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จุฬาลงกรณ์มหาวิทยาลัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

SYNTHESIS OF CHEMICALLY MODIFIED SILICA GEL FOR PRECONCENTRATION OF HEAVY METALS

Mr. Wittaya Ngeontae

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

A Thesis Submitted in Partial Fulfillment of the Requirements
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Field of Study	Chemistry
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ทำสังการสังเคราะห์ซิลิกาเจลชนิดใหม่ 3 ชนิด โดยการดัดแปรทางเคมีด้วยหมู่ฟังก์ชันเป็นเอมิโดเอมิดอกซึม (เฟส A) เบนโซไทอาโซลิล อะซิเทต (เฟส B) และ อะมิโนไทโอเอมิโดแอนทราควิโนน (เฟส Q) เพื่อใช้สำหรับสกัดโลหะหนักอย่างเลือกจำเพาะและเพื่อเพิ่มความเข้มข้นของโลหะหนักจากตัวอย่างน้ำ ทำการพิสูจน์เอกลักษณ์ของซิลิกาเจลที่สังเคราะห์ได้ด้วยคาร์บอน-13 เอ็นเอ็มอาร์ การวิเคราะห์ปริมาณธาตุองค์ประกอบ อินฟาเรดสเปกโทรสโกปี การวิเคราะห์พื้นที่ผิวจำเพาะด้วยการดูดซับไนโตรเจน และเทอร์มอกัลวิเมทริกอะนาไลซิส ศึกษาการสกัดที่มีผลมาจากค่าพีเอชของสารละลาย และระยะเวลาที่ใช้ในการสกัด ในระบบแบทช์กับไอออนตะกั่ว นิกเกิล แคดเมียม ทองแดง และโคบอลต์ พบว่าค่าพีเอชของสารละลายที่เหมาะสมอยู่ในช่วง 4-7 และสามารถเข้าสู่สมดุลการสกัดได้อย่างรวดเร็วพฤติกรรมการดูดซับบนพื้นผิวเป็นไปตามโมเดลของแลงเมียร์ เฟส A มีความจำเพาะต่อไอออนทองแดง ในขณะที่เฟส B สามารถสกัดได้ทั้งไอออนทองแดงและตะกั่ว ส่วนเฟส Q สามารถสกัดได้ทุกไอออนที่ทำการศึกษา สำหรับระบบไหล ทำการบรรจุซิลิกาเจลที่ดัดแปรในคอลัมน์ขนาดเล็กที่สร้างขึ้นเอง แล้วศึกษาอัตราการไหลของสารละลายที่เหมาะสม ใช้สารละลายกรดไนตริกเข้มข้น 1 เปอร์เซ็นต์โดยปริมาตร 5 มิลลิลิตร เป็นตัวชะจากสารละลายตัวอย่างเริ่มต้น 100 มิลลิลิตร พบว่าการสกัดโลหะหนักด้วยซิลิกาที่สังเคราะห์ขึ้นทั้งสามชนิดไม่ได้ถูกรบกวนจากไอออนโซเดียม โพแทสเซียม แมกนีเซียม แคลเซียม คลอไรด์ และซัลเฟต ได้นำวิธีการที่ได้ไปทดลองใช้กับการเพิ่มความเข้มข้นของโลหะหนักในตัวอย่างน้ำจากสระน้ำธรรมชาติ น้ำประปา และน้ำดื่ม ให้ความแม่นและความเที่ยงของวิธีที่สูง มีค่าดีเทคชันลิมิตของวิธีการเป็น 22, 3.0, 1.2, 1.0 และ 0.95 นาโนกรัมต่อมิลลิลิตร สำหรับไอออนตะกั่ว นิกเกิล แคดเมียม ทองแดง และโคบอลต์ ตามลำดับ

จุฬาลงกรณ์มหาวิทยาลัย

ภาควิชา	เคมี	ลายมือชื่อนิสิต	วิทยา เงินแท้
สาขาวิชา	เคมี	ลายมือชื่ออาจารย์ที่ปรึกษา	วัลภา เอื้องไมตรีภิรมย์
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KEY WORD : PRECONCENTRATION / CHEMICALLY MODIFIED SILICA / HEAVY METAL / FLAME ATOMIC ABSORPTION SPECTROMETRY

WITTAYA NGEONTAE: SYNTHESIS OF CHEMICALLY MODIFIED SILICA GEL FOR PRECONCENTRATION OF HEAVY METALS. THESIS ADVISOR: ASST. PROF. WANLAPA AEUNGMAITREPIROM, Ph.D., THESIS CO-ADVISOR: ASSOC. PROF. THAWATCHAI TUNTULANI, Ph.D., 118 pp.

Three types of novel chemically modified silica with amidoamidoxime (phase A), ethyl 2-benzothiazoly acetate (phase B) and aminothioamido anthraquinone (phase Q) were synthesized for selective extraction and preconcentration heavy metals from water sample. The modified silica's were characterized by ^{13}C-NMR, elemental analysis, FT-IR, N_2-Adsorption and TGA. The extraction studied towards Pb(II), Ni(II), Cd(II), Cu(II) and Co(II) were carried out in batch extraction as function of pH and extraction time. The optimum pH ranges 4-7 with fast extraction equilibrium. The metal adsorption behaviors well agreed with Langmuir's adsorption model. Phase A showed highly selective towards Cu(II) while phase B preferred both Cu(II) and Pb(II). Phase Q, on the other hand, can be applied for all metal ion extraction. In flow system, the modified silica were packed by lab-made mini-columns. The solution flow rates were optimized. Nitric acid (1%, 5 mL) was used as an eluent from the initial sample volume of 100 mL. No interferences from Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^- and SO_4^{2-} was observed. Spiked pond water, tap water and drinking water were chosen as real samples. The proposed method provided high accuracy and precision. The method detection limits were 22, 3.0, 1.2, 1.0, 0.95 ng mL^{-1} for Pb(II), Ni(II), Cd(II), Cu(II) and Co(II), respectively.

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LIST OF SYMBOLS AND ABBREVIATIONS

LOD	Limit of detection
FAAS	Flame atomic absorption spectroscopy
EA	Elemental analysis
LLE	Liquid liquid extraction
SPE	Solid phase extraction
^{13}C-NMR	Carbon nuclear magnetic resonance
^{1}H-NMR	Proton nuclear magnetic resonance
FT-IR	Fourier transforms infrared spectroscopy
TGA	Thermal gravimetric analysis
GFAAS	Graphite furnace atomic absorption spectroscopy
ICP	Inductively coupled plasma
ICP-MS	Inductively coupled plasma-Mass spectrometry
RSD	Relative standard deviation
δ	Chemical Shift
Å	Angstrom
b	Langmuir constant related to energy of adsorption (L mol^{-1})
BET	Brunauuer Emmett Teller
$^{\circ}$C	Degree Celsius
s	Singlet (^{1}H-NMR spectrum)
d	Doublet (^{1}H-NMR spectrum)
t	Triplet (^{1}H-NMR spectrum)
q	Quartet (^{1}H-NMR spectrum)
ESI-MS	Electrospray ionization-Mass spectrometry
g	Gram
HCL	Hollow cathode lamp
J	Coupling constant

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K	Kelvin
LOQ	Limit of quantitation
MDL	Method detection limit
PMDL	Practical method detection limit
mg	Milligram
N_f	Amount of metal adsorbed per gram of sorbent
N_f^s	Maximum amount of metal adsorbed per gram of sorbent
ppm	Parts per million
ppb	Parts per billion
µg	Microgram
µL	Microliter
mL	Milliliter
mL min^{-1}	Milliliter per minute
mA	Milliampare
nm	Nanometer
MHz	Megahertz
mmol	Millimole
Hz	Hertz
M	Molarity
ν	Frequency
cm^{-1}	Percentimeter
BJH	Barrett Joyner Halenda pore volume
rpm	Round per minute

CHAPTER I

INTRODUCTION

Nowadays, new technology is developed rapidly in parallel with the generation of pollutants. Clean and green environment is needed for a good life. Scientists are a key of this matter. If the scientists create new technology they also should concern and control the pollutant generated. Certain pollutants are heavy metals that have been used in many technologies. Heavy metals are always present as a contamination in environment at very low concentration, but toxicity seems to be serious. Determination and quantification of the contamination of trace metals in the environment seem to be difficult because the concentration of analyte is lower than the limit of detection (LODs) of the instrument.

Flame atomic absorption spectroscopy (FAAS) is one of the most common techniques for heavy metal determination because it offers many advantages, for example, high sample throughput, low cost operation, high precision and selectivity. Unfortunately, this technique suffers from some drawbacks such as chemical interference from the sample matrix and its high detection limit. Therefore, the trace concentration level cannot be determined by this technique. However, these problems can be resolved by the preconcentration technique because this technique can both remove the sample matrix and enrich concentration simultaneously.

There are many techniques available for metal preconcentration such as liquid-liquid extraction (LLE) [1] and solid-phase extraction (SPE) [2-14]. Advantages of SPE over LLE are (i) easy to use, (ii) high preconcentration factor and (iii) flexibility to choose the solid phase for optimum results. However, the main drawback of SPE is the lack of selectivity [2], which leads to high interference of the other existing species with the target metal ion. Increasing selectivity of the sorbent is thus the main goal of research.

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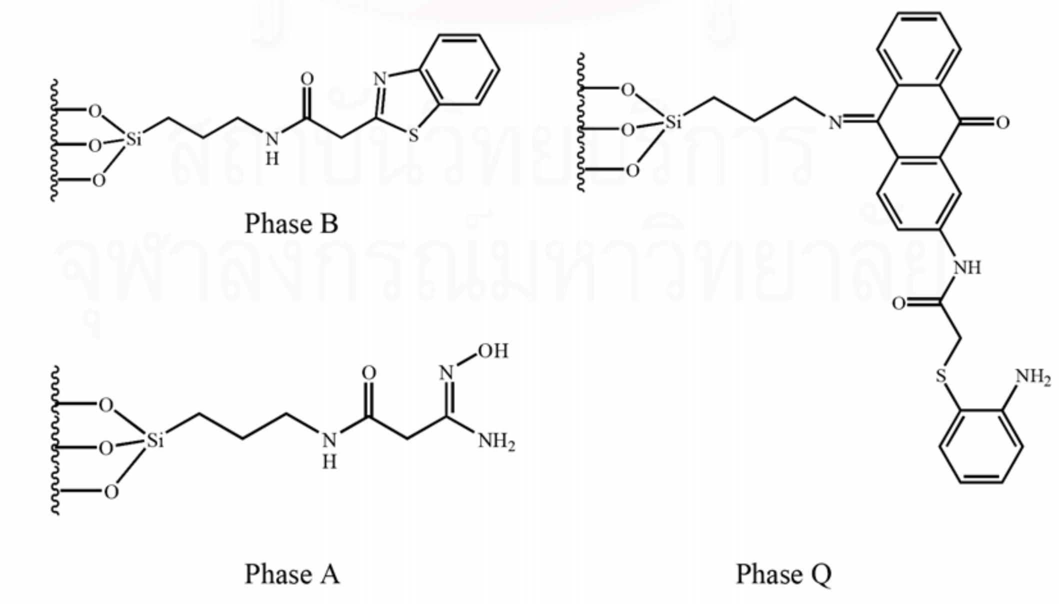
Among many types of sorbent used in SPE, silica gel immobilized with various organic compounds as metal chelating agent has received great attention. The selectivity of the immobilized surface towards metal ions depends on various factors such as size of the modifier, activity of the loaded groups and characteristic of the hard-soft acid-base. The modified silica gel generally exhibits higher sorption capacities than those of organic polymer-based resins. Silica gel has many reactive sites. Therefore, a number of organic molecules can be immobilized for a good sorption capacity towards metal ions.

1.1 Research Objectives

The aims of the research are focused on the chemically modified silica gel having chelating molecules which are selective to heavy metals. Then, the modified silica was applied for heavy metal extraction and preconcentration in order to improve the detection limit of flame atomic absorption spectroscopy. Furthermore, the method development at optimized parameters was applied for real sample analysis.

Three types of chemically modified silicas with different chelation groups, amidoamidoxime silica (phase A), benzothiazoleamido silica (phase B) and aminothioamido-anthraquinone silica (phase Q), were synthesized. The modified silicas were characterized by solid state ^{13}C-NMR, FT-IR spectroscopy, nitrogen adsorption, thermal gravimetric analysis (TGA) and elemental analysis.



Phase B

Phase A

Phase Q

Figure 1.1 Structures of modified silicas synthesized in this research

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Extraction properties of the modified silica towards several heavy metals in batch mode were studied. The parameters that can affect the extraction efficiency such as solution pH and extraction time were evaluated. The adsorption behaviors of metal ions on the modified silica were explored by using the adsorption isotherm model. Consequently, the modified silica was packed in the column in order to investigate the adsorption behaviors in the flow system. The parameters that affected the efficiency in column such as solution flow rate, desorption solution, sample volume and interfering ions were optimized. Finally, the methods optimized in column mode were used in real samples in order to present validity of the proposed method.

1.2 Scope of This Research

This dissertation focused on solid phase extraction and preconcentration of heavy metal in order to improve the detection limit of FAAS by using three novel solid supports. Herein, the chelating ligand are covalently attached to the silica gel and characterized by several techniques. The novel modified silica with different ligand types are expected to have different selectivity towards heavy metal ions without competition from major abundant ions. Parameters affecting the extraction efficiency and the adsorption behaviors between solid and aqueous phase will be explored. The modified silica will then be used to increase the concentration of heavy metal ions in order to improve the sensitivity and detection limit of analyte ions by the FAAS.

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1.3 Expected Benefits of This Research

We expected that the novel chelating modified silica synthesized in this dissertation will show the selectivity for heavy metals and improve detection limit of flame atomic absorption spectroscopy.

CHAPTER II

THEORY AND RELATED RESEARCH

2.1 Flame Atomic Absorption Spectroscopy (FAAS)

Flame atomic absorption spectroscopy is one of the trace metal determination techniques. It provides several interesting characteristics especially the specificity towards analytes. Unfortunately, this technique has some drawbacks such as poor detection limit. In addition, sample preparation may be required. In practical, diluted solution is aspirated into a flame to atomize the sample. An element-specific or resonance wavelength of light passes through the atomized sample. The quantity of light absorbed by the sample is directly proportional to the concentration of the interested element in that aspirated sample.

Flame atomic absorption spectroscopy, either air/acetylene or nitrous oxide/acetylene flame, is used to evaporate the solution solvent and dissociate the sample into free ground state atoms. For air/acetylene flame, the element such as alkali metals and heavy metals or transition metals are all atomized with good efficiency. A typical FAAS detection limits are in the sub-ppm range. However, there are a number of refractory elements like V, Zr, Mo and B that cannot be detected very well with a flame source. This is due to the maximum temperature reached, even with the N_2O/acetylene flame, is insufficient to break down compounds of these elements.

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Table 2.1 Typical detection limits of flame atomic absorption spectrometry*

Element	Detection Limit ($ng\ mL^{-1}$)	Element	Detection Limit ($ng\ mL^{-1}$)
Ag	1.5	Au	9.0
Cd	0.8	Co	9.0
Cr	3.0	Cu	1.5
Fe	5.0	Ni	6.0
Zn	1.5	Pb	15.0
Sn	150	Mn	1.5

*Guide to Atomic Spectroscopy Techniques and Applications, Perkin Elmer D-5139A KG120010 Printed in USA © 2000 PerkinElmer, Inc.

The detection instrument for single element atomic spectroscopy (Flame atomic absorption spectrometry and graphite furnace atomic absorption spectroscopy (GFAAS) is generally less costly than the multi-element techniques such as inductively coupled plasma (ICP) and inductively coupled plasma-mass spectrometry (ICP-MS) due to less complex systems. There is a cost difference among instruments for the same technique. Instruments which offer versatility frequently offer a greater degree of automation than that of the basic instrument. Cost of the instrument is the first issue to look at regarding the total cost of data determined. Other things may include cost of down time, the value of real time data in solving critical excursions, cost of sample delivery and cost of maintenance.

2.2 Limitations to FAAS Sensitivity [3]

Flame atomic absorption is a rapid and precise method of analysis. Determinations of analyte concentrations can be achieved in mg L^{-1} concentration level. This technique is usually used in the routine work for most of elements. However, this technique has a detection limit in higher order than other elemental analysis techniques. For atomic absorption, free ground state atoms must be placed in a beam of the resonance wavelength light that corresponding to an appropriate electronic transition of the analyte.

Any sampling process conceived must therefore address the process of creating ground state atoms and directing them to the spectrometer light path. In examining the FAAS process, we can find a number of areas limiting the sensitivity of the technique. The absorbance depends on the number of atoms in the optical path of the spectrometer at a given instant.

The nebulization process, which provides sample solution into the burner chamber at approximately 3-8 mL min^{-1}, limits the sample introduction. The sample introduction part is the main effect to the amount of sample to the atomizer. This is actually contributed to the pre-mix burner design which has the undesirable characteristic of being very wasteful of sample. Only a small fraction of the sample nebulized ever reaches the flame. On the other hand most of the sample solution is directed to the drain. Hence, the population of free atom in the flame is low and directly effect the sensitivity of this technique due to the absorbance is directly proportional to the quantity of the free atom in the atomizer.

Moreover, the sample which is introduced into the flame resides in the light path for only a fleeting moment as it is propelled upwards through the flame. The sensitivity of atomic absorption can be improved by addressing the limitations of flame sampling. Other direction may be applied by increasing the concentration of the analyte with the sample preparation stage such as preconcentration by using solid-phase extraction.

2.3 Chelating Sorbent in Inorganic Chemical Analysis

Right now, research on developing sorbent for solid phase extraction involves two main types of sorbents: inorganic and organic materials. The sorbent used for the extraction of the heavy metals contains two important parts: the sorbent base and the chelate functional groups. Different materials normally used for chelating group immobilization can be classified as shown in Figure 2.1.

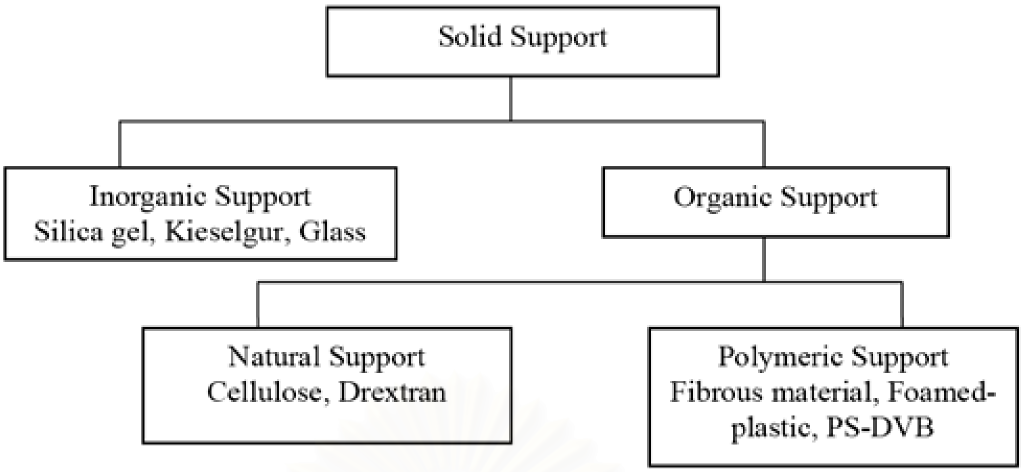

Figure 2.1 Chelating sorbents used in inorganic chemical analysis

2.4 Method of Preparation

There are several procedures for preparing chelating sorbents: (i) direct physical adsorption of a complexing agent onto the sorbent surface, (ii) electrostatic interactions between an ion exchange resin and charge chelating ligands and (iii) covalently bond on an organic complexing agent to the sorbent.

2.4.1 Modification through impregnation

Impregnation deals with physical interactions between the chelating ligand and solid support by either inclusion into the pores of the support material or adhesion process [4]. For example, 8-hydroxyquinoline was immobilized onto silica gel for preconcentration of metal ions by simply stirring with the activated silica [5]. Moreover, the modification of silica gel with Eriochrome black T (ECB-T) was reported by stirring a mixture of ethanolic solution of ECB-T and silica gel for 30 minutes [6]. Mahmoud used silica gel immobilized with the indicator ECB-T as a solid phase extractor for Zn(II), Mg(II) and Ca(II) ions [7]. Beside that, lucigenin (bis-N-methylacridinium nitrate), a chemiluminescent reagent was immobilized onto silica surface from its aqueous solution for determination of Fe(II) and V(IV) with a detection limit of 0.8 and 0.7 mg L^{-1}, respectively [8].

Activated carbon is a useful sorbent for the physically adsorption of the complexing agent. Dowratanachai et al. [9] used ammonium pyrrolidine dithiocarbamate impregnated to activated carbon (APDC-AC) for simultaneous preconcentration of Cd(II), Cu(II), Ni(II), and Zn(II) from aqueous solution by packing in column solid phase extraction. Trace metal ions in aqueous solution were quantitatively sorbed onto APDC-AC packed in a SPE column. The sorbed metals were eluted with 1 *M* nitric acid in acetone solution and analyzed by flame atomic absorption spectrometry. The methodology gave recoveries from 90 to 106% and RSD from 0.6 to 5.5%. Narin et al. [10] determined Cu(II), Mn(II), Cd(II), Pb(II), Ni(II), and Cr(III) in natural water by FAAS after complexation with pyrocatechol violet and adsorption on activated carbon with good recovery. Giacomelli et al. [11] determined molybdenum and bismuth in steels by GFAAS after complexation with ammonium salt of dithiophorsphoric acid *o,o*-diethylester and sorption on activated carbon. APDC was employed as complexing agent to determine trace silver and bismuth from cobalt, nickel and their nitrate anions.

2.4.2 Ion exchangers modified with chelating agents

Charge complexing ligand can be adsorbed onto ion exchanger resins by exchanging with counter ion of the ion exchanger. The ion exchange resin is equilibrated with solutions of complexing reagents that must be organic molecules with a strongly dissociated, anionic (for example sulfonic) or cationic (quaternary nitrogen) groups. There are several chelate forming reagents such as thoron sulfosalicylic acid, 8-hydroxyquinolin-5-sulfonic acid, Eriochrome Black T dyes, bismuthiol II-sulfonic acid, pyrocatechol violet have been immobilized on anionic resins [12].

2.4.3 Modification through covalent grafting

Basically good solid supports should be inert towards chemicals. Therefore, modifications of solid support via the chemical bonding seem to be difficult. However, this problem can be achieved after surface activation/modification [13-14]. For example, polystyrene divinyl benzene (PS-DVB) and Amberlite XAD-2 can be activated by adding the nitro group to the sorbent via nitration reaction. After that nitro groups are reduced by the reducing agent such as $SnCl_2$ convert to amine group. Amine groups are active functionality and can be used for further reactions. Kumar et al. [15] functionalized Amberlite XAD-2 by using *o*-aminothiophenol through –N=N– linked group and applied for preconcentration of Cu(II), Cd(II), Co(II), Ni(II), Zn(II) and Pb(II), prior to be analyzed by FAAS. Moreover, they applied for simultaneous enrichment of six metal ions in water sample. The preconcentration factors were found to be 50, 50, 100, 65, 40 and 40, respectively. Kumar et al. [16] modified Amberlite with pyrocatechol through an azo spacer for preconcentration and determination of heavy metals by FAAS. The modified sorbent can be used for preconcentration of Cd(II), Co(II), Cu(II), Fe(II) Ni(II) and Zn(II). The enrichment factor was found to be 200 for all metals except Fe(II) and Cu(II) for which the values were 10 and 100, respectively.

2.5 Silica: A Solid Support in Metal Ion Preconcentration [1]

2.5.1 Chemistry of silica gel

Silica gel is an inorganic polymer consisting of inter-linked SiO_4 in tetrahedral fashion, with the ratio of Si:O of 1:2. Silica gel is a porous, granular material. Silica gel was usually synthesized from sodium silicate, silicon tetrachloride or substituted chlorosilane/orthosilicate solution [17]. The major component is the silicon atom linked with oxygen, so called siloxane bond. The surface of silica is terminated by silanol group. Silanol group is the silicon atom with hydroxyl group which may be single, germinal or vicinal form. A single silanol is an isolated hydroxyl group. Moreover, vicinal silanol possess two hydroxyls on an adjacent silicon atom, while germinal silanol possess two hydroxyl groups on the same silicon atom.

