensing films with different concentrations of Cu(II).

Table 2.2 Effects of detection time on the linear range and detection limit.

Time (min)	Equation	Linear range (ppm)	R^2	LOD (ppm)
2	y = 0.0201x - 0.0118	0.5 - 8.0	0.9624	0.108
5	y = 0.0423x - 0.0106	0.5 - 8.0	0.9976	0.051
10	y = 0.0680x + 0.0002	0.1 - 8.0	0.9975	0.032
15	y = 0.0863x + 0.0065	0.1 - 8.0	0.9945	0.026
20	y = 0.0975x + 0.0162	0.1 - 8.0	0.9895	0.023
25	y = 0.1202x + 0.0055	0.1 - 5.0	0.9833	0.018

2.4.6 Reproducibility and repeatability

Reproducibility in the film preparation procedure was evaluated with sensing films prepared from 4 different batches. For each batch, 4 films were chosen to use for the absorbance measurement in 5.0 ppm Cu(II) solution. The %RSD of the response signals was 4.27%.

Repeatability was studied by dipping 12 films of the same batch in 5.0 or 8.0 ppm Cu(II) solution and the results are shown in Figure 2.10. The %RSDs were 4.40% for 5.0 ppm and 1.74% for 8.0 ppm. This indicates good repeatability.

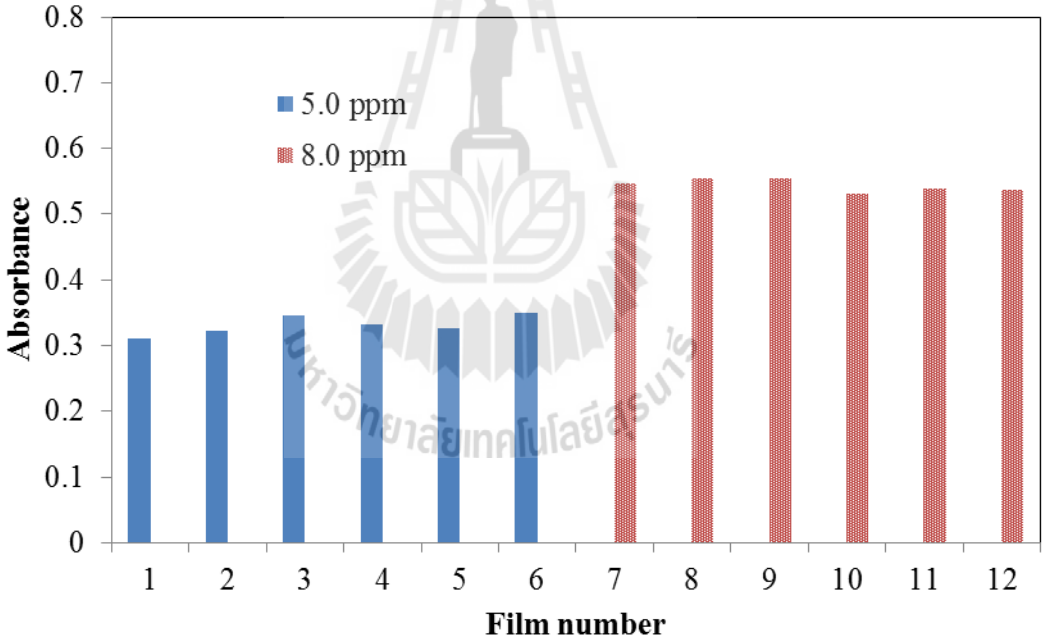


Figure 2.10 Repeatability study of sensing films with 5.0 and 8.0 ppm Cu(II) solution.

2.4.7 Regeneration of the sensing films

Reagents including HCl, EDTA and NaCl with various concentrations were utilized for regeneration of the sensing films. A sensing film after exposed to Cu(II) was dipped into a regeneration reagent bath with stirring for different times and a signal was recorded. The regeneration of the sensing film was successful with 1.0 M HCl and 1.0 M EDTA that is the signal returned to the initial value as before the sensing film was exposed to the Cu(II) solution. The regeneration time was 3 min for HCl and 5 min for EDTA. The effect of concentration was studied by using solutions of 0.01, 0.1 and 1.0 M HCl. At the concentration <1.0 M the regeneration time increased. Therefore, 1.0 M HCl and the regeneration time of 3 min were selected for further study.

The reversibility of sensing films was investigated by dipping a sensing film in 5.0 ppm Cu(II) solution and alternatively regenerated with 1.0 M HCl. The results are shown in Figure 2.11. The relative standard deviations of baseline signals and analytical signals were 1.01% and 1.95%, respectively. This suggests that the sensing film can be regenerated at least 11 times with no significant change in the response signal.

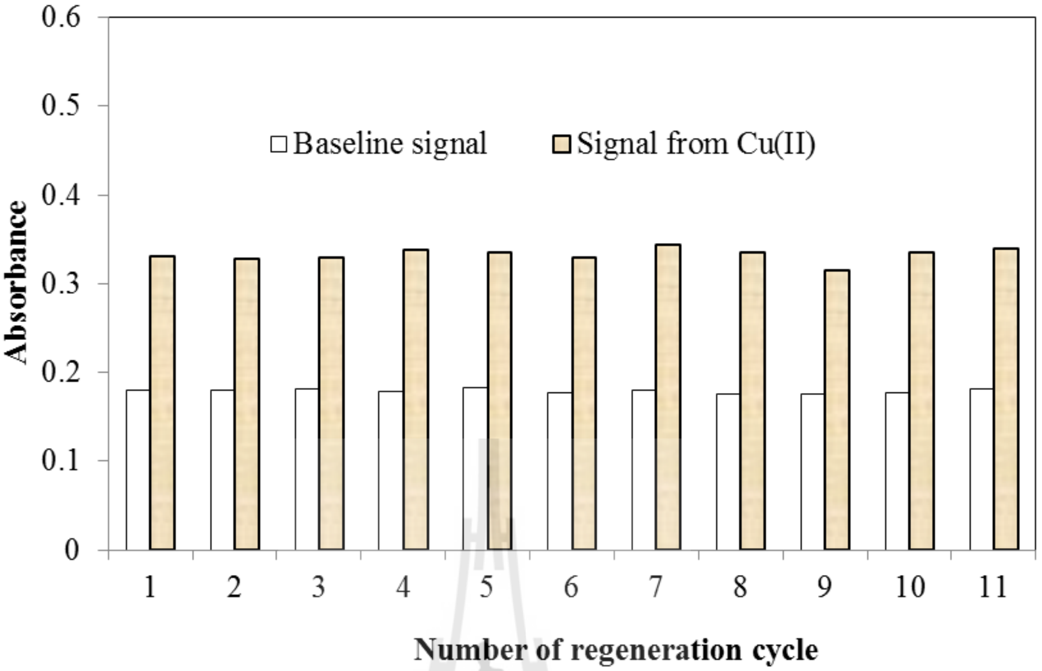


Figure 2.11 Reversibility of the sensing film studied with 5.0 ppm Cu(II) and 1.0 M HCl.

2.4.8 Stability of the sensing films

Stability of the sensing films in aqueous solutions was tested by soaking sensing films in 1.0 M HCl and phosphate buffer pH 4.0 for 4 h with the absorbance recorded every 5 min. Figure 2.12 shows that the sensing film has good stability in both solutions and the calculated %RSD of the monitored signals were 1.36% and 0.48% for the films in HCl and buffer, respectively. Moreover, no significant change of the signal at 555 nm was observed after the sensing film was immersed in phosphate buffer pH 4 for 3 weeks.

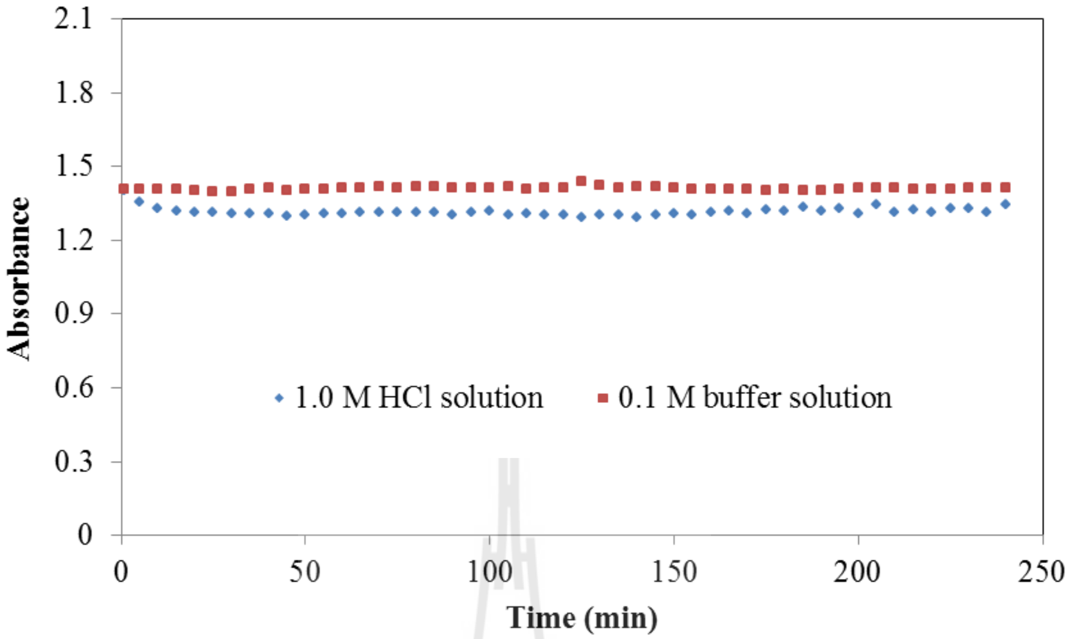


Figure 2.12 Stability of the sensing film in 1.0 M HCl and phosphate buffer pH 4.0.

2.4.9 Selectivity

The effect of foreign species on the determination of Cu(II) was studied at the optimum condition. The sensing film was tested with 7.87 x 10^{-5} M Cu(II) and with the solution mixture of 7.87 x 10^{-5} M Cu(II) and 7.87 x 10^{-5} M investigated metal ion. Table 2.3 shows that major interferences were Zn(II) and Hg(II). However, when the concentration of Zn(II) and Hg(II) was reduced to half of the Cu(II) concentration, the %errors decreased to 3.77% and -3.67%, respectively.

Table 2.3 Interference study using the proposed sensing film.

Investigated ion	**%Error**
Ni(II)	-1.16
Fe(II)	-2.65
Co(II)	-4.05
Fe(III)	4.59
Mn(II)	2.78
Zn(II)	6.81
Cd(II)	4.81
Cr(III)	-1.30
Hg(II)	-10.56
Pb(II)	2.96
Ag(I)	-4.87

2.4.10 Analysis of water samples

To test the analytical validity of the proposed sensing film, the developed sensing film was applied for the determination of Cu(II) in tap water samples. Tap water samples were collected from two locations in Suranaree University of Technology, Thailand. All water samples were used without previous treatment. In addition, the Cu(II) content in the samples was measured using flame atomic absorption spectrometry for comparison purpose. Table 2.4 shows the results of this study. The results from the proposed method agreed well with those from the FAAS method.

Table 2.4 Results from the determination of Cu(II) in water samples by the proposed and FAAS method.

Sample	Concentration of Cu(II) (ppm)			%Error[b]
	Added	Proposed method	FAAS	
tap water 1	-	<detection limit	<detection limit	-
	2	2.26 ± 0.06[a]	2.24 ± 0.02	0.76
	5	5.29 ± 0.02	5.63 ± 0.01	-6.01
tap water 2	-	<detection limit	<detection limit	-
	2	2.23 ± 0.08	2.24 ± 0.01	-0.58
	5	5.38 ± 0.04	5.48 ± 0.03	-1.82

[a] average value ± standard deviation, n = 3

[b] $\%\text{error} = \dfrac{[\text{Cu(II)}]_{\text{sensingfilm}} - [\text{Cu(II)}]_{\text{FAAS}}}{[\text{Cu(II)}]_{\text{FAAS}}} \times 100$

2.5 Conclusion

A sensing film was fabricated by using 5MTAR embedded in chitosan. The sensing film responded to Cu(II) with a change of the absorbance signal at 555 nm. The sensing film has a linear response in the concentration range 0.1-8.0 ppm and the limit of detection was 0.032 ppm. The sensor can be regenerated readily with 1.0 M HCl more than 10 times and has a long lifetime. The response of the sensing film was reproducible. The developed sensing film was applied successfully for the determination of Cu(II) in tap water samples.

2.6 References

Abdullah, J., Ahmad, M., Karuppiah N., Heng, L. Y., Sidek, H. (2006) Immobilization of tyrosinase in chitosan film for an optical detection of phenol. **Sensors and Actuators B: Chemical**. 114: 604-609.

Ahmad, M., Narayanaswamy, R. (1995) Development of an optical fibre Al(III) sensor based on immobilized chrom azurol S. **Talanta**. 42: 1337-1384.

Chamjangali, M. A., Soltanpanah, S., Goudarzi, N. (2009) Development and characterization of a copper optical sensor based on immobilization of synthesized 1-phenyl-1,2-propanedione-2-oxime thiosemicarbazone on a triacetylcellulose membrane. **Sensors and Actuators B: Chemical**. 138: 251-256.

Chung. Y., Chung. W. (2003) Determination of Co(II) Ion as a 4-(2-thiazolylazo)resorcinol or 5-methyl-4-(2-thiazolylazo)resorcinol chelate by reversed-phase capillary high-performance liquid chromatography. **Bulletin of the Korean Chemical Society**. 24: 1781-1784.

Loo-Teck, N., Yong, J. Y., Huijun, Z. (1998) Natural polymer-based sulfite biosensor. **Electroanalysis**. 10: 1521-4109.

Likussar, W., Boltz, D. F. (1971) Theory of continuous variation plots and a new method for spectrophotometric determination of extraction and formation constant. **Analytical Chemistry**. 43:1265-1272.

Karipcin, F., Dede, B., Percin-Ozkorucuklu, S., Kabalcilar, E. (2010) Mn(II), Co(II) and Ni(II) complexes of 4-(2-thiazolylazo)resorcinol: Syntheses, characterization, catalase-like activity, thermal and electrochemical behaviour. **Dyes and Pigments**. 84: 14-18.

Mahendra, N., Gangaiya, P., Sotheeswaran, S., Narayanaswamy, R. (2003) Investigation of a fibre optic copper sensor based on immobilized α-benzoinoxime (cupron). **Sensor and Actuators B: Chemical**. 90: 118-123.

Ngah, W. W. S., Endud, C. S., Mayanar, R. (2002) Removal of copper(II) ions from aqueous solution onto chitosan and cross-linked chitosan beads. **Reactive and Functional Polymers**. 50: 181-190.

Rinaudo, M. (2006) Chitin and chitosan: Properties and applications. **Progress in Polymer Science**. 31: 603-632.

Sombatsri, S., Wittayakun, J., Sanai, K., Kajsanthia, K., Prayoonpokarach, S. (2012) An optical sensing film for the determination of Co(II) based on disodium-1-nitroso-2-naphthol-3,6-disulfonate immobilized in chitosan film. **Sensors and Actuators B: Chemical**. 166-167: 772-776.

Wada, H., Nakagawa, G. (1975) Some 4-(2-thaiazolyazo)resorcinol derivatives as analytical reagents. **Bunseki Kagaku**. 24: 758-761.

Yusof, N. A., Ahmad, M. (2002) A flow cell optosensor for determination of Co(II) based on immobilised 2-(4-pyridylazo)resorcinol in chitosan membrane by using stopped flow, flow injection analysis. **Sensors and Actuators B: Chemical**. 86: 127-133.

Yusof, N. A., Ahmad, M. (2002) A flow cell optosensor for lead based on immobilized gallocynin in chitosan membrane. **Talanta**. 58: 459-466.

CHAPTER III

A COPPER(II) SENSOR BASED ON 5-METHYL-4-(2-THIAZOLYLAZO)RESORCINOL IMMOBILIZED ONTO AMINO-SILICA NANO PARTICLES EMBEDDED IN A CHITOSAN FILM

3.1 Abstract

A chromogenic sensing reagent, 5-methyl-4-(2-thiazolylazo)resorcinol, was immobilized onto a film composed of 5 %w/v of amino-silica particles suspended in chitosan and the film was used for the determination of Cu(II) in water samples. Parameters that affect the response of the sensing film to Cu(II) were investigated. In the presence of Cu(II), the sensing film had the absorption maximum at 555 nm and the optimum response was achieved in a solution of pH 4.5. A linear calibration curve was obtained with Cu(II) concentration in the range of 0.10 to 40 ppm with a response time of 10 min and the linear equation was –log (absorbance) = -0.5691log C_{cu} + 2.667 with r^2 = 0.9959. The limit of detection was 20 ppb calculated based on three times the standard deviation of the blank signal. No significant interferences from the studied metal ions were observed. The sensing film exhibited good stability with the operational lifetime of 3 weeks. Regeneration of the sensing film after exposed to Cu(II) was succeeded by soaking the film in 1.0 M HCl for 2 min.

3.2 Introduction

One class of optical chemical sensors based on the absorbance measurement can be constructed by immobilizing a sensing reagent that binds selectively with an analyte to give distinctive change in absorbance, into a polymer supporting matrix. The supporting matrix is a crucial part of the sensor. It should be compatible with the sensing reagent, somewhat transparent, and physically and chemically stable. Nafion (Mohammad, Tahereh, Jafar and Mohammad, 2004), silica sol–gel (Paula, Alberto and Conceição, 2004), chitosan (Xu and Zhujun, 2008), poly(vinyl alcohol) (Nur, Emur, Ibrahim and Alaaddin, 2008) and cellulose (Ensafi and Masoud, 2008), for example, have been used as supporting materials for constructing optical chemical sensors.

Sensitivity is one of the analytical figures of merit of concern in developing an analytical method based on a chemical sensor. The amount of the immobilized sensing reagent is one of the factors that influences the sensor sensitivity. In general, the maximum amount of the reagent immobilized will result in the optimum signal response from the analyte (Yusof and Ahmad, 2002). For chemical sensors constructed in the form of a thin film, the amount of the immobilized sensing reagent is limited depending upon the amount of functional groups of the polymer that can bind with the sensing reagent. One alternative to mitigate the limitation is to incorporate microparticles or nanoparticles with appropriate functional groups on the particle surface into the polymer film.

Silica with particles in a nanosize range is widely used in many applications such as biosensing and biomedical research because of its high surface area, high mechanical stability and low toxicity. The -OH rich surface of silica provides the ability to be functionalized with a range of molecules such as organosilane with amino or mercapto functional groups.

In this work, nanosilica particles were synthesized with the surface modified with amino functional group. The particles were incorporate into chitosan films and immobilized with methyl-4-(2-thiazolylazo)resorcinol (5MTAR). The developed sensing films were used for determination of Cu(II) in water samples with absorption measurement.

3.3 Experimental

3.3.1 Preparation of solutions

A stock solution of chitosan was prepared by dissolving 1.0 g chitosan powder (MW > 600,000, Fluka) in 100 mL of 1 %v/v acetic acid solution with stirring provided for 3 h. The obtained solution was filtered, sonicated for 30 min to remove air bubbles and kept at 4 ^{o}C for further used.

A stock solution of 500 ppm Cu(II) was prepared by dissolving 0.1901 g $Cu(NO_3)_2 \cdot 3H_2O$ in phosphate buffer pH 4.5 and the volume of the solution was made up to 100 mL in a volumetric flask. Cu(II) solutions with lower concentrations were prepared by appropriate dilution of the stock solution.

Phosphate buffer solutions pH 3-9 were prepared by mixing appropriate volumes of 0.1 M phosphoric acid (85%, UNIVAR) and 0.1 M sodium phosphate (99.5%, Riedel-de Haën). A pH-meter (Metler Delta 320) was used to measure the pH of the solutions.

A 0.25 %v/v glutaraldehyde solution was prepared by dilution of 10 mL 25% v/v glutaraldehyde (Unilab) in a 1000-mL volumetric flask using DI water as a solvent. This solution was used for crosslinking the prepared chitosan films.

A stock solution of 5MTAR was prepared by dissolving 0.0250 g of 5MTAR in 40% ethanol in phosphate buffer. The volume of the solution was adjusted to 100 mL.

3.3.2 Preparation of silica and modified silica particles

Silica particles were synthesized using a method modified from a literature (Shina, Lee, Lee, Ahn and Kim, 2008). Tetraethylorthosilane (TEOS, 98%, Acros Organics) was used as a silica source. The surface of silica particles was modified with 3-aminopropyltetraethylsilane (APTES, 99%, Acros Organics). A solution of TEOS in ethanol was made by mixing 33.5 mL TEOS and 266.5 mL ethanol with stirring provided for 20 min. This solution is called solution (I). A solution of NH_3 was prepared using 7.6 mL 28 wt% NH_3, 81.6 mL DI water and 210.9 mL ethanol. After mixing, stirring was provided for 20 min. This solution is called solution (II). Solution (I) was quickly added to solution (II) and the mixture was stirred for 3.5 h. After that, 1.4 mL APTES was added into the mixture which was further stirred for 15 h. Silica particles were removed from the mixture by centrifugation, washed with ethanol and dried at 100 ^{o}C for 12 h.

3.3.3 Characterization of silica and modified silica particles

Transmission electron microscope (TEM) (JSM6400, JSM) was used to obtain morphologies of synthesized silica particles. A solid sample was distributed on a carbon tape attached onto a Cu-grid sample holder. The sample morphologies were recorded with the magnification of 1000-5,000x.

Scanning electron microscope (SEM) (JEOL2010, TEOL) was used to observe the morphologies of the films that contained silica particles. The observations of solid samples were prepared by placing solid particles on a stub sample holder with a carbon tape. The samples were dried under a UV lamp for 15 min and coated with gold for 5 min in a Balzer sputtering coater. The samples morphologies were recorded with 10 kV accelerating voltage and 100-10,000x magnification.

Fourier transform infrared spectroscopy (FTIR) (IR-Prestrige-21, Shimadzu) was used to confirm the successfulness of the modification of the particle surface. A KBr technique was selected for preparation of the samples. A sample and KBr powder were mixed with the weight ratio of 1:100. The mixture was ground in a mortar. The ground powder was transferred to a pellet master and a pressure of 150 psi was applied to form a pellet. IR spectra were obtained in the wavenumber range of 400-4000 cm^{-1} with the resolution of 4 cm^{-1}.

3.3.4 Preparation of amino-silica particles/chitosan sensing films

The prepared amino-silica particles were suspended in 1 %w/v chitosan solution. An aliquot of 200 µL mixture was spread on a transparent film into the size 0.8 x 2.5 cm^2 and dried in an oven at 60 ^oC for 12 h. The amino-silica particles/chitosan film was crosslinked by soaking the film in 30 mL 0.25% glutaraldehyde solution for 7.5 min.

To immobilize 5MTAR, the amino-silica particles/chitosan film was immersed in a dye solution for 4 h under stirring. After that, the film was washed 2 times with 10 mL of 0.1 M NaCl and then with DI water. The film was dried at room temperature and kept in a desiccator. The concentration of the dye solution was varied in the range of 0.001-0.1 %w/v.

Immobilization time that yield the optimum signal for the sensing films was investigated by varying the contact time from 10 to 240 min using the optimum dye concentration. After the immobilization process, the film was washed 2 times with 10 mL 0.1 M NaCl and with 20 mL DI water. All of the prepared films were washed with with 1.0 M HCl for 10 min and DI water. The absorbance of the films at 467 nm was measured.

The effect of amino-silica particles loading on the response of the sensing films with Cu(II) was studied by mixing various amount of amino-silica particles on chitosan films. Silica particles were suspended in 1%w/v chitosan solution to obtain 1, 5 and 10 %w/v mixture solutions. The mixture solutions were cast on transparent films and crosslinked with 0.25% glutaraldehyde for 7.5 min. Immobilization of 5MTAR was later carried out with the derived films by soaking the films in 0.05% 5MTAR solution for 2 h. After that, the films were washed 2 times with 10 mL of 0.1 M NaCl and then with 20 mL DI water. All of the sensing films were washed with with 1.0 M HCl for 10 min and DI water. The films were dried at room temperature and kept in a desiccator.

3.3.5 Response measurement of the sensing films to Cu(II)

Absorbance of a sensing film with the size of 0.8 x 2.5 cm^2 was measured with a fiber optic spectrometer (CHEMUSB4-VIS/NIR, Ocean Optics). The film was dipped in 2.0 mL of a studied solution that was contained in a 10-mm cuvette. A signal obtained from the sensing film dipped in 2.0 mL of phosphate buffer was used as a blank signal. The wavelength range in this study was 350-800 nm.

3.3.6 Effect of the pH on the response of sensing films to Cu(II)

The effect of pH on the response of the sensing films to Cu(II) was studied by using phosphate, acetate and citrate buffer to control the pH of the solutions. The pH of the solutions was varied in the range of 2.0-7.0. The concentration of Cu(II) was fixed at 5.0 ppm and the absorbance at 555 nm was measured at 10 min after the film was immersed into the Cu(II) solution.

3.3.7 Regeneration and reversibility

The regeneration of the sensing films after exposed to 5.0 ppm Cu(II) solution was studied using solutions of 0.1 and 1.0 HCl as regenerating reagents. An investigated film was dipped into 20 mL regenerating reagent contained in a beaker with stirring provided. At a given time, the film was removed from the reagent and washed with DI water followed by a phosphate buffer pH 4.5. The absorbance of the films was measured at 555 mm and compared with the signal of the film before exposed to the Cu(II) solution. To determine the number of the regeneration cycles, the regeneration process was conducted repeatedly on the same film using 1.0 M HCl as the regenerating reagent. The relative standard deviations of the background signal and the signal from Cu(II) were calculated.

3.3.8 Reproducibility and repeatability

To evaluate the reproducibility of the film preparation procedure, total 16 films obtained from 4 batches (4 films/batch) were used in the study. The films were tested in 2.0 ppm Cu(II) solution at the optimum measurement condition. %RSDs of the response signals were calculated.

Repeatability was evaluated using 12 sensing films obtained from the same batch and tested with the solutions of 1.0 and 10.0 ppm Cu(II) (6 films/solution). The measurement was made at the optimum condition. %RSDs of the response signals were calculated.

3.3.9 Stability and lifetime

Stability of the sensing films were tested by soaking a sensing film in 1.0 M HCl and phosphate buffer pH 4.5. The absorbance of the film at 467 nm was measured every 5 min for 4 h.

Lifetime of sensing films were tested by using three sensing films. The films were immersed into 2.0 ppm Cu(II) in phosphate buffer pH 4.5. The measurement was made every 7 days for 5 weeks.

3.3.10 Selectivity

The effect of foreign species was studied at an equivalent molar ion concentration of Cu(II) and the investigated species with the optimum measurement condition for Cu(II). The sensing film was tested with 3.15×10^{-5} M Cu(II). The response signal obtained with Cu(II) was denoted as A. The absorbance of the sensing film tested with a solution mixture of 3.15 x 10^{-5} M Cu(II) and 3.15 x 10^{-5} M foreign metal ion was denoted as A_i. Percent error was calculated according to equation (3.1)

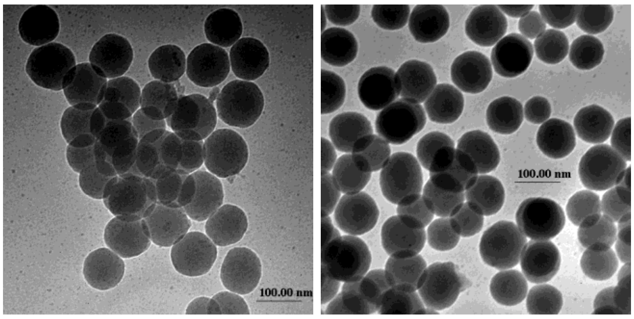
$$\%\text{error} = \frac{A - A_i}{A} \times 100 \qquad (3.1)$$

An investigated ion was considered as an insignificant interferent when the %error was within ±5%.

3.4 Results and discussion

3.4.1 Characterization of synthesized silica particles and the particles modified with APTES

In this work, APTES was used to modify the silica particles to provide amino functional groups on the particle surface. TEM images of the synthesized silica particles are shown in Figure 3.1. Silica and amino-silica particles have relatively uniform spherical shape and sizes in the range of 60-100 nm. The method used to synthesize amino-silica particles was similar to that used by Wu, Xiang, Kim, Chun and Lee (2006) and the researchers reported that amino-silica particles had larger size than silica particles.



a) b)

Figure 3.1 TEM images of a) silica particles and b) amino-silica particles.

IR spectroscopy was used to confirm the success of the silica surface modification and the results are shown in Figure 3.2. Si-O-H stretching at 3300-3600 cm^{-1} was observed for silica and amino-silica particles. CH_2 stretching at 2925 and 2853 cm^{-1} showed up in the spectrum of the modified silica indicating that the chemical modification of the surface of the silica particles was successful.

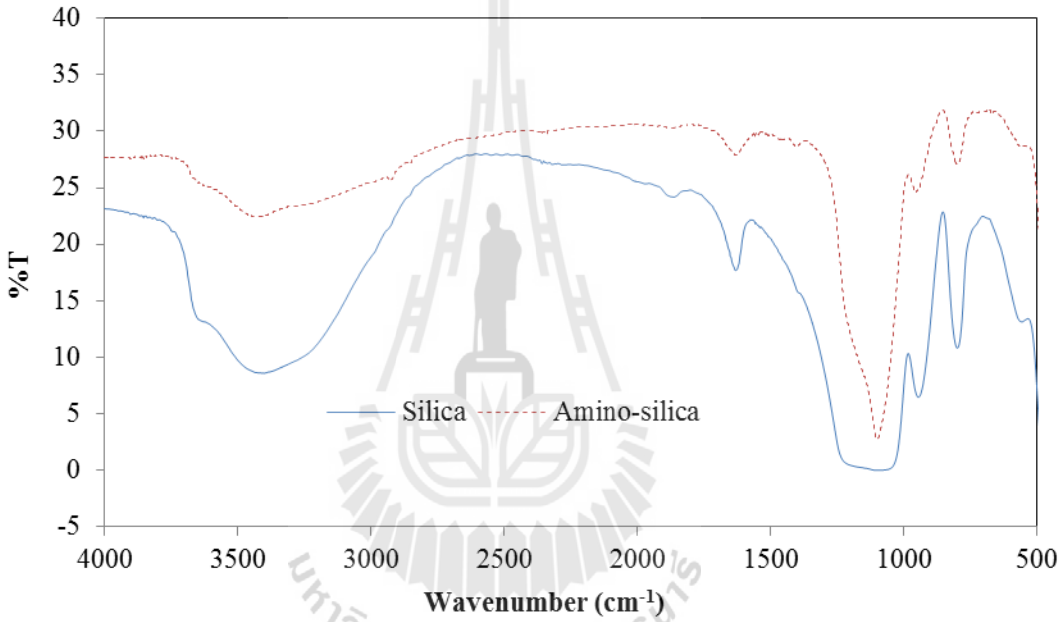


Figure 3.2 IR spectra of silica and amino-silica particles.

3.4.2 Morphology of amino-silica particles/chitosan films

Figure 3.3 shows an SEM image of the 5 %w/v amino-silica particles/chitosan film. Amino-silica particles were well dispersed in the film. This could be attributed to the positive charge formed on the amino groups of chitosan and silica particles by protonation resulting in a repulsive force between silica particles and chitosan. Therefore, the film was used for immobilization of 5MTAR to make the sensing film.

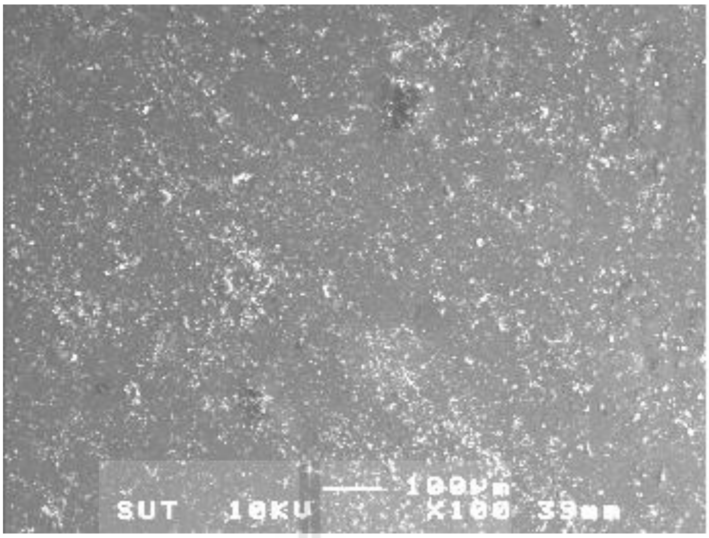


Figure 3.3 SEM image of 5 %w/v amino-silica particles/chitosan film.

3.4.3 Influence of amino-silica particles on the response of the sensing films

The sensing films were prepared with different compositions including chitosan, 5 %w/v silica particles/chitosan film and 1, 5 and 10 %w/v amino-silica particles in chitosan. Immobilization of 5MTAR was later carried out with the derived films by soaking the films in 0.05% 5MTAR solution for 2 h. The prepared sensing films were tested with 5.0 ppm Cu(II) and the results are shown in Figure 3.4. The signal from the films incorporated with amino-silica particles increased with %amino silica loading of 1 and 5 %w/v. But at 10 %w/v amino silica loading, the signal was lower than those of 1 and 5%w/v loading, but the signal was still higher than that of the film without particles. This could be due to the densely packed of the particles in the film that could hinder the adsorption of the dye. For the films incorporated with the unmodified silica particles, the signal obtained is the lowest. From this result, it is clear that higher amount of the sensing reagent is immobilized in the films with the surface modified silica particles compared to the film without the particles. The mixture of 5 %w/v amino silica-particles/chitosan films was selected for further study.

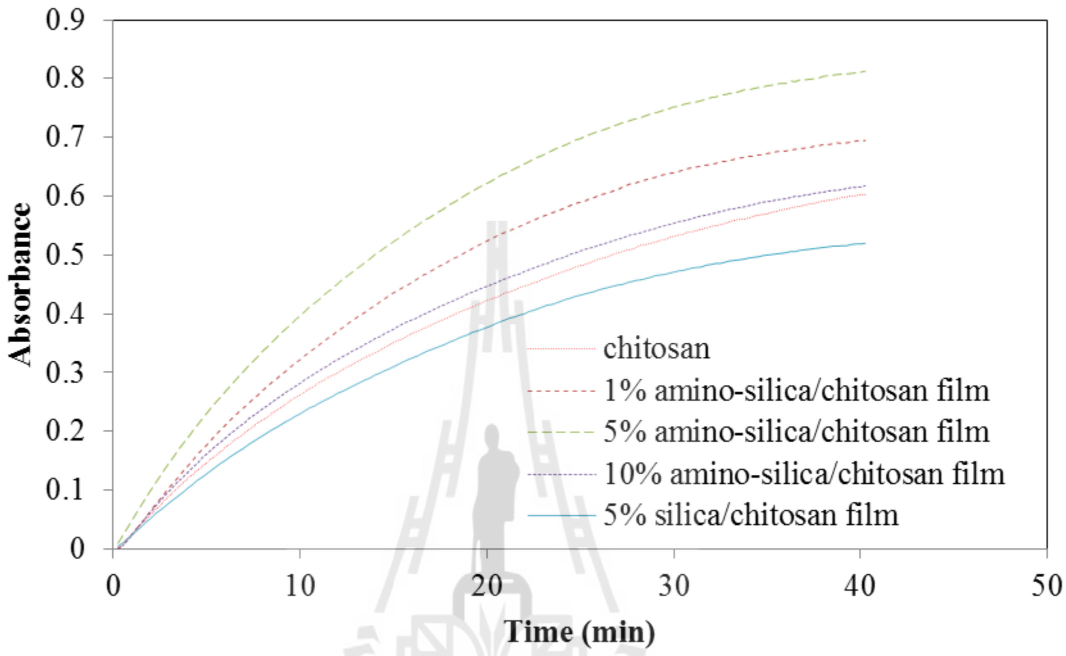


Figure 3.4 Time dependent responses of films with different compositions to 5.0 ppm Cu(II) at pH 4.0.

3.4.4 Study of immobilization conditions

3.4.4.1 Optimization of 5MTAR concentration

To evaluate the suitable concentration of 5MTAR solution used for the preparation of sensing films, dye solutions at various concentrations were used in the immobilization process. A film was immersed in a solution of 5MTAR and the solution was shaken for 4 h. The film was washed with 0.1 M NaCl and DI water. The absorbance of the washed solution was monitored until the absorbance signal of the dye was not observed.

The results from this study are shown in Figure 3.5**.** The absorbance of the dye-immobilized films increased with the concentration of the dye. The absorbance reached the plateau when the concentration of the dye was 0.05 %w/v. Therefore, the solution of 0.05 %w/v 5MTAR was selected to use further in the immobilization process.

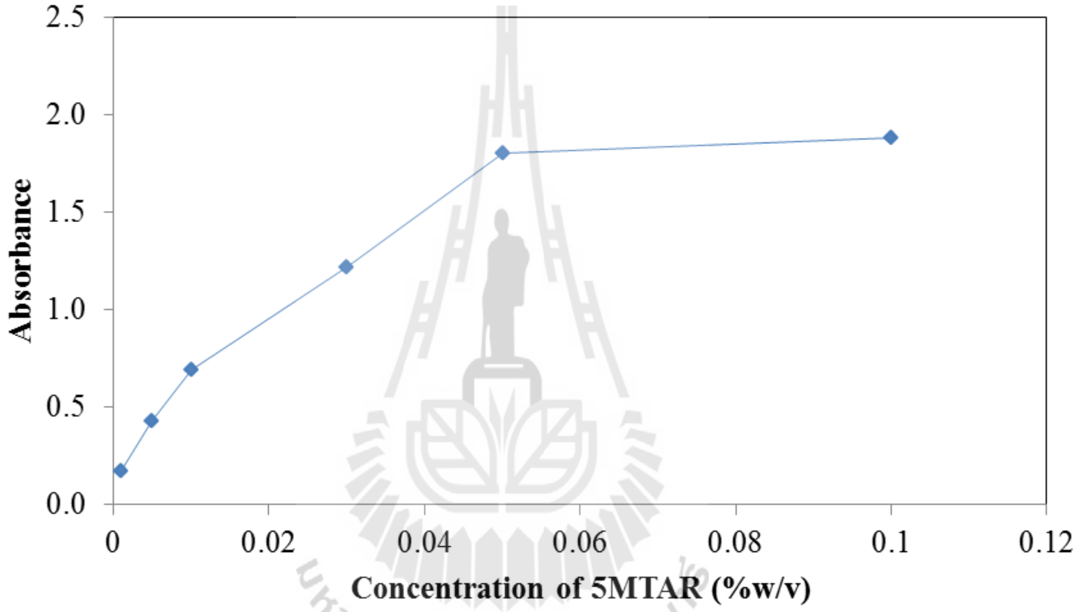


Figure 3.5 Absorbance of films immobilized with different concentrations of 5MTAR. The absorbance of the dye in the films was measured at 450 nm.

3.4.4.2 Optimization of immobilization time

To determine the optimized time for the immobilization process, the films were soaked in 0.05 %w/v 5MTAR solution with the time varied in the range of 10 min to 4 h. The absorbance of the films was recorded at the maximum absorption wavelength of the dye at 450 nm and the results are shown in Figure 3.6. The absorbance increased with time and leveled off after 120 min. Therefore, immobilized time of 120 min was chosen for further study.

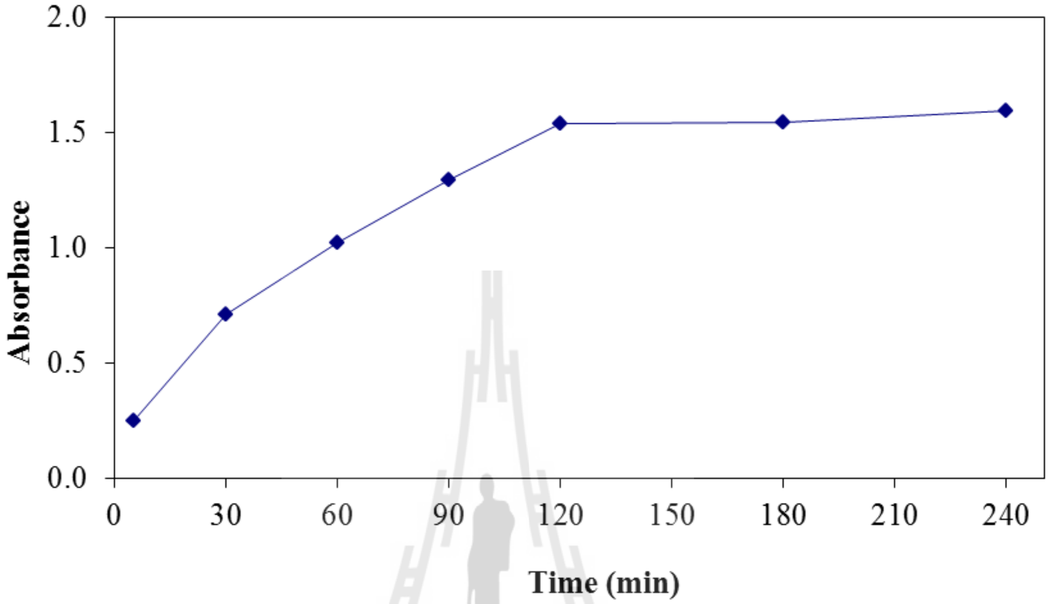


Figure 3.6 Absorbance of films immobilized with 0.05 %w/v 5MTAR at different times.

3.4.5 Effect of pH on the response of sensing films to Cu(II)

Influence of pH on the response of sensing films to Cu(II) was studied by dipping a sensing film into 10.0 ppm Cu(II) solution which the pH of the solution was controlled using phosphate buffer. The pH of the solutions was varied in the range of 3.0-7.0. Figure 3.7 shows the results from this study. The sensing film had the highest response signal at pH 4.5. The response signal decreased when the pH was lower than 4.5. This could be from H^+ competing with Cu(II) for binding sites on 5MTAR. In addition, at the pH higher than 10.0, the response signal decreased because Cu(II) could form complexes with hydroxide ions (Chamjangali, Soltanpanah, Goudarzi, 2009). Therefore, in further study the pH of Cu(II) solutions was controlled at 4.5 with phosphate buffer.