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A pair of vicinal or germinal silanols can form a so-called bonded pair because of these abilities to form hydrogen bond with each other. Ong et al. [18] investigated the dissociation of the hydroxyl group and found that the pKa values of the silanol group were 4.9 and 8.5 at the silica/water interfaces. Silanol group with no hydrogen bonding can be isolated from normal silanol groups possessing low pKa values. The fact for considering them as isolated silanol is due to easy dissociation of hydroxyl hydrogen compared to other silanols coupled through hydrogen bond. Other type of silanols with higher pKa value (8.5) is believed to be those connected to each other through H-bonding directly or via the bridging water molecule. Allen et al. [19] also reported that the silanol groups on the surface of silica sol particles, where isolated silanol and bridged silanols with pKa values of 5.5 and 9.0. The significance of these groups is that three types of underivatized silanols may be reactive, with at least three different strengths for hydrogen bonding or weak cation exchange with any solute that may be bound to the bonded phase of the sorbent [20].

2.5.2 Surface modification of silica gel

Modification of silica surface is the process that the chemical composition on the surface can be changed without damage or change in original silica structure. Surface can be modified either by physical treatment such as thermal or hydrothermal that change the ratio of silanol and siloxane on the silica surface. Beside that, the chemical treatment can be changed in the chemical properties of silica surface such as covalent bond with the silanol group. Covalently bond of the chelating molecules on silica surface provides several advantages such as immobility, mechanical stability and water insolubility, thereby increases the efficiency, sensitivity and selectivity of the separation [21].

Silica gel surface is always modified by two processes. That is organo-functionalization when the modifying agent is an organic group. The other is inorgano-functionalization, in which the group anchored on the surface can be an organometallic composite or a metallic oxide.

The possibilities of target directed variation of the chemical and analytical properties of the sorbent by modifying them is almost limitless. Chemical modification of silica surface by organic chelating group can provide a greater selectivity for the analyte than that offered by traditional ion-exchangers.

The most convenient way to develop a chemically modified surface is achieved by simple immobilization (or fixing) of the group on the surface by adsorption or electrostatic interaction or hydrogen bond formation or other type of interactions. Simple impregnation of the solution of modifiers or covalent binding, so called, covalent grafting [22] of the chelating molecule to the silica matrix is the common practice of developing a functionalized silica surface.

2.6 Selection and Design of Chelating Sorbent

In order to achieve the best sorbent for each purpose, the chelating sorbent should have the following criteria.

1. The chelating group should extract the target metal ions selectively. On the other hand, common metal ion such as Na^+, Mg^{2+}, and Ca^{2+} should not be extracted or affect to the extraction process.
2. The metal-chelate complex should be stable and should not break up especially when the concentration of metal ions in the sample is low.
3. Desorption mechanism of metal complex should not be complicate. For example, only protonation by the acid solution should give back the free ligand and free metal.

If the chelating ligands can provide these required criteria, it doesn’t mean the synthesized material will be the best material for every metal ion. The best material still need some other criteria especially in the selectivity point of view. Hence, the other principle such as hard-soft acid-base may be applied for better specificity.

2.7 The Chemically Modified Silica by Organic Ligands

The chemically modified silica is a process for adding a chelating ligand onto the silica surface. Due to the inert property of silica gel, the activation of the silanol surface is required. Then the activated silica surface can be further reacted like a normal organic synthesis reactions.

In the activation step, organic molecule containing the desired organic functional group is directly attached to the support. In addition, the organic ligand can be modified on the original chain bonded to the support. The silylization process is the most usefulness method for activating the silica surface. This reaction is employed to generate products of coupling agents to permit the fixation of a reagent that is able to fulfill predetermined specific applications. The silylated organofunctional groups play an important role in co-ordination chemistry. It provides many possibilities to synthesis new ligands [18].

A silanol group on the silica surface reacts with the silane compound to yield a covalent bond on the silica surface [17]. By the introduction of organic functional groups to silica surface there is a partial conversion of surface silanol to a new organofunctional surface that acquires organophilic properties. Thus, ligand-grafted silica gives a set of properties to the surface, which differs considerably from the original matrix [23]. The chemical linker provides attachment to silica surface where as the tailored donor atoms in the complexing groups maximize the affinity towards the metal ions.

The immobilization of organo-functional groups has been reported in the past three decades. The main material is silica because this support offers pronounced advantages over other materials. There are several silylating agents that contain different functional groups. The further modification can be obtained from the organic functional group that covalently attached on the inorganic framework [24], on the reaction between a silylating agent and a silanol group is more effective than organic polymeric supports, which contain a high number of cross-linking and requiring a longer reaction time for surface activation [25], on the silica gel contains higher surface area with homogeneous composition giving higher adsorption capability towards metal ions.

Indeed, it is a great resistance material to organic solvents [17], silica gel has high mass exchange characteristics and no swelling [25], silica has very high thermal and chemical resistance [26]. However, irreversible binding of metal ions and lack of selectivity are the main disadvantages of silica bound ligands in their repeated cyclic use and elution process [27-28]. If the limitation or drawback of the modified silica gel can be improved, this material will be useful in analytical applications such as metal preconcentration.

2.8 Theory of Solid-Phase Extraction (SPE) [29]

The basic principle of SPE is similar to liquid–liquid extraction (LLE). The extraction involved a partitioning of analytes between two immiscible phases. However, instead of two immiscible liquid phases as in LLE, SPE involves partitioning between a liquid and a solid phase. This approach is always passing the liquid sample through a column that can retain analytes. The retained analytes are subsequently recovered by elution with an appropriate solvent. The basic procedure makes this technique very attractive. This approach is a sample preparation technique which simultaneously provides both concentration and purification of analytes from the solution.

2.8.1 Basic principles

In practical, SPE can be divided into four main steps: (i) conditioning, (ii) adsorption, (iii) washing and (iv) elution as is illustrated in Figure 2.2.

First of all, the solid sorbent should be conditioned by using an appropriate solvent. The conditioning solvent must be compatible with the sample solution. The nature of the conditioning solvent depends on the nature of the solid sorbent. The condition step is needed in order to remove possible impurities that initially contained in the sorbent and fills the void volume with solvent. The solid sorbent should not allow to dry especially between the conditioning and the sample loading steps, otherwise the analytes will not be efficiently retained and poor recoveries will be obtained.

The second step is the loading of the sample through the sorbent. The solution flow rate should be considered. It must be low enough in order to have an efficient retention of the analyte. However, it should be fast enough to avoid excessive duration. Beside that, the solution flow rate should be constant during the loading or elution step. The analytes are concentrated on the sorbent during the sample percolation step. In the meantime, the sample matrix may also be partial retained on the solid sorbent. For the perfect sorbent, the sample matrix should not be retained on the sorbent. This step provides the analyte separation and concentration.

The third step is called washing step. This step is very important for the system that sample matrix is also partially retained on the sorbent. In practical, the appropriate solvent with low elution strength that cannot elute the sorbed analyte but high enough to remove the retained matrix will be used. This step may be ignored and assume that the retained matrix will not interfere the determination step. The main reason is the loss of the retain analyte mostly occurred during the washing step due to no suitable solvent for elution.

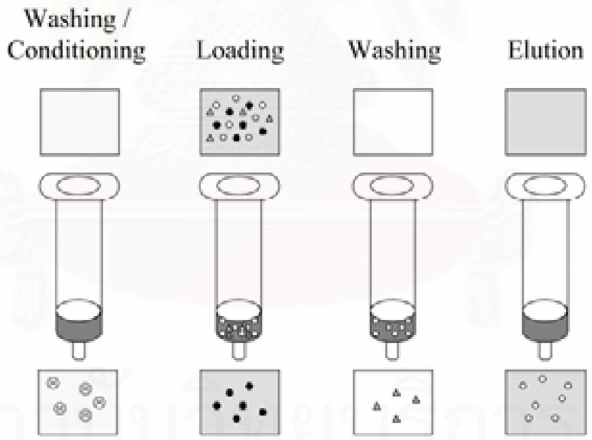

Figure 2.2 SPE operation steps: ☹ contamination • non-retained compound Δ interfering compound ○ analyte [29]

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The final step is the elution of the retained analyte by an appropriate solvent. The eluting solution should be chosen carefully. The most important concern is the elution solvent must be quantitatively eluting the retained analyte. In order to achieve higher preconcentration factor, the sample volume must be as small as possible but should be enough for such determination technique. In addition, the solution flow rate of the eluent should be adjusted to ensure efficient elution. The other precaution is the eluting solvent must be compatible with the analytical measurement technique and should be free from impurities. Moreover, it should be low in cost and non toxic.

2.8.2 Retention of trace elements on the sorbent

The important thing is the analyte must be reversible retained on the sorbent with at least one retention mechanism. There are several mechanisms for sorption of metal ions on the solid support depending on the nature of the sorbent.

Adsorption

The adsorption mechanism stems from van der Waals forces or hydrophobic interactions. This interaction is a non-directional interaction and depends on the polarity or hydrophilic/hydrophobic behaviors of the sorbent and the analyte. Therefore, this mechanism cannot be used for separating the analytes which have the same polarity or hydrophilicity. Basically the retention factor for SPE is slightly high therefore analyte can be only retained without separation. Non polar sorbent material is the most useful for this mechanism. For example silica C-18 (reversed phase) is always used for the hydrophobic analyte. However, our purpose is to preconcentrate trace metal ions that cannot be retained by such sorbents.

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Chelation

There are several factors which effect the binding ability of metal ions onto the chelating ligand that can be summarized as follows.

(1) The polarizability of the metal ion (charge and size).

(2) The nature of donor atoms in the ligand such hard or soft base property.

(3) Extraction condition such as optimum pH or competition ion in the solution.

(4) Solid support effect the extraction such as hydrophilicity of sorbent (wetability) should be used when the samples are in the water.

In some cases, the behavior of immobilized chelating sorbents towards metal ions may be predicted by using the formation constants of the metals with the chelating agent before grafting on the sorbent [7]. On the other hand, the other interaction should be considered because the environment of the ligand changed will effect the chelation ability or selectivity.

Several functional groups which different donor atoms are used. In order to achieve selectivity towards soft metal ions such as Ag(I) or Hg(II), donor atoms most frequently used are nitrogen, oxygen and sulfur. The nature of the functional groups will give an idea of the selectivity of the ligand towards trace elements. According to the hard-soft acid-base principle, metal ions can be divided into three groups as below:

(1) ‘Hard’ acid: The characteristic of the cation in this group are small size and low charge polarizability. This group included alkali and alkaline-earth metals.

(2) ‘Borderline’ acid: Cations in this group have an intermediate character in both size and charge (polarizability) such as Fe(II), Co(II), Ni(II), Cu(II), Zn(II), Pb(II) and Mn(II). They possess affinity for both hard and soft ligands and the suitable geometry of the complex.

(3) ‘Soft’ acid: The characteristics of this group are large size and high charge polarizability. These tend to form covalent bonds with the donor ligand. Hence, Cd(II) and Hg(I) possess strong affinity for intermediate (N) and soft (S) ligands.

Lewis base is also classified in the same manner as acid. The order of donor atom affinity for soft acid is observed as O < N < S. A reversed order can be applied for hard acid. In the case of bidentate chelating ligand which contains two donor sites provide a various level of the softness. The couple of the donor site makes the selectivity of the chelating ligand better than the monodentate ligand. The softness of the bidentate donor site is (O, O) < (O, N) < (N, N) < (N, S). Basically, the competition between Group 1 and Group 2 metals is observed in the case of O donor sites. Beside metal ions in Group 2 and Group 3 competition always present in the case of N and S donor sites. However, the competition between Group 1 and Group 3 is weak. This principle can be applied to the ligand design for specific analytes in different cases.

Ion-pairing

The retention of the metal ion by ion paring mechanism is basically based on the interaction between the hydrophobic sorbent and hydrophobic part of the metal and ion-paring ligand. The sorbent is normally silica-C18 that well known as a hydrophobic sorbent [30]. The metal ions are modified in the first step with such reagents contain a nonpolar part (such as a long aliphatic hydrocarbon chain) and a polar part (such as an acid or a base). Typical ion-pair reagents are quaternary ammonium salts and sodium dodecylsulfate (SDS) [31-32].

Ion exchange

The classical mechanism of ion-exchange is normally based on the exchange between analyte ions and the counter ion of the ion-exchange sorbent. Therefore, this mechanism does not provide any selectivity between same charge species. The commercial available of the ion-exchange sorbent provide both cation and anion-ion exchanger with strong or weak dissociation. Strong is Sulfonic acid groups and quaternary amines are used as cation and anion exchangers, respectively. Weak ion exchangers are usually carboxylic acid groups for cation-exchange and primary, secondary and tertiary amines for anion-exchange.

Strong ion exchanger is always independent of the solution pH, On the other hand, weak ion exchanger is strongly dependent on the solution pH.

2.9 On-line Coupling to Atomic Absorption Spectrometry

In order to combine sample preparation step to determination step for the automated system, on-line flow injection system is connected directly to the preconcentration column. Olsen et al. [33] and Fang et al. [34-35] were the first to describe an on-line FI sorbent extraction preconcentration system for FAAS by using micro-columns packed with a cation-exchanger. After that, they also proposed a system for on-line flow injection sorbent extraction preconcentration with electrothermal vaporization AAS (ET-AAS) using lead as a model [36]. The special characteristic of the sorbent required for this system are rapid sorption and desorption of the analytes [37].

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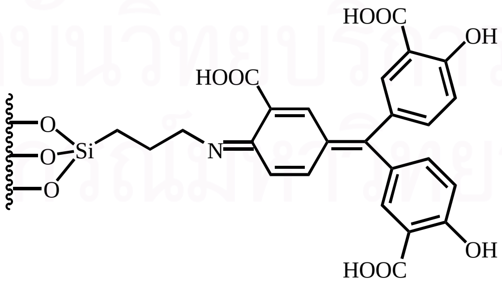
2.10 Literature Review

In recent years, many papers reported the solid phase extraction and preconcentration of heavy metals by chemically modified silicas. Modifications of the silica surface by various chelating ligands provide different selectivity towards metal ions. According to the hard-soft acid-base principle, donor atoms of the chelating ligand play an important role to achieve selectivity. Moreover, the orientation or the ligand geometry also affected the selectivity of the modified silicas. Here, we summarized previous papers by focusing on the donor types, the selectivity of each modified structures and the unique properties of each synthesized materials.

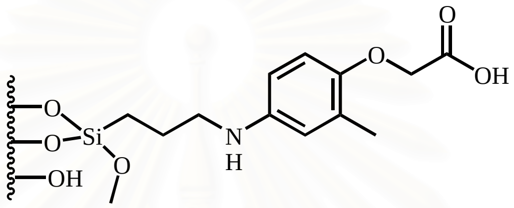
Type I Oxygen donor sites

The modified silicas with oxygen as donor sites such as carboxylate, cabamate and hydroxyl group have been synthesized. According to the hard-soft acid-base principle, these functional groups can be coordinated to the borderline acid. Moreover, it can also act as a weak cation exchanger. Therefore, the chelating ligand with oxygen donor sites can be applied for metal extraction and preconcentration of such borderline hard acid. However, due to the hard base and the ion exchange property of the oxygen donor atom, the selectivity of this chelating type is not very good. Sharma et al. [38] prepared the modified silica gel with aurin tricarboxylic acid. The modified silica **I-I** showed selective chelation for Cr(III) at pH 3.8–5.5.



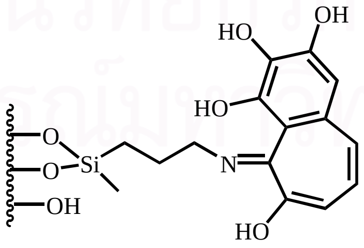
I-I aurin tricarboxylic acid chemically bonded on the silica gel

Prado et al. [39] interested in the properties of the herbicide to the metal chelation, because the structure of the herbicide contained carboxylic group. They used 2,4-dichlorophenoxyacetic acid as a chelating group covalently bonded with aminopropyl silica **I-II**. The modified silica was used for Cu(II), Ni(II), Zn(II) and Cd(II) extraction from aqueous and ethanolic solutions. The retention of cation from aqueous solutions were null at pH ≤ 3, however the adsorption increased up to pH 7. The modified silica was applied for preconcentration and quantification of trace level metal ions present in water and ethanol samples.



I-II 2,4-dichlorophenoxyacetic acid chemically bonded on the silica.

Mahmoud et al. [40] prepared the modified silica by using purpurogallin as a chelating group. Purpurogallin is a compound containing 4 hydroxyl groups that can be probably coordinated to hard metal ions. The silica gel chemically bonded with purpurogallin **I-III** showed strong affinity and selectivity as well as fast equilibration towards Fe(III) compared to other metal ions. The potential applications of this phase as a selective solid extractor for Fe(III) from natural tap water samples and real matrices were also studied. The results revealed good extraction and the preconcentration factor was 500.



I-III purpurogallin chemically bonded on the silica gel

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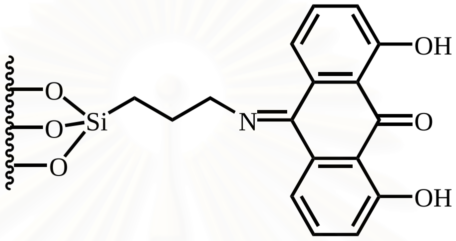
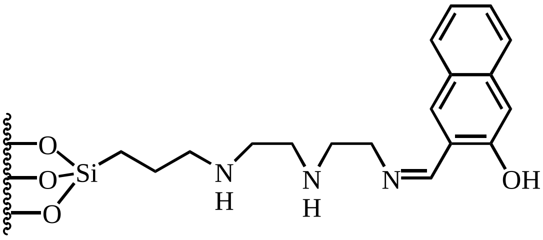
Goswami et al. [41] synthesized the silica gel modified with 1,8-dihydroxy anthraquinone **I-IV**. It was used to preconcentrate Pb(II), Cd(II) and Zn(II). All of the test metal ions were desorbed with HCl or HNO_3 solutions. The sorption capacity were found to be 76.0, 180.0 and 70.2 mmol g^{-1} for Pb(II), Zn(II) and Cd(II), respectively, with the preconcentration factor of 200. The interfering ions were studied and the results found that no interferences were observed. The lowest concentration of metal ions was 5.0 ng mL^{-1}.



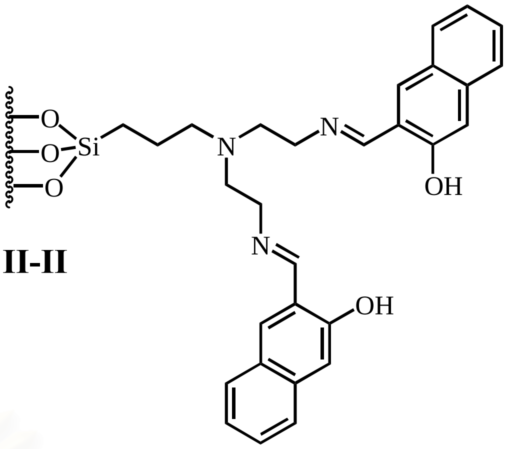
I-IV 1, 8-dihydroxyanthraquinone chemically bonded on the silica gel

Type II Oxygen-Nitrogen donor sites

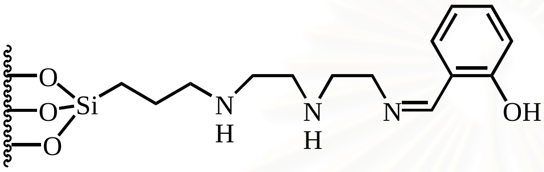
The chelating ligand can be added two different donor atoms in order to achieve different selectivity. Here are some examples of the papers that described modified ligands contain O and N as donor sites. Soliman et al. [42] modified four types of Schiff’s base ligands and attach to the silica surface. Diethylenetriamine, mono-naphthaldehyde and mono-salicyaldehyde Schiff’s bases (**II-I** and **II-III**) were synthesized via the reaction of silica gel modified diethylenetriamine with naphthaldehyde and salicyaldehyde, respectively. Phases **II-II** and **II-IV** arose from the reaction of bis-naphthaldehyde and bis-salicylaldehyde with diethylenetriamine and 3-chloropropyltrimethoxysilane, respectively. The extraction study with Fe(III), Ni(II), Cu(II), Zn(II), Cd(II) and Pb(II) ions were evaluated in both batch and column methods. Phases **II-III** and **II-I** showed high performance towards Cu(II) extraction, where their Cu(II) sorption found to be 0.957 and 0.940 mmol g^{-1}, respectively. However, phases **II-IV** and **II-II** showed the great affinity towards Fe(III) extraction.



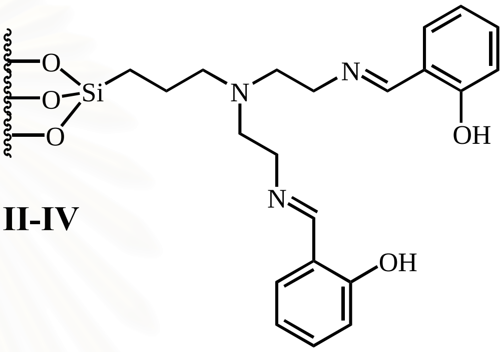
II-I



II-II

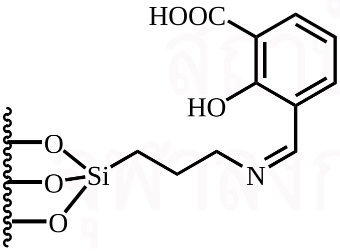


II-III

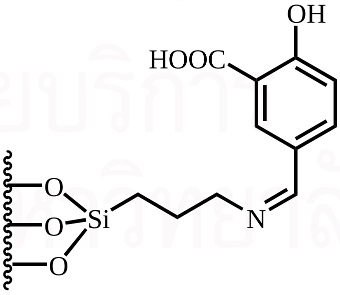


II-IV

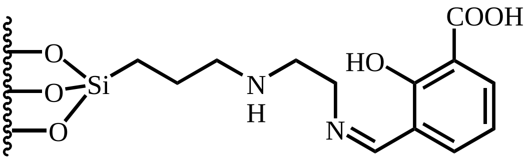
Mahmoud et al. [43] prepared the immobilized formylsalicylic acid on the silica gel surface (Phase **II V-VIII**). The resulting phases were used for the extraction of Fe(III). The capacity was found to be 0.95-0.96 mmol g^{-1}. The selectivity of the silica modified with formylsalicylic acid for Fe(III) extraction was observed from the extraction mixture of other metal ions. The other metal ions showed lower capacity compared with Fe(III).



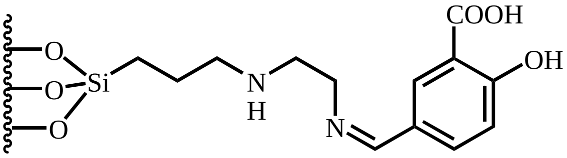
II-V



II-VI



II-VII



II-VIII

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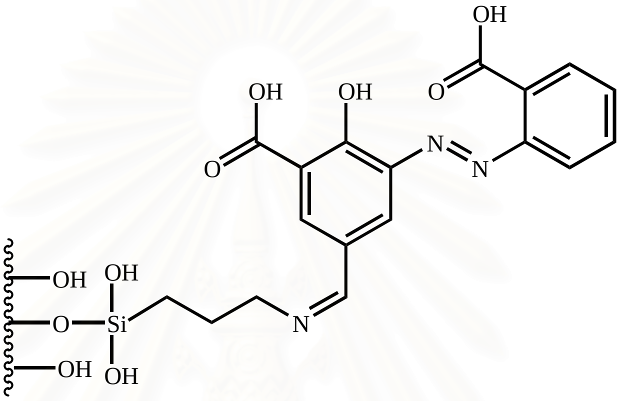
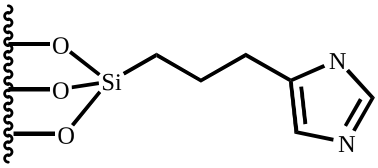
Akl et al. [44] synthesized the chelating sorbent having 5-formyl-3-(1′-carboxyphenylazo) salicylic acid **II-IX**. The adsorption characteristics of the synthesized silica were studied. Nine trace metals, Cd(II), Zn(II), Fe(III), Cu(II), Pb(II), Mn(II), Cr(III), Co(II) and Ni(II), can be quantitatively adsorbed from natural aqueous systems at the optimum pH 7.0–8.0. The sorbed metal ions can be readily desorbed with 1 *M* HNO_3 or 0.05 *M* Na_2EDTA. This method can be determined reliably with a preconcentration factor of 100.



II-IX silica gel chemically bonded with 5-formyl-3-(1′-carboxyphenylazo) salicylic acid

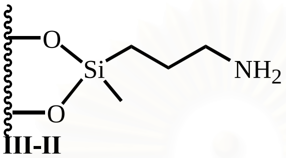
Type III Nitrogen donor sites

Silva et al. [45] synthesized the chemically modified silica gel with 3-(1-imidazolyl)propyl groups **III-I** for determination of trace levels of copper(II) in an aqueous matrix. The synthesized silica was packed in a column and connected to an on-line flow injection (FI) system. After the preconcentration stage, the analytes were eluted with a HNO_3 solution and determined by flame atomic absorption spectrometry. The detection limits obtained were 0.4 µg L^{-1} of Cu(II), with a preconcentration time of 90 seconds.