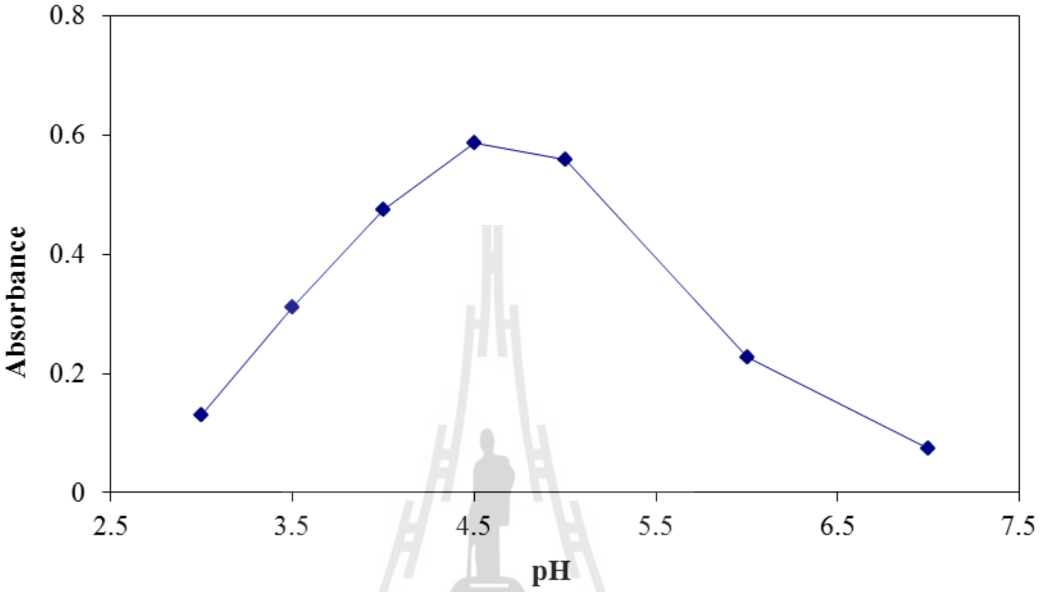


Figure 3.7 Effect of pH on the response of the sensing films studied in 10.0 ppm Cu(II) solution**.**

3.4.6 Calibration and detection limits

Time profile response curves of the sensing films in Cu(II) solutions with different concentrations at pH 4.5 are shown in Figure 3.8. The signal approached 95% of the steady state signal in 10-25 min depending on the concentration of Cu(II). To optimize the analysis time, calibration graphs were constructed using signals at different times in the range of 5-20 min. Calibration equations are summarized in Table 3.1. A larger linear range was obtained with a shorter response time. The calibration curve obtained using the signals at 10 min shown in Figure 3.9 had the highest r^2 and the widest linear range. The limit of detection was 0.020 ppm (based on three times the standard deviation of the blank signal). Therefore, the response time of 10 min after soaking the sensing film in a Cu(II) solution was selected as the optimum time for the determination of Cu(II).

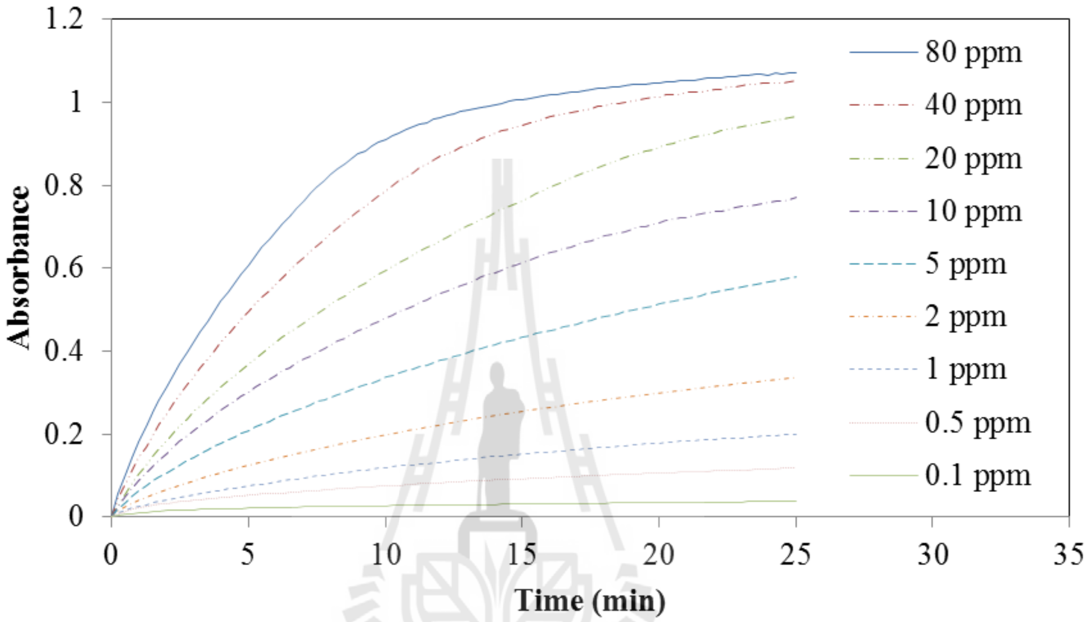


Figure 3.8 Time dependent signals of sensing films with different concentrations of Cu(II).

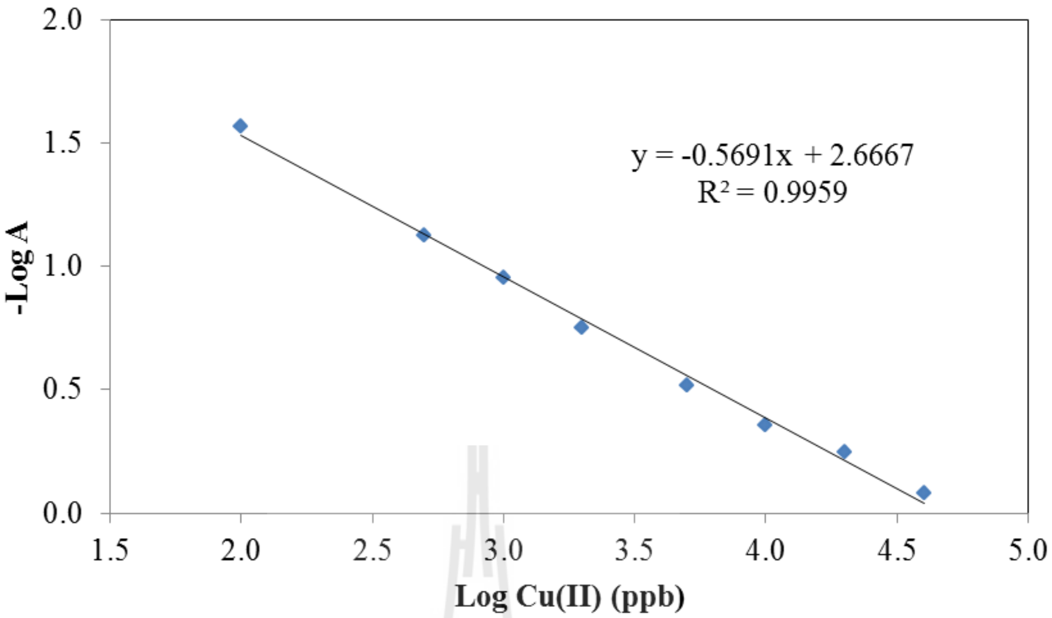


Figure 3.9 Calibration curve for Cu(II) at 10 min response time.

Table 3.1 Calibration equations, linear ranges and limits of detection obtained from various response times.

Time (min)	Equation	Linear range (ppm)	r^2	LOD (ppm)
5	-logA = -0.5051log[Cu(II)] + 2.4610	0.5-40	0.9923	0.0231
10	-logA = -0.5691log[Cu(II)] + 2.6667	0.1-40	0.9959	0.0204
15	-logA = -0.6140log[Cu(II)] + 2.7178	0.1-20	0.9917	0.0199
25	-logA = -0.5565log[Cu(II)] + 2.1711	0.5-10	0.9850	0.0028

3.4.7 Reproducibility and repeatability

To evaluate the reproducibility of the film preparation procedure, total 16 films obtained from 4 batches (4 films/batch) were used in the study. The films were tested in 5.0 ppm Cu(II) solution at the optimum condition. The response signals were recorded and the %RSD calculated from the response signals was 3.11% which indicated good reproducibility in the film preparation procedure.

Repeatability was evaluated using the films obtained from the same batch and tested with the solutions of 1.0 and 10.0 ppm Cu(II) and the results are shown in Figure 3.10. The calculated %RSDs were 3.51% for the study with 1.0 ppm Cu(II) and 1.15% for the study with 10.0 ppm Cu(II). This suggested that good repeatability was obtained.

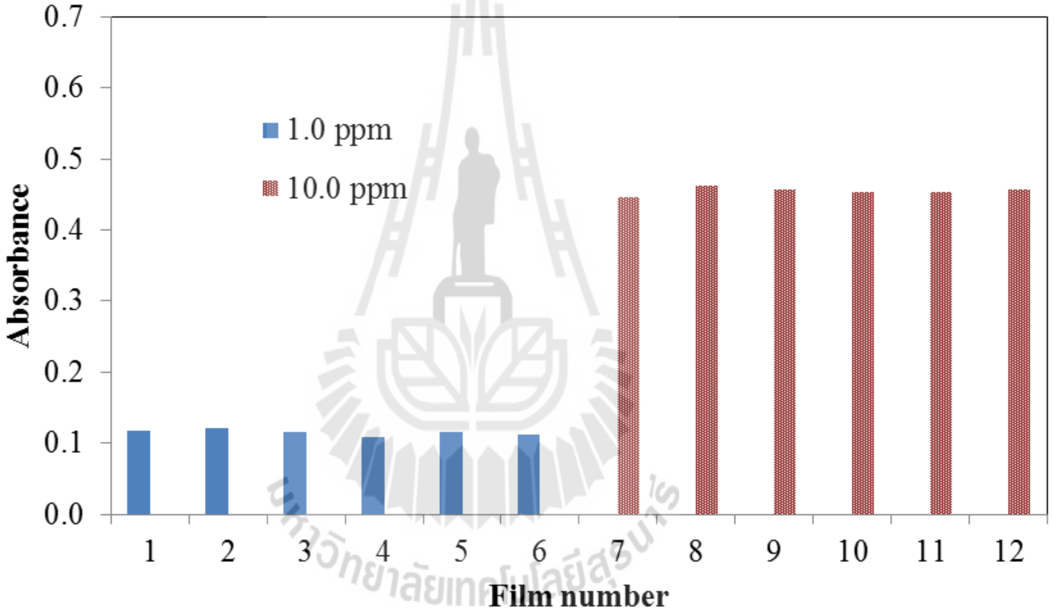


Figure 3.10 Repeatability study of 12 sensing films with 1.0 and 10.0 ppm Cu(II) solution.

3.4.8 Regeneration and reversibility

The regeneration of the sensing film was studied by using 0.1 and 1.0 M HCl. The sensing film after exposed to 10.0 ppm Cu(II) was dipped into 20 mL HCl solution with stirring for different times and the signals were recorded. The absorbance of the film returned to its baseline signal when the film was put in 1.0 M

HCl for 2 min as shown in Figure 3.11. Regeneration time of 3 min was selected for this work to make sure that the regeneration was complete.

The reversibility of the sensing film was investigated by dipping the sensing film in 5.0 ppm Cu(II) solution and alternatively regenerated with 1.0 M HCl. The results are shown in Figure 3.12. The relative standard deviations of baseline signals and analytical signals were 2.94 % and 4.12 %, respectively. This suggested that the sensing film can be reused at least 10 times with no significant change in the response signals.

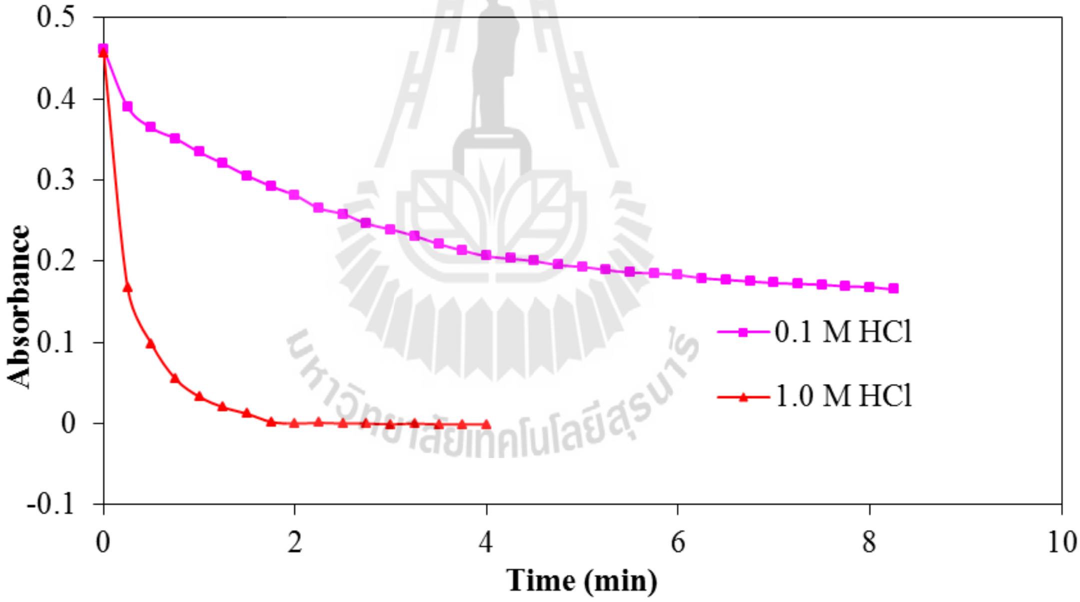


Figure 3.11 Time dependent of response signals of the sensing film in 0.1 M and 1.0 M HCl after the sensing film was exposed to 5.0 ppm Cu(II) solution.

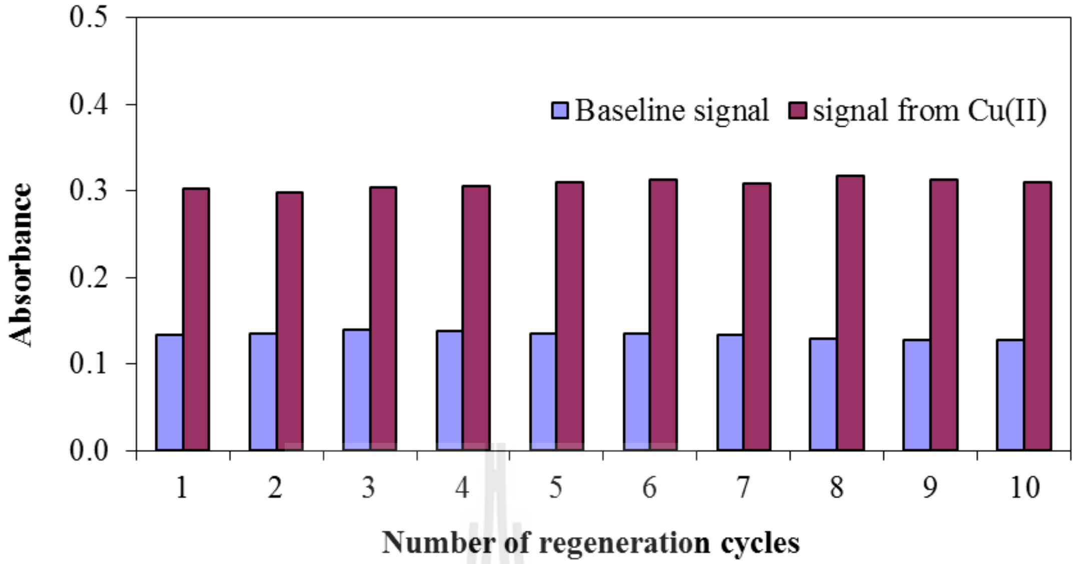


Figure 3.12 Reversibility of the sensing film studied with 5.0 ppm Cu(II) and 1.0 M HCl.

3.4.9 Stability and lifetime of the sensing films

Stability of sensing films was tested by soaking a sensing film in 1.0 M HCl and phosphate buffer pH 4.5 for 4 h. The signal was recorded every 5 min. The sensing film showed good stability in both solutions as the %RSD of the signal were less than 5%. Moreover, 3 sensing films were tested with 10.0 ppm Cu(II) in phosphate buffer pH 4.5 every 7 days over the period of 35 days and the results are shown in Figure 3.13. The %RSD of the signals was 3.44% indicated that the sensing films can be used for at least one month after preparation with no significant change of the analytical signal.

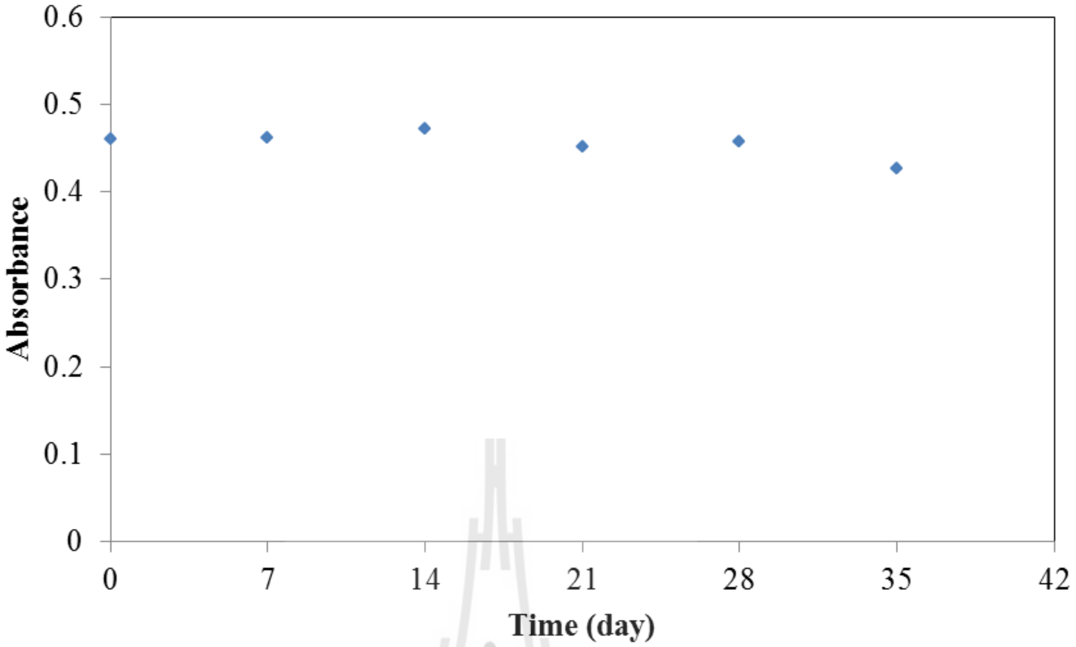


Figure 3.13 Lifetime of the sensing films with 10.0 ppm Cu(II) in phosphate buffer pH 4.5 every 7 days.

3.4.10 Interference study

The effect of foreign species on the determination of Cu(II) was studied at the optimum condition and the results are summarized in Table 3.2. The sensing film was tested with 7.87 x 10^{-5} M Cu(II) and with the solution of Cu(II) mixed with an investigated metal ion. The concentration of both ions was 7.87 x 10^{-5} M. From the results in Table 4.2, %errors obtained with the investigated ions were less than ±5% which suggested that these ions are not significant interferents.

Table 3.2 Interference study using the proposed sensing film.

Investigated ion	%Error
Fe(II)	2.87
Zn(II)	-4.64
Cd(II)	1.46
Cr(III)	3.81
Hg(II)	3.34

3.5 Conclusions

Optical sensing films for Cu(II) determination based on amino-silica particles suspended in chitosan immobilized with 5MTAR were successfully fabricated with relatively simple procedure. The films had linear response for Cu(II) in the concentration range of 0.1-40 ppm with the pH 4.5 solutions and the response time of 10 min. The detection limit for Cu(II) was 20 ppb. The film could be regenerated readily with 1.0 M HCl at least 10 times and had a long lifetime. Good repeatability and reproducibility were obtained in the study using the developed sensing films.

3.6 References

Chamjangali, M. A., Soltanpanah, S., Goudarzi, N. (2009). Development and characterization of a copper optical sensor based on immobilization of synthesized 1-phenyl-1,2-propanedione-2-oxime thiosemicarbazone on a triacetylcellulose membrane. **Sensors and Actuators B: Chemical**. 138: 251-256.

Ensafi, A. A., Masoud, F. (2008) Development of a spectrophotometric optode for the determination of Hg(II). **IEEE Sensors Journal**. 8: 347-353.

Mohammad, K. A., Tahereh, M. I., Jafar, H. K., Mohammad, P. (2004) Development of an optical chemical sensor based on 2-(5-bromo-2-pyridylazo)-5 -(diethylamino)phenol in Nafion for determination of nickel ion. **Talanta**. 63: 713-720.

Nur, A., Emur, H., Ibrahim, Y., Alaaddin, C. (2008) Selective optical sensing of copper(II) ions based on a novel cyclobutane-substituted Schiff base ligand embedded in polymer films. **Sensors and Actuators B: Chemical**. 134: 510-515.

Paula, C. A. J., Alberto, N. A. M., Conceição, B. S. M. M. (2004) Development of a sol–gel optical sensor for analysis of zinc in pharmaceuticals. **Sensors and Actuators B: Chemical**. 103: 169-177.

Shina, Y., Lee, D., Lee, K., Ahn, K. H., Kim, B. (2008) Surface properties of silica nanoparticles modified with polymers for polymer nanocomposite applications. **Journal of Industrial and Engineering Chemistry**. 14: 515-519.

Wu, Z., Xiang, H., Kim, T., Chun, M., Lee, K. (2006) Surface properties of submicrometer silica spheres modified with aminopropyltriethoxysilane and phenyltriethoxysilane. **Journal of Colloid and Interface Science**. 304: 119-124.

Xu, H., Zhujun, Z. (2008) Electrogenerated chemiluminescence sensor for itopride with $Ru(bpy)_3^{2+}$-doped silica nanoparticles/chitosan composite films modified electrode. **Sensors and Actuators B: Chemical**. 131: 403-410.

Yusof, N. A., Ahmad, M. (2002) A flow cell optosensor for lead based on immobilized gallocynin in chitosan membrane. **Talanta**. 58: 459-466.

CHAPTER IV

AN OPTICAL CHEMICAL SENSOR FOR THE DETERMINATION OF COPPER(II) BY USING 5-METHYL-4-(2-THIAZOLYAZO) RESORCINOL IMMOBILZED IN NAFION FILM

5.1 Abstract

A chromogenic sensing reagent, 5-methyl-4-(2-thiazolylazo)resorcinol (5MTAR), was immobilized in Nafion film and the film was used for the determination of Cu(II). Parameters that affected the response of the sensing film to Cu(II) were investigated. In the presence of Cu(II) in a solution of pH 3.5, the sensing film had the absorption maximum at 562 nm. The sensing film responded linearly to Cu(II) in the concentration range of 0.1 to 5.0 ppm with a response time of 7 min and the limit of detection was 13 ppb. Hg(II) was the major interference. Regeneration of the sensing film after exposed to Cu(II) could be simply done by soaking the sensing film in 0.1 M HCl for 1 min. The sensing film was used for the determination of Cu(II) in water samples and Thai whisky with satisfactory results.

5.2 Introduction

Nafion is a sulfonated tetrafluoroethylene based fluoropolymer-copolymer. It is classified as an ionic polymer because of the sulfonic groups that exhibit the ionic character. The structure of Nafion is shown in Figure 4.1. Nafion is used in many applications such as fuel cell, metal and organic dye adsorption, solid phase extraction, electrochemical, and optical chemical sensor.

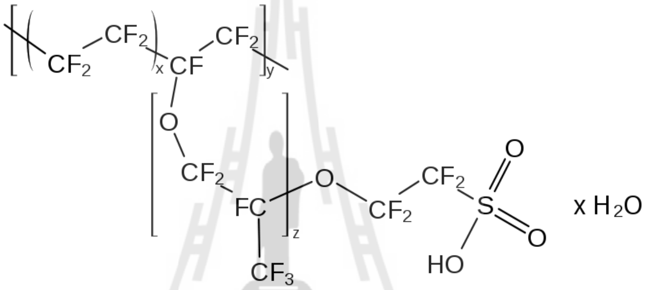


Figure 4.1 Nafion chemical structure.

Nafion has some interesting features that can be applied as a supporting matrix for optical chemical sensors such as optically transparent, high permeability for ions, high mechanical strength and good film forming property. Madden, Cardwell, Cattrall and Deady (1995) prepared a sensor for metal ions by immobilization of 1-(2-pyridylazo)-2-naphthol (PAN) on a Nafion film. Sands, Cardwell, Cattrall, Farrell, Iles and Kolev (2002) reported a highly versatile stable optical sensor based on 4-decyloxy-2-(2-pyridylazo)-1-naphthol in Nafion for the determination of Cu(II). Coo and Belmonte (2002) fabricated a Cu(II) sensor baesd on PAN immobilized in Nafion and incorporated the sensor in a flow injection system. Bener, Özyürek, Güçlü and Apak (2013) used cupric–neocuproine immobilized in a Nafion film to screen the total antioxidant capacity in food extracts and biological samples Amini, Momeni-Isfahani, Khorasani and Pourhossein, (2004) developed Ni(II) sensor based on immobilization of 2-(5-bromo-2-pyridylazo)-5-(diethylamino)phenol (Br-PADAP) in a Nafion film.

In this work, Nafion was used as a supporting matrix for immobilization of 5MTAR to fabricate an optical sensor for determination of Cu(II). The factors that affected the response of the sensor were studied. The performance characteristics of the sensor were evaluated and the analyses of Cu(II) in water and Thai whisky samples were demonstrated.

5.3 Experimental

4.3.1 Preparation of solutions

A stock solution of 500 ppm Cu(II) was prepared by dissolving 0.1901 g of copper nitrate trihydrate ($Cu(NO_3)_2 \cdot 3H_2O$ 99.99%, Merck) in DI water and the volume of the solution was adjusted in a 100-mL volumetric flask.

Phosphate buffer solutions of pHs 3-9 were prepared by mixing 0.1 M phosphoric acid (85%, UNIVAR) and 0.1 M dibasic sodium phosphate (99.5%, Riedel-de Haën). The amount of the phosphate salt and the volume of phosphoric acid solution were varied to obtain the desired pH and a pH-meter was used to monitor the pH of the solutions.

4.3.2 Preparation of Nafion sensing film

Solutions of the sensing reagent in the concentration range of 1-7% (w/v) were prepared by dissolving the suitable amount of 5MTAR in 2 mL Nafion solution. The solutions were sonicated for 30 min. After sonication, an aliquot of 4 μL was spread on the area of 0.8 x 1.0 cm^2 of a transparent film and the solvent was evaporated at room temperature for 1 day. The prepared films were then kept in a desiccator.

Effect of film thickness was studied by varying the volume of the 3 %w/v 5MTAR in Nafion solution for spreading on the area of 0.8 x 1 cm^2. The volume of the solution was varied within the range of 3-50 µL.

4.3.3 Response of the sensing films to Cu(II)

Absorbance of a sensing film with the size of 0.8 x 1.0 cm^2 was measured with a UV-Vis spectrometer (CHEMUSB4-VIS/NIR, Ocean Optics). The film was dipped in a 2 mL of a studied solution that was contained in a 10-mm cuvette as shown in Figure 4.2. A signal obtained from the sensing film dipped in 2 mL of phosphate buffer was used as a blank signal. The wavelength range in this study was 350-800 nm.

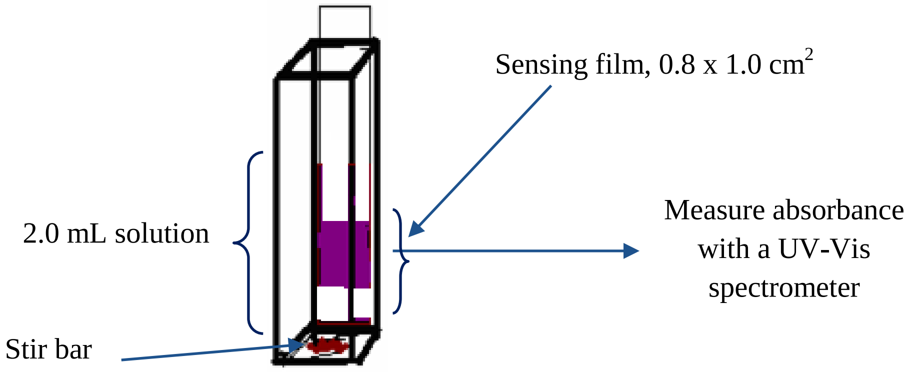


Figure 4.2 A setup for absorbance measurement with the sensing film.

4.3.4 Effect of the pH on the response of sensing films to Cu(II)

The effect of pH of the Cu(II) solution to the response of sensing films to was studied at different values from 3.0-7.0. Three buffer systems, acetate, citrate and phosphate buffer, were investigated for controlling the pH of solutions. The concentration of Cu(II) was fixed at 5.0 ppm and the absorbance at 562 nm was measured at 10 min after the film was exposed to the Cu(II) solution.

4.3.5 Regeneration study

The regeneration of sensing films after exposed to Cu(II) was studied using solution of HCl and EDTA as regenerating reagents. The sensing film after exposed to 2.0 ppm Cu(II) solution was placed into a beaker containing 20 mL of the regenerating reagent. At a given time interval, the film was removed from the regenerating reagent, washed with DI water and buffer solution and the absorbance was measured. The obtained signal was compared to the signal of the film before exposure to the Cu(II) solution.

4.3.6 Reproducibility and repeatability

To evaluate the reproducibility of the film preparation procedure, total 16 films obtained from 4 batches (4 films/batch) were used in the study. The films were tested in 2.0 ppm Cu(II) solution at the optimum measurement condition. The response signals were recorded and used for the calculation of %RSD.

Repeatability was evaluated using the 10 sensing films obtained from the same batch and tested with the solutions of 1.0 and 5.0 ppm Cu(II) (5 films/solution). The obtained response signals were used for the calculation of %RSDs.

4.3.7 Stability of sensing films

Stability of sensing films was tested by continuously soaking sensing films in 0.1 M HCl and phosphate buffer pH 3.5 for 4 h. The absorbance at 443 nm was measured every 5 min.

Lifetime of sensing films were tested by using 21 sensing films. Each time 3 films were tested with 2.0 ppm Cu(II) in phosphate buffer pH 3.5 every 7 days for 35 days.

4.3.8 Selectivity

The effect of foreign species was studied at equivalent molar ion concentration of Cu(II) and the investigated species at the optimum measurement condition for Cu(II). The sensing film was tested with 3.15 x 10^{-5} M Cu(II). The response signal obtained with Cu(II) was denoted as A. The absorbance of the sensing film tested with a solution mixture of 3.15 x 10^{-5} M Cu(II) and 3.15 x 10^{-5} M foreign metal ion was denoted as A_i. The two signals were used for the calculation of percent error as shown in equation (4.1).

$$\%\text{error} = \frac{A - A_i}{A} \times 100 \qquad (4.1)$$

When the calculated %error was within ±5%, the tested metal ion was considered not significantly interfere the analysis of Cu(II).

4.3.9 Analysis water and Thai whisky samples

Tap water samples were collected at F1 building, Suranaree University of Technology, Thailand. Water samples were used without any treatment except for the pH adjustment with phosphate buffer for the measurement with the sensing film. Two Thai brand whisky samples which contained 40 %v/v ethanol were used in this study. Whisky samples were prepared by removing of the alcohol content in 50 mL whisky sample in a water bath with the temperature controlled at 90 ^{o}C for 3 h. Each treated sample was transferred into a 25 mL volumetric flask and the volume of the solution was made up with phosphate buffer. For comparison purpose, the determination of Cu(II) with an atomic absorption spectrometer (AAnalyst 100, Perkin-Elmer) was conducted using the AOAC method no. 974.24 for tap water samples and method no. 28.1.23 for Thai whisky samples (AOAC International. 1995).

5.4 Results and discussion

4.4.1 Response of the sensing films to Cu(II)

Figure 4.3 shows absorption spectra of the sensing film that soaked in phosphate buffer at pH 3.5 with the maximum absorption wavelength at 443 nm. When the sensing film was dipped in 10 ppm Cu(II) solution, pH 3.5 and stirred, a new peak occurred at 562 nm as a result of the formation of Cu(II)-5MTAR complex. Therefore, in this work the wavelength at 562 nm was used for Cu(II) determination.

The concentration of the dye solution used for immobilization was varied in the range of 1-7 %w/v. It was found that immobilization with 3 %w/v resulted in the film with homogenous distribution of dye and optimum response with Cu(II). When the amount of 5MTAR was lower than 3 %w/v, a lower response signal with Cu(II) was obtained. At the concentration of 5MTAR higher than 3 %w/v inhomogeneous distribution of the dye was observed.

Effect of film thickness was studied by varying the volume of the 3 %w/v 5MTAR in Nafion solution for spreading on the area of 1 x 1 cm^2. The volume of the solution was within the range of 3-50 µL. A film obtained with 4 $\mu L/cm^2$ appeared homogenous and had short response time. When the volume of the solution was lower than 4 $\mu L/cm^2$, it could not be spread to completely cover the specified area. When the thickness of the sensing film increased from the increasing the volume of solution, the response signal of the sensing film to Cu(II) increased, but it took longer for the signal to reach the equilibrium state. This similar situation was also reported in the literature (Wansapura, Seliskar and Heineman, 2007). Therefore, 4 µL of 3 %w/v 5MTAR was used for fabrication of the sensing film.

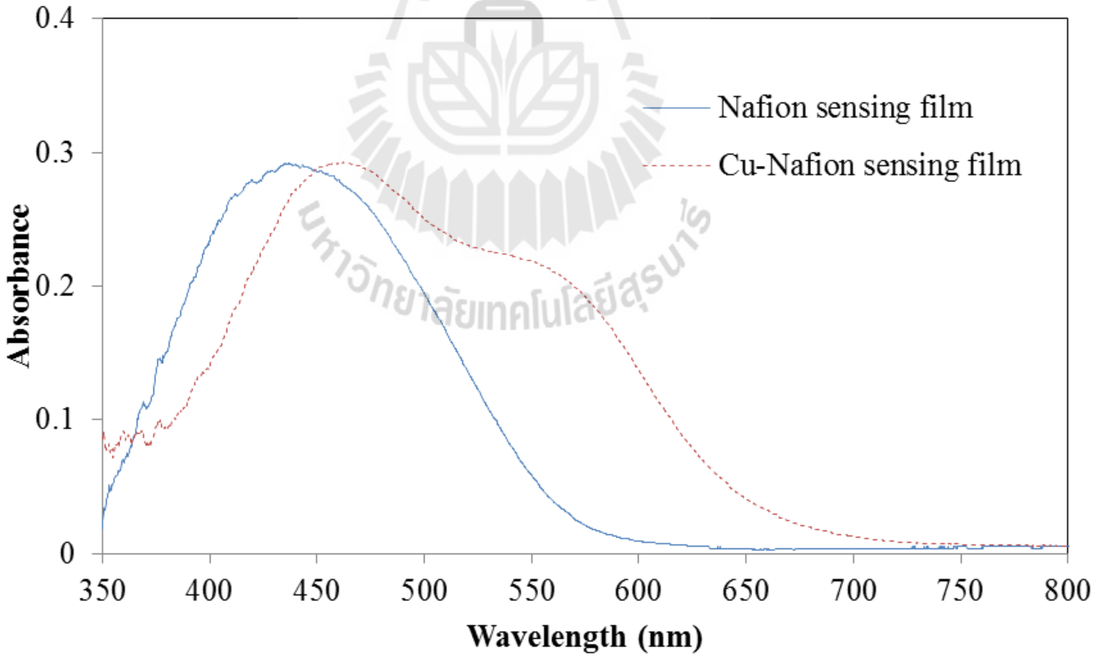


Figure 4.3 Absorption spectra of the sensing film and the sensing film in 10 ppm Cu(II) solution in 0.1 M phosphate buffer pH 3.5.

4.4.2 Effect of pH on the sensing film response

The influence of the pH on the response of the sensing film was investigated using three buffer systems which were phosphate, acetate, and citrate buffer. Figure 4.4 shows that the optimum response of the sensing film was obtained in phosphate buffer at pH 3.5. In addition, the response signal decreased at the pH <3.5 because of deprotonation of 5MTAR was lower. At the pH >5.0 the response signal decreased because Cu(II) could form complex with hydroxide ions (Chamjangali, Soltanpanah and Goudarzi, 2009).

Response signals obtained using acetate buffer were about the same as those obtained in the phosphate buffer. Moreover, the lowest responses were obtained with citrate buffer because citrate could form complex with Cu(II) (Parry and Dubois, 1952., Field, McCourt, McBryde, 1974). Therefore, phosphate buffer was further used to control the pH of the studied solutions.

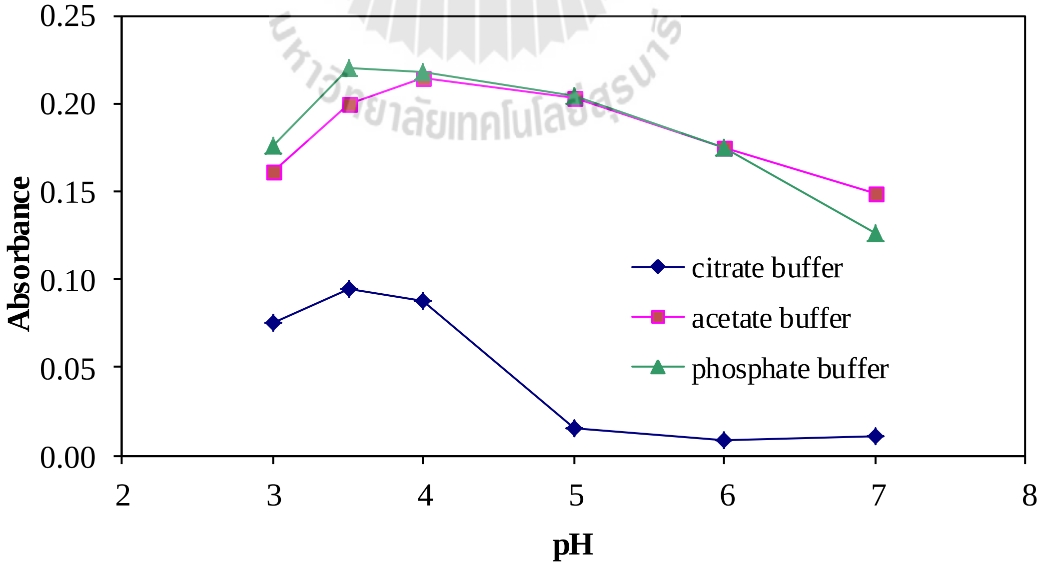


Figure 4.4 Influence of buffer types and pH to the response of the sensing films with 5.0 ppm Cu(II) solution.

4.4.3 Response time, calibration curve and detection limit

Time profile response curves of the sensing films in Cu(II) solutions with different concentrations at pH 3.5 are shown in Figure 4.5. The signal approached 95% of the steady state signal in 2-10 min depending on the concentration of Cu(II). A faster response was obtained in solutions with higher concentration of Cu(II), this could be from the attribute of the analyte diffusion from the bulk solution to the film interface and the association of the analyte with 5MTAR driven by the concentration gradient.

To optimize the analysis time, calibration graphs were constructed using signal at different times in the range of 5-8 min. The results are shown in Table 4.1. All calibration graphs at different times have linear relationship in the same concentration range and the detection limits obtained (based on three times the standard deviation of the blank signal) were about the same. However, the calibration curve obtained using the signals at 7 min has the highest r^2. Therefore, the response time of 7 min after soaking the sensing film in a Cu(II) solution was selected as the optimum time for the determination of Cu(II).

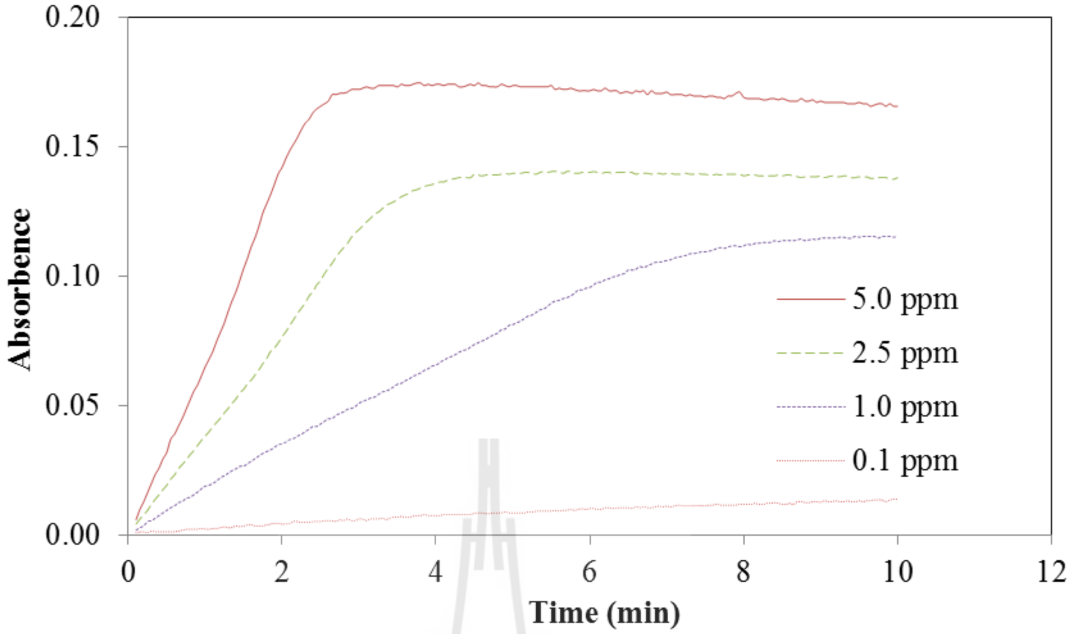


Figure 4.5 Response curves of the sensing film in solutions with different concentrations of Cu(II).

Table 4.1 Calibration equations, linear ranges, and detection limits at various response times.

Time (min)	Equation	r^2	LOD (ppm)
5	y = 0.0964log[Cu(II)] - 0.191	0.9740	0.012
6	y = 0.0945log[Cu(II)] - 0.181	0.9959	0.013
7	y = 0.0934log[Cu(II)] - 0.175	0.9997	0.013
8	y = 0.0919log[Cu(II)] - 0.170	0.9964	0.013

4.4.4 Reproducibility and repeatability

To evaluate the reproducibility of the film preparation procedure, total 16 films obtained from 4 batches (4 films/batch) were used in the study. The films were tested in 2.0 ppm Cu(II) solution at the optimum condition. The response signals were recorded and the %RSD of response signals was calculated to be 4.73%. The result indicated good reproducibility in the film preparation method.

Repeatability was evaluated using the films obtained from the same batch and tested with the solutions of 1.0 and 5.0 ppm Cu(II). The results are shown in Figure 4.6. The calculated %RSDs were 3.13% for the response with 1.0 ppm Cu(II) and 2.13% for 5.0 ppm Cu(II). This suggested that good repeatability in the measurement method was obtained.