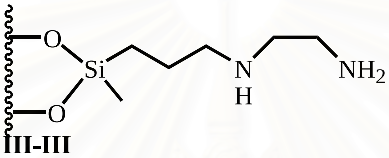


III-I silica gel chemically bonded with 3(1-imidazolyl) propyl groups

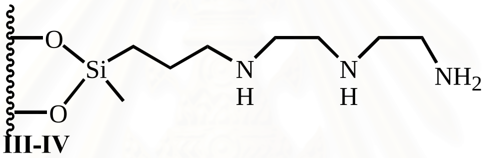
Mahmoud et al. [46] synthesized four modified silica gel having different number of N or amine groups (Phase **III II–V**). Structure characterization related to immobilization of the amine derivatives was accomplished and evaluated. A series of bi- and trivalent metal ions were selected to test the selectivity. Moreover, the binding and interactions with these metals based on distribution coefficient and the separation factor were also evaluated. The results of these evaluation processes showed that these modified silica provided higher selectivity towards Pb(II) and Cd(II).



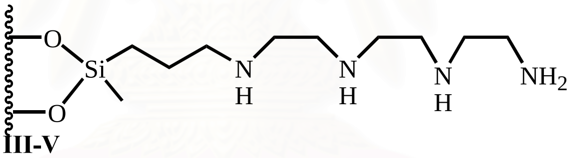
III-II



III-III

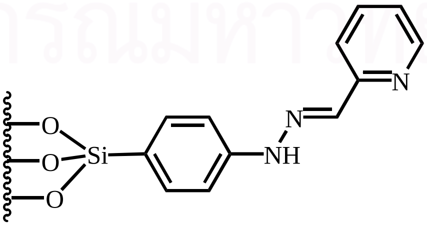


III-IV



III-V

Watanesk et al. [47] reported chemically bonded 2-pyridinecarboxaldehyde phenyl-hydrazone onto the silica surface **III-VI**. The synthesized silica was studied on the extraction ability towards Fe(III), Co(II), Ni(II), and Cu(II). The sorption behaviors of the studied metals were fit with Langmuir's adsorption isotherm: Fe(II) < Co(II) < Ni(II) < Cu(II).



III-VI silica gel chemically bonded with 2-pyridinecarboxaldehyde phenyl-hydrazone

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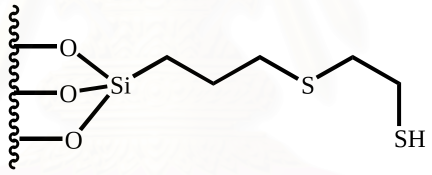
Type IV Sulfur donor sites

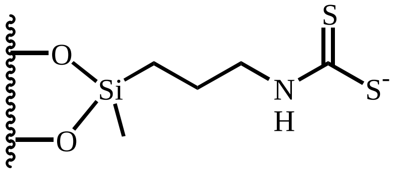
Sulfur atom is a soft base that can be used as the donor site for soft acids such as Hg(II), Ag(I) and Cd(II). For this reason, S as a donor site provides a better selectivity to heavy metal over hard acid such as alkali metals. Therefore, this type of the chelating ligand looks promising to the application in real natural water sample that contains high concentration of hard metal ions.

Arakaki et al. [48] synthesized a thiol sorbent via two steps: the synthesis of silica and 3-mercaptopropyltrimetoxysilane followed by adding ethylene sulfide **IV-I**. The modified silica contained two sulfur atoms act as the coordinating sites. This material was employed for extracting divalent cations (Co(II), Cu(II), Ni(II), Cd(II), Pb(II) and Hg(II)) from aqueous solution. The studies showed that the adsorption isotherms of the interested ions agreed with Langmuir’s type. Moreover, Hg(II) had a highest maximum number of mole adsorbed (3.3 mmol g^{-1}).

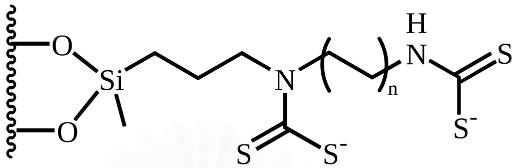


IV-I silica gel chemically bonded with ethylene sulfide

Mahmoud et al. [49] synthesized four novel solid phase extractors with imino-dithiocarbamate derivatives (Phase **IV II–V**). The four modified silica were found to exhibit excellent affinity towards selective extraction of Hg(II) in the presence of interfering metal ions. Moreover, low interference of Cu(II) ion was observed. The results illustrated the excellent extraction of Phase **IV-IV** and **IV-V** towards Hg(II). The extraction of Hg(II) from sample solution was insignificant due to the matrix effects. This stemmed from the high selectivity to Hg(II) over other metal ions.



IV-II

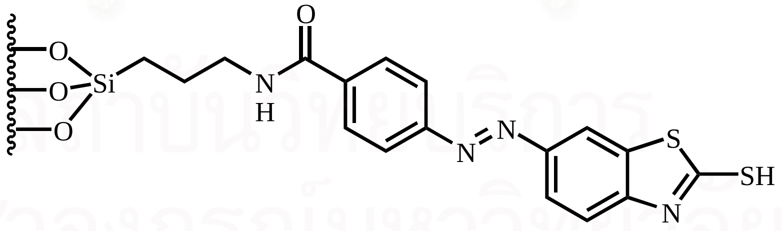


IV-III, n=1
IV-IV, n=2
IV-V, n=3

Silica gel chemically bonded with imino dithiocarbamate derivatives

Type V Sulfur-Nitrogen donor sites

Ma et al. [50] prepared the modified silica using aminopropylbenzoylazo-2-mercaptobenzothiazole. The synthesized silica **V-I** showed high selectivity for the preconcentration and separation of trace Hg(II). The modified silica provided an exchange capacity to be 41.4 mmol g^{-1}. The method was applied for the determination of Hg(II) in the polluted soil, incinerated biological material, and spiked natural water.



V-I silica gel chemically bonded with aminopropylbenzoylazo-2-mercaptobenzothiazole

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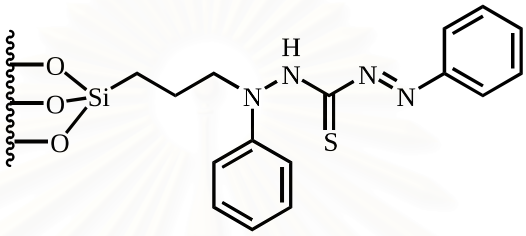
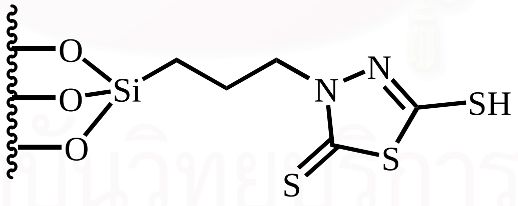
Mahmoud et al. [51] prepared the modified silica with dithizone via two routes, based on the chemical binding (Phase **V-IIA**) and the physical adsorption (Phase **V-IIB**). The capacity of Phase **V-IIA** towards metal ions was higher than Phase **V-IIB**. The highest distribution coefficient (K_d) was reported for Hg(II) by Phase **V-IIB**. The potential applications of Phase **V-IIB** for selective extraction of Hg(II) from aqueous solution were successfully accomplished as well as pre-concentration of low concentration of Hg(II) from natural tap water with a preconcentration factor of 200.



V-II A, B silica gel chemically bonded with dithizone

Pedro Lessia et al. [52] synthesized the chemically modified silica with 2, 5-dimercapto-1,3,4-thiadiazole **V-III** as chelating ligand for metal ions adsorption from ethanol. The studied metal ions were Zn(II), Cd(II), Ni(II), Pb(II), Co(II) and Fe(III). Elution step was done with a mixture of acetone and hydrochloric acid. Moreover, the synthesized silica was applied to preconcentration of metal ions from commercial ethanol.



V-III silica gel chemically bonded with 2, 5-dimercapto-1,3,4-thiadiazole

Safavi et al. [53] described a new reaction to activate the silanol group by using thionyl chloride in order to add chloro groups (as a leaving group) on the silica surface. This method can be directly used for bonding the chelating molecule to silica surface. 2-Mercaptobenzothiazole was chosen as the ligand containing S and N as donor sites. This modified silica showed an effective sorbent for preconcentration of silver ion from aqueous solution. The preconcentration factor was found to be 300.

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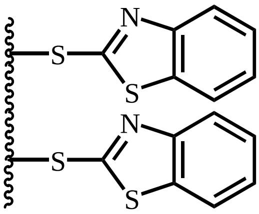
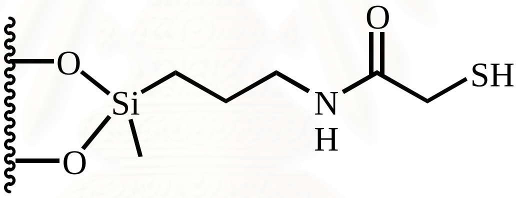


V-IV silica gel chemically bonded with 2-mercaptobenzothiazole

Type VI Sulfur-Oxygen donor sites

Machado et al. [54] prepared the modified silica gel with thioglycolic acid as a chelating group **VI-I**. The thioglycolic acid is a ligand containing S and O as donor sites. The ion adsorption properties of the modified silica were studied using Cu(II) in aqueous/ethanolic solutions. The new modified silica showed good sorption ability for Cu(II) at lower temperature and its reuse capacity was demonstrated.

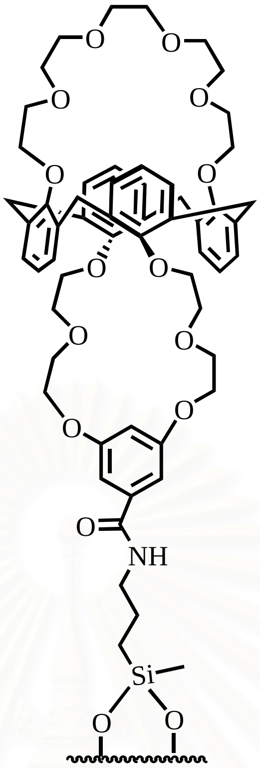


VI-I silica gel chemically bonded with thioglycolic acid

Type VII Macrocyclic chelating group

As mentioned above, donor atoms are based on hard-soft acid base principle. However, the geometry of the chelating group is one of the important factors that can promote the specificity of the metal and ligand. The supramolecular chemistry principle has been applied for designing the ligand in order to add the macrocyclic chelating group onto the modified silica.

Duhart et al. [55] synthesized the covalently bonded calix[4]arene-biscrown-6 containing two different crown loops by a sol–gel process onto polysiloxane backbone **VII-I**. The synthesized material can be used to selectively remove Cs^+ from acidic and high concentration of Na^+ content in nuclear waste water. Moreover, this material was applied to facilitate membrane diffusion process.



VII-I silica gel chemically bonded with calix[4]arenebiscrown-6

Barbette et al. [56] studied an extraction of U(VI) using a novel modified silica substituted of tetra-aza-macrocycles (Phase **VII II-V**). The new phase can be used to extract U(VI) as well as a commercially available acid-type chelating resin. It was found that total removal of U(VI) from a contaminated solution can be achieved by using a column packed with such tetra-aza-macrocycles-bound silica gel. Finally, modified silica gel was applied to a pilot scale device allowing the total decontamination of real effluents containing traces of uranium, plutonium, and americium.

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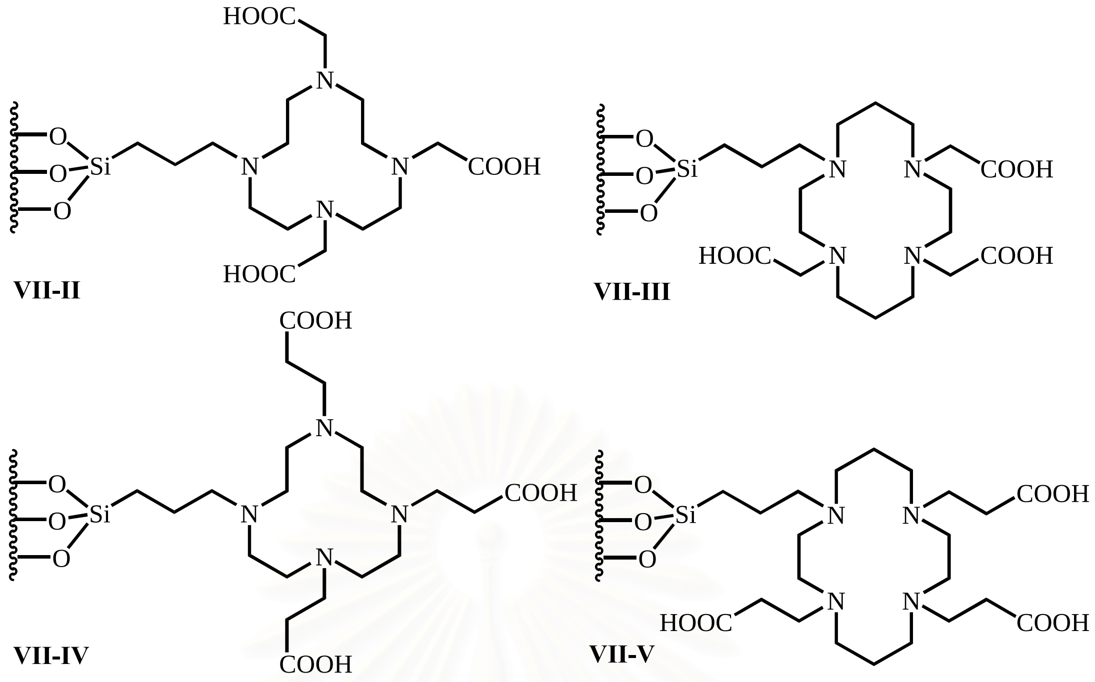

VII-II **VII-III** **VII-IV** **VII-V**

VII-II-V silica gel chemically bonded with substituted tetra-aza-macrocycles

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CHAPTER III

EXPERIMENTAL SECTION

3.1 Apparatus

- Flame atomic absorption spectrometer

Atomic absorption spectrometer model AAnalyst 100 (Perkin-Elmer) was used to determine metal concentrations. Instrumental parameters are listed in Table 3.1.

Table 3.1 FAAS conditions for the determination of heavy metals

Operating condition	Cu	Pb	Cd	Co	Ni
Wavelength (nm)	324.8	283.3	228.8	240.7	232.0
Slit width (nm)	0.7	0.7	0.7	0.2	0.2
Lamp type	HCL*	HCL*	HCL*	HCL*	HCL*
Lamp current (mA)	15	10	4	30	25

*Hollow cathode lamp

- pH meter

A pH meter (Hanna instruments pH 211) was used for all pH measurements.

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- Peristaltic pump

The solution flow rates of all column experiments were controlled by peristaltic pump (model ISMATEC with Tygon tubing R 3607 i.d. 2.79 mm wall 0.86 mm).

- Minicolumn

Modified silica was packed into a 2.0-3.5 cm of stomach tubing to which the end of column was closed with cotton and hollow plastic tube.

- Nuclear magnetic resonance (NMR)

Characterizations of the synthesized molecules were carried out by nuclear magnetic resonance (Bruker 400 MHz). All chemical shifts were reported in part per million (ppm) using the residual proton in deuterated solvent as internal reference.

Solid state ^{13}C-NMR (DPX-300 Brucker Biospin) was used to characterize modified silicas.

- Elemental analyses (EA)

Elemental analyses were carried out on a Perkin-Elmer CHON/S analyzer (PE 2004 series II) using ignition combustion gas chromatography separated by frontal analysis and quantitatively detected by thermal conductivity detector.

- Infrared spectroscopy (IR)

Infrared spectra were obtained on a Nicolet Impact 410 using KBr pellet.

- Glasswares

Class A glasswares were used.

3.2 Chemicals

All chemicals were used without further purification. Commercial grade solvents such as dichloromethane, hexane, methanol and ethyl acetate were purified by distillation. Acetonitrile and toluene were dried over CaH_2 and freshly distilled under nitrogen prior to use. Other chemicals were shown in Table 3.2.

Table 3.2 Chemicals used in this research

Chemicals	Supplier
Aminopropyl triethoxy silane	Acros Organic
2-Aminothiophenol	Merck
2-Aminoanthraquinone	Fluka
Calcium nitrate	Merck
Chloroacetyl chloride	Merck
Ethyl cyano acetate	Fluka
Hydroxylamine hydrochloride	Carlo Erba
Magnesium nitrate	Merck
Methyl cyanoacetate	Fluka
Nitric acid	Merck
Potassium hydroxide	Merck
Potassium nitrate	BDH
Pyridine	Fluka
Silica gel 60	Fisher Scientific
Single standard solution Cu, Pb, Cd, Co and Ni 1000 mg L^{-1}	Fisher Scientific
Sodium carbonate	Merck
Sodium chloride	Carlo Erba
Sodium nitrate	Carlo Erba
Sodium sulfate anhydrous	Carlo Erba

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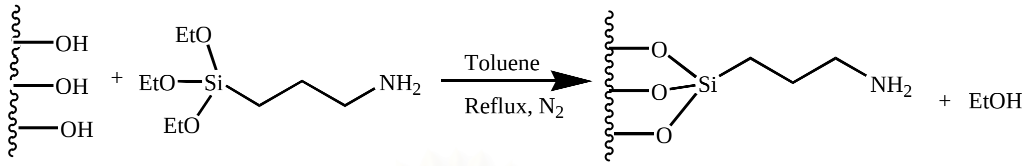
3.3 Synthesis of Modified Silica Gel

3.3.1 Synthesis of aminopropyl silica [57]



Silica gel (50 g) was suspended in 200 mL of dried toluene, refluxed and mechanically stirred under nitrogen for 2 hours. To this suspension, 20 mL of aminopropyl triethoxysilane was added dropwise and the mixture was kept at reflux for 24 hours. The solid was filtered, washed with ethanol and dried in vacuo. The immobilized silica gel was named aminopropyl silica.

Characterization data for aminopropyl silica

^{13}C-NMR spectrum (solid state NMR): δ (in ppm)

δ = 43.4 ($H_2N\mathbf{C}H_2CH_2$), 26.2 ($CH_2\mathbf{C}H_2CH_2$), 10.1 ($CH_2\mathbf{C}H_2OSi$)

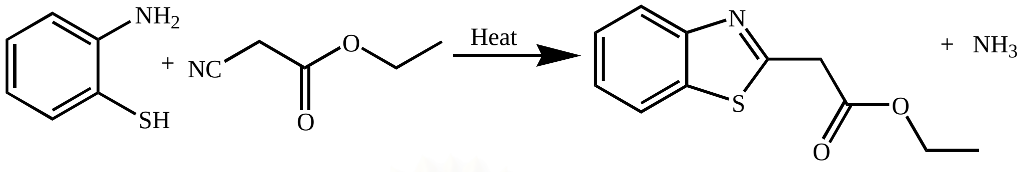
FT-IR spectrum (KBr disk): ν (in cm^{-1})

ν = 3000-3400 (O-H stretching), 1637 (N-H bending, scissoring), 1229 (Si-O-Si stretching), 1011 (Si-C stretching), 933 (Si-O stretching)

Elemental Analysis: Found C 5.89, H 0.98, N 2.35

3.3.2 Synthesis of benzothiazoleamido silica

3.3.2.1 Synthesis of benzothiazole ethyl acetate [58]



A mixture of ethyl cyanoacetate (6.0 g, 53.0 mmol) and 2-aminothiophenol (6.6 g, 53.0 mmol) was stirred at 120 °C for 2 hours under nitrogen. After the mixture was cooled to room temperature, the product was dissolved in dichloromethane and purified by column chromatography with dichloromethane as an eluent. The product was a yellow oil (11.07 g, 50.0 mmol, 94% yield).

Characterization data for benzothiazole ethyl acetate

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz.): δ (in ppm)

δ = 8.08 (d, 1H, J = 8.4 Hz, ArH), 7.88 (d, 1H J = 8.4 Hz, ArH), 7.48 (t, 1H, J = 7.6 Hz, ArH), 7.39 (t, 1H, J = 7.4 Hz, ArH), 4.26 (q, 2H, J = 7.2 Hz, OCH_2CH_3), 4.16 (s, 2H, CH_2CO), 1.31 (t, 3H, J = 7.2 Hz, OCH_2CH_3)

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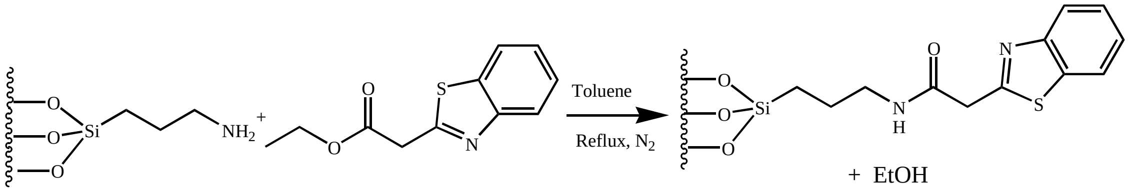
3.3.2.2 Synthesis of benzothiazoleamido silica (Phase B)



A suspension of 25 g aminopropyl silica in 150 mL of dried toluene and 1 g of benzothiazole ethyl acetate were added into a 250 mL two-necked round bottom flask. The mixture was stirred and heated at 100 °C under nitrogen for 24 hours. The suspension was allowed to cool to room temperature. The silica was filtered and washed with dichloromethane (200 mL ×2), methanol (200 mL ×2) and dichloromethane (200 mL ×2). The product was named Phase B, kept in desiccator and dried in vacuo.

Characterization data for benzothiazoleamido silica

^{13}C-NMR spectrum (solid state NMR): δ (in ppm)

δ = 188.5, 168.46, 153.1, 136.7, 129.8, 122.9, 42.9, 22.8, 10.3

FT-IR spectrum (KBr disk): ν (in cm^{-1})

ν = 3000-3400 (O-H stretching), 1653 (C=O bending), 1555 (N-H bending, C-N stretching), 1077 (Si-C stretching), 804 (Si-O stretching)

Elemental Analysis: Found C 8.93, H 1.61, N 2.43

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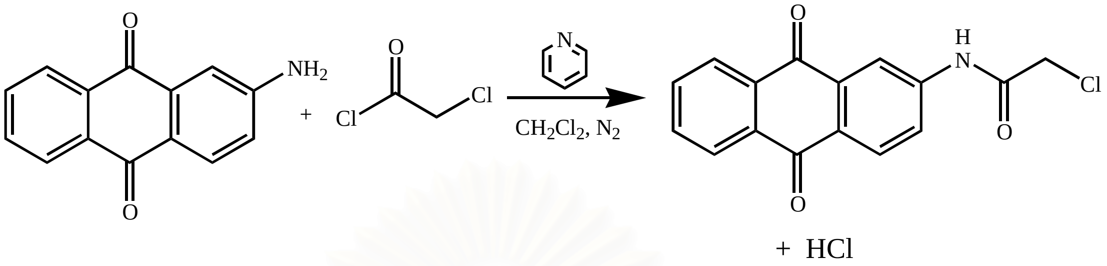
3.3.3 Synthesis of aminothioamido anthraquinone silica

3.3.3.1 Synthesis of chloroamido anthraquinone



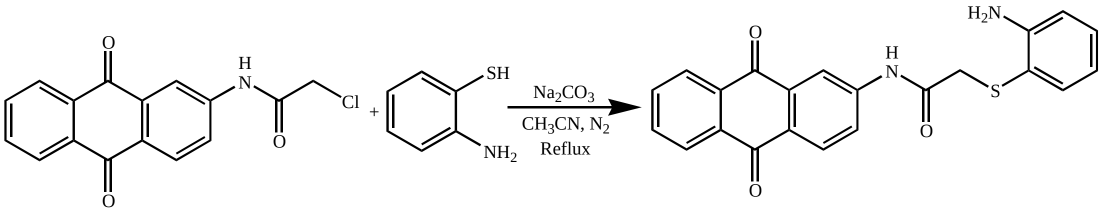
Into a 250 mL two-necked round bottom flask equipped with a reflux condenser, 2-aminoanthraquinone (1.0 g, 4.5 mmol), pyridine (0.5 mL, 6.2 mmol) and dichloromethane (60 mL) were mixed and stirred under nitrogen at 0 °C. Chloroacetyl chloride (0.4 mL, 5.4 mmol) in dichloromethane (20 mL) was then added dropwise. After stirring for 4 hours, the solution was transferred to a separatory funnel and extracted with 3 *M* HCl. The dichloromethane layer was washed with saturated sodium chloride solution. The organic layer was then dried over anhydrous sodium sulfate, filtered and evaporated to dryness to obtain a dark green residue. The residue was further dissolved in dichloromethane, and hexane was then slowly added. The chloroamido anthraquinone precipitated as dark green solid (1.2 g, 85%). The product was dried in vacuum and used immediately for the next step.