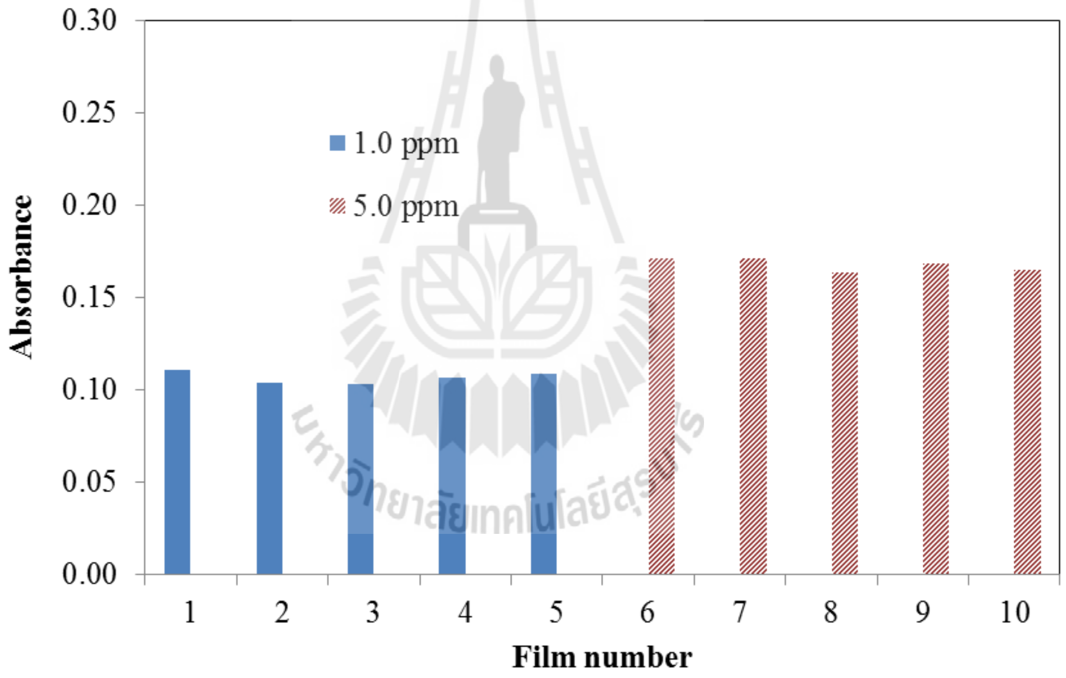


Figure 4.6 Repeatability study of the measurements with the sensing films in solutions of 1.0 and 5.0 ppm Cu(II).

4.4.5 Regeneration and reversibility

The regeneration of the sensing film after exposed to Cu(II) was studied using solution of HCl and EDTA as regenerating reagents. The sensing film after exposed to Cu(II) was immersed into a beaker containing 20 mL of the regenerating reagent with stirring provided. At a given times, the film was removed from the reagent, washed with DI water and buffer solution and then the absorbance was measured. Figure 4.7 shows the results of this study. Regeneration was completed when HCl was used. Faster removal rate was obtained with increase concentration of HCl. For economically reason, 0.1 M was selected as the regenerating reagent and the regeneration time was fixed at 2 min to make sure that the regeneration was complete.

The reversibility of the sensing film was investigated with 2.0 ppm Cu(II) solution and 0.1 M HCl. The results are shown in Figure 4.8. The relative standard deviation of the baseline signals and the analytical signals (n = 8) were 6.77 and 2.40%, respectively.

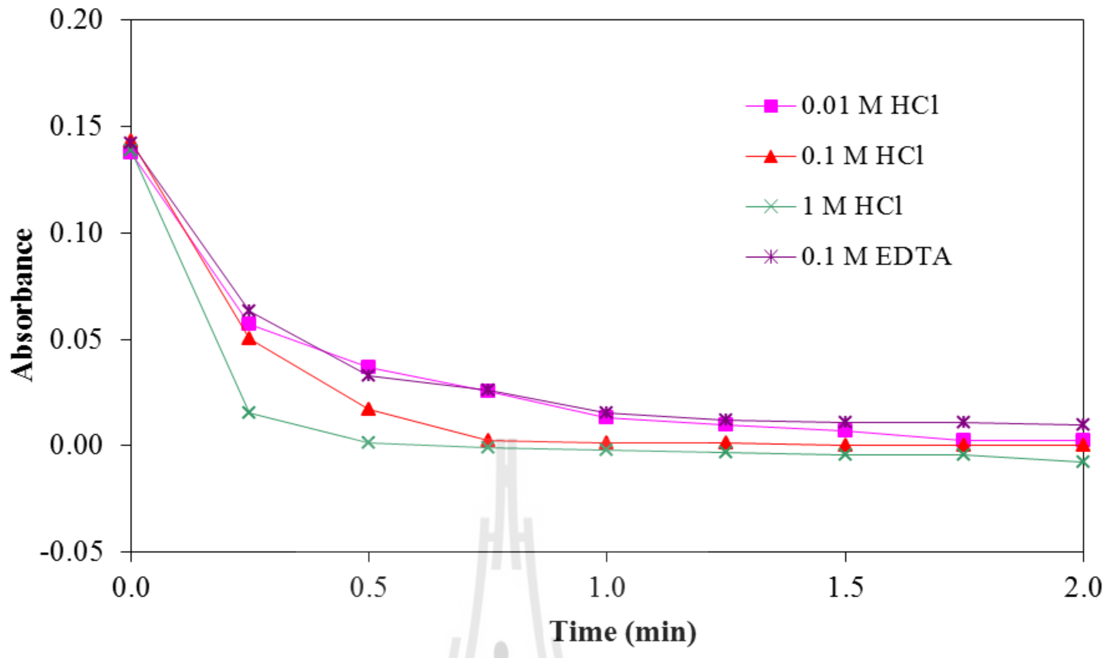


Figure 4.7 Regeneration of sensing film with different types and concentrations of regenerating reagents.

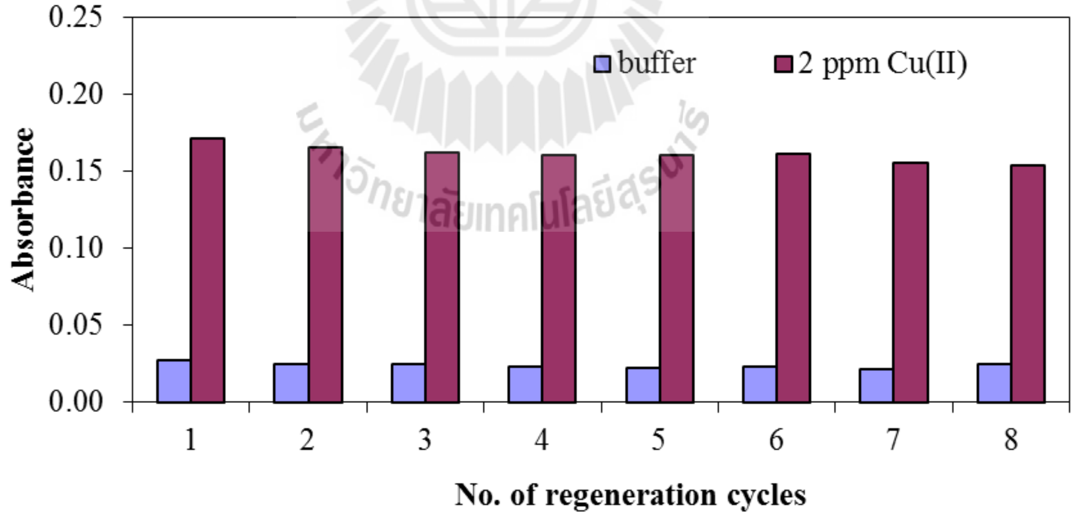


Figure 4.8 Reversibility study of the sensing film with 2.0 ppm Cu(II) and 0.1 M HCl as the regenerating reagent.

4.4.6 Stability and lifetime

Stability of the sensing film was tested by soaking the sensing film in 0.1 M HCl and phosphate buffer pH 3.5 for 4 h. The absorbance signals were recorded every 5 min. The sensing film showed good stability in both solutions as suggested by %RSD of 0.85%.

With three sensing films tested in 2.0 ppm Cu(II) at the optimum condition every 7 days over a period of 35 days, the results are shown in Figure 4.9. No significant change of the analytical signals at 562 nm was observed over the studied period (%RSD = 3.55%).

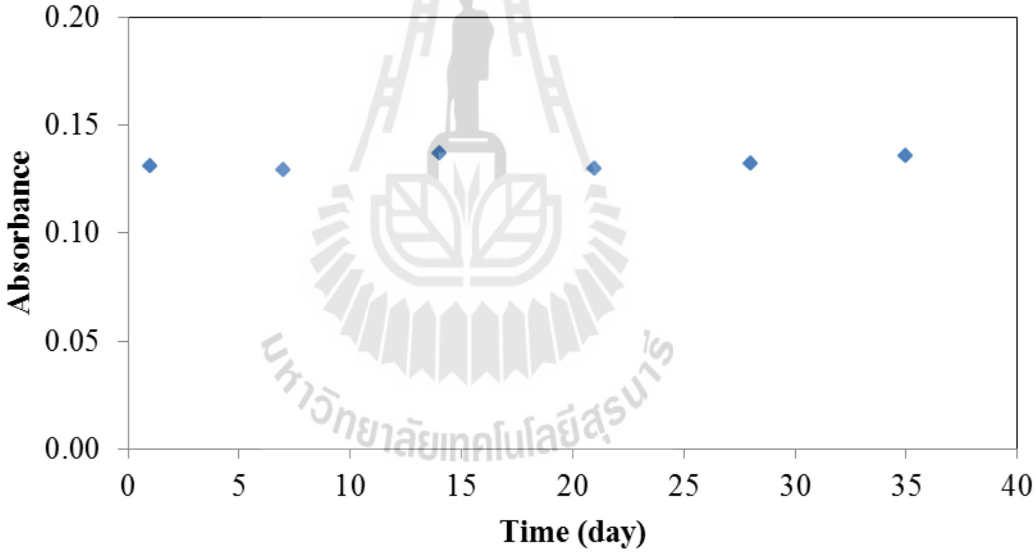


Figure 4.9 Lifetime of the sensing film with 2.0 ppm Cu(II).

4.4.7 Selectivity

The effect of foreign species on the determination of Cu(II) was studied at optimum condition and the results are shown in Table 4.2. Most tested metal ions have %error within ±5.0% except for Hg(II). Therefore, removal of Hg(II) should be of concern for the analysis of samples containing Hg(II) at the concentration higher than that of Cu(II).

Table 4.2 Interference effect of the determination of 3.15×10^{-5} M Cu(II) by using the proposed sensing film.

Interference	%Error
Ni(II)	2.17
Fe(II)	3.42
Fe(III)	2.22
Co(II)	-0.20
Zn(II)	4.27
Ag(I)	3.68
Pb(II)	-1.25
Cd(II)	3.47
Cr(III)	4.14
Mn(II)	1.22
Hg(II)	5.22
Na(I)	1.15

4.4.8 Analysis of real samples

To examine the analytical validity of the proposed sensor, determination of Cu(II) in tap water and Thai whisky samples was conducted. The results from the analyses are shown in Table 4.3 and 4.4. The calculation of %recovery and t-test results are evident that the sensor is suitable for determination of Cu(II) in such samples with a good precision and accuracy.

Table 4.3 Determination of Cu(II) in water samples by using the proposed sensing film compared with FAAS.[a]

Samples	Cu(II) added (ppm)	Sensing film found (ppm)	AAS found (ppm)	Recovery (%)
Tap water	-	<DL	<DL	-
	1.00	0.94 ± 0.03	0.95 ± 0.05	101.1
	2.50	2.55 ± 0.10	2.48 ± 0.12	97.4

[a]The reported values are means of 3 replicate measurements and the standard deviations.

Table 4.4 Determination of Cu(II) in Thai whisky samples.[a]

Samples	Sensing film found (ppm)	AAS found (ppm)	t-test
Thai whisky 1	<DL	<DL	-
Thai whisky 2	0.366 ± 0.013	0.375 ± 0.015	0.790

[a]The reported values are means of 3 replicate measurements and the standard deviations.

4.4 Conclusions

A chemical sensor for Cu(II) detection based on 5MTAR immobilized in Nafion film was successfully developed. Procedure used for preparation of the sensor was relatively simple. Response of the sensor to Cu(II) resulted in the change of the absorbance at 562 nm, which can be correlated to the concentration of Cu(II) in the range of 0.1-5.0 ppm. The optimum response was obtained in solutions at pH 3.5 of phosphate buffer with the response time of 7 min. The proposed method for the determination of Cu(II) with the developed sensor provided the limit of detection in ppb level which should be sufficient for the analysis of Cu(II) at the environmental relevant level. The sensor can be regenerated readily with a solution of HCl more than 7 times and has a long lifetime. The developed sensor was applied for the determination of Cu(II) in tap water and Thai whisky samples with satisfactory results.

4.5 References

Amini, M. K., Momeni-Isfahani, T., Khorasani, J. H., Pourhossein, M. (2004) Development of an optical chemical sensor based on 2-(5-bromo-2-pyridylazo)-5-(diethylamino)phenol in Nafion for determination of nickel ion. **Talanta**. 63: 713-720.

Bener, M., Özyürek, M., Güçlü, K., and Apak, R. (2013) Novel optical fiber reflectometric CUPRAC sensor for total antioxidant capacity measurement of food extracts and biological samples. **Journal of Agricultural and Food Chemistry**. 61: 8381-8388.

Chamjangali, M. A., Soltanpanah, S., Goudarzi, N. (2009). Development and characterization of a copper optical sensor based on immobilization of synthesized 1-phenyl-1,2-propanedione-2-oxime thiosemicarbazone on a triacetylcellulose membrane. **Sensors and Actuators B: Chemical**. 138: 251-256.

Coo, L. D., Belmonte, C. J. (2002). Nafion-PAN optical chemical sensor: optimization by FIA. **Talanta**. 58: 1063-1069.

Field, T. B., McCourt, J. L., McBryde, W. A. E. (1974). Composition and stability of iron and copper citrate complexes in aqueous solution. **Canadian Journal of Chemistry**. 52: 3119-3124.

Madden, J. E., Cardwell, T. J., Cattrall, R. W., Deady, L. W. (1995) Nafion-based optode for the detection of metal ions in flow analysis. **Analytica Chimica Acta**. 319: 129-134.

Parryan, R. W., Dubois, F. W. (1952) Citrate complexes of copper in acid solutions. **Journal of the American Chemical Society**. 74: 3749–3753.

Sands, T. J., Cardwell, T. J., Cattrall, R. W., Farrell, J. R., Iles, P. J., Kolev S. D. (2002). A highly versatile stable optical sensor based on 4-decyloxy-2-(2-pyridylazo)-1-naphthol in Nafion for the determination of copper. **Sensors and Actuators B: Chemical**. 85: 33-41.

Wansapura, C. M., Seliskar, C. J., Heineman, W. R. (2007) Spectroelectrochemical sensing based on multimode selectivity simultaneously achievable in a single device. 20. Detection of metal ions in different oxidation states. **Analytical Chemistry**. 79: 5594-5600.

CHAPTER V

ADSORPTION OF COPPER(II) BY USING 5-METHYL-4-(2-THIAZOLYLAZO)RESORCINOL IMMOBILZED ON MCM-41

5.1 Abstract

A chromogenic reagent, 5-methyl-4-(2-thiazolylazo)resorcinol (5MTAR), was immobilized on MCM-41 and used for the adsorption of Cu(II) in water. Parameters that affected the adsorption capacity were investigated. 5MTAR was adsorbed on non-calcined MCM-41 and calcined MCM-41 with the adsorption capacities 131.6 and 7.0 mg/g, respectively. The immobilization time and the pH of 5MTAR solution were 30 min and 6.0 for noncalcined MCM-41 and 1 h and 3.0 for calcined MCM-41. Non-calcined MCM-41 immobilized with 5MTAR was selected for further studies for the adsorption of Cu(II). Adsorption of Cu(II) on 5MTAR-immobilized non-calcined MCM-41 adsorbent followed Langmuir adsorption model and pseudo-second-order kinetic equation. The adsorption capacity for Cu(II) was 24.2 mg/g with the contact time of 15 min at pH 5.0. The adsorbent showed high selectivity to Cu(II) and could be regenerated at least 5 times by soaking in 0.2 M EDTA for 30 min with the %RSD of 5.0%.

5.2 Introduction

Copper contaminated in water from many industries such as electroplating, mining, paints and pigments and tanning can be a potential toxic pollutant. Removal of copper in such water sources is important to improve the water quality before discharging to the environment.

Adsorption is a one of the methods that has been used for the removal of heavy metal ions from contaminated water. The method is simple and economical. A variety of adsorbents, such as clays (Karapinar and Donat, 2009), zeolites (Wu, Zhao and Yang, 2011), silica (Bois, Bonhomme, Ribes, Pais, Raffin, Tessier and Franck, 2003), SBA-15 (Aguado, Arsuaga, Arencibia, Lindo and Gascn, 2009) and MCM-41 (Parida, Mishra and Dash, 2012) have been used for Cu(II) removal.

MCM-41 is widely used as an adsorbent because it has high surface area, high stability in acid medium. The surface of MCM-41 can be modified with various functional groups to be selective for a certain adsorbate. For metal ion adsorption, parent MCM-41 has low adsorption capacity and selectivity. Therefore, many researchers modified the surface of MCM-41 by using amine or thiol group to enhance the adsorption capacity. However, the modification with amine and thiol exhibited low selectivity (Benhamoua, Baudub, Derrichea, and Baslyb, 2009).

In this work, 5MTAR which is a ligand that can selectively form complex with Cu(II) was immobilized into MCM-41 and the adsorbent was used for Cu(II) adsorption in water samples. Selectivity and regeneration of the prepared adsorbent were evaluated. Kinetic model and adsorption isotherm were investigated.

5.3 Experimental

5.3.1 Preparation of solutions

A stock solution of 500 ppm Cu(II) was prepared by dissolving 0.1901 g $Cu(NO_3)_2 \cdot 3H_2O$ in phosphate buffer and the volume of the solution was made up to 100 mL in a volumetric flask. Cu(II) solutions with lower concentrations were prepared by appropriate dilution of the stock solution.

5MTAR stock solution was prepared by dissolving 0.0250 g of 5MTAR in 40% ethanol in phosphate buffer. The volume of the solution was adjusted to 100 mL.

Phosphate buffer solutions pH 3-9 were prepared by mixing appropriate volume of 0.1 M phosphoric acid (85%, UNIVAR) and 0.1 M dibasic sodium phosphate (99.5%, Riedel-de Haën) and a pH-meter was used to measure the pH of the solutions.

5.3.2 Preparation of MCM-41

To extract silica, 89 g rice husk was mixed with 250 mL 3 M HCl in a round bottom flask and the mixture was refluxed at 90 °C for 3 h. The treated rice husk was separated from the mixture and washed with water until the pH of the washed solution was ~7. The treated rice husk was later calcined in a muffle furnace at 550 °C for 3 h with the heating rate of 5 °C/min. The obtained white solid was rice husk silica.

MCM-41 was prepared using a method from the literature (Rintramee, Föttinger, Rupprechter and Wittayakun, 2012). A 3.0 g rice husk silica was dissolved in 30 mL of 5 M NaOH with stirring provided for 4 h or until the silica dissolved completely. The silica solution was added into a CTAB solution, which was prepared by dissolving 10 g CTAB powder in 250 mL DI water. The pH of the solution mixture was adjusted to 10-11 using 0.3 M H_2SO_4, transferred to a Teflon-lined stainless steel autoclave and kept in an oven at 110 °C for 3 days. A solid product obtained was separated by centrifugation and washed with DI water until the pH of the washed solution was ~7. This solid product was called non-calcined MCM-41 (MCM-41-NC). To remove CTAB, MCM-41-NC was calcined at 540 °C for 6 h with the heating rate of 2 °C/min. The obtained white solid was called calcined MCM-41 (MCM-41-C).

5.3.3 Characterizations of adsorbents

X-ray diffraction (XRD) patterns of MCM-41-NC, MCM-41-C and MCM-41-NC immobilized with 5MTAR were obtained with a Bruker diffractometer (AXS D5005). X-ray was from CuKα radiation with the source operated at 40 kV and 40 mA. Scan speed was 0.02^o per min and a scan range was 1.5 to 10^o (2θ).

Fourier transform infrared spectra of MCM-41-NC and MCM-41-C were obtained using a Bruker infrared spectrometer (TENSOR 27). KBr technique was selected for preparation of the samples. A sample and KBr powder were mixed with a weight ratio of 1:100 before making the pellet. The IR spectra were recorded in the range of 400-4000 cm^{-1}.

Textual properties of MCM-41-NC and MCM-41-C were determined by nitrogen adsorption-desorption analysis at -196 °C using a Micrometrics ASAP 2010. MCM-41-NC was degassed at 250 °C for 2 h. (Jabariyan and Zanjanchi, 2012) and MCM-41-C was degassed at 300 °C for 12 h (Rintramee et, al., 2012) before the measurement. A method of Brunarer-Emmatte-Teller (BET) was used to calculate the specific surface area of the samples using the data obtained from the isotherms in the relative adsorption pressure (P/P_0) range of 0.001-0.990. A method of Barrett-Joyner-Halenda (BJH) was used to calculate the pore size of the samples using the desorption branches of isotherms.

5.3.4 Immobilization of 5MTAR on non-calcined and calcined MCM-41

A 0.0050 g MCM-41-NC or MCM-41-C was placed into 25 mL plastic bottle and 10 mL of 5MTAR solution was added and stirred for 30 min. The adsorbent was separated by centrifugation. The solution was analyzed for the nonadsorbed 5MTAR by a UV-Vis spectrometer. The solid separated was washed with DI water several times and dried in an oven at 80 ^{o}C for 12 h. The effect of pH, contact time and 5MTAR concentration were investigated.

5.3.5 Cu(II) adsorption

Adsorption of Cu(II) was conducted using 10 mL Cu(II) solution and 0.0050 g adsorbent. The mixture was placed in a 25 mL plastic bottle with stirring provided at 400 rpm. After 1 h adsorption the solid and liquid phases were separated by centrifugation at 3000 rpm for 10 min. The amount of Cu(II) after adsorption was determined using an atomic absorption spectrometer (AAanalyst 100, Perkin-Elmer).