Characterization data for chloroamido anthraquinone

^{1}H-NMR spectrum ($CDCl_3$, 400 MHz.): δ (in ppm)

δ = 10.32 (s, 1H, *H*NCO), 8.10 (m, 4H, Ar*H*), 7.64 (m, 2H, Ar*H*), 7.30 (s, 1H, Ar*H*), 4.03 (s, 2H, OCCH_2Cl)

3.3.3.2 Synthesis of aminothioamido anthraquinone



Into a 100 mL two-necked round bottom flask equipped with a reflux condenser, aminothiophenol (0.1 g, 0.9 mmol), sodium carbonate (0.2 g, 2.0 mmol) and acetonitrile (30 mL) were mixed and stirred under nitrogen at room temperature for 30 minutes. Chloroamido anthraquinone (0.25 g, 0.8 mmol) in acetonitrile (20 mL) was then added dropwise. The solution was refluxed for 12 hours. Sodium carbonate was filtered off and acetonitrile was evaporated to obtain a dark yellow solid. The residue was dissolved in dichloromethane, and hexane was then slowly added. The aminothioamido anthraquinone was precipitated as dark yellow solid (0.3 g, 91%). The product was dried in vacuum and kept in a desiccator.

Characterization data for aminothioamido-anthraquinone

^{1}H-NMR spectrum (DMSO-d_6, 400 MHz): δ (in ppm)

δ = 10.72 (broad, 1H, *H*NCO), 8.41 (s, 1H, Ar*H*NHCO), 8.18 (m, 3H, OCAr*H*), 8.01 (d, *J* = 8.8 Hz, 1H, OCAr*H*CNH), 7.90 (m, 2H, OCAr*H*), 7.26 (d, *J* = 8 Hz, 1H, SAr*H (o)*), 7.03 (t, *J* = 8 Hz, 1H, SAr*H (m)*), 6.69 (d, *J* = 8 Hz, 1H, SAr*H(p)*), 6.47 (t, *J* = 8 Hz, 1H, H_2NAr*H(m)*), 3.62 (s, 1H, COCH_2S)

Mass spectrometry: ESI-MS m/z = 389.3 [$C_{22}H_{16}N_2O_3S+H^+$]

Elemental analysis: Anal. Cal. for $C_{22}H_{16}N_2O_3S$; C 68.02, H 4.15, N 7.21

Found C 68.06, H 4.07, N 7.10

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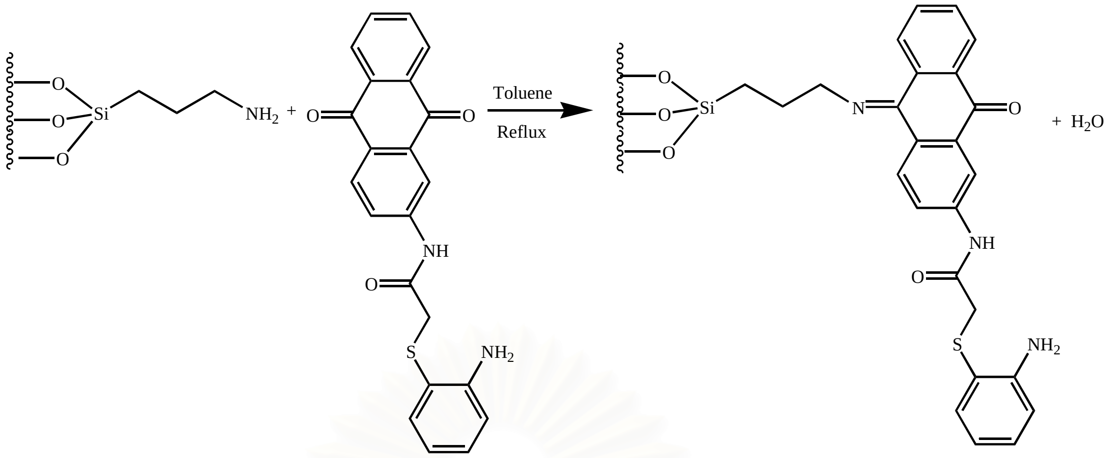
3.3.3.3 Synthesis of aminothioamido anthraquinone silica (Phase Q)



Into a 250 mL two-necked round bottom flask containing a suspension of 25 g of aminopropyl silica in 150 mL of dried toluene, 0.25 g of aminothioamido anthraquinone was added. The mixture was stirred and heated at 100 °C under nitrogen for 24 hours. The suspension was allowed to cool to room temperature. The silica was filtered and washed with dichloromethane (200 mL ×2), methanol (200 mL ×2), and dichloromethane (200 mL ×2) to give an orange solid aminothioamido anthraquinone silica named phase Q. The product was dried in vacuo and kept in a desiccator.

Characterization data for aminothioamido anthraquinone silica

^{13}C-NMR spectrum (solid state NMR): δ (in ppm)

δ = 199.7, 192.9, 184.4, 181.6, 168.4, 146.4, 133.7, 128.3, 126.4, 121.7, 118.2, 46.5, 26.5, 9.5

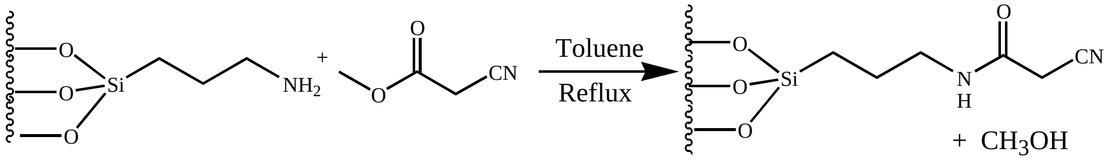
FT-IR spectrum (KBr disk): ν (in cm^{-1})

ν = 3000-3400 (O-H stretching), 1649 (C=O bending), 1552 (N-H bending, C-N stretching), 1077 (Si-C stretching), 797 (Si-O stretching)

Elemental Analysis: C 6.94, H 1.65, N 2.68

3.3.4 Synthesis of amidoamidoxime silica

3.3.4.1 Synthesis of cyanoamide silica



Into a 250 mL two-necked round bottom flask containing a suspension of 20 g of aminopropyl silica gel in of 150 mL dried toluene and cyano methyl acetate (6.2 g, 62.8 mmol) was added. The mixture was stirred at 100 °C under nitrogen for 24 hours. The suspension was allowed to cool to room temperature. The silica was filtered and washed with dichloromethane (200 mL ×2), methanol (200 mL ×2) and dichloromethane (200 mL ×2). The product was named cyanoamide silica and dried in vacuo and kept in a desiccator.

Characterization data for cyanoamide silica

^{13}C-NMR spectrum (solid state NMR): δ (in ppm)

δ = 169.4, 164.0, 116.2, 42.7, 22.2, 10.0

FT-IR Spectrum (KBr disk): ν (in cm^{-1})

ν = 3000-3400 (O-H stretching), 1676 (C≡N stretching), 1626 (C=O bending), 1548 (N-H bending, C-N stretching), 1049 (Si-C stretching), 800 (Si-O stretching)

Elemental Analysis: Found C 9.07, H 1.06, N 3.85

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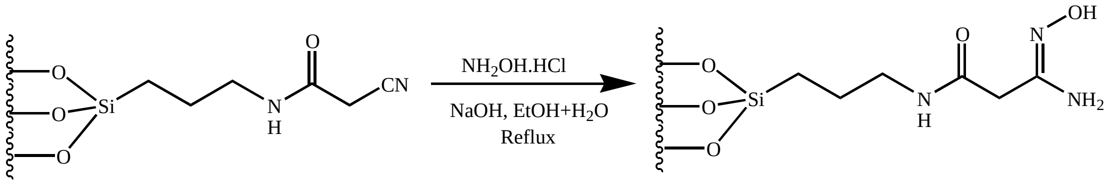
3.3.4.2 Synthesis of amido amidoxime silica



Into a 250 mL two-necked round bottom flask, 20 g of cyanoamide silica was suspended in ethanol 150 mL. Hydroxylamine hydrochloride (4.6 g, 67.2 mmol) and sodium hydroxide (2.68 g, 67.2 mmol) in water were then added, and the pH was adjusted to pH 7. The mixture was stirred and heated at 80°C under nitrogen for 24 hours. The suspension was allowed to cool at room temperature. The silica was filtered and washed with water (200 mL ×2), ethanol (200 mL ×2) and dichloromethane (200 mL ×2). The product was kept in a desiccator and dried in vacuo. The modified silica was named amido amidoxime silica or phase A.

Characterization data for amidoamidoxime silica

^{13}C-NMR spectrum (solid state NMR): δ (in ppm)

δ = 170.0, 163.6, 151.9, 42.8, 21.9, 10.1

FT-IR Spectrum (KBr disk): ν (in cm^{-1})

ν = 3000-3400 (O-H stretching), 1660 (C=N stretching), 1552 (N-H bending, C-N stretching), 1081 (Si-C stretching), 800 (Si-O stretching)

Elemental Analysis: Found C 5.97, H 1.57, N 2.76

3.4 Preparation of Solutions

All solutions were prepared using Milli-Q water 18 MΩ cm^{-2}. Metal ion solutions were prepared by stepwise dilution of 1000 mg L^{-1} stock standard solution (with another stock will be reported).

3.5 Optimization Condition

3.5.1 Batch extraction

All experiments ware performed in 3 replicates.

3.5.1.1 Effect of pH

The effect of solution pH was evaluated by monitoring percentages of metals sorption at difference pHs. Experiments were performed by using 5 mg L^{-1} of Cu(II), Co(II), Ni(II), 10 mg L^{-1} of Pb(II) and 2 mg L^{-1} of Cd(II). Solutions of metal ions were adjusted to the appropriate pH (from 1-6) using 1% HNO_3 and 1% KOH. Modified silica (20 mg) was added into test tubes and 5.0 mL of metal solutions was added to mix with the modified silica. The suspension was stirred for 30 minutes and then centrifuged at 3500 rpm for 5 minutes. The supernatants was separated and determined by FAAS.

3.5.1.2 Effect of contact time

The influent of the contact time on the sorption efficiency and adsorption kinetic were evaluated. The experiments were performed by using 5 mg L^{-1} of Cu(II), Ni(II) and Co(II), 10 mg L^{-1} of Pb(II) and 2 mg L^{-1} of Cd(II) at the optimized pH. For phase Q all metal ions were studied, but in the case of phase B only Cu(II) and Pb(II) were studied. In phase A, only Cu(II) was evaluated. The modified silica (20 mg) was added into the test tube and 5.0 mL of each metal solution was mixed with the silica. The suspension was stirred with different contact time from 5-60 minutes.

The suspension was separated by centrifugation at 3500 rpm for 5 minutes. The residual metal ion concentrations were determined by FAAS.

3.5.1.3 Adsorption isotherm

In this study, the concentrations of each metal ion were varied at fixed silica amount. The equilibrium concentrations at different initial concentration were determined. The experiments were carried out on the optimized pH and extraction time. For phase Q, the concentrations of metal ions were 5-100 mg L^{-1} pH 3.5 for Cu(II), 50-300 mg L^{-1} pH 3.5 for Pb(II), 2-50 mg L^{-1} pH 3.5 for Cd(II), 5-100 mg L^{-1} pH 4.5 for Co(II) and 5-100 mg L^{-1} pH 4.5 for Ni(II). For phase B the metal concentration were 5-100 mg L^{-1} pH 4.5 for Cu(II) and 50-300 mg L^{-1} pH 4.5 for Pb(II). In the case of phase A, only Cu(II) at concentration range of 5-100 mg L^{-1} pH 4.5 was studied.

Modified silica (20 mg) was then added into the test tubes, except in the case of Pb(II) only 10 mg, 5.0 mL of each metal solutions was added. The suspensions were stirred and then centrifuged at 3500 rpm for 5 minutes. The supernatant was separated and determined by FAAS.

3.5.2 Column extraction

3.5.2.1 Effect of flow rate

Home-made mini-columns were prepared by packing the modified silica (about 20-70 mg) in a plastic tube and connected to a peristaltic pump. Experiments were studied at various flow rate controlled by the peristaltic pump. 5.0 mL of test solutions containing 10 mg L^{-1} for Pb(II), 5 mg L^{-1} for Cu(II), Ni(II), Co(II) and 2 mg L^{-1} for Cd(II) were passed through the mini-column packed with a sorbent at various flow rates between 0.5 to 5 mL min^{-1}. The solutions were collected, and concentrations of metal ions were determined by FAAS.

3.5.2.2 Effect of the stripping concentration

The effect of HNO_3 concentration on striping efficiency of the retained metals in the mini-column was evaluated. Mini-columns were prepared by using the different amount of modified silica. Phase Q (20 mg) was used for Cu(II), Pb(II), Cd(II) but in the case of Co(II) and Ni(II) 40 and 60 mg of phase Q were used, respectively. In addition, 20 and 40 mg of sorbent were packed in the mini-column for phase B and phase A, respectively. 5.0 mL of individual metal solution containing 5 mg L^{-1} for Pb(II) or 2.5 mg L^{-1} for Cu(II), Co(II), Ni(II) or 1 mg L^{-1} for Cd(II) was passed through a mini-column at an optimum flow rate controlled with a peristaltic pump. The stripping of sorbed metal ion was investigated using 5.0 mL of HNO_3 solution, of which the concentration were 1, 3, 5 and 7%v/v, passed through the mini-column at a flow rate of 0.5 mL min^{-1}. The residual concentration and amount of metal ion in the stripped solution were determined by FAAS.

3.5.2.3 Effect of the sample volume

Model solutions of different solution volume (25, 50 and 100 mL) spiked with 25 μg for Pb(II), 10 μg for Cu(II), Co(II), Ni(II) and 5 μg for Cd(II) were adjusted to appropriate pH. The solutions were passed through a mini-column at an optimum flow rate controlled with a peristaltic pump. The sorbed metal was eluted by 5.0 mL of 1% HNO_3 at a flow rate of 0.5 mL min^{-1}. The amount of metal in the effluent was determined by FAAS.

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3.5.2.4 Minimum concentration and linear range of method

A stock metal solutions (5 mg L^{-1} for Pb(II), 2 mg L^{-1} for Cu(II), Co(II) and Ni(II) and 1 mg L^{-1} for Cd(II)) were prepared. An individual working solution was prepared by diluting the stock metal solution at appropriate concentration under the optimum pH in a 100 mL of volume metric flask (but a 50 mL of volumetric flask was used in the case of Ni(II)). The individual working metal solution was passed through a mini-column packed with an appropriate amount of modified silica at an optimum flow rate controlled with a peristaltic pump. The sorbed metal ions were eluted by 5.0 mL of 1% HNO_3 at the solution flow rate 0.5 mL min^{-1}. The sorbed metal in the effluent was determined by FAAS.

3.5.2.5 Interfering ion study

Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-} were individually mixed with the studied metal ions in order to observe the effect of the coexist ion on the extraction efficiency of the modified silica. The binary systems of each metal ion and interfering ion concentration levels were evaluated. The ionic salts were prepared using NO_3^- or Na^+ as the counter ion. The interfering ions were studied in 3 concentration levels at 10, 100 and 1000 mg L^{-1}. Stock metal solution (2.5 mL) at an appropriate concentration was added into a 100 mL of volumetric flask and then diluted with the interfering ion solution and adjusted to appropriate pH. The flow optimized conditions were used.

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3.5.2.6 Application to natural real samples

All optimized parameters as summarized in Table 3.3 were used for real samples. The proposed method was applied to the determination of metal ions in real water samples: (i) Chulalongkorn University’s pond water (ii) tap water (ii) drinking water. All samples were collected with polyethylene bottle and used without storage. The Chulalongkorn University’s pond water was filtered through a nylon 0.45 μm membrane. Tap water and drinking water were used without other pretreatments.

The sample solution (100 mL) was adjusted to appropriate pH and passed through a mini-column at an optimum flow rate controlled with a peristaltic pump. The sorbed metal was eluted with 1% HNO_3 (5.0 mL) at a flow rate of 0.5 mL min^{-1} and determined by FAAS. The accuracy of the proposed method was performed by adding 0.5, 5.0, 5.0 and 50 μg of Cd(II), Co(II), Cu(II) and Pb(II), respectively into the water sample. The accuracy and precision were reported in term of %recovery and %RSD, respectively. The study of Ni(II) was performed in the same manner using 50 mL of sample solution spiked with 5.0 μg of Ni(II).

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Table 3.3 Summary of the optimized parameters in flow system

Parameter	Optimized conditions				
	Cu(II)	Pb(II)	Cd(II)	Co(II)	Ni(II)
Phase Q					
- Amount of sorbent (mg)	20	20	50	60	60
- Working metal Concentration (mg L^{-1})	2.5	5	1	2.5	2.5
- Sample volume (mL)	100	100	100	100	50
- Sample pH	3.5	3.5	3.5	4.5	4.5
- Sample flow rate (mL min^{-1})	4.0	4.0	4.0	4.0	2.5
Phase B					
- Amount of sorbent (mg)	20	70	-	-	-
- Working metal Concentration (mg L^{-1})	2.5	5	-	-	-
- Sample volume (mL)	100	100	-	-	-
- Sample pH	4.5	4.5	-	-	-
- Sample flow rate (mL min^{-1})	4.0	1.6	-	-	-
Phase A					
- Amount of sorbent (mg)	40	-	-	-	-
- Working metal Concentration (mg L^{-1})	2.5	-	-	-	-
- Sample volume (mL)	100	-	-	-	-
- Sample pH	4.5	-	-	-	-
- Sample flow rate (mL min^{-1})	4.0	-	-	-	-

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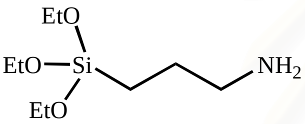
CHAPTER IV

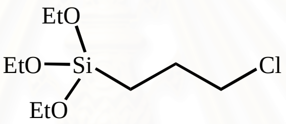
RESULTS AND DISCUSSION

4.1 Synthesis and Characterization of Chemically Modified Silica

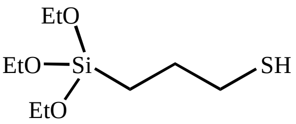
The silica surface contains silanol groups, which can act as weak cation exchangers especially in the basic solution. Moreover, the silanol groups are chemically inert. This means reactions between this group and other chemicals are difficult. However, it can react with organosilane compound [2], such as 3-aminopropyltriethoxy silane **I**, 3-chloropropyltriethoxy silane **II**, 3-mercaptopropyl silane **III** as shown below.



I



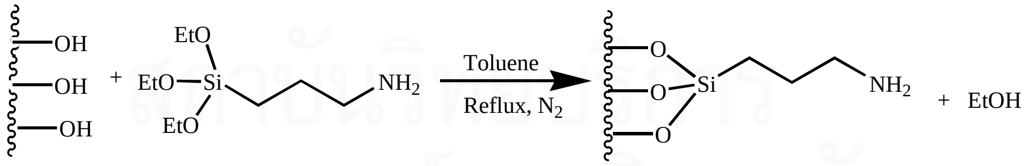
II



III

The reaction between organosilane compound and silanol group provides an opportunity for further reactions on modified functionality.

4.1.1 Synthesis of aminopropyl silica: Activation of the silica surface



Aminopropyl silica was synthesized by the reaction between silanol groups on silica surface and 3-aminopropyltriethoxy silane. The silanol group can react with the silane compound by the silylation reaction or nucleophilic substitution reaction in dried toluene. The reaction was refluxed in toluene for 24 hours under nitrogen. Then, aminopropyl silica was washed with dichloromethane to separate aminopropyl silica from unreacted compounds.

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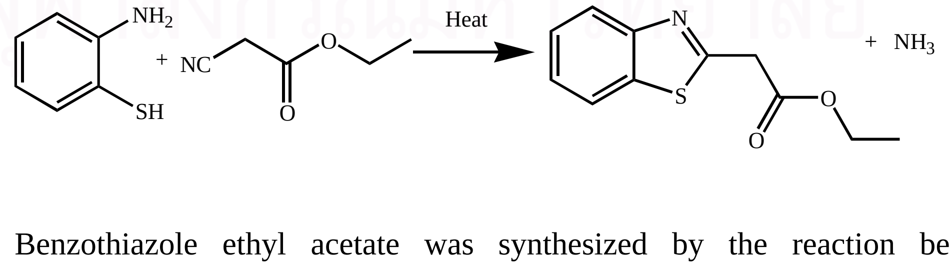
The aminopropyl silica was characterized by solid state ^{13}C-NMR spectroscopy. The spectrum showed 3 peaks of propyl carbons at 43.4, 26.2 and 10.1 ppm indicated that the modified silica gel contained the aminopropyl group on the silica surface. The elemental analysis showed that the composition of original silica gel and aminopropyl silica were different. Pure silica did not have any C, H or N but aminopropyl silica showed C 5.89%, H 0.98% and N 2.35% with C/H ratio of 6.02. This result showed that an organic functional group was attached to the silica. Main functional groups of aminopropyl silica were determined by FT-IR spectroscopy as KBr pellet. The wavenumber 3000 - 3400 cm^{-1} is contributed to O-H stretching and the adsorbed water molecules. The N-H bending of amino group was observed at 1637 cm^{-1}. The siloxane (Si-O-Si, stretching) band appeared at a board intense peak centered at 1229 cm^{-1}. The band due to Si-C bond stretching and Si-O stretching of the silanol group was observed at 1011 and 933 cm^{-1}, respectively. Thermal and chemical stability of modified silica compared with pure silica was evaluated by using thermal gravimetric analyzer (TGA). This technique provided the stability informations and the immobilized organic amount on the silica. The TGA curve of pure silica showed only one mass change around 25 – 210 °C. This mass loss became the loss of the absorbed water or the moisture. The TGA spectrum of aminopropyl silica (the first step modification) showed 4.8% mass loss at 25 – 170 °C due to adsorbed water or the moisture and 11.6% mass loss at 170 – 800 °C corresponding to the loss of the aminopropyl group.

4.1.2 Synthesis of benzothiazoleamido silica

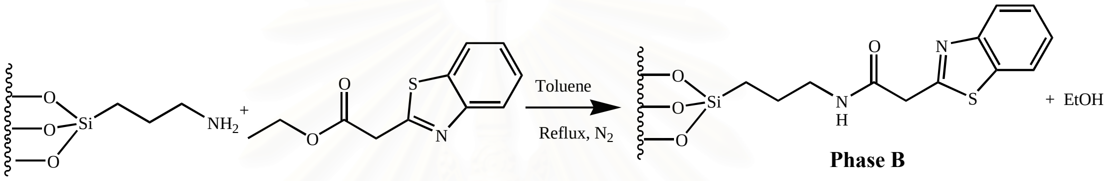
4.1.2.1 Synthesis of benzothiazole ethyl acetate



Benzothiazole ethyl acetate was synthesized by the reaction between aminothiophenol and methylcyano acetate. This reaction yielded the heterocyclic compound containing S and N donor sites and ammonia as by product.

This reaction did not need any solvent, the reaction setup was done in one pot. The mixture of two reactants was heated at 120 °C under nitrogen in order to protect disulfide formation from oxidation reaction by oxygen in air. The product was practically pure and confirmed by ^{1}H-NMR. The ^{1}H-NMR spectrum showed that the signals of –NH_2 and –S*H* at 3.68 ppm disappeared. The splitting pattern of aromatic become *d*, *d*, *t*, *t* pattern with coupling constants of 8.4, 8.4, 7.6 and 7.4 Hz, respectively. These results indicated a cyclic formation on aminothiophenol molecule.

4.1.2.2 Modified aminopropyl silica with benzothiazole ethyl acetate



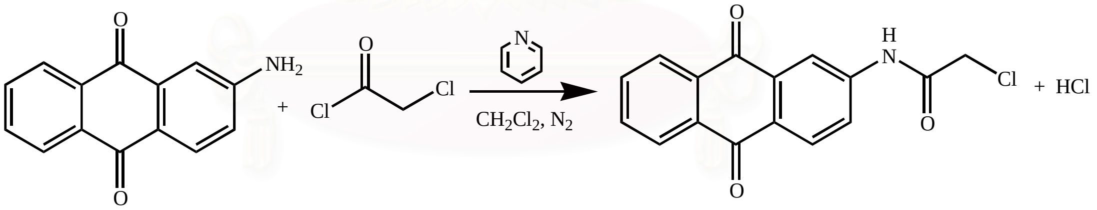
Benzothiazole ethyl acetate reacted with the amine group on the aminopropyl silica yielded an amide linkage. This reaction is nucleophilic substitution and the reaction occurred in the toluene under nitrogen atmosphere at refluxed temperature of toluene. The benzothiazoleamido silica was washed with methanol and dichloromethane to remove the unreacted reagents from the modified silica. The product changed color from white to bright yellow.

Characterization of the modified organic structure was carried out by several techniques. Solid state ^{13}C-NMR spectrum indicated aminopropyl silica changed, according to the presence of new signals at 120-190 ppm corresponding to the benzothiazole group. Elemental analysis results showed that the benzothiazoleamido silica contained C 8.93%, H 1.61% and N 2.43% (C/H = 5.55) which seemed to be different from the amount of C/H in the aminopropyl silica (C/H = 6.02). The different functional groups of the reaction product and reactant silica can be observed by using FT-IR spectroscopy. The FT-IR spectrum of the modified silica showed an absorption peak at 3000-3400 cm^{-1} that corresponded to O-H stretching of the silanol group. Moreover, C=O bending appeared at 1653 cm^{-1} and the peak at 1555 cm^{-1} was corresponded to N-H bending and C-N stretching. Si-C and Si-O stretching showed at 1077 cm^{-1} and 804 cm^{-1}, respectively. The specific surface area (S_{BET}) [59] of phase B was obtained by nitrogen adsorption at several pressures.

The S_{BET} of SiB being 269.4 m^2 g^{-1} was smaller than that of non-functionalized silica gel, S_{BET} = 421.1 m^2 g^{-1} reported by Yaemplai [60]. A decrease in S_{BET} is probably due to the presence of the organic moieties that can block the access of nitrogen to the silica base [61-63]. The average pore diameter of phase B was 55.7 Å. Thermal and chemical stability of phase B compared with pure silica base was evaluated by using TGA. This technique provides the stability informations and the immobilized organic amount on the silica. The TGA curve of the original silica showed only one mass change around 25 – 100 °C. This mass loss was the loss of the adsorbed water or moisture. Moreover, the TGA curve of phase B seemed to be different when compared with the original silica, this because phase B showed 3 steps of mass loss. The first weight loss of 4% was also similar to the original silica. Follow by 8% mass loss around 180 – 460 °C, this corresponded to the loss of the benzothiazole group. Moreover, the last mass loss at 460 – 800 °C was due to the loss of modified silane compound (7%).

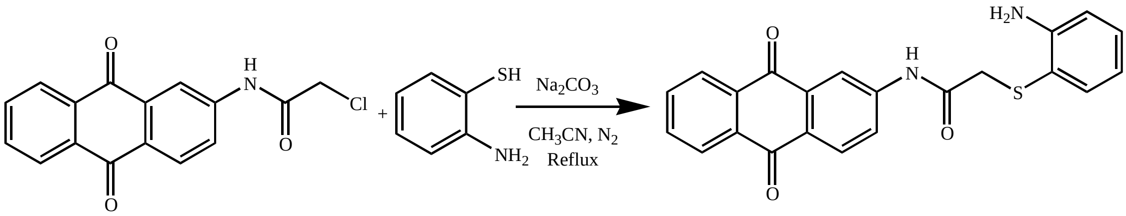
4.1.3 Synthesis of aminothioamido anthraquinone silica

4.1.3.1 Synthesis of chloroamido anthraquinone



This compound is an intermediate of the target molecule. The chloroamido anthraquinone was synthesized via 2-aminoanthraquinone and chloroacetyl chloride, in the presence of pyridine as base at ice bath temperature. The reaction is a nucleophilic substitution yielding amide linkage called chloroamido anthraquinone. The chloroacetyl chloride is very moisture sensitive, so moisture and air must be avoided. The product was dissolved in dichloromethane and precipitated in hexane. The product was characterized by ^{1}H-NMR spectroscopy. The ^{1}H-NMR spectrum showed the characteristic signal of *H*NCO at 10.32 ppm indicating that the amide linkage was formed.

4.1.3.2 Synthesis of aminothioamido anthraquinone



The aminothiophenol derivative was synthesized by the reaction of chloroamido anthraquinone and aminothiophenol. The reaction occurred in the present of sodium carbonate as base in dried acetonitrile. Aminothiophenol was mixed with sodium carbonate to generate strong nucleophile (S^-). The nucleophile attacked the chloro-carbon of chloroamido anthraquinone yielded C-S bond. The product was purified by dissolving in dichloromethane, following by adding hexane to precipitate the product. The product was characterized by ^{1}H-NMR, elemental analysis and ESI-MS. The ^{1}H-NMR spectrum showed characteristic pattern of the aminothiophenol at 6.5-7.3 ppm (*d, d, t, t*) indicating that the chemical shift, spitting pattern and coupling constant were different from reactants. The spectrum did not show -S*H* peak but the present of -NH_2 signal at 4.39 ppm still occurred indicating that $-S^-$ is an attack nucleophile. Elemental analysis and ESI-MS showed the %C, %H, %N or molecular weight corresponded well with the calculated results.

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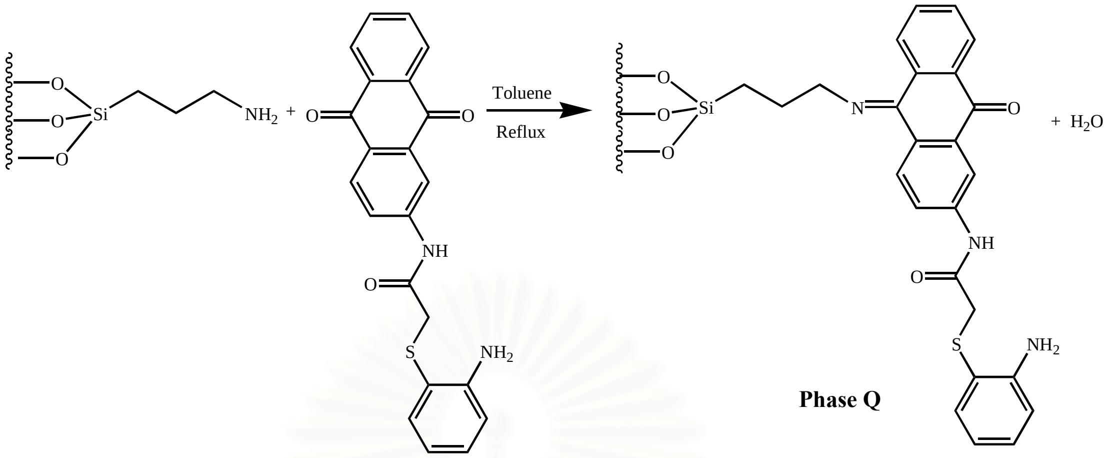
4.1.3.3 Modified aminopropyl silica with aminothioamido anthraquinone



Aminothioamido anthraquinone silica was synthesized by the reaction between aminopropyl silica and aminothioamido anthraquinone to form a Schiff's base linkage. The previous research used Schiff's base linkage between 1,8-dihydroxy anthraquinone and aminopropyl silica [41]. Structure characterizations were carried out using solid state ^{13}C-NMR spectroscopy, elemental analysis, FT-IR spectroscopy, N_2 adsorption and TGA. Results indicated that aminothioamido anthraquinone was connected to amino groups of the aminopropyl silica.

The signals of solid state ^{13}C-NMR observed at 9.5, 26.5 and 46.5 ppm have been assigned to the linkage carbons between aminothioamido anthraquinone and silica gel, Si-**C**H_2, -**C**H_2- and –**C**H_2-N, respectively. Other signals at 118 – 200 ppm were corresponding to carbons of aminothioanthraquinone moieties. Furthermore, the elemental analysis showed the aminothioamido anthraquinone silica contained C 6.94%, H 1.65% and N 2.68%. The C/H ratio was found to be 4.20 which was lower than aminopropyl silica (C/H = 6.02).

The FT-IR spectrum confirmed the presence of aminothioamido anthraquinone bonded to the silica surface. The absorption peaks at 1649, 1552 cm^{-1} were due to C=C and C-N stretching. A large and broad band near 3400 cm^{-1}, was corresponding to the silanol group stretching and the adsorbed water molecule.

The specific surface area (S_{BET}) of phase Q was obtained by nitrogen adsorption at several pressures. The S_{BET} of phase Q, 308.4 m^2 g^{-1}, was smaller than those of non-functionalized silica gel, S_{BET} = 421.1 m^2 g^{-1} reported by Yaemplai [60]. A decrease in S_{BET} is probably due to the presence of the organic moieties that can block the access nitrogen to the silica base [61-63].

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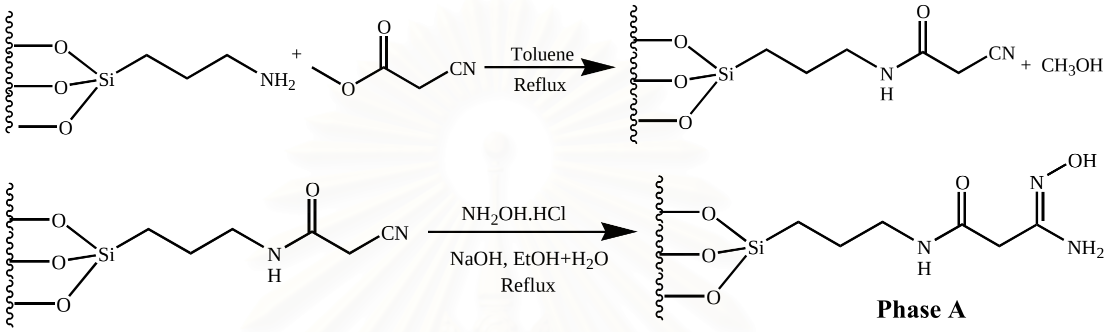
The BJH average pore diameter of phase Q was 55.6 Å. According to TGA results, the mass loss in phase Q could be observed in 2 steps. 4.22% loss was firstly observed stage presented at around 25-160 °C, following by 11.7% mass loss at 160 – 800 °C indicating the loss of modified organic compound on the silica.

4.1.4 Synthesis of amidoamidoxime silica



Cyano group was immobilized on silica gel in the first step. The cyano group was linked to the aminopropyl silica via the amide linkage. The product was characterized by solid state ^{13}C-NMR spectroscopy, FT-IR spectroscopy and elemental analysis. The ^{13}C-NMR spectrum showed the characteristic peaks at 169.43 ppm (N**C**OCH$_2$) corresponding to the amide carbon. Furthermore, the carbon of cyanine group signal showed at 164.00 ppm. The characteristic of the important functional groups such as C≡N stretching showed at 1676 cm^{-1} and C=O bending at 1626 cm^{-1}. The ratio of C/H was 8.58. This value was significantly higher than aminopropyl silica (C/H = 6.02).

The cyanoamido silica was converted to the amidoxime group using hydroxylamine hydrochloride. The reaction was refluxed in the mixture of ethanol and water. The hydroxylamine salt was activated by neutralizing with sodium hydroxide and was adjusted to neutral before adding to the reaction.

The product was washed with water, ethanol and dichloromethane for removing the residual hydroxylamine. The silica product was characterized by solid state ^{13}C-NMR spectroscopy, elemental analysis, N$_2$ adsorption and TGA.

The ^{13}C-NMR spectrum showed the important signal of carbon at 163.5 ppm corresponding to CH$_2$**C**NH$_2$ and the amide carbon shifted to 170 ppm. The FT-IR spectrum showed the new functional group at 1660 cm^{-1} corresponding to C=N stretching and N-H bending at 1552 cm^{-1}.

The specific surface area (S_{BET}) of phase A was obtained by nitrogen adsorption at several pressures. The S_{BET} of phase A being 300.3 m^2 g^{-1} was smaller than that of non-functionalized silica gel, S_{BET} = 421.1 m^2 g^{-1} reported by Yaemplai [60]. A decrease in S_{BET} is probably due to the presence of the organic moieties that can block the access of nitrogen to the silica base. The BJH average pore diameter of phase A was 57.1 Å. The TGA curve of the pure silica showed only one mass change around 25 – 210 °C. This mass loss is due to the absorbed water or the moisture. Phase A showed 3 steps of mass loss. The first weight loss is similar to pure silica with 4.8 % loss. Following by 6.5% mass loss around 210 – 480 °C, this should be the loss of the benzothiazole group. The last mass loss at 480 – 800 °C was the loss of modified silane compound about 4.8%. This behavior signified that the organic moiety was clearly immobilized on the silica.

4.2 Solid Phase Extraction and Preconcentration Study

4.2.1 Batch extraction study

4.2.1.1 Effect of solution pH

The acidity of a solution has two effects on metal sorption. Firstly, protons of acid solution can protonate binding sites of the chelating molecules. Secondly, hydroxide in basic solution may complex and precipitate many metals. Therefore, pH of a metal solution is the first parameter to be optimized. The optimum pH is actually dependent on the various effects especially the pK_a of donor site. Therefore, the pH of solution must be larger than pK_a of the donor site and not higher than K_{sp} of the metal-hydroxide formation.

In order to obtain the optimum pH, the solution pHs were varied from 1-6. Higher pH values were neglected because the functionalized silica was not stable in alkali solutions due to the breaking of Si-O-Si bond by hydroxide ion attack [64]. The results are shown in the Figures 4.1-4.3.

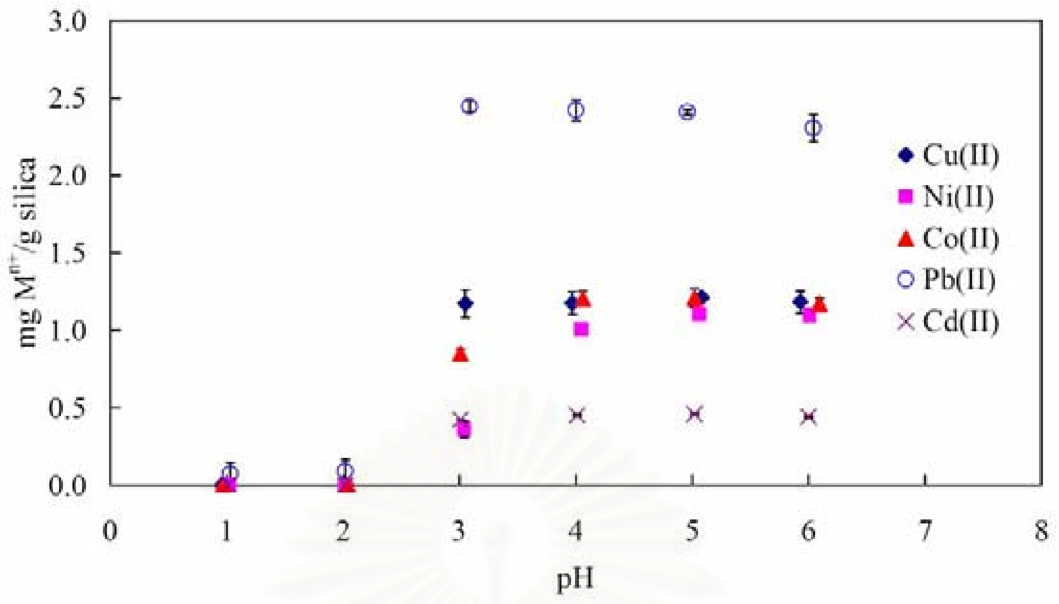


Figure 4.1 Effect of pH solution on extraction efficiency of phase Q

The effect of solution pHs on phase Q adsorption is shown in Figure 4.1. According to the structure of the modified ligand, the aromatic amine is a weak base; therefore H$^+$ in the solution can protonate the basic site and affect the coordination ability toward metal ions. The optimum pH for the maximum sorption of Pb(II), Cu(II) and Cd(II) occurred at pH ≥ 3 and at pH ≥ 4 for Co(II) and Ni(II). Lower extraction efficiencies at low pH are due to the protonation of the amine groups. According to the size of metal ions and hard-soft acid base principle, we proposed that Pb(II), Cu(II) and Cd(II) would bind to the N_2S moiety of the aminothioamido anthraquinone while Co(II) and Ni(II) would bind to the OS moiety of the host compartment as illustrated in Figure 4.2. Moreover, maximum sorption capacity order obtained is Pb(II) > Cu(II) > Ni(II) ≈ Co(II) ≈ Cd(II).

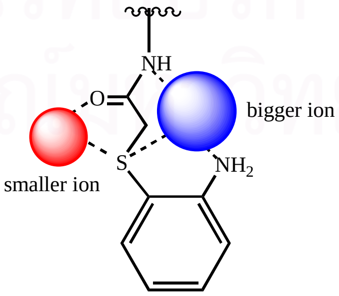


Figure 4.2 Proposed mode of chelation for adsorption of investigated metal ions onto aminothioamido moieties [Ionic radius (in pm): Pb(II) = 133, Cd(II) = 109, Cu(II) = 87, Ni(II) = 83, Co(II) = 79] [78]

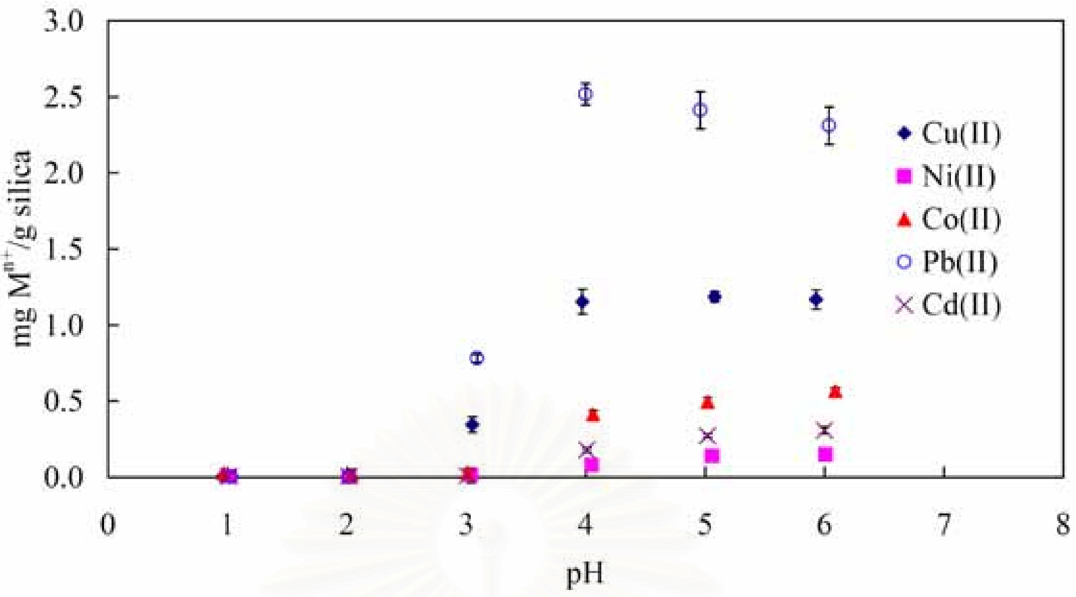


Figure 4.3 Effect of pH solution on extraction efficiency of phase B

The effect of solution pH on the sorption of Cu(II), Pb(II), Ni(II), Cd(II) and Co(II) on phase B is illustrated in Figure 4.3. Cu(II) and Pb(II) were quantitatively sorbed at pH > 4 while the sorption of other three metal ions at pH 3-6 was low. The sorption of Cu(II) and Pb(II) increased when solution pH increased. This behavior was also observed from the extraction of Cd(II), Ni(II) and Co(II) at pH > 3. This effect can be explained by the protonation of chelating sites which inhibited their binding ability to metal ions. These results indicated that N in the benzothiazole group played an important role as the donor sites. Indeed, N is a Lewis base and could be protonated at pH ≤ 4. The geometry of the coordination sites plays an important role in the selectivity of the metal ions towards immobilized ligand.

Moreover, this result was described by the size of the Pb(II) which is larger than Cu(II) or Pb(II) and a softer acid than Cu(II). In addition, Pb(II) basically preferred the amide linkage. For phase B, the coordination site was proposed as shown in the Figure 4.4.

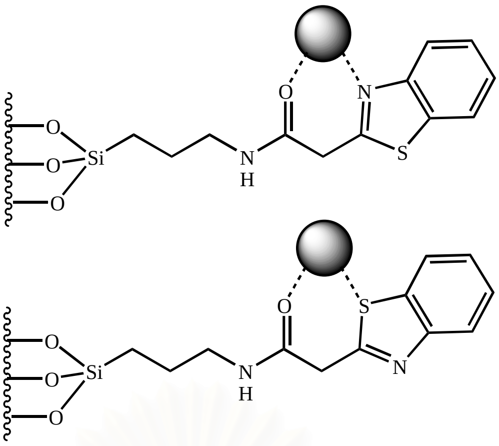


Figure 4.4 Proposed mode of chelation for adsorption of investigated metal ions onto benzothiazole moieties

According to the pH effect on Cu(II), Pb(II), Co(II), Cd(II) and Ni(II) sorption on phase B, we proposed to study only Cu(II) and Pb(II) for other parameters.

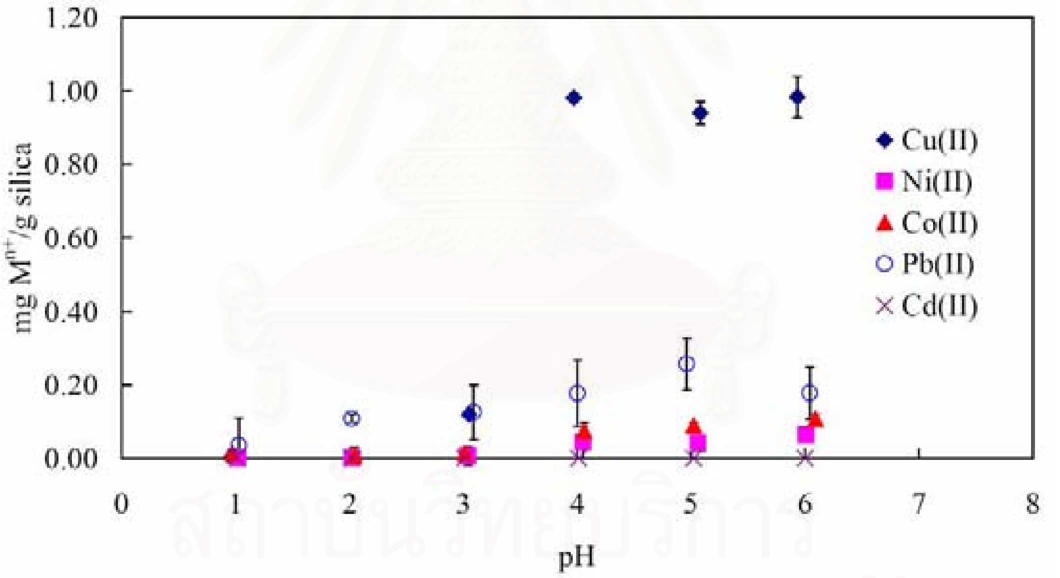


Figure 4.5 Effect of pH on extraction efficiency of phase A

For phase A, surprisingly only Cu(II) was retained at pH ≥ 4, on the other hand low sorption capacities of Pb(II), Cd(II), Co(II) and Ni(II) were obtained at the interval pH range 1-6 (Figure 4.5). Low extraction efficiency at low pH could be explained in the same manner as phase Q and phase B.

This result indicated that phase A was highly selective towards Cu(II) probably due to the suitable geometry of amidoamidoxime group and polarizability of Cu(II). This result indicated that Cu(II) have suitable size and polarizability with the chelating site that contained a moderate hard base. The proposed structures of the complex are shown in Figure 4.6.

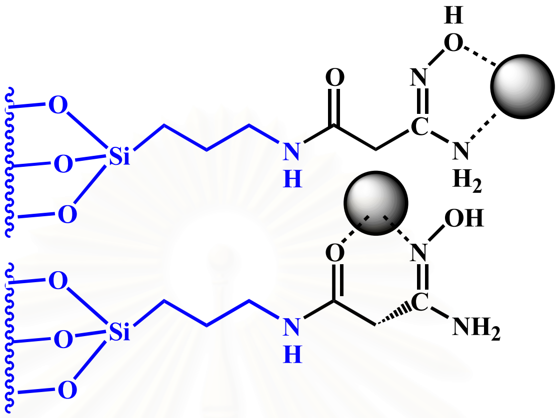


Figure 4.6 Proposed mode of chelation for adsorption of investigated metal ion onto amidoamidoxime moieties

According to the pH effect on Cu(II), Pb(II), Co(II), Cd(II) and Ni(II) sorption on phase A, we proposed to study only Cu(II) for further parameter.