The effect of pH on the adsorption of Cu(II) was investigated in the pH range of 2-7 using phosphate buffer solution to control the pH of the solutions. The mass of adsorbents used in the adsorption study was 0.0050 g. The concentration of Cu(II) was fixed at 15 ppm. The adsorption time was 1 h.

Influence of time on the adsorption capacity was investigated using 15 ppm Cu(II) solution with pH 5.0 and 0.0050 g adsorbent. The adsorption time was varied within the range of 0-120 min.

5.3.6 Regeneration and reversibility

Regeneration of 5MTAR/MCM-41 after the adsorption of Cu(II) was investigated. The adsorption of Cu(II) was conducted using 15 ppm Cu(II) solution. After the adsorption, the adsorbent was separated by centrifugation at 3000 rpm for 10 min. Then the adsorbent was soaked in 20 mL EDTA solution at pH 5 and the adsorbent was separated by centrifugation at 3000 rpm for 10 min. The adsorbent was washed with DI water and buffer solution before being used in the adsorption again. The amount of Cu(II) after adsorption was determined using the atomic absorption spectrometer . The EDTA concentration was varied from 0.2 to 1.0 M. The time used for the regeneration was investigated from 5 to 60 min.

Adsorption reversibility was investigated with MCM-41-NC immobilized with 5MTAR. The adsorbent was mixed with 10 mL 15 ppm Cu(II) solution at the optimum condition for 30 min, separated from the solution and placed in 20 mL 0.2 M EDTA. After that the adsorbent was put into the phosphate buffer. These procedures were repeated until the significant change in the adsorption capacity was observed.

5.3.7 Selectivity

The effect of foreign species on the adsorption of Cu(II) was studied at the optimum condition for adsorption. A solution of Cu(II) mixed with an investigated ion was prepared with the molar ratio of both ions equals to 1:1. The concentration of ions was 2.36 x 10^{-4} M. Adsorption capacity of the adsorbent in Cu(II) solution was compared with that of the adsorbent in the solution with Cu(II) mixed with the investigated ion. The amount of Cu(II) after adsorption was determined using the atomic adsorption spectrometer.

5.4 Results and discussion

5.4.1 Characterization of MCM-41 samples

MCM-41-C and MCM-41-NC were successfully synthesized with a silica source from rice husk as confirmed by XRD patterns in Figure 5.1. For the MCM-41-NC characteristic peaks appeared at 2θ about 2.3°, 3.7°, 4.5° and 5.7°. These correspond to reflection planes 100, 110, 200 and 210, respectively. For MCM-41-C, the peaks were shifted slightly to higher 2θ because of the shrinkage of the structure after calcination (Ariapad, Zanjanchi and Arvand, 2012).

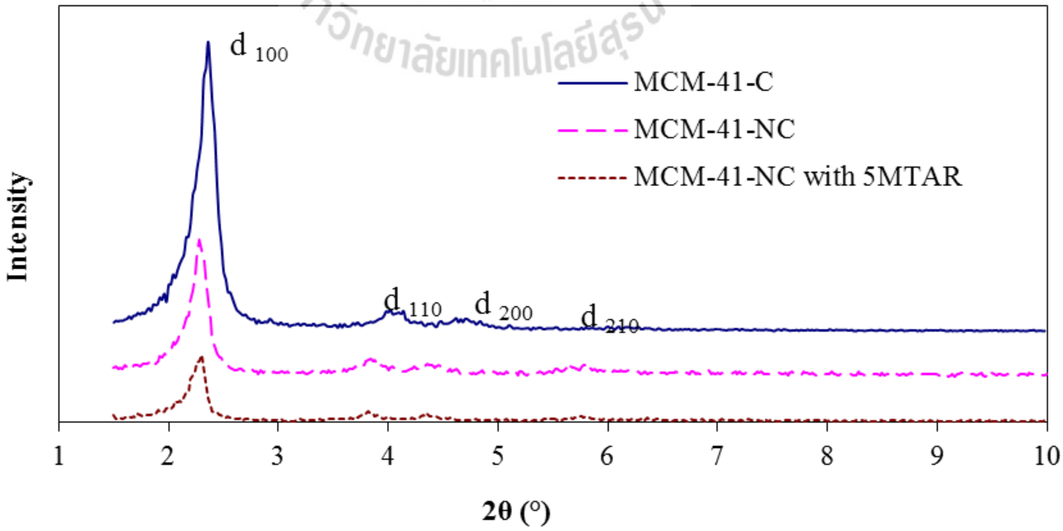


Figure 5.1 XRD patterns of MCM-41-C, MCM-41-NC and MCM-41-NC immobilized with 5MTAR.

IR spectra of MCM-41-C and MCM-41-NC are shown in Figure 5.2. IR spectrum of MCM-41-NC showed two bands at 2852 and 2921 cm^{-1} which were assigned to C-H stretching of -CH_3 and -CH_2 (Ariapad et al., 2012). These two bands disappeared after the MCM-41-NC was calcined at 540 °C for 6 h.

From N_2 adsorption-desorption analysis, specific surface areas of MCM-41-C and MCM-41-NC were 1288 and 434 m^2/g, respectively. Total pore volumes were 0.972 cm^3/g for MCM-41-C and 0.316 cm^3/g for MCM-41-NC. Larger surface area and pore volume were expected as the template was removed from the MCM-41-NC.

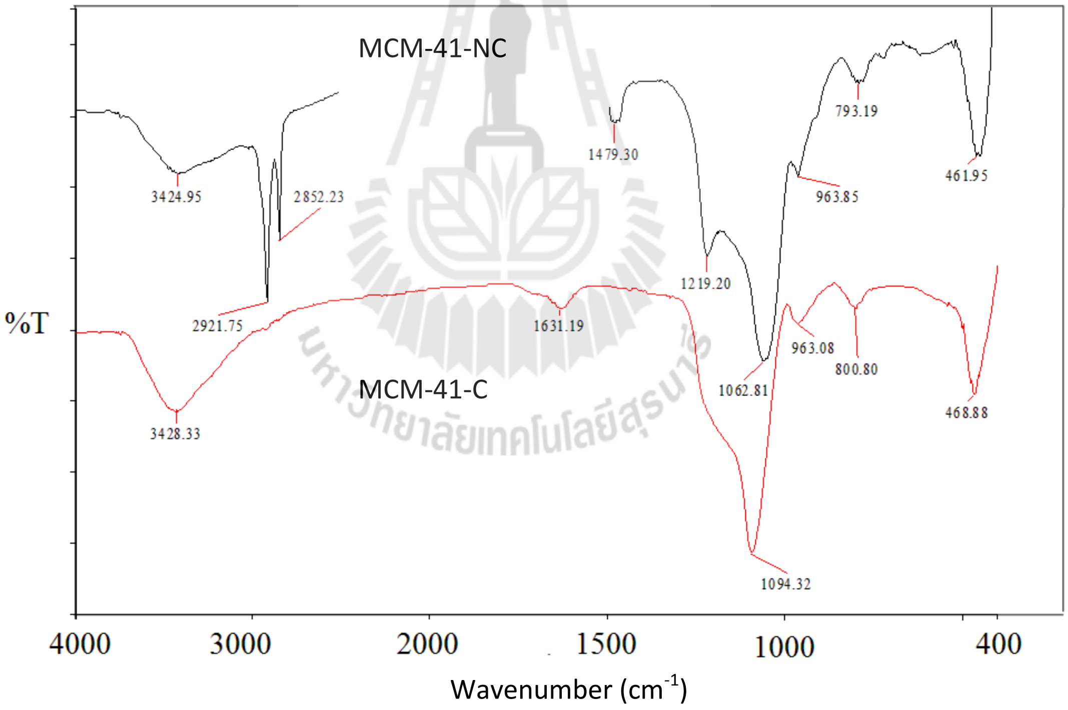


Figure 5.2 IR spectra of MCM-41-C and MCM-41-NC.

5.4.2 Immobilization of 5MTAR on MCM-41-NC and MCM-41-C

5.4.2.1 Effect of pH

Effect of pH on the immobilization of 5MTAR on MCM-41-NC and MCM-41-C was investigated in 100 ppm 5MTAR solution with the pH of solutions varied in the range of 2-8. From Figure 5.3, MCM-41-C had very low adsorption capacity at all pHs. This could be explained by the fact that the surface of MCM-41-C is populated with hydroxyl groups (Braga, Costa, Macedo, Ghesti, Souza, Dias and Dias, 2011) which is less favorable to interact with 5MTAR. However, for MCM-41-NC, the surfactant that was used as a template and not removed from the adsorbent could interact with 5MTAR and consequently increased the amount of 5MTAR adsorbed. Adsorption capacity reduced at the pH < 6.0, which could result from the protonation of 5MTAR. Similar trend was observed when the pH > 6.0. This could be from the deprotonation of 5MTAR. The ionic species of 5MTAR could interact less strongly with the template molecules compared to the nonionic 5MTAR.

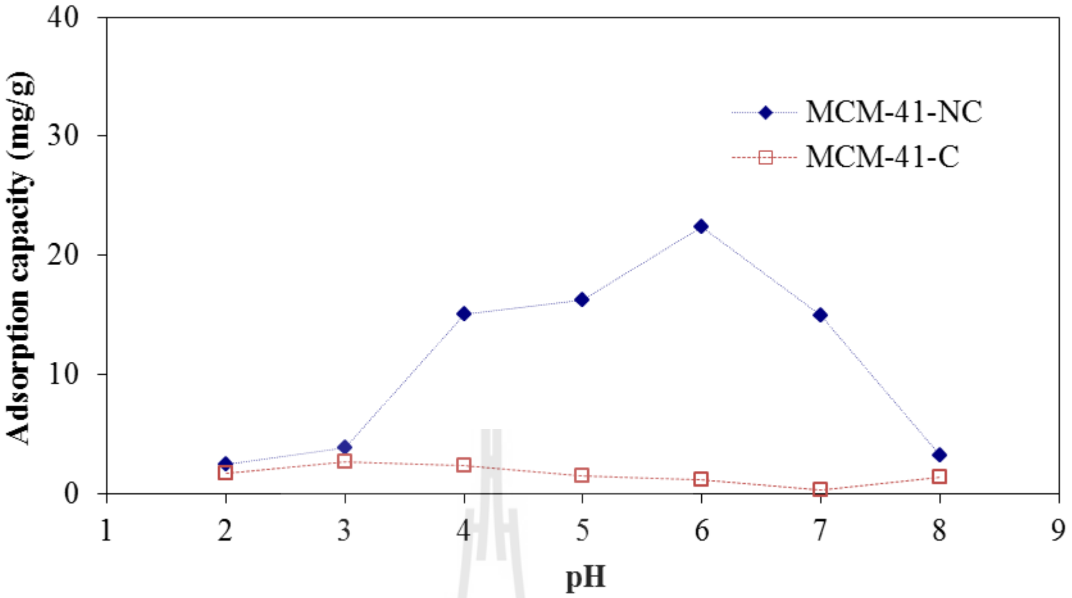


Figure 5.3 Effect of pH on the immobilization of 5MTAR on MCM-41-NC and MCM-41-C.

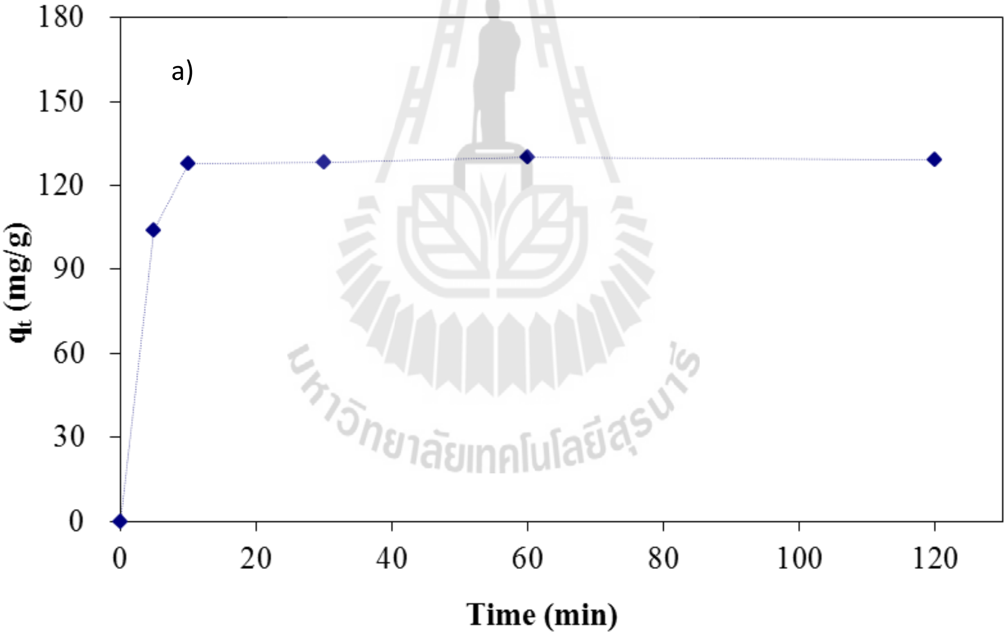
5.4.2.2 Effect of contact time

Effect of contact time on the immobilization of 5MTAR on MCM-41 was optimized. Adsorption study was conducted at various times in the range of 15-750 min. 5MTAR was adsorbed rapidly on the MCM-41-NC compared with the MCM-41-C. It took ~ 10 min for the adsorption to reach the equilibrium state for MCM-41-NC, whereas the adsorption on the MCM-41-C took > 1 h as shown in Figure 5.4. In addition, higher adsorption capacity was obtained with the MCM-41-NC compared to that with the MCM-41-C. This could be attributed to favorable interaction of 5MTAR with the surfactant inside the pores of the MCM-41-NC.

The adsorption data for both MCM-41 adsorbents were fitted with pseudo-second-order kinetic model as follows,

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \tag{5.1}$$

Where; q_t is the amount of Cu(II) adsorbed at time t (mg/g). k_2 is the pseudo-second-order rate constant (g/mg. min). q_e is the equilibrium adsorption capacity (mg/g). The fitted equation of MCM-41-NC adsorbent was y = 0.0076x + 0.0240 with a coefficient of correlation, R^2 = 0.9970. Values of k_2 and q_e calculated from the plot of t/q_t against t, were 2.4 x 10^{-3} g/mg. min and 131.6 mg/g, respectively. For the MCM-41-C adsorbent, the data was not fitted with the model.



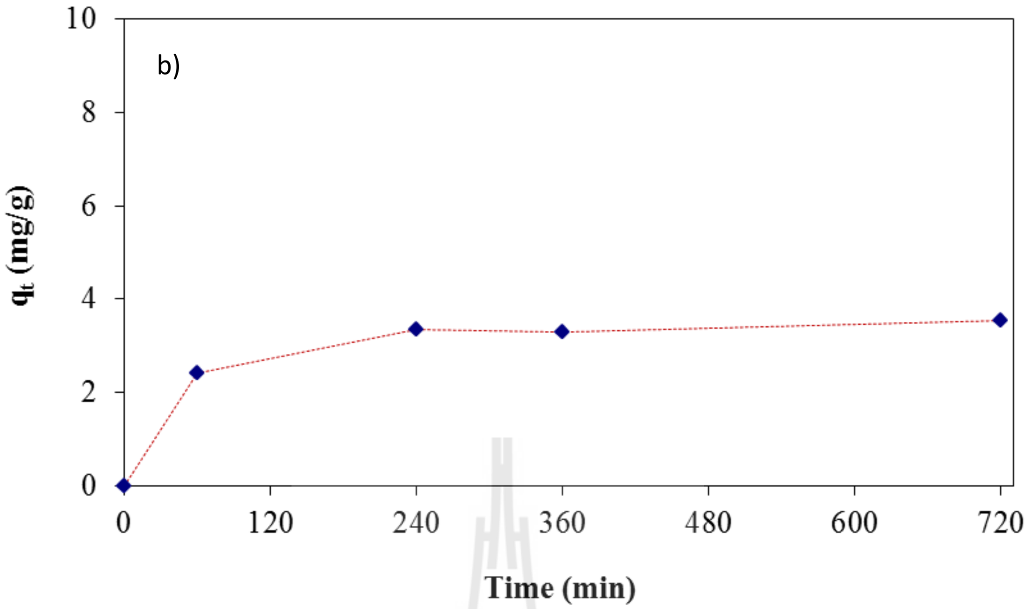


Figure 5.4 Influence of time for the immobilization of 5MTAR on a) MCM-41-NC and b) MCM-41-C.

5.4.2.3 Optimization of 5MTAR concentration

To evaluate the optimum concentration of 5MTAR to be used in the immobilization process for both adsorbents, the concentration of 5MTAR was varied in the range of 50-750 ppm. For MCM-41-NC, the pH of the solutions was fixed at 6.0 and the adsorption time was 30 min. For MCM-41-C, the pH of the solutions was 3.0 and the adsorption time was 240 min.

Adsorption capacities at different 5MTAR concentrations for both MCM-41 adsorbents are shown in Figure 5.5. The results were correlated with Langmuir adsorption model that often used to describe adsorption of a solute in a liquid solution. Values of q_m and K_A were found from the plot of X_e/q against X_e as shown in Figure 5.6. On MCM-41-NC, the K_A and q_m were calculated to be 3.9 mg^{-1} and 128.2 mg/g, respectively. The linear correlation coefficient was 0.9912. It is clear that MCM-41-NC has significantly higher amount of 5MTAR immobilized and therefore, it was selected for adsorption of Cu(II) in the next study. For MCM-41-C, the results were not fitted with neither Langmuir nor Freundlich adsorption isotherm.

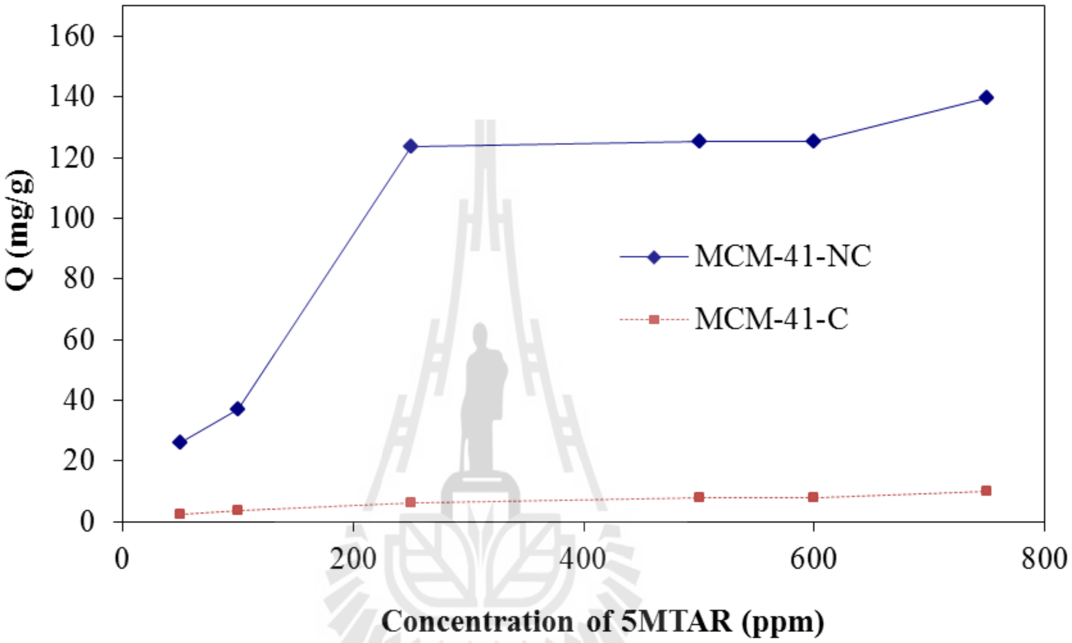


Figure 5.5 Adsorption capacities of MCM-41-NC and MCM-41-C at different concentrations of 5MTAR.

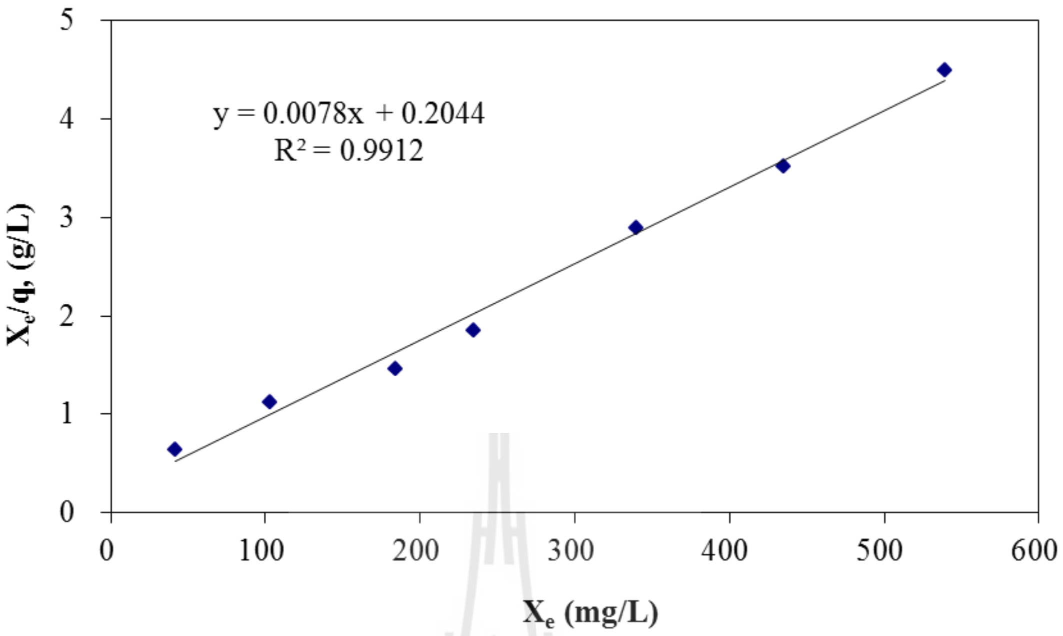


Figure 5.6 Langmuir adsorption isotherm of 5MTAR on MCM-41-NC.

5.4.3 Cu(II) adsorption

5.4.3.1 Effect of pH

The pH of a solution is one of an important factor that has influence on an adsorption capacity of Cu(II) on 5MTAR-immobilzed MCM-41-NC. As shown in Figure 5.7, high adsorption capacities were obtained in the pH range 5.0-7.0. Adsorption capacity at the pH < 5.0 was decreased due to the competition between H^+ and Cu(II) reacting with 5MTAR. At the pH >7.0, the hydrolysis of Cu(II) occurred which reduced the amount of Cu(II) adsorbed and caused the precipitation of Cu(II) (Chamjangali, Soltanpanah and Goudarzi, 2009). Further adsorption studies were conducted with solutions that the pH was controlled at 5.0.

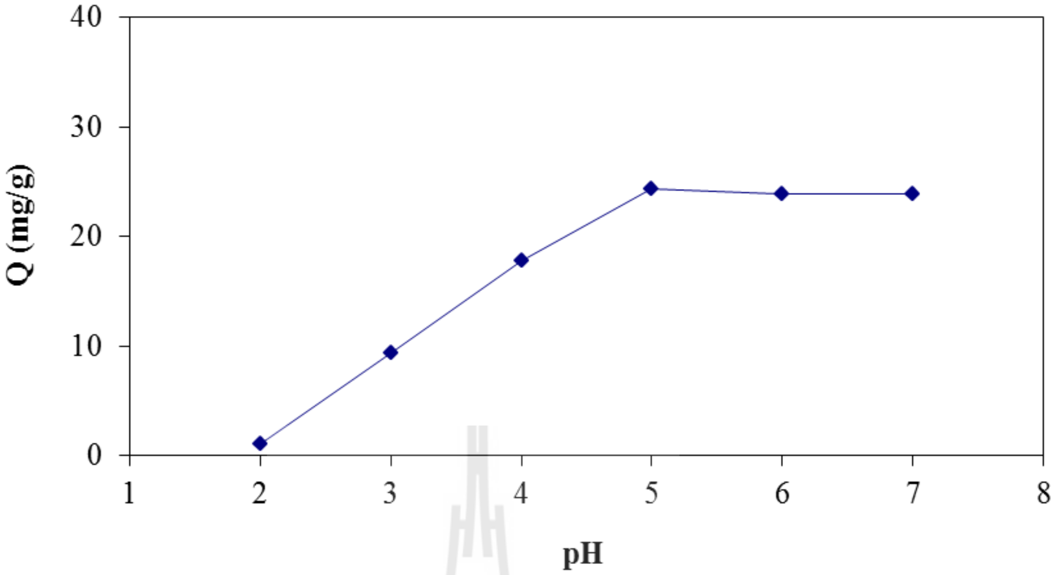


Figure 5.7 Effect of pH on the adsorption of Cu(II) on the 5MTAR-immobilized MCM-41-NC.

5.4.3.2 Effect of contact time

To investigate the influence of adsorption time, adsorption of Cu(II) with 5MTAR-immobilized MCM-41-NC was done using 15 ppm Cu(II) at pH 5.0 and the adsorption time was varied from 2 to 120 min. The amount of the adsorbent used was 0.0050 g and temperature was 27±1 °C. The equilibrium adsorption was obtained in 20 min as shown in Figure 5.8.

The pseudo-second-order equation (1) was used to fit the adsorption data. Values of k_2 and q_2 were calculated from the plot of t/q_t against t, Figure 5.9, and were 4.7×10^{-2} g/mg. min and 25.9 mg/g, respectively. The linear correlation coefficient was 0.9990.

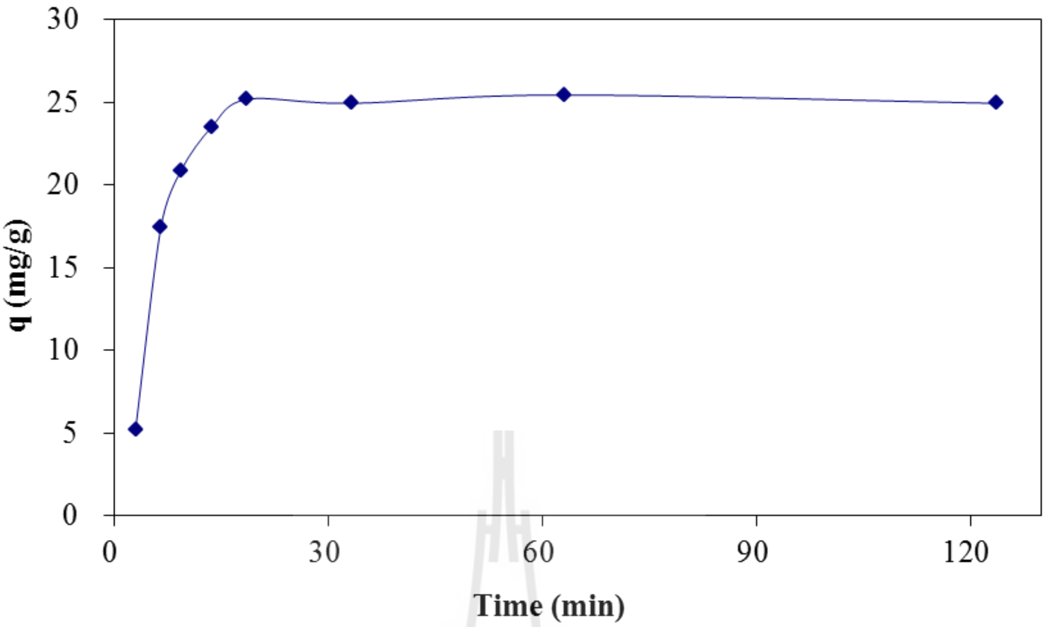


Figure 5.8 Adsorption of Cu(II) on 5MTAR-immobilized MCM-41-NC at different times.

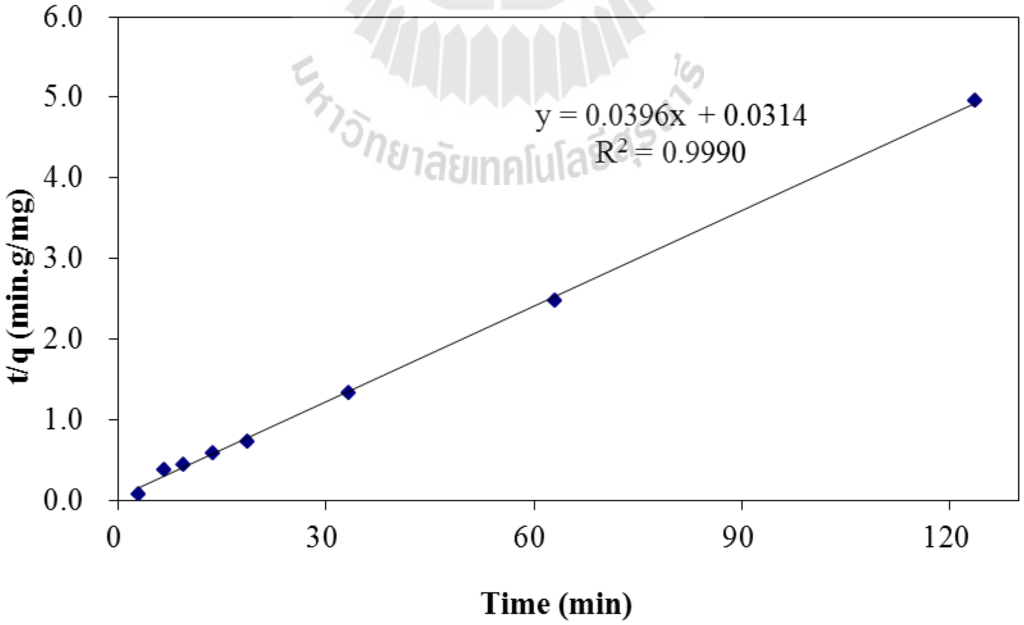


Figure 5.9 Pseudo-second-order adsorption model fitting for data obtained from the adsorption of Cu(II) on 5MTAR-immobilized MCM-41-NC.

5.4.3.3 Isotherm study

Equilibrium relationships between adsorbates and adsorbents can be explained by adsorption isotherms. In this study, an isotherm for Cu(II) was obtained from batch reactions with Cu(II) concentration range of 0.1 to 20 ppm. The adsorption temperature and time were fixed at 27 °C and 30 min, respectively. Figure 5.10 shows the adsorption isotherm of Cu(II) on 5MTAR-immobilized MCM-41-NC. The results were correlated with a Langmuir adsorption model is often used to describe adsorption of a solute in a liquid solution. The model equation is show as follows,

$$\frac{X_e}{q} = \frac{X_e}{q_m} + \frac{1}{K_A q_m} \tag{5.2}$$

Where X_e is the concentration of Cu(II) in the solution at equilibrium (mg/L). q is the amount of Cu(II) adsorbed per unit mass of the adsorbent (mg/g). K_A is the Langmuir constant (mg^{-1}). q_m is the maximum adsorption capacity (mg/g). Values of q_m and K_A were found from the plot of X_e/q against X_e, Figure 5.11, which were 24.2 mg/g and 413.0 mg^{-1}, respectively. The linear correlation coefficient was 0.9980.

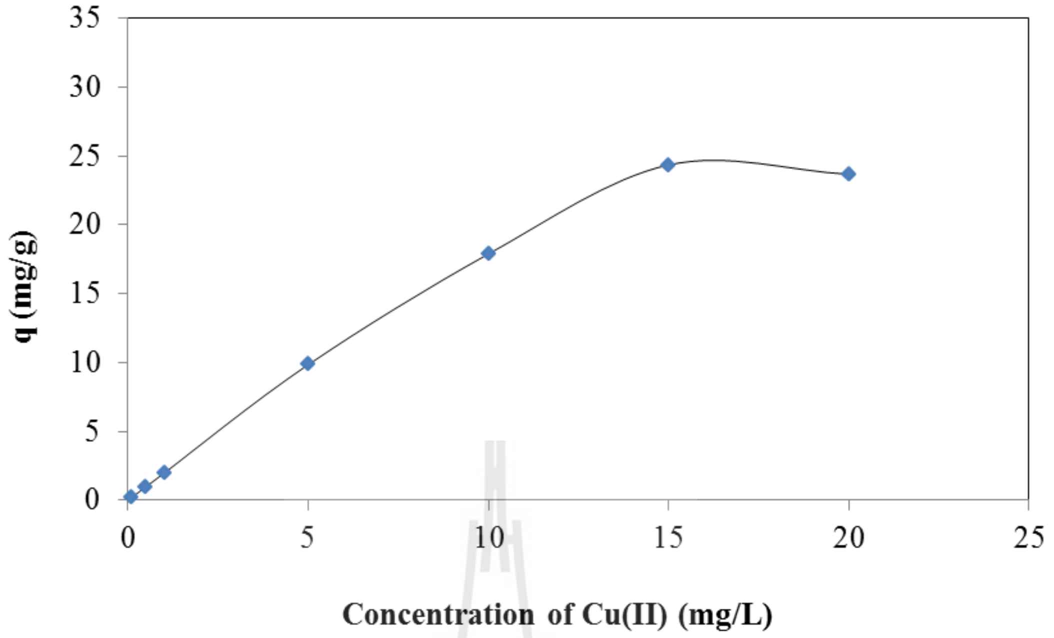


Figure 5.10 Adsorption of Cu(II) on 5MTAR-immobilized MCM-41-NC at different initial concentrations of Cu(II).

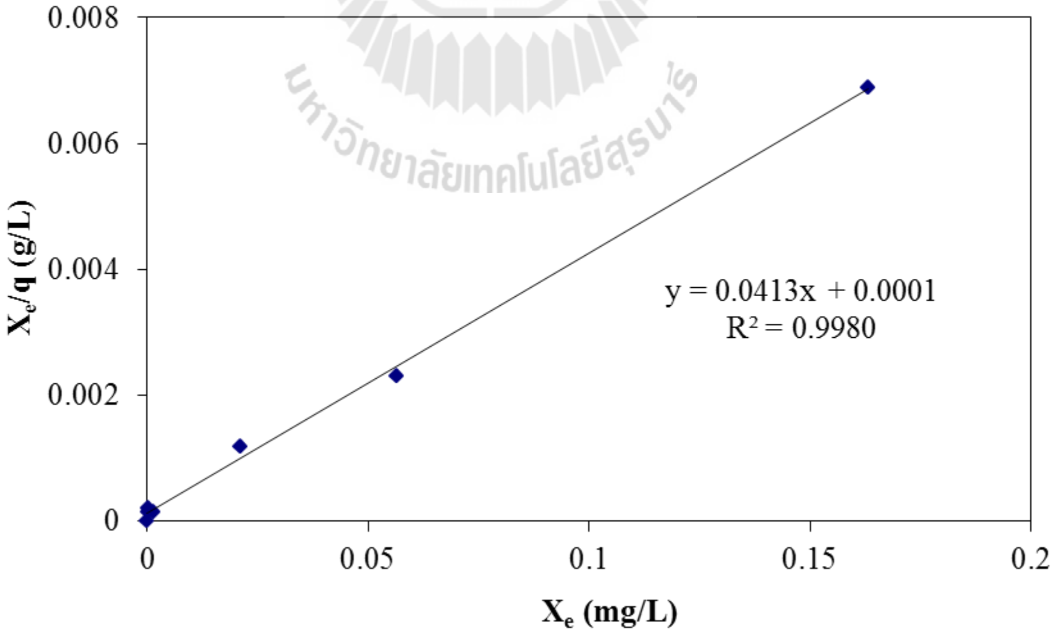


Figure 5.11 Langmuir adsorption isotherm of Cu(II) adsorbed on 5MTAR immobilized MCM-41-NC.

5.4.4 Regeneration study

Regeneration of 5MTAR-immobilized MCM-41-NC after exposed to 15 ppm Cu(II) was investigated by using EDTA solutions at pH 5.0. The EDTA concentration and contact time were optimized. The results show that the optimum EDTA concentration was 0.2 M at pH 5.0 due to the % adsorption after regeneration compared to the first adsorption approached 100% as shown in Table 5.1. When higher EDTA concentrations were used, the adsorption capacity decreased. This could be due to the leaching of 5MTAR from the adsorbent after washing excess EDTA with DI water and buffer solution.

With the optimum regenerating condition, 0.2 M EDTA and 30 min soaking time (Figure 5.12), the adsorbent was repeatedly tested for the adsorption of 15 ppm Cu(II) solution and the results are shown in Figure 5.13. Within the first three regeneration cycle, the %Cu(II) adsorption was >98.3%. After the third regeneration cycles, %Cu(II) adsorption dropped to ~ 90.3%. This could be contributed to the leaching of 5MTAR from the adsorbent.

Table 5.1 Effect of EDTA concentration on the regeneration study of 5MTAR-immbilized MCM-41-NC exposed to Cu(II).

Concentration of EDTA (M)	Adsorption capacity, (mg/g)	% adsorption compared with first adsorption[a]
0.2	24.5	101.2
0.5	21.8	91.0
1.0	20.0	83.4

[a] Adsorption capacity for first adsorption was 24.0 mg/g.

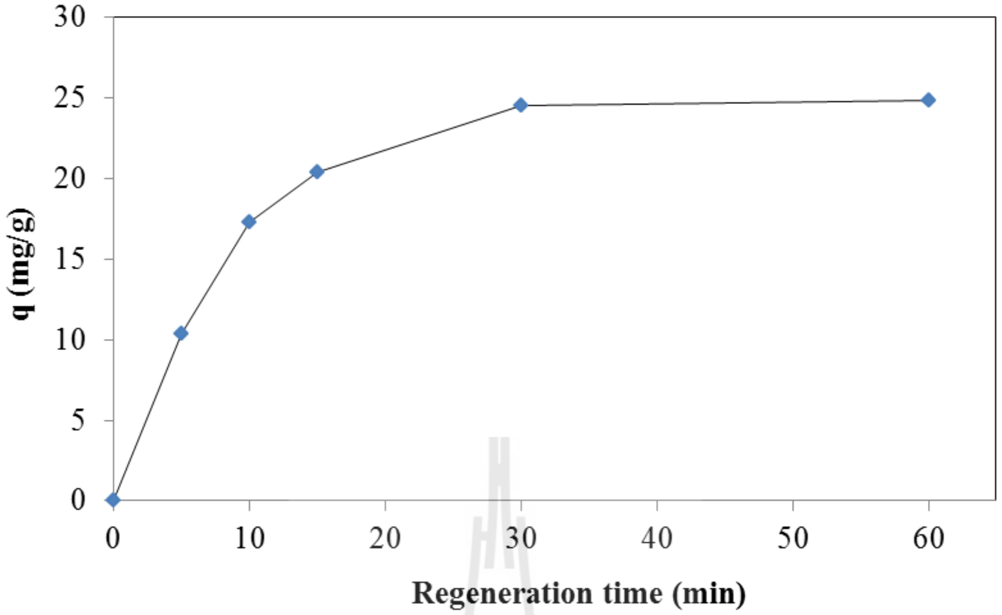


Figure 5.12 Effect of soaking time of on the regeneration of 5MTAR-immobilized MCM-41-NC exposed to Cu(II). The studied condition was 15 mL 0.2 M EDTA at pH 5.0 and 0.0050 g adsorbent.

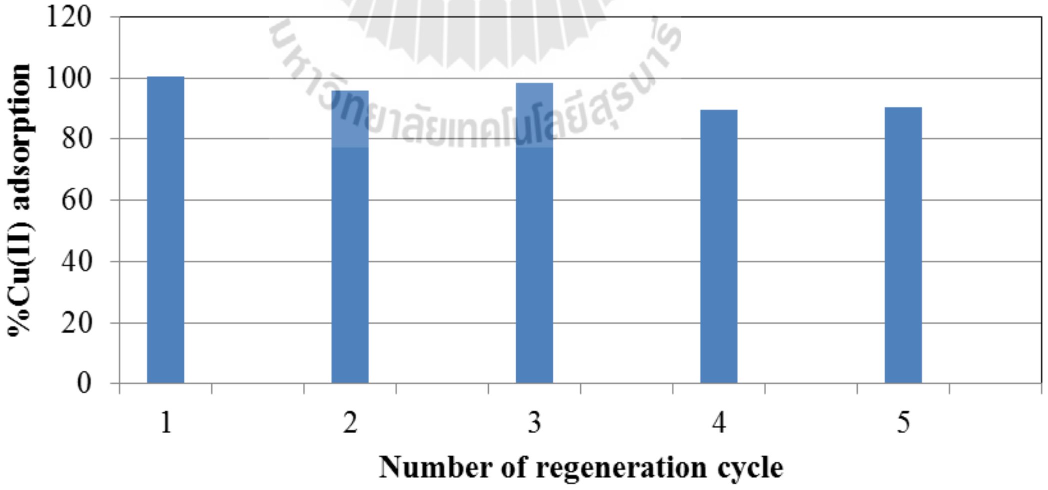


Figure 5.13 Repeated regeneration study of 5MTAR-immobilized MCM-41-NC exposed to Cu(II). Regeneration condition was 15 mL 0.2 M EDTA at pH5, 0.0050 g adsorbent and 30 min soaking time.

5.4.5 Selectivity study

The effect of foreign species on the adsorption of Cu(II) was studied at the optimum adsorption condition for Cu(II). Adsorption capacities between the Cu(II) and Cu(II) mixed with foreign species on 5MTAR-immobilized MCM-41-NC were compared. Figure 5.14, shows that the adsorbent selectivity adsorbed Cu(II) in the presence of other tested metal ions, as the %Cu(II) adsorption was >90% for most of the tested metal ions except for Hg(II).

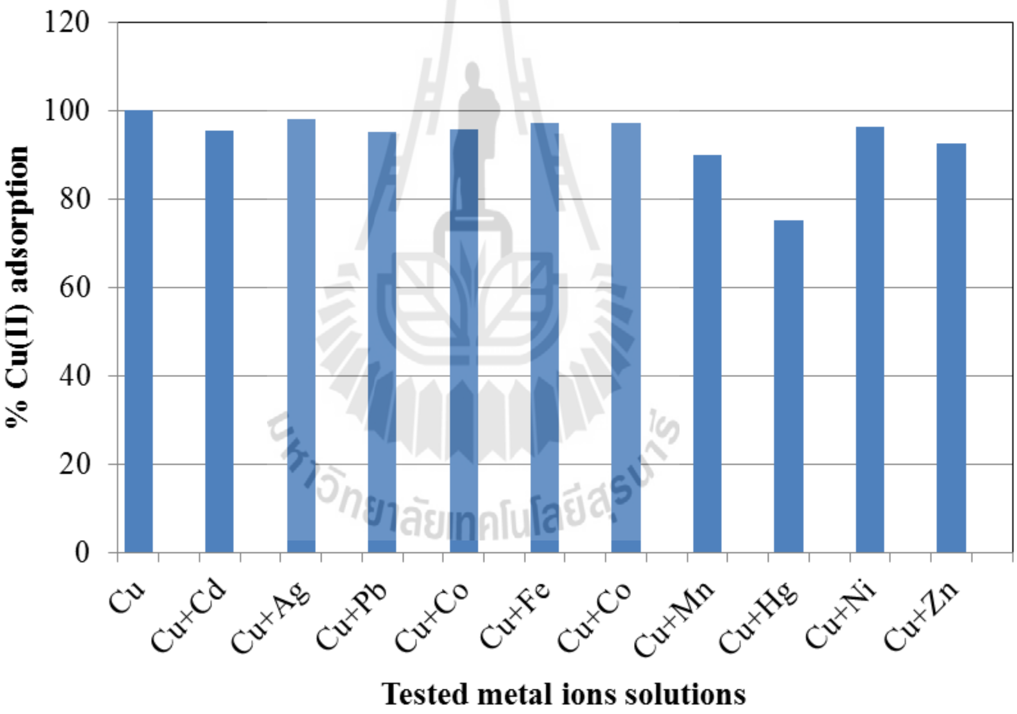


Figure 5.14 Effect of foreign species on the adsorption of Cu(II) with 5MTAR-MCM-41-NC.