4.2.1.2 Effect of contact time

Adsorption kinetic of metal ion towards modified silica was an interesting parameter, fact that the fast complexation is basically required in analytical applications. The adsorption kinetic of the metal ion depends on several effects. The main factor is the hydrophilicity of the modified ligand. If modified ligands are more lipophilic, the time needed to extract metal ion from aqueous solution will be longer than the hydrophilic ligands. This experiment is important especially in a flow system because a shorter contact time between modified silica and metal ions in the solution in the flow system was observed. The rapid adsorption is thus suitable for the flow system. On the other hand, the slow kinetic should not be applied in the flow system because the analyte cannot be retained quantitatively on the modified silica.

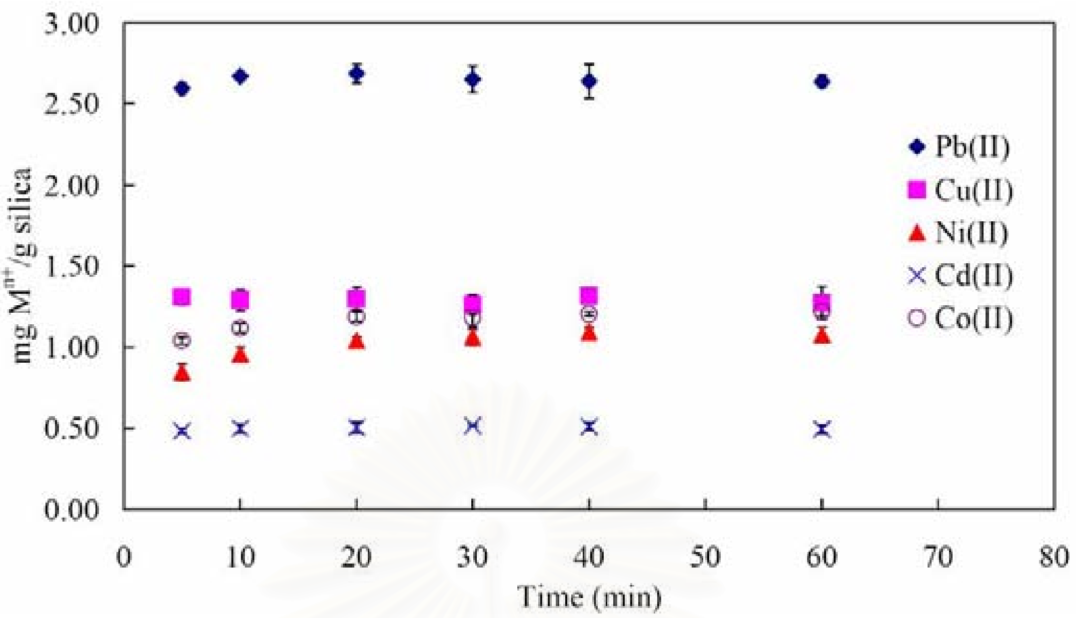


Figure 4.7 Effect of contact time on metal sorption onto phase Q at pH = 3.5 for Cu(II), Pb(II) and Cd(II), pH = 4.5 for Co(II) and Ni(II)

For phase Q, all mentioned metal ions were studied. The contact times were varied from 5-60 minutes. The results are illustrated in Figure 4.7. It took only 5 minutes for Pb(II), Cu(II) and Cd(II) and 10 minutes in the case of Ni(II) and Co(II) to reache the maximum sorption. These results indicated that phase Q had a rapid adsorption kinetics. Therefore, it was suitable for an application in a flow system and can be used in the preconcentration of trace metal ions.

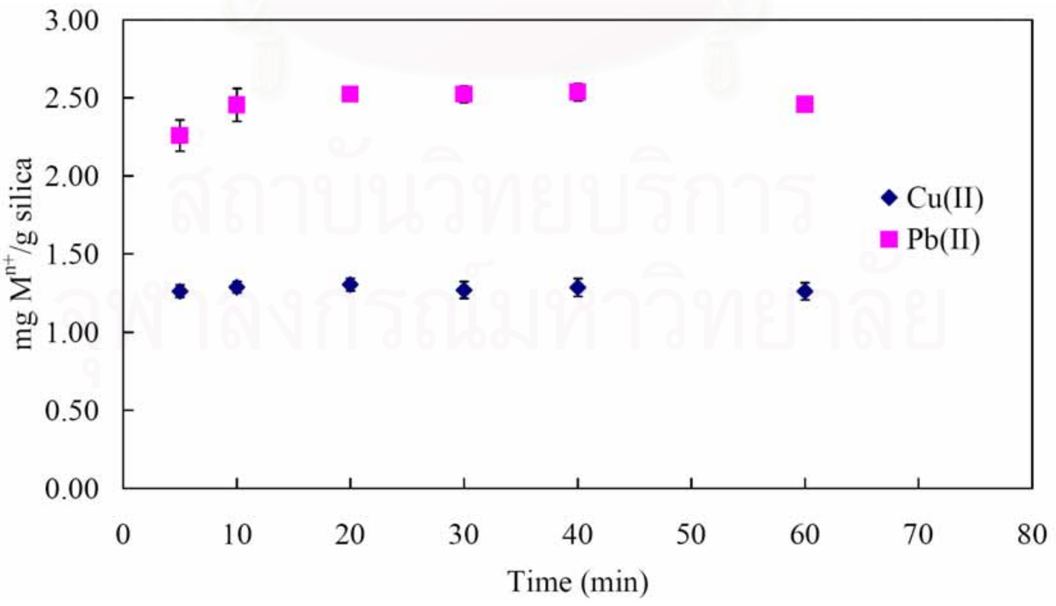


Figure 4.8 Effect of contact time on Cu(II) and Pb(II) sorption onto phase B at pH = 4.5

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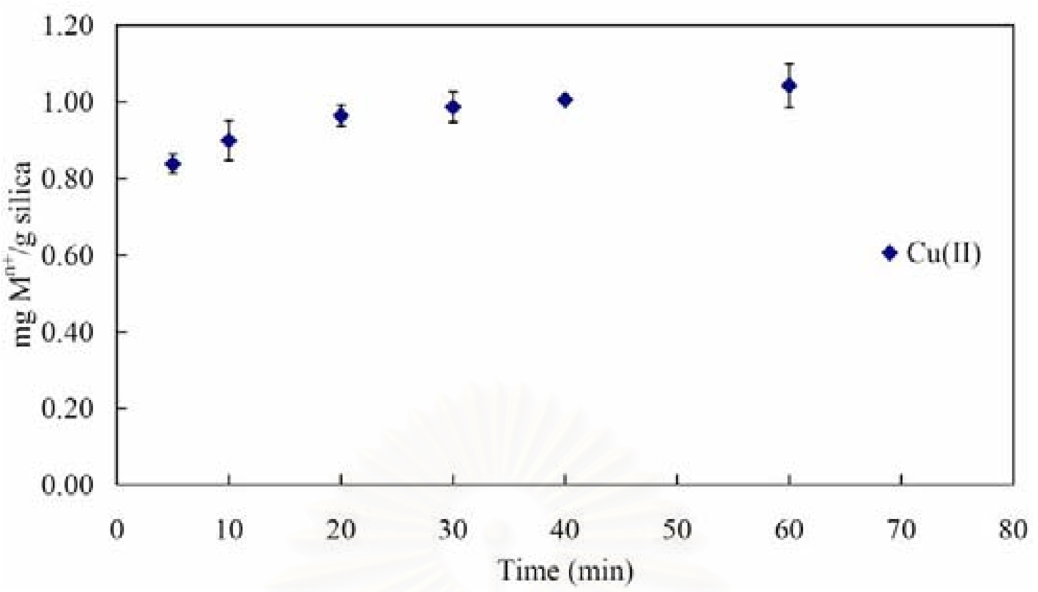

Figure 4.9 Effect of contact time on Cu(II) sorption onto phase A at pH = 4.5

For phase B and phase A, we interested only Cu(II) and Pb(II) and only Cu(II), respectively because other metal ions had low sorption capacities. The results are shown in Figure 4.8 and Figure 4.9 for phase B and phase A, respectively. The contact time required for reaching maximum sorption was 5 and 10 minutes for adsorption of Cu(II) and Pb(II) onto phase B, respectively. For phase A, Cu(II) needed 20 minutes to reach the maximum sorption. Rates of Pb(II) and Cu(II) adsorption onto phase B and phase A were moderately rapid. These results indicated that both phases were also suitable for using in the flow system.

4.2.1.3 Adsorption isotherm

In this experiment, thermodynamic parameters of metal ions adsorption on silica surface were studied. The behavior of the metal ions towards silica surface at equilibrium conditions was used to evaluate the adsorption mechanism. Several models of adsorption isotherm have been described [65].

Sorption is a physical and/or chemical process in which a substance is accumulated at an interface between phases. A number of different equations can be used to predict theoretical adsorption capabilities for different adsorbents. For example, Freundlich isotherm and Langmuir isotherm have been widely used to predict adsorption capabilities of metals on the solid sorbent.

- Freundlich isotherm

Freundlich isotherm is the oldest and most widely used adsorption equation for solid–liquid system. The empirically derived Freundlich isotherm can be defined as follows:

$$N_f = K_f C^{\frac{1}{n}} \tag{4.1}$$

Where N_f is the unit of metal ion added per unit of modified silica (mol g^{-1}), C is equilibrium concentration in solution (mol L^{-1}). K_f and n can be determined experimentally by determining the degree of adsorption N_f at different concentrations C. The information can then be plotted using the following equation.

$$\log N_f = \log K_f + \frac{1}{n}\log C$$

(4.2)

In a plot of the above equation, log K_f is the intercept of the line when C is 1 mol L^{-1}, and it indicates the adsorption capacities of the silica, 1/n is the gradient assuming a linear relationship.

- Langmuir's isotherm

Langmuir proposed the first isotherm model which assumed monolayer coverage of the adsorbent surface. The most commonly used expression of the Langmuir equation for describing adsorption data for solid–liquid systems is

$$N_f = \frac{bN_f^s C}{1 + bC}$$

(4.3)

Where N_f is the amount or concentration of the solute adsorbed (mol) per gram of modified silica, C is the equilibrium concentration of the metal ion in mol L^{-1}, b and N_f^s are constants. The linearized expression of this equation is

$$\frac{C}{N_f} = \frac{1}{bN_f^s} + \frac{C}{N_f^s}$$

(4.4)

This equation is called the “Double-Reciprocal Langmuir Equation” and more suitable in situations in which the distribution of equilibrium concentrations tends to be skewed towards the lower end of the range of the equilibrium concentrations. An EPA report [66] concluded that the Langmuir constant or affinity parameter (*b*) is related to the bonding energy between the metal ion and the adsorbent, but that specific functional relationship is uncertain. Kinniburgh [67] noted that *b* was estimated from the slope of the adsorption isotherm at very low concentrations. The constant N_f^s is accepted as the adsorption maximum of the adsorbent with respect to the specific solute and is interpreted as the maximum amount or concentration that an adsorbent can retain. The results are illustrated in Figure 4.10 - 4.17.

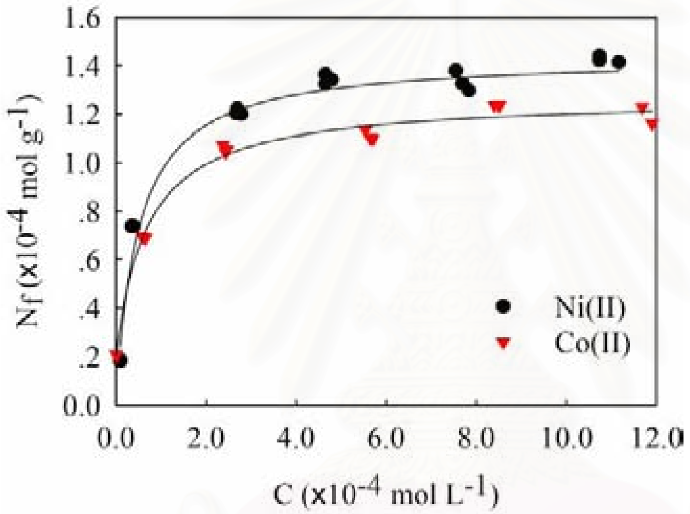

Figure 4.10 Adsorption isotherm of Ni(II) and Co(II) onto phase Q at 298±1 K

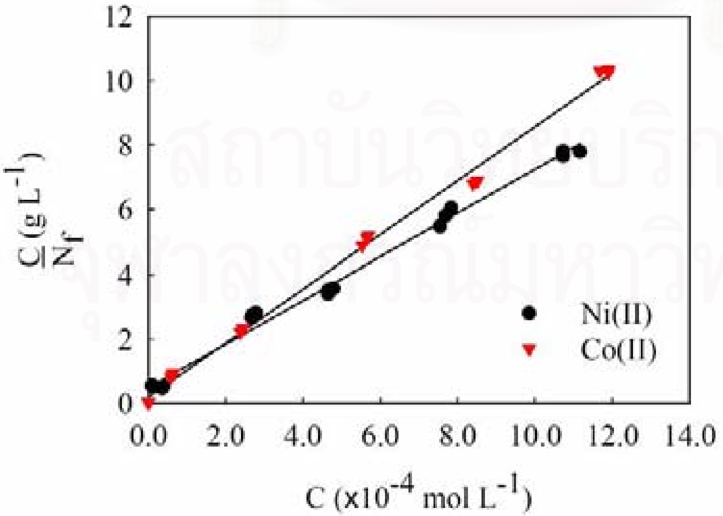

Figure 4.11 Langmuir adsorption model of Ni(II) and Co(II) onto phase Q at 298±1 K

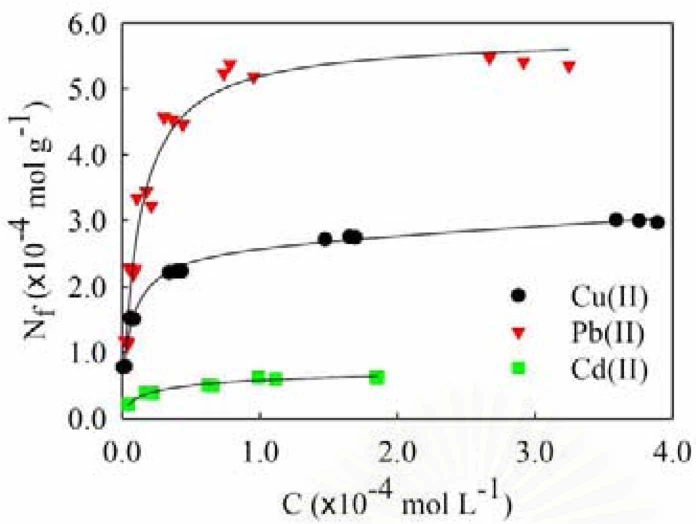

Figure 4.12 Adsorption isotherm of Cu(II), Pb(II) and Cd(II) onto phase Q at 298±1 K

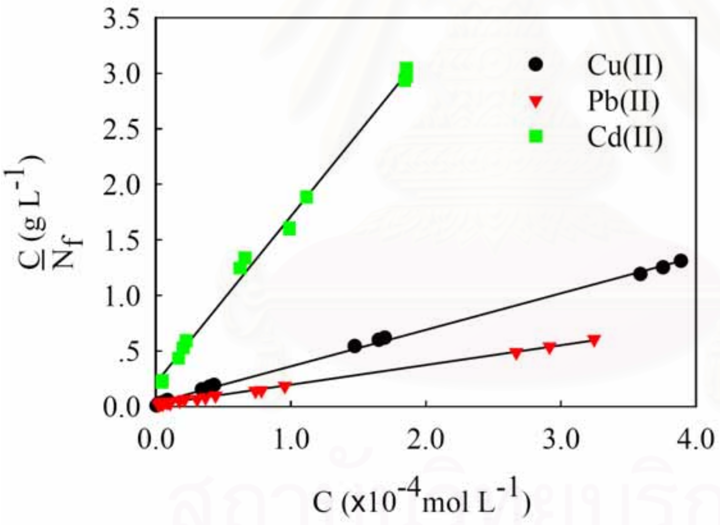

Figure 4.13 Langmuir adsorption model of Cu(II), Pb(II) and Cd(II) onto phase Q at 298±1K

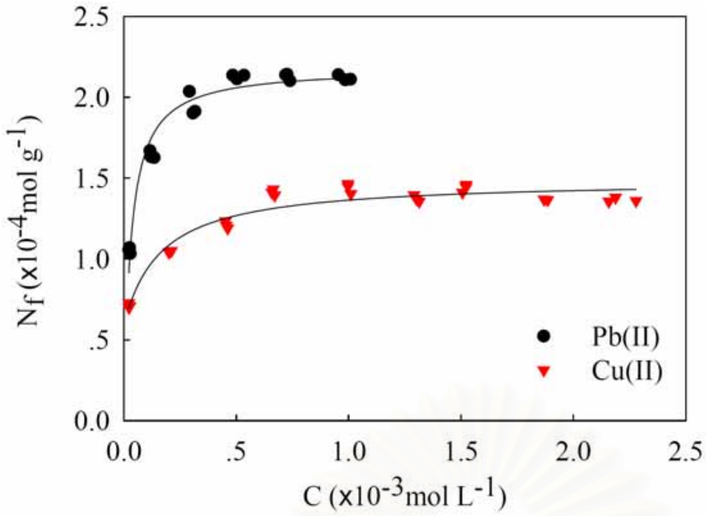

Figure 4.14 Adsorption isotherm of Cu(II) and Pb(II) onto phase B at 298±1 K

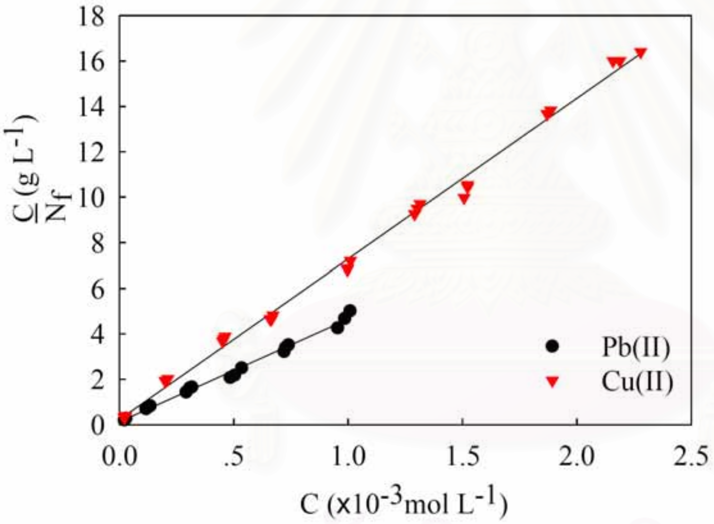

Figure 4.15 Langmuir adsorption model of Cu(II) and Pb(II) onto phase B (Remark: weight of B-Pb(II) = 10 mg, B-Cu(II) = 20 mg) at 298±1 K

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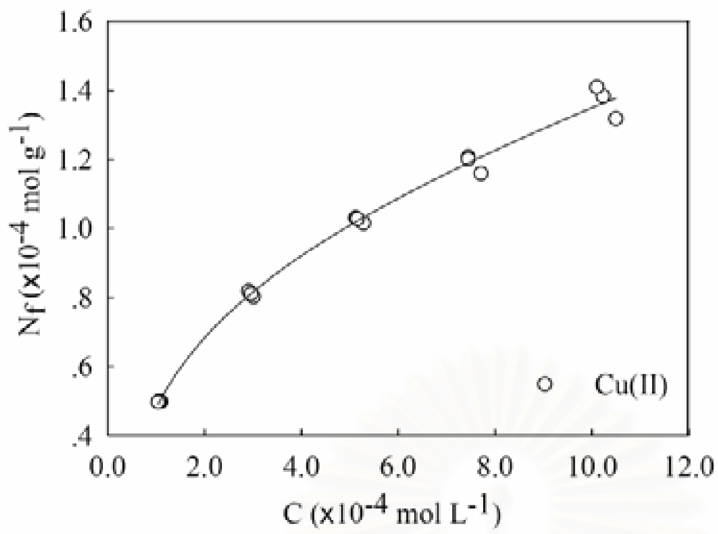

Figure 4.16 Adsorption isotherm of Cu(II) onto phase A at 298±1 K

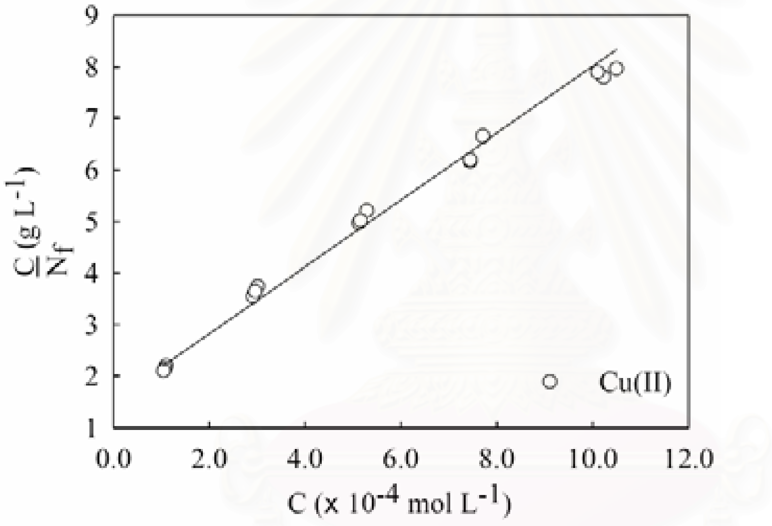

Figure 4.17 Langmuir adsorption model of Cu(II) onto phase A at 298±1 K

Langmuir's equation give many advantages not only illustrated the mechanism of the adsorption and adsorption pattern but also provides important thermodynamic parameters of the adsorption. The slope of the linear model represents a maximum sorption capacity (slope $=\frac{1}{N_f^s}$) and the intercept indicates the equilibrium constant (intercept $=\frac{1}{bN_f^s}, b=\frac{K_{eq}}{a}, a = activity$) high K_{eq} value indicates directly coordination between metal ion and the chelating moiety on silica surface. Moreover, K_{eq} does not depended on kinetic of metal ion adsorption. The conclusions of thermodynamic parameter are shown in the Table 4.1.

Table 4.1 Thermodynamic parameters from Langmuir's isotherm at 298±1 K

Element	linear equation	r	N^f_s ($\times 10^{-4}$ mol g^{-1} silica)	b ($\times 10^{4}$ L mol^{-1})
Phase Q				
Cu(II)	y = 3317.1x + 0.2440	0.9990	3.0	13.6
Pb(II)	y = 1778.6x + 0.0187	0.9992	5.6	9.5
Ni(II)	y = 6745.4x + 0.4949	0.9961	1.5	1.4
Co(II)	y = 8355.5x + 0.2031	0.9978	1.2	4.1
Cd(II)	y = 15006x + 0.2120	0.9975	0.67	7.1
Phase B				
Cu(II)	y = 6795x+0.3006	0.9943	1.4	4.2
Pb(II)	y = 4518x+0.1278	0.9921	2.1	3.5
Phase A				
Cu(II)	y = 6139.4x + 1.6998	0.9964	1.6	0.36

All results showed the linearity of the Langmuir's adsorption isotherm with the correlation coefficient (r) ≥ 0.99 indicating that the adsorption isotherm of these three phases for metal ions obeyed Langmuir's adsorption model. The adsorption is chemisorption by coordination of chelating moiety on the silica surface and metal ions. The main characteristic of the experiment corresponded with Langmuir's model in which the monolayer coverage of metal ions on the silica surface. Other mechanisms for example precipitation, second adsorption on the first layer were not occurred [62, 68]. Moreover, the sorption capacities of each metal towards modified silica were different, indicating the different selectivity. Different structures of the modified ligands provide different selectivities of the modified silica towards metal ions. This is clearly due to the role of the modified chelating ligand onto the silica based adsorbent.

The *b* value critically depended on the activity of the solution. Therefore, the *b* value reported here cannot compare with different metal ions or same ions but different concentration range. On the other hand, the maximum sorption capacity is the absolute value for each phase. It can be compared to another phase or metal ion type.

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The order of maximum sorption capacity of Cu(II) was phase Q > phase A ≈ phase B. This result was probably described by the affinity of the donor sites (N_2S) and flexible geometry of aminothiophenol moiety on phase Q which are more suitable than phase A and phase B. Moreover, in the comparison with the maximum capacity of Pb(II), the order of phase Q > phase B was observed. The result can be described in the same analogy with the case of Cu(II).

4.2.2 Column extraction

4.2.2.1 Effect of solution flow rate

In a flow system, flow rate of metal ion solutions are a critical parameter [2]. The contact time must be long enough to complete adsorption, however, it should be short enough for efficient preconcentration step. Mini-columns were prepared by using an easy package equipped with a peristaltic pump in order to control the solution flow rate.

The sorptions on phase Q packed in mini-column were studied at various flow rates. The solutions were prepared by using 5.0 mL of each metal solutions with concentration 10 mg mL^{-1} for Pb(II), 5 mg mL^{-1} for Cu(II), Ni(II), Co(II) and 2 mg mL^{-1} for Cd(II). Test solutions were passed through the minicolumn at various flow rates between 0.5 to 5 mL min^{-1}. The results are illustrated in Figure 4.18. The sorptions of Pb(II), Cu(II) and Cd(II) were not affected by the solution flow rate.

On the other hand, the decrease in sorption of Ni(II) and Co(II) with increasing flow rate were observed. This behavior was undesirable for the flow system because when the flow rate change would cause significant change in extraction efficiency. However, sorption efficiency can be improved by increasing amount of phase Q to 60 mg for Ni(II) and to 40 mg for Co(II) as shown in Figures 4.19 and 4.20, respectively.

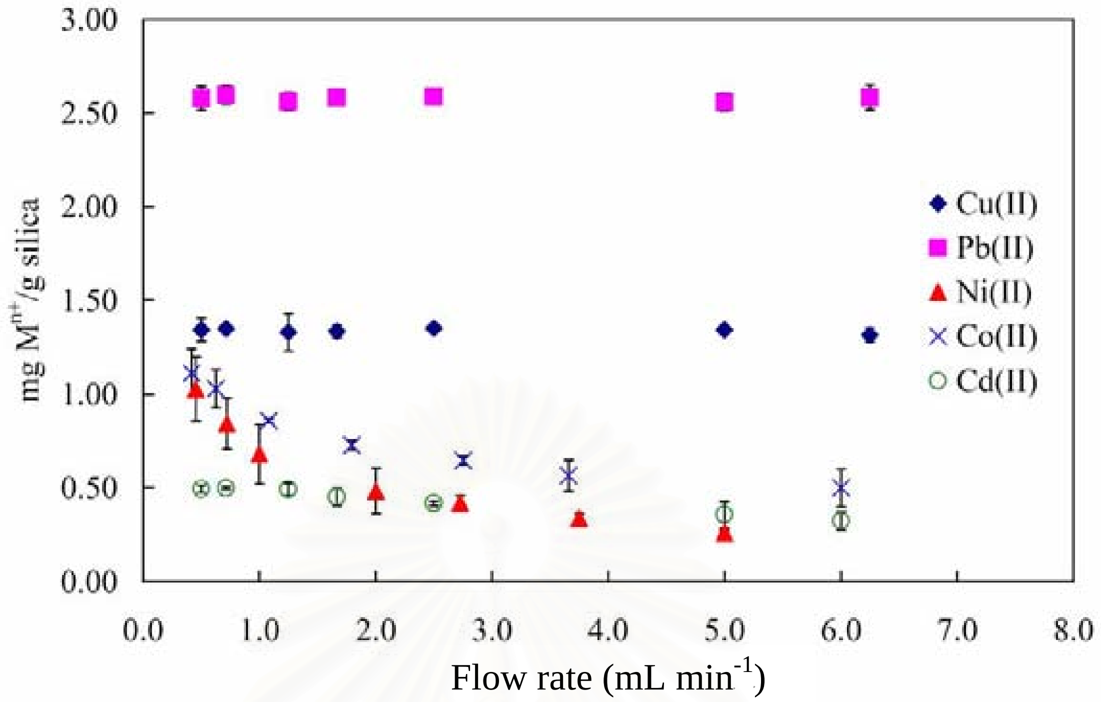

Figure 4.18 Effect of solution flow rates on column packed with 20 mg of phase Q

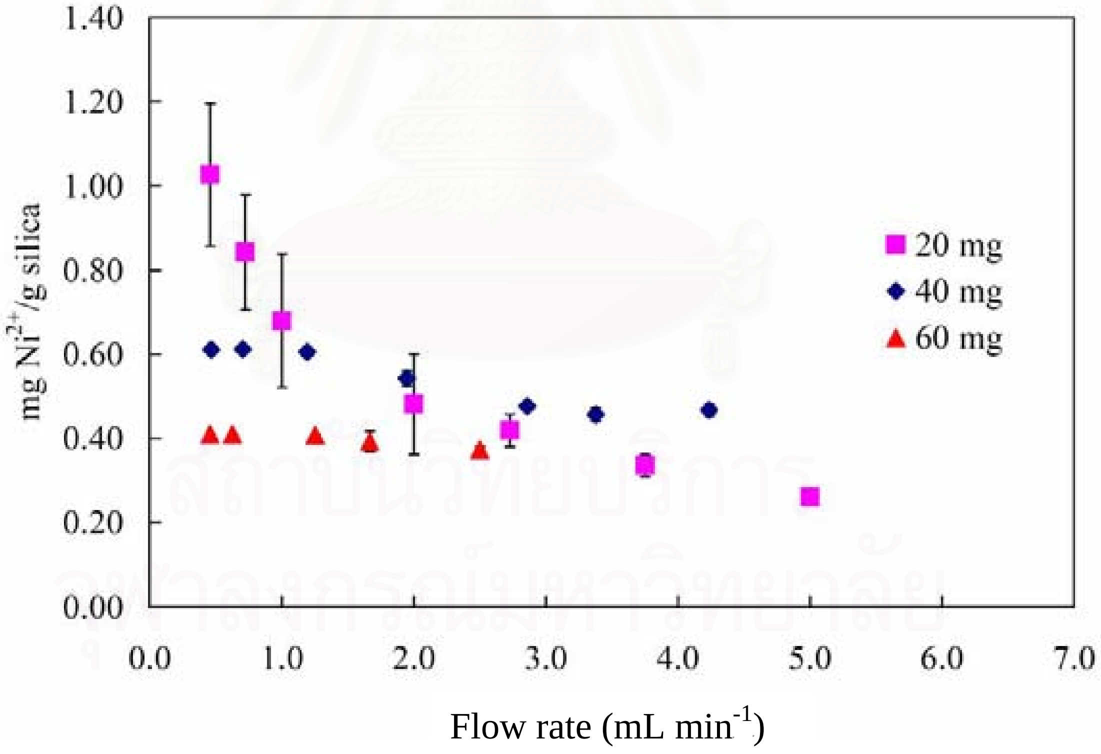

Figure 4.19 Effect of solution flow rates on column packed with various amounts of phase Q onto Ni(II) extraction

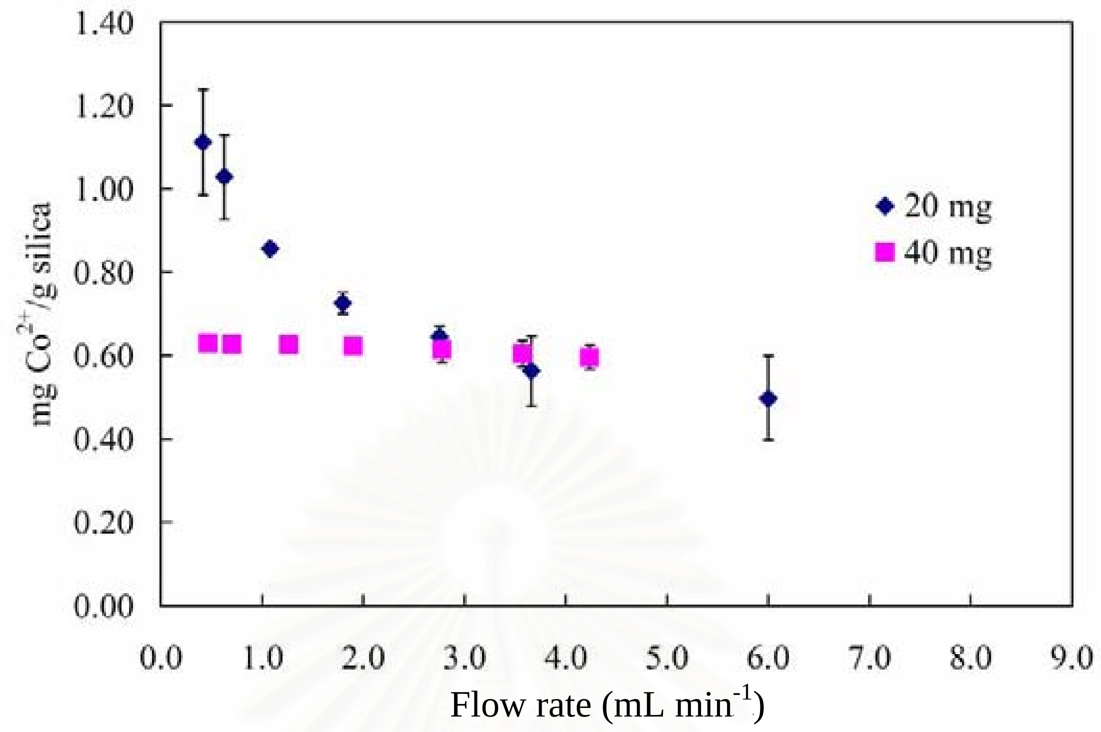

Figure 4.20 Effect of solution flow rates on column packed with 20 mg and 40 mg of phase Q towards Co(II) extraction

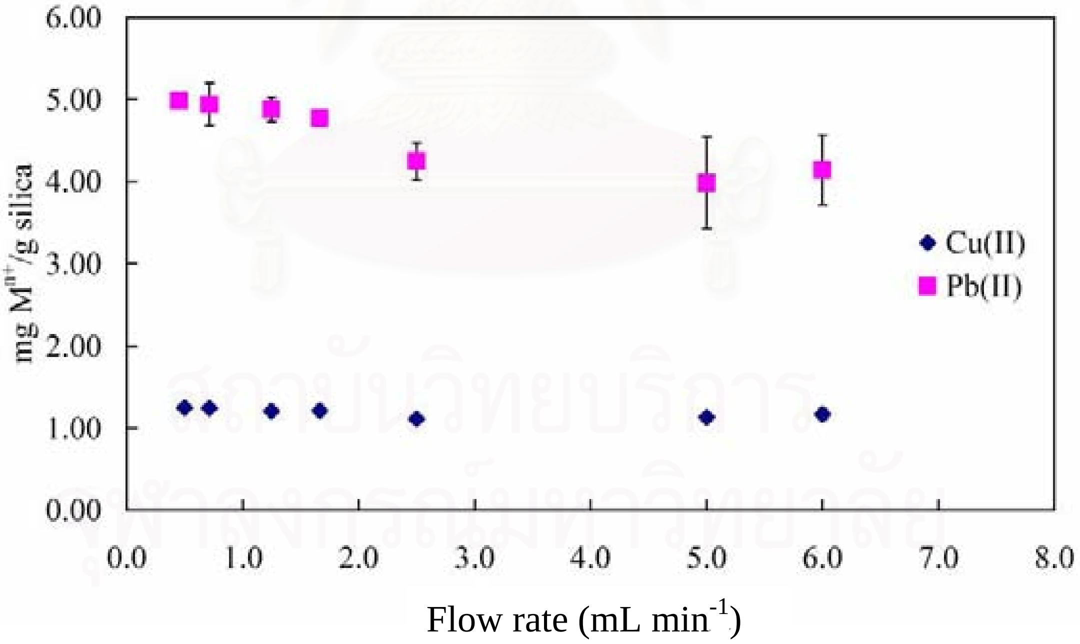

Figure 4.21 Effect of solution flow rates on column packed with 20 mg of phase B

The effect of the solution flow rate on the extraction efficiency of phase B was also investigated. The results are illustrated in Figure 4.21. The studies showed that the solution flow rate did not affect Cu(II) extraction while using only 20 mg of phase B. This indicated that phase B can be applied to extract Cu(II) without flow rate effect in this studied range. On the other hand, the extraction efficiencies of Pb(II) were constant at the flow rate lower than 1.6 mL min^{-1}. Pb(II) sorption decreased at high flow rates due to the increase in the flow rate of metal solution that reduced the contact time. Therefore, the solution flow rate of 1.6 mL min^{-1} was chosen as the optimum flow rate for Pb(II).

The experiments were also explored for phase A in the same manner as phase Q and phase B. The solution flow rates were varied in the range of 0.5 - 5 mL min^{-1}. Solution flow rates versus various amount of modified silica are shown in Figure 4.22. In the case of 20 mg of phase A, the rapidly decrease in Cu(II) extraction efficiency in increasing the solution flow rate was found. These results confirmed the observation from the previous section that the contact time of Cu(II) onto phase A was moderately rapid. When 40 mg of phase A was used, the extraction efficiencies were independent from the solution flow rate in this studied range. Therefore, the optimum flow rate of Cu(II) onto phase A for the next experiment was 4 mL min^{-1}.

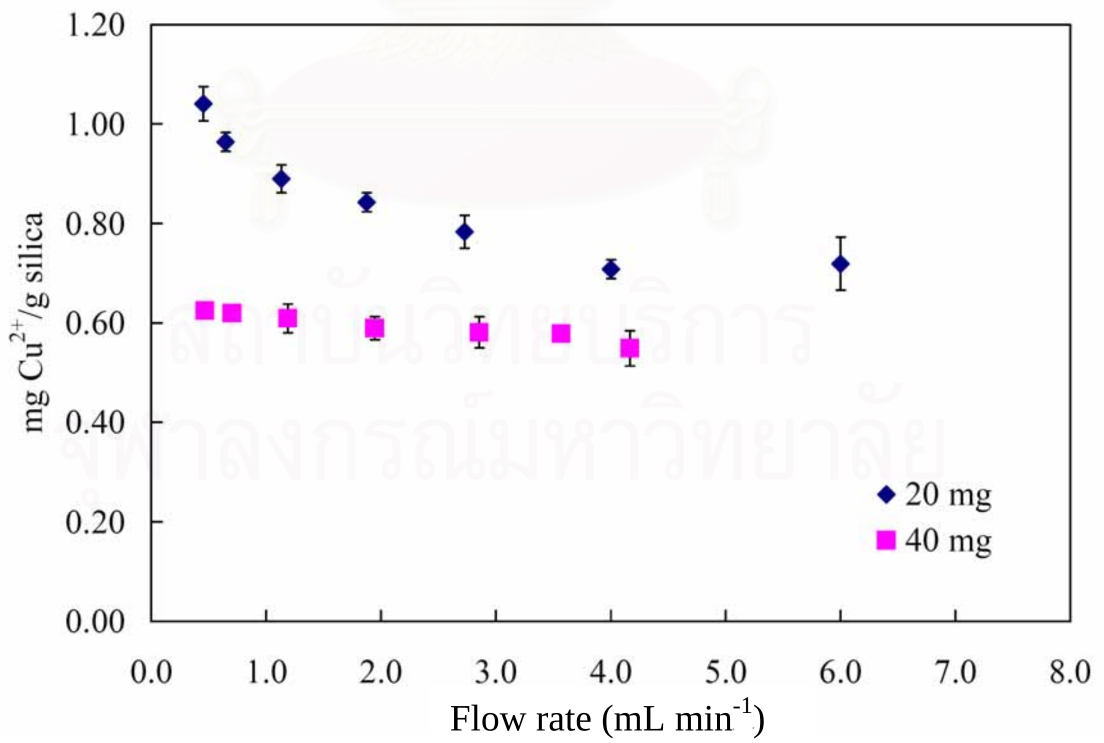

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Figure 4.22 Effect of solution flow rates on column packed with 20 mg and 40 mg of phase A

4.2.2.2 Effect of striping concentration

The stripping solution in both type and concentration should be accounted. The types of the solution have to be compatible with the determination technique. Furthermore, the concentration of the solution should not be used in high concentration that would affect the determination step. Several types of the stripping solution were used depending on the adsorption-desorption mechanism and how strong of the metal and ligand interactions. For example, Jamali et al. [69] used thiourea for eluting Pd(II) from the thiophene-2-cabaldehyde-modified mesoporous silica with the best recovery. Moreover, Fan et al. [70] studied the adsorption of Hg(II) onto the chemically modified silica with xylenol orange and used $HClO_4$ as eluent, followed by determination of metal ion with spectrophotometry without interference from the eluent. In addition, for the determination of eluted metal from the sorbent using FAAS, acids such as nitric acid seem to be compatible with this technique. However, the acid concentration was an important factor. The effects of HNO_3 concentration are shown in Table 4.2. It was found that the % recovery of each metal ion using different concentration of HNO_3 was not significantly different.

Therefore, 1% HNO_3 was chosen because at this concentration the chelation sites of ligand would not be protonated. Moreover, the lowest concentration of acid may contain the smallest amount of other contaminations.

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Table 4.2 Effect of HNO_3 concentration on metal desorption

HNO_3 (%)	%Recovery*				
	Cu(II)	Pb(II)	Cd(II)	Co(II)	Ni(II)
Phase Q					
1	95±3	92±2	92±2	95±1	94±2
3	97±1	94±3	93±2	91±1	96±1
5	96±1	92±2	89±2	93±1	95±1
7	96±2	91±1	89±4	91±1	94±1
Phase B					
1	89±2	90±2	-	-	-
3	94±1	93±2	-	-	-
5	95±1	91±2	-	-	-
7	93±1	89±1	-	-	-
Phase A					
1	96±1	-	-	-	-
3	97±1	-	-	-	-
5	97±1	-	-	-	-
7	95±2	-	-	-	-

* Mean value $\pm SD$, n = 3

4.2.2.3 Effect of sample volume

In order to achieve high preconcentration factor, metal ions in a large sample volume has to be quantitatively sorbed and desorbed by a small stripping volume. However, incomplete retention of metal ions will possibly occur because the sample itself can act as eluent or less contact of analyte and the sorbent. Breakthrough volume is the maximum sample volume that should be percolated through a given mass of sorbent after which analyte start to elute from the sorbent resulting in non-quantitative recoveries. In these experiments, breakthrough volume is the maximum solution volume that an analyte can be retained with the acceptable recovery range. The recoveries of metal ion in the function of sample volume are shown in Table 4.3. The recoveries obtained were compared with the acceptable range of estimated recovery.

Table 4.3 Effect of sample volume on recovery of metal ions

Elements	%Recovery[*]		
	25 mL	50 mL	100 mL
Phase Q			
Cu (20 mg)	105±1	106±2	104±1
Pb (20 mg)	89±2	72±2	82±2
Ni (60 mg)	97±1	92±1	57±3
Co (40 mg)	97±1	88±2	62±1
Co (60 mg)	94±2	92±2	87±2
Cd (20 mg)	95±1	83±1	48±2
Cd (50 mg)	96±1	97±1	94±1
Phase B			
Cu (20 mg)	92±2	93±1	93±1
Pb (20 mg)	76±2	53±1	70±2
Pb (50 mg)	80±2	50±2	78±2
Pb (70 mg)	97±1	98±2	102±1
Phase A			
Cu (40 mg)	75±1	83±2	88±2

[*] Mean value ± *SD*, n = 3

The effect of sample volume on metal sorption was studied by passing 25-100 mL of a metal solution through the column packed with 20-60 mg of phase Q at a flow rate of 4 mL min^{-1} (for Ni(II) = 2.5 mL min^{-1}). It was found that the adsorption of Cu(II) and Pb(II) with 20 mg of phase Q were not affected by the sample volume. High recovery of Ni(II) was obtained when the sample volume of 50 mL and 60 mg of phase Q was used. In the case of Co(II), the results showed good recoveries onto 40 mg of phase Q with 50 mL of sample volume. According to this problem, by increasing an amount of phase Q up to 60 mg, it was found that the recoveries could be improved to 87% with 100 mL of sample volume. In the case of Cd(II), when 20 mg of phase Q was used, the % recovery decreased when sample volume increased, especially at 100 mL of sample volume. As the same analogy, phase Q was increased to 50 mg. The result showed the increase in %recovery of 94% with 100 mL of solution volume.

For phase B, it was found that the recoveries of Cu(II) onto 20 mg of sorbent was not affected by the sample volume between 25 – 100 mL. A sample volume in this range could be used in Cu(II) preconcentration. However, low recoveries of Pb(II) were obtained with 20 mg of phase B. The increase in an amount of phase B to 50 mg could not be enough to improve the recoveries of Pb(II) for any sample volumes. The good recoveries of Pb(II) were obtained when 70 mg of phase B was used with no influence of sample volume in the range of 25- 100 mL.

In addition, the recoveries of Cu(II) onto 40 mg of phase A were not significantly affected by the sample between 25 – 100 mL.

Table 4.4 Analyte recovery and precision at different concentration [71]

Analyte, %	Analyte ratio	Unit	Mean recovery, %	RSD, %
100	1	100%	98-102	1.3
10	10^{-1}	10%	98-102	1.8
1	10^{-2}	1%	97-103	2.7
0.1	10^{-3}	0.1%	95-105	3.7
0.01	10^{-4}	100 ppm	90-107	5.3
0.001	10^{-5}	10 ppm	80-110	7.3
0.0001	10^{-6}	1 ppm	80-110	11
0.00001	10^{-7}	100 ppb	80-110	15
0.000001	10^{-8}	10 ppb	60-115	21
0.0000001	10^{-9}	1 ppb	40-120	30

4.2.2.4 Method detection limit and linear range of the method

Linearity of this particular method is an important parameter used to validate the method. This result gives the information of ability of the working concentration range that can be carried out by this method. Here, we demonstrated a practical working range and method detection limit in order to show the exact ability of the method. Metal ions were diluted as low concentration as the signal of FAAS cannot be detected. The lowest concentration that still gave an acceptable recovery is “practical method quantitation limit” (PMQL). Acceptable values are both good in %recovery and high precision (% RSD) as shown in Table 4.4.

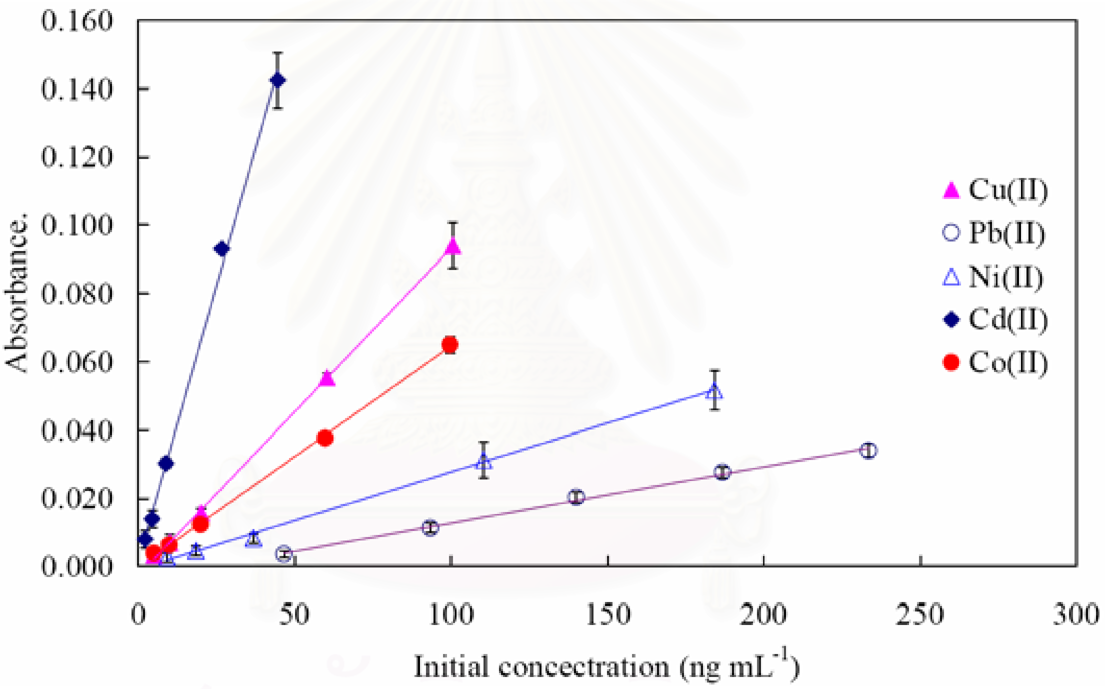

Figure 4.23 Linear range of the proposed method of phase Q towards Cu(II), Pb(II), Cd(II), Co(II) and Ni(II) at solution volume of 100 mL (50 mL for Ni(II)), n = 3

The linear working ranges of the method for phase Q are shown in Figure 4.23. Each metal ion showed different working ranges. The linear working range of 5-100, 93-233, 18-184, 2-40 and 5-100 ng mL^{-1} were obtained for Cu(II), Pb(II), Ni(II), Cd(II) and Co(II). Moreover, sensitivity of the method could be obtained from the slope of linear range. Indeed, the sensitivity of the method is mainly depended on the sensitivity of the instrument. The lowest concentrations are limited by several factors, for example instrument detection limit, sample volume (before preconcentration), or striping volume. Therefore, the lowest concentration could be improved by adjusting several parameters.