5.5 Conclusions

5MTAR was immobilized on the MCM-41-NC and MCM-41-C. MCM-41-NC had higher adsorption capacity for 5MTAR at pH 6.0 with the adsorption time of 30 min. The equilibrium adsorption capacity was 131.6 mg/g and the adsorption conformed well with pseudo-second-order kinetic model. 5MTAR-immobilized MCM-41-NC was selected as a adsorbent for Cu(II) adsorption.

The 5MTAR-immobilized MCM-41-NC was used for adsorption of Cu(II). The adsorbent provided the adsorption capacity to be 24.2 mg/g at pH 5.0 and the adsorption equilibrium was established rapidly within 15 min. The adsorption behavior was fitted with pseudo-second-order kinetic model and Langmuir adsorption isotherm.

The 5MTAR-immobilized MCM-41-NC provided high adsorption selectivity and reproducibility. The adsorbent can be regenerated with a solution of EDTA and can be reused at least 5 times.

5.6 References

Aguado, J., Arsuaga, J. M., Arencibia, A., Lindo, M., Gascn, V. (2009) Aqueous heavy metals removal by adsorption on amine-functionalized mesoporous silica. **Journal of Hazardous Materials**. 163: 213-221.

Ariapad, A., Zanjanchi, M.A., Arvand, M. (2012) Efficient removal of anionic surfactant using partial template-containing MCM-41. **Desalination**. 284: 142-149.

Bois, L., Bonhomme, A., Ribes, A., Pais, B., Raffin, G., Tessier, Franck. (2003) Functionalized silica for heavy metal ions adsorption. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**. 221: 221-230.

Braga, P. R.S., Costa, A. A., Macedo, J. L., Ghesti, G. F., Souza, M. P., Dias, J. A., Dias, S. C. L. (2011) Liquid phase calorimetric-adsorption analysis of Si-MCM-41: Evidence of strong hydrogen-bonding sites. **Microporous and Mesoporous Materials**. 139: 74-80.

Benhamoua, A., Baudub, M., Derrichea, Z., Baslyb J.P. (2009) Aqueous heavy metals removal on amine-functionalized Si-MCM-41 and Si-MCM-48. **Journal of Hazardous Materials**. 171: 1001-1008.

Chamjangali, M. A., Soltanpanah, S., Goudarzi, N. (2009). Development and characterization of a copper optical sensor based on immobilization of synthesized 1-phenyl-1,2-propanedione-2-oxime thiosemicarbazone on a triacetylcellulose membrane. **Sensor and Actuators B: Chemical**. 138: 251-256.

Jabariyun, S., Zanjanchi, M. A. (2012) A simple and fast sonication procedure to remove surfactant templates from mezoporus MCM-41. **Ultrasonics Sonochemistry**. 19: 1087-1093.

Karapinar, N., Donat, R. (2009) Adsorption behaviour of Cu(II) and Cd(II) onto natural bentonite. **Desalination.** 249: 123-129.

Rintramee, K., Föttinger, K., Rupprechter, G., Wittayakun, J. (2012) Ethanol adsorption and oxidation on bimetallic catalysts containing platinum and base metal oxide supported on MCM-41. **Applied Catalysis B: Environmental**. 115-116: 225-235.

Parida, K., Mishra, K. G., Dash, S. K. (2012) Adsorption of copper(II) on NH_2-MCM-41 and its application for epoxidation of styrene. **Industrial & Engineering Chemistry Research**. 51: 2235-2246.

Wu, X. L., Zhao, D., Yang, S. T. (2011) Impact of solution chemistry conditions on the sorption behavior of Cu(II) on Lin'an montmorillonite. **Desalination**. 269: 84-91.

CHAPTER VI

CONCLUSIONS

This research was directed toward the development of optical sensing films for Cu(II) determination based on 5-methyl-4-(2-thiazolylazo)resorcinol (5MTAR) immobilized in chitosan, amino-silica particles mixed with chitosan and Nafion. The studies concerned about selection of the compatible supporting matrices for 5MTAR, optimization of parameters for fabrication of the sensing films and evaluation of parameters affecting the response of the sensing films to the analyte. The application of the developed sensing for real samples analysis was also demonstrated.

The reaction of 5MTAR with Cu(II) in a solution produced a complex that gave a distinctive change in the absorbance signal at 550 nm. When the reaction occurred in the developed films, a red shift in the absorption spectra was obtained. The change in the absorbance signal cold be correlated to the concentration of Cu(II) in sample solutions.

Procedures used to prepare chitosan and amino-silica suspended in chitosan based sensing films were almost the same and time consuming, besides the extra steps in the preparation of amino-silica particles that took much longer time to produce nanosize amino-silica particles. A procedure for fabrication of Nafion based sensing films was much less complicate. A film could be simply formed by dissolving 5MTAR in a solution of Nafion, spreading and drying the cocktail solution on a transparent support.

It has been demonstrated that the developed sensing films in combination with the optimized measurement conditions could be used for the determination of Cu(II) at part per million (ppm) levels. The largest linear range, 0.1-40 ppm, was obtained with amino-silica suspended in chitosan based sensing film and the shortest linear range, 0.1-5.0 ppm, was obtained with Nafion based sensing film. Samples with the analyte concentrations outside the working range could be subjected to appropriate dilution or preconcentration before the analysis with the sensors. The sensors after exposed to Cu(II) could be regenerated readily with a solution of HCl and had a long lifetime. Good repeatability and reproducibility were obtained in the study using the developed sensing films. The chitosan based sensing film was applied successfully for the determination of Cu(II) in tap water samples. Nafion based sensing film was applied for the determination of Cu(II) in tap water and Thai whisky samples with satisfactory results.

In the study of adsorption of 5MTAR on MCM-41, non-calcinced MCM-41 (MCM-41-NC) had higher adsorption capacity than calcined MCM-41. The adsorption conformed well to pseudo-second-order kinetic model. The 5MTAR-immobilized MCM-41-NC was used for adsorption of Cu(II). The adsorption capacity for Cu(II) of the adsorbent was 24.2 mg/g in a solution pH 5.0. The adsorption equilibrium was established rapidly within 15 min. The adsorption behavior was fitted with pseudo-second-order kinetic model and Langmuir adsorption isotherm. The 5MTAR-immobilized MCM-41-NC had high adsorption selectivity toward Cu(II). The adsorbent can be regenerated with a solution of EDTA and can be reused at least 5 times. The developed adsorbent could be potentially applied for the extraction of Cu(II) in aqueous samples and the adsorbed Cu(II) could be later leached out and subjected to an appropriate detection scheme.

APPENDICES

APPENDIX A

DETERMINATION OF STOICHIOMETRY OF Cu(II)-5MTAR COMPLEX

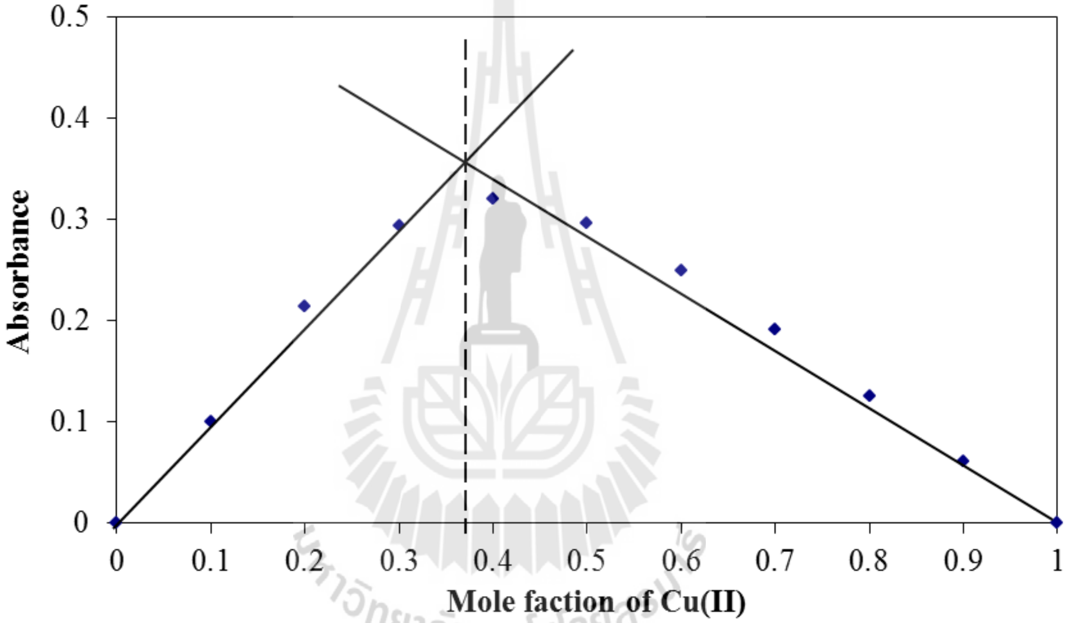


Figure A.1 Determination of a stoichiometric ratio of Cu(II)-5MTAR complex using the continuous variation method. Phosphate buffer pH 3.5 mixed with acetonitrile with the volume ratio of 70:30 was used as a solvent for the preparation of solutions.

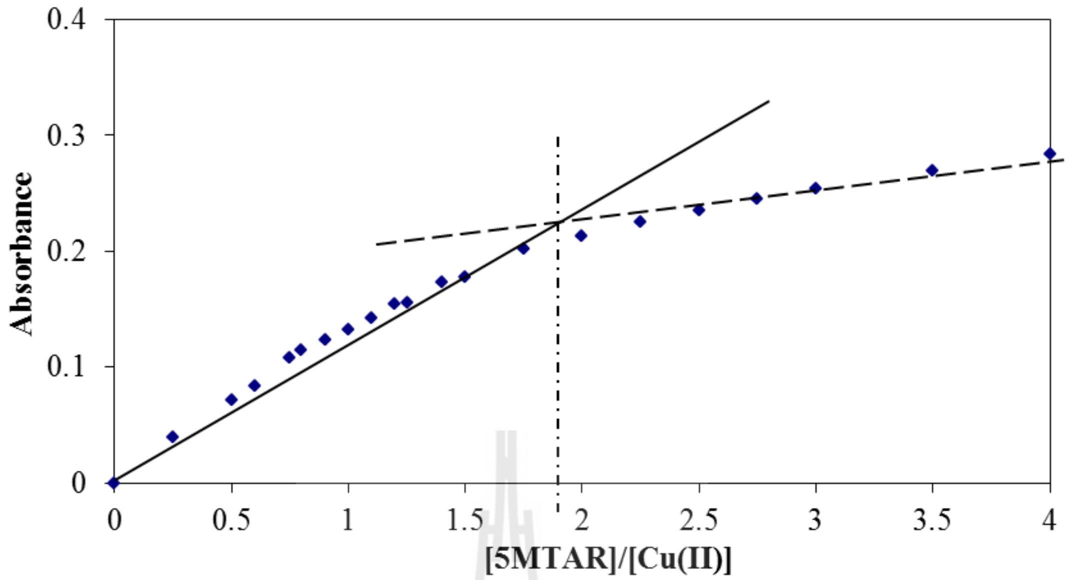


Figure A.2 Determination of a stoichiometric ratio of Cu(II)-5MTAR complex using the mole ratio method. The concentration of Cu(II) used in the study was 2.72 x 10^{-5} M. Phosphate buffer pH 3.5 mixed with acetonitrile with the volume ratio of 70:30 was used as a solvent.

APPENDIX B

ABSORPTION SPECTRA OF SOLUTIONS OF 5-METHYL-4-(2-THIAZOLYAZO)RESORCINOL AT VARIOUS pHs

Solutions of 5-methyl-4-(2-thiazolyazo)resorcinol (5MTAR) were prepared using acetonitrile mixed with DI water with the volume ratio of 30:70 as a solvent. The pH of the solution was adjusted using solutions of HCl or NaOH.

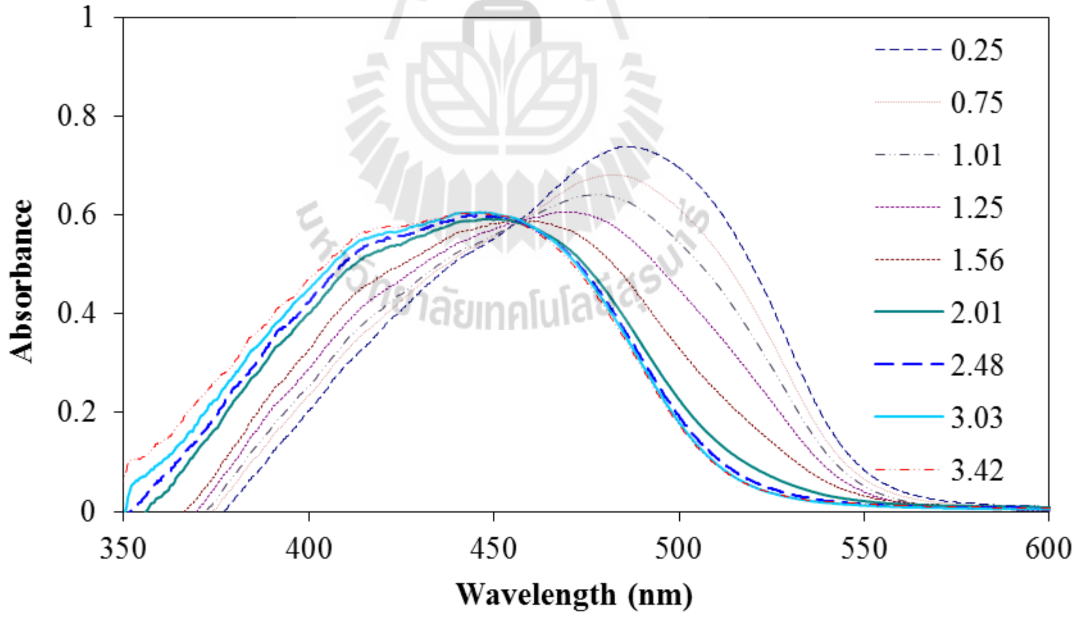


Figure B.1 Absorption spectra of solutions of 5MTAR in the pH range of 0.25-3.42.

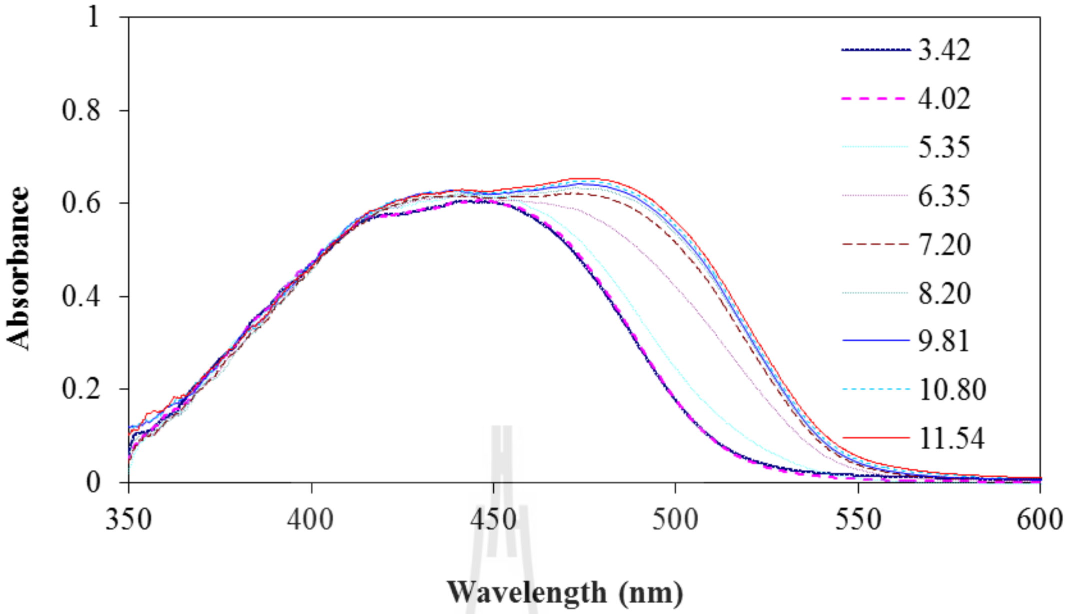


Figure B.2 Absorption spectra of solutions of 5MTAR in the pH range of 3.42-11.54.

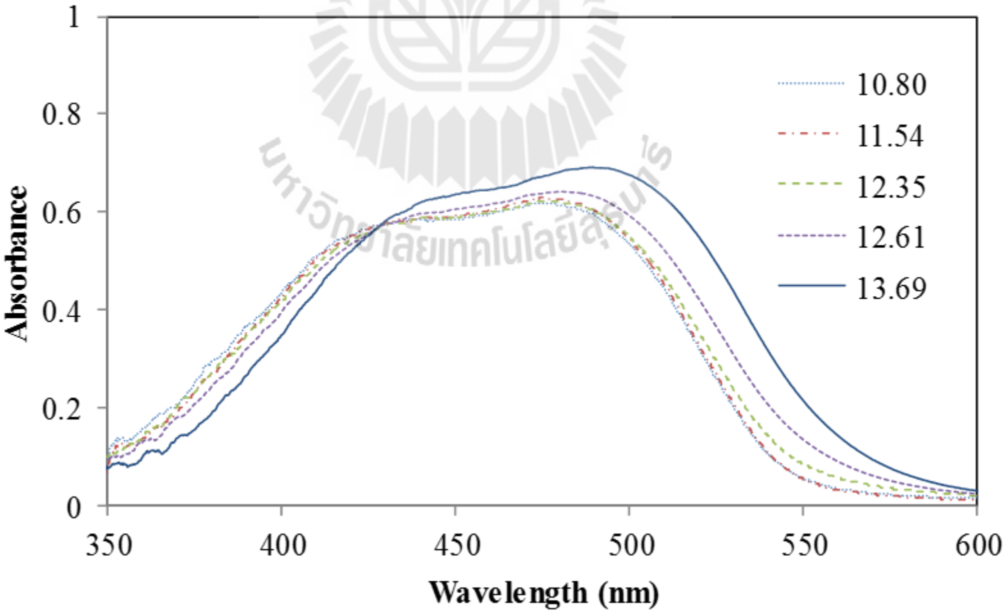


Figure B.3 Absorption spectra of solutions of 5MTAR in the pH range of 10.80-13.69.

APPENDIX C

PROTON DISSOCIATION EQUILIBRIA OF 5-METHYL-4-(2-THIAZOLYAZO)RESORCINOL IN AQUEOUS SOLUTIONS

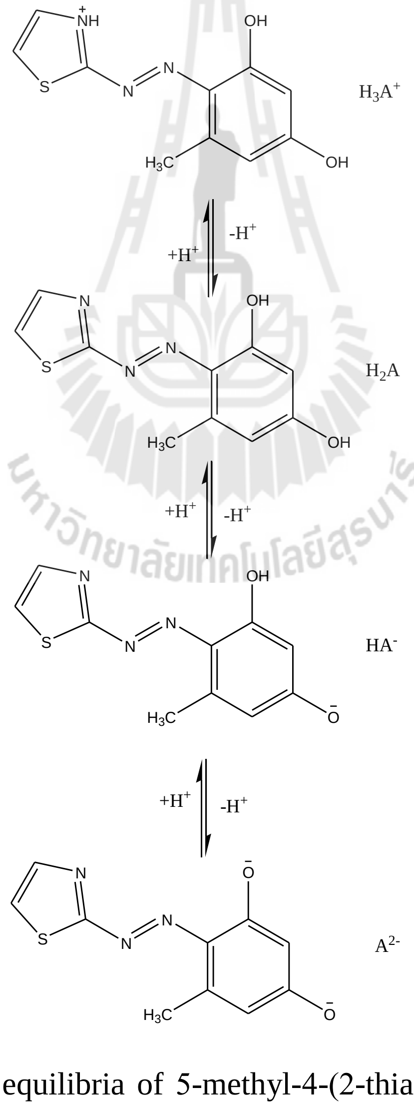


Figure C.1 Protolytic equilibria of 5-methyl-4-(2-thiazolyazo)resorcinol in aqueous solution (Wada and Nakagawa, 1975).

Reference

Wada, H., Nakagawa, G. (1975) Some 4-(2-thaiazolyazo)resorcinol derivatives as analytical reagents. Bunseki Kagaku. 24: 758-761.

CURRICULUM VITAE

KOTCHAKRIANGKRAI SANAI

Born: 24 August, 1985 in Ubon Ratchathani, Thailand

Citizenship: Thai

Education:

2004-2007 B. Sc. Chemistry

Ubon Ratchathani University, Ubon Ratchathani, Thailand

Presentationin international conferences:

Sanai, K., Wittayakun, J., and Prayoonpokarach, S. Preparation of Submicrometer Silica Particles/Chiosan Films for Optical Chemical Sensor Applications, The 38th CHEMECA 2010 Conference, 26-29 September 2011, Hilton Hotel, Adelaide, Australia.

Publication:

Sombatsri, S., Wittayakun, J., **Sanai, K.**, Kajsanthia, K., and Prayoonpokarach, S. (2012) An optical sensing film for the determination of Co(II) based on disodium-1-nitroso-2-naphthol-3,6-disulfonate immobilized in chitosan film. Sensors and Actuators B: Chemical. 166: 772-776. IF = 3.840