Table 4.5 %Recoveries of Cu(II), Pb(II), Ni(II), Cd(II) and Co(II) onto phase Q preconcentration at various initial concentrations

Conc.	%Recovery*				
(ng mL^{-1})	Cu(II)	Pb(II)	Ni(II)	Cd(II)	Co(II)
5	119±10	-	-	-	-
10	99± 5	-	-	-	-
20	90± 3	-	-	-	-
60	94± 1	-	-	-	-
100	94± 2	-	-	-	-
46	-	39±17	-	-	-
93	-	69± 3	-	-	-
140	-	85± 2	-	-	-
186	-	88± 5	-	-	-
233	-	83± 1	-	-	-
9	-	-	96±20	-	-
18	-	-	84±10	-	-
36	-	-	75± 5	-	-
110	-	-	94± 6	-	-
184	-	-	93± 4	-	-
2	-	-	-	68±6	-
4	-	-	-	74±2	-
9	-	-	-	92±1	-
26	-	-	-	101±6	-
40	-	-	-	89±3	-
5	-	-	-	-	97±1
10	-	-	-	-	84±8
20	-	-	-	-	87±4
60	-	-	-	-	92±1
100	-	-	-	-	97±1

* Mean value $\pm SD$, n = 3

The practical method quantitation limits (PMQL) of phase Q were evaluated under the optimum conditions of each metal with a preconcentration factor of 20 (sample volume = 100 mL, striping solution = 5 mL for Cu(II), Pb(II) Co(II) and Cd(II)) and the preconcentration factor of 10 for Ni(II) (sample volume = 50 mL, striping solution = 5 mL for Ni(II)). The results are shown in Table 4.5. The recoveries and %RSD which were in the acceptable range by comparison with Table 4.4 were found to be at the concentrations of 5, 93, 18, 2 and 5 ng mL^{-1} for Cu(II), Pb(II), Ni(II), Cd(II) and Co(II), respectively. The low PMDL indicated that the preconcentration step was required for determination of these metal ions at trace level because they cannot be directly measured by FAAS.

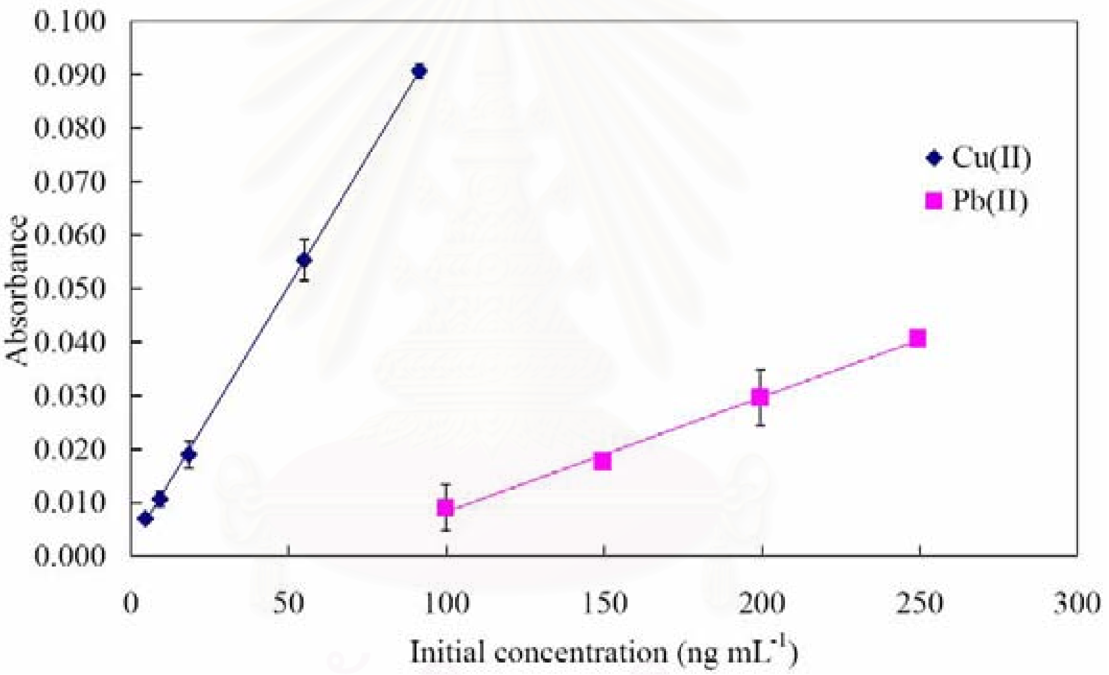

Figure 4.24 Linear range of the proposed method of phase B towards Cu(II) and Pb(II) at solution volume of 100 mL

Linear working ranges of the method for phase B are shown in Figure 4.24. Each metal ion showed different working ranges. The linear working range of 4-91 and 200-250 ng mL^{-1} were obtained for Cu(II) and Pb(II), respectively. Remarkably, the working range shown in this experiment cannot be directly measured by FAAS. The results indicated that the preconcentration step was required when the concentrations of analytes are lower than the detection limit of the instrument. Sensitivities of the method were also observed from the slope of this experiment. According to the results, Cu(II) showed better sensitivity than Pb(II) with calibration sensitivity of 0.97 and 0.21 for Cu(II) and Pb(II), respectively.

Actually, when the preconcentration factors of both ions are equal, the sensitivities are exactly depended on the instrument. The practical method quantitation limits of phase B were evaluated under the optimum conditions of each metal with a preconcentration factor of 20 (sample volume = 100 mL, striping solution = 5 mL). The results are shown in Table 4.6. The recoveries and %RSD which were in the acceptable range by comparison with Table 4.4 were found to be at the concentrations of 4 and 200 ng mL^{-1} for Cu(II) and Pb(II), respectively.

Table 4.6 %Recoveries of Cu(II) and Pb(II) of phase B at various initial concentrations

Conc.	%Recovery*	
(ng mL^{-1})	Cu(II)	Pb(II)
4	76±1	-
9	75±6	-
18	80±5	-
54	88±2	-
91	88±1	-
50	-	37±13
100	-	51±11
150	-	71± 2
200	-	92± 2
250	-	102± 1

* Mean value ± *SD*, n = 3

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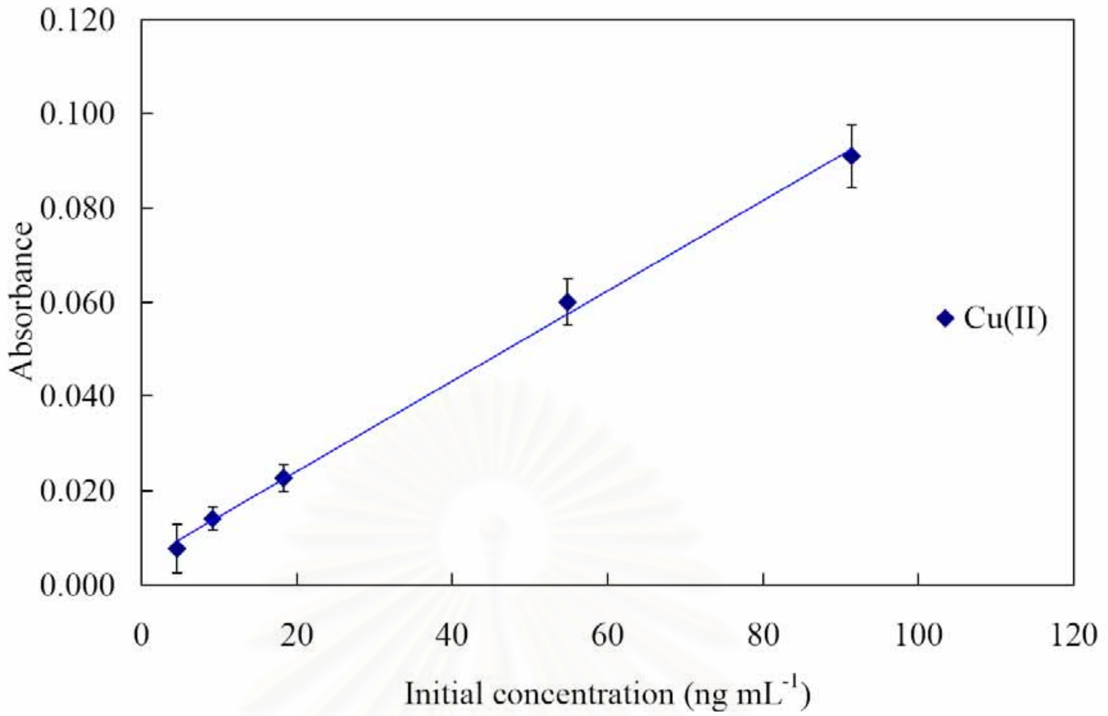

Figure 4.25 Linear range of the proposed method of phase A towards Cu(II) at solution volume of 100 mL

Table 4.7 %Recoveries of Cu(II) of phase A at various initial concentrations

Conc. (ng mL^{-1})	%Recovery*
	Cu(II)
4	34±12
9	83±10
18	86± 6
54	93± 3
91	88± 2

* Mean value ± *SD*, n = 3

The linear working ranges of the method for phase A is shown in Figure 4.25. The linear working range of Cu(II) was found to be 9-91 ng mL^{-1}. The practical method quantitation limits of phase A were evaluated under the optimum conditions of each metal with a preconcentration factor of 20 (sample volume = 100 mL, striping solution = 5 mL). The results are shown in Table 4.7. The recoveries and %RSD which were in the acceptable range by comparison with Table 4.4 were found to be at the concentrations of 9 ng mL^{-1}.

4.2.2.5 Effect of the interfering ions

According to the objective of this research, the selectivity of the modified silica towards heavy metals was a crucial concerning point. The modified material should have specificity to only heavy metals. Moreover, alkali or alkali earth metals should not be interfered. The hypothesis was to evaluate the recovery of the system contained interfering ions and without interfering ions. If the recovery is reduced significantly, it means added ions should interfere the extraction system.

Cations and anions used in this study were chosen from their major abundance in nature, for example Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^- and SO_4^{2-}. Salts used were nitrate salts and sodium salts for cation and anion interfering ions, respectively. Experiments were performed in 3 levels of interfering ion concentrations under the optimum conditions of each metal. The results obtained are shown in Table 4.8 – 4.10 for phase Q, phase B and phase A, respectively.

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Table 4.8 Effect of the interfering ions to %recoveries of phase Q

Element	Interfering ion concentration (mg L^{-1})	%Recovery*		
		10	100	1000
Cu (94±4)**	Na^+	101±3	107±1	104±6
	K^+	105±1	108±2	105±1
	Mg^{2+}	103±1	108±4	107±1
	Ca^{2+}	100±3	106±2	105±1
	Cl^-	100±1	107±1	109±3
	SO_4^{2-}	103±2	106±1	107±1
Pb (89±1)**	Na^+	89±3	95±1	97±3
	K^+	87±3	95±1	97±3
	Mg^{2+}	93±4	89±6	93±4
	Ca^{2+}	89±7	96±3	98±1
	Cl^-	89±6	89±3	93±3
	SO_4^{2-}	94±3	89±3	101±3
Cd (97±2)**	Na^+	102±1	106±1	102±2
	K^+	102±1	101±1	104±1
	Mg^{2+}	101±1	100±1	97±2
	Ca^{2+}	103±1	101±2	103±1
	Cl^-	99±1	104±1	106±1
	SO_4^{2-}	94±2	103±1	106±1
Co (91±1)**	Na^+	92±1	96±1	92±1
	K^+	90±3	92±1	91±1
	Mg^{2+}	90±1	89±1	91±1
	Ca^{2+}	99±1	99±1	94±4
	Cl^-	96±1	99±1	98±2
	SO_4^{2-}	96±1	99±1	98±2
Ni (94±4)**	Na^+	100±3	100±3	96±1
	K^+	98±6	100±2	95±2
	Mg^{2+}	98±1	97±1	93±2
	Ca^{2+}	98±1	98±1	90±2
	Cl^-	97±2	97±2	92±2
	SO_4^{2-}	100±2	104±2	99±2

*Mean value±SD, n = 3

**%Recovery±SD, no interference system, n = 3

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Table 4.9 Effect of the interfering ions to % recoveries of phase B

Element	Interfering ion concentration (mg L^{-1})	%Recovery*		
		10	100	1000
Cu (94±2)**	Na^+	97±7	100±1	90±2
	K^+	98±1	94±2	102±2
	Mg^{2+}	93±1	102±2	105±3
	Ca^{2+}	93±2	100±1	100±1
	Cl^-	89±2	98±1	97±3
	SO_4^{2-}	94±1	103±1	103±3
Pb (82±3)**	Na^+	84±6	88±7	102±1
	K^+	83±4	85±5	100±1
	Mg^{2+}	80±7	81±1	85±1
	Ca^{2+}	78±6	88±5	105±4
	Cl^-	98±4	98±2	102±2
	SO_4^{2-}	84±3	91±3	97±3

*Mean value±SD, n = 3
**%Recovery±SD, no interference system, n = 3

Table 4.10 Effect of the interfering ions to %recoveries of phase A

Element	Interfering ion concentration (mg L^{-1})	%Recovery*		
		10	100	1000
Cu (88±4)**	Na^+	91±7	97±1	107±1
	K^+	92±9	108±4	105±1
	Mg^{2+}	92±3	107±3	104±1
	Ca^{2+}	102±2	107±2	103±1
	Cl^-	98±1	100±1	103±1
	SO_4^{2-}	101±1	102±2	106±1

*Mean value±SD, n = 3
**%Recovery±SD, no interference system, n = 3

It was found that all interfering ions did not significantly interfere. Recoveries were not less than those in the absence of interfering ions. These results indicated that phase Q, phase B and phase A have high selectivity towards soft metals and have potentials to apply to real sample that contains various ions. The results also supported the mechanism of the metal-modified silica interactions.

According to ion-exchange mechanism, upon increasing ion concentration, the extraction efficiency should be decreased.

On the other hand, for the chelation mechanism, the increase of salt concentration will affect the extraction efficiency because high salt ions can keep a variation of the ionic strength in the solution. The binding mechanism of sorbents may be only chelation between metal ions and the binding sites.

4.3 Method Detection Limit

The method detection limit of the method is limited by the instrument detection limit. The lower detection limit is obtained by the higher preconcentration factor. Basically, the instrument detection limit can be determined by using blank solution of the measurement system. The limit of detection is defined as the concentration of the analyte in yielding signal equivalent to three times of the signal of blank solution. Moreover, the limit of quantitation is the point where concentration at the signal of blank solution is ten times higher than signal of blank solution. However, most of the times the signal from blank solution cannot be addressed by such instrument especially FAAS because the instrument cannot detect anything in the solution. Therefore, the estimation of the detection limit by calibration curve was proposed [72]. The detection limit can be calculated by following equations:

$$LOD_{(signal)} = y_{B(signal)} + 3S_{B(signal)} \tag{4.5}$$

$$LOQ_{(signal)} = y_{B(signal)} + 10S_{B(signal)} \tag{4.6}$$

Where $y_{B(signal)}$ = blank signal; calculated from y-intercept of the calibration curve

$S_{B(signal)}$ = standard deviation of y-intercept; calculated from (4.7)

$$S_{B(signal)} = S_{y/x} = \sqrt{\frac{\sum_i (y_i - \hat{y}_i)^2}{n-2}} \tag{4.7}$$

Where n = number of calibration points

y_i = y value measured at given x value

$\hat{y}_i$ = y value on regression line

The $\mathrm{LOD_{concentration}}$ and $\mathrm{LOQ_{concentration}}$ can be calculated by using the calibration curve. In addition, method detection limit (MDL) and method quantitation limit (MQL) of the method can be calculated by relating with the preconcentration factor (P.F.) as the following equation:

$$\text{Preconcentration Factor (P.F.)} = \frac{\text{Sample Volume (mL)}}{\text{Elution Volume (mL)}} \tag{4.8}$$

$$\text{MDL} = \frac{\text{LOD}}{\text{P.F.}} \tag{4.9}$$

$$\text{MQL} = \frac{\text{LOQ}}{\text{P.F.}} \tag{4.10}$$

The calculated results are summarized in Table 4.11. The results indicated that the method detection limit or method quantitation limit are strongly dependent of the instrument detection limit. The preconcentration factor is the important role in lowering concentration where the regular instrument cannot perform. The proposed method can be used at the method quantitation limit in the ng mL^{-1} range that normally cannot be obtained from the regular FAAS.

Table 4.11 Summary of the detection limit of the proposed method

Elements	Detection limit (ng mL^{-1})				
	Preconcentration Factor (Theoretical)	LOD	LOQ	MDL	MQL
Cu(II)	20	20	60	1.0	3.0
Pb(II)	20	450	1500	22	75.0
Cd(II)	20	23	78	1.2	3.9
Co(II)	20	19	63	0.95	3.2
Ni(II)	10	30	100	3.0	10

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4.4 Applications to Natural Real Samples

The optimized parameters were used for real samples. The objective of this experiment is to demonstrate that the modified silicas can be used in real situations. The pond water is a representative of natural water sample. In real samples the heavy metals are present in trace amount among various substances, for example, alkali and alkali earth metals, organic matters, humic acid, etc. These matters are obstacles in the determination of heavy metal. The accuracy and precision of the method were evaluated using spiked samples.

The spiked real sample was performed using Chulalongkorn University's pond water, tap water and drinking water. The results are shown in Tables 4.12– 4.14.

For Cu(II), Pb(II), Cd(II), Co(II) and Ni(II) determination in Chulalongkorn University's pond water by phase Q column preconcentration, the found values were 4.1±0.4, 40.2±2.2, 2.4±0.1, 4.9±0.1 and 5.0±0.1 μg, respectively (Table 4.12). The recoveries obtained of 80% were in the acceptable range. The %RSD of 6 replicates were less than 10% indicating that this proposed method gave a high precision and could be used with high repeatability.

Table 4.12 Cu(II), Pb(II), Cd(II), Co(II) and Ni(II) in Chulalongkorn University pond water by phase Q column preconcentration

Element	Added, μg	Found [a], μg	Recovery [a], %	RSD, %
Cu	0	n.d.	-	-
	5	4.1±0.4	85±8	9
Pb	0	n.d.	-	-
	50	40.2±2.2	80±4	5
Cd	0	n.d.	-	-
	2.5	2.4±0.1	97±2	2
Co	0	n.d.	-	-
	5	4.9±0.1	98±1	1
Ni	0	n.d.	-	-
	5	5.0±0.1	100±2	2

[a] Mean value $\pm \frac{t_{0.05}s}{\sqrt{n}}$, n = 6, nd = not detectable

[pH 3.5 for Pb(II), Cu(II) and Cd(II); pH 4.5 for Ni(II) and Co(II); sample volume = 100 mL (50 mL for Ni(II)); sorbent = 20 mg for Pb(II) and Cu(II), 50 mg for Cd(II), 60 mg for Ni(II) and Co(II); 5 mL of 1%HNO_3 as stripping solution at flow rate 0.5 mL min^{-1}]

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Table 4.13 Cu(II), Pb(II), Cd(II), Co(II) and Ni(II) in tap water by phase Q column preconcentration

Element	Added, µg	Found [a], µg	Recovery [a], %	RSD, %
Cu	0	n.d.	-	-
	5	4.5±0.1	91±2	2
Pb	0	n.d.	-	-
	50	41.4±2.2	83±4	5
Cd	0	n.d.	-	-
	2.5	2.6±0.1	102±2	2
Co	0	n.d.	-	-
	5	4.8±0.1	97±1	1
Ni	0	n.d.	-	-
	5	5.1±0.1	101±1	1

[a] Mean value $\pm \frac{t_{0.05}s}{\sqrt{n}}$, n = 6, nd = not detectable

[pH 3.5 for Pb(II), Cu(II) and Cd(II); pH 4.5 for Ni(II) and Co(II); sample volume = 100 mL (50 mL for Ni(II)); sorbent = 20 mg for Pb(II) and Cu(II), 50 mg for Cd(II), 60 mg for Ni(II) and Co(II); 5 mL of 1%HNO_3 as stripping solution at flow rate 0.5 mL min^{-1}]

Table 4.14 Cu(II), Pb(II), Cd(II), Co(II) and Ni(II) in drinking water by phase Q column preconcentration

Element	Added, µg	Found [a], µg	Recovery [a], %	RSD, %
Cu	0	13.6±0.3	-	-
	2.5	16.1±0.2	101±9	9
Pb	0	n.d.	-	-
	50	37.5±2.6	75±5	7
Cd	0	n.d.	-	-
	2.5	2.6±0.1	103±4	4
Co	0	n.d.	-	-
	5	4.9±0.2	97±3	4
Ni	0	n.d.	-	-
	5	4.7±0.1	94±2	2

[*] Mean value $\pm \frac{t_{0.05}s}{\sqrt{n}}$, n = 6, nd = not detectable

[pH 3.5 for Pb(II), Cu(II) and Cd(II); pH 4.5 for Ni(II) and Co(II); sample volume = 100 mL (50 mL for Ni(II)); sorbent = 20 mg for Pb(II) and Cu(II), 50 mg for Cd(II), 60 mg for Ni(II) and Co(II); 5 mL of 1%HNO_3 as stripping solution at flow rate 0.5 mL min^{-1}]

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The application of phase Q to tap water and drinking water gave similar results (Table 4.13 and Table 4.14). The recoveries of Cu(II), Pb(II), Cd(II), Co(II) and Ni(II) in tap water were 91, 83, 102, 97 and 101%, respectively and in drinking water were found to be 101, 75, 103, 97 and 94%.

Recoveries of each metal were in the acceptable range, indicating a good accuracy of the proposed method. The exception was the recovery of Pb(II) in drinking water which was only at 75%. This may be due to the presence of 13.6 µg Cu(II) in drinking water. Cu(II) may compete with the extraction of Pb(II). In the precision point of view, the %RSD of 6 replicates were less than 6% in tap water sample and less than 9% in drinking water.

Table 4.15 Cu(II) and Pb(II) in water samples by phase B column preconcentration

Element	Added, µg	Found [a], µg	Recovery [a], %	RSD, %
CU pond water				
Cu	0	n.d.	-	-
	5	4.3±0.3	85±5	6
Pb	0	n.d.	-	-
	50	37.1±1.4	74±3	4
Tap water				
Cu	0	n.d.	-	-
	5	4.6±0.1	91±2	2
Pb	0	n.d.	-	-
	50	44.9±1.9	90±3	4
Drinking water				
Cu	0	16.9±0.3	-	-
	5	19.5±0.1	104±3	3
Pb	0	n.d.	-	-
	50	45±3	90±6	7

[a] Mean value $\pm \frac{t_{0.05}s}{\sqrt{n}}$, n = 6, n.d. = not detectable

[pH 4.5, sample volume = 100 mL, sorbent = 20 mg for Cu(II) and 70 mg for Pb(II); 5 mL of 1%HNO_3 as stripping solution at flow rate 0.5 mL min^{-1}]

The application of phase B to determine Cu(II) and Pb(II) in spiked real samples is shown in Table 4.15. It was found that the recoveries of Cu(II) in Chulalongkorn University’s pond water, tap water and drinking water were 85, 91 and 104%, respectively. In addition, the recoveries of Pb(II) were found to be 74, 90 and 90% for Chulalongkorn University’s pond water, tap water and drinking water, respectively. The precision of this method was quite good.

The %RSD of 6 replicates were less than 7%. The method detection limits were found to be 22 and 1.0 ng mL^{-1} for Pb(II) and Cu(II), respectively.

Table 4.16 Cu(II) in water samples by phase A column preconcentration

Element	Add, μg	Found [a], μg	Recovery [a], %	RSD, %
CU pond water				
Cu	0	n.d.	-	-
	5	5.0±0.2	101±4	4
Tap water				
Cu	0	n.d.	-	-
	5	4.8±0.1	96±3	3
Drinking water				
Cu	0	18.2±0.4	-	-
	5	20.5±0.1	91±6	7

[a] Mean value $\pm \frac{t_{0.05}s}{\sqrt{n}}$, n = 6, nd = not detectable

[pH 4.5, sample volume = 100 mL, sorbent = 40 mg, 5 mL of 1%HNO_3 as stripping solution at flow rate 0.5 mL min^{-1}]

The application of phase A to determined Cu(II) in real samples is illustrated in Table 4.16. The recoveries of Cu(II) in Chulalongkorn University pond water, tap water and drinking water were 101, 96 and 91%, respectively. Recoveries of each sample type were in the acceptable range. The recoveries in all samples are remarkably high. This may be due to high selectivity of Cu(II) onto phase A. Other metal ions or substances in the sample composition could not compete with the extraction of Cu(II) ion onto phase A. The %RSD of 6 replicates were less than 8% indicating that this particular method is highly precise.

4.5 Comparison of the Performance Characteristic with Literatures

Three phases of the modified silica were compared in term of the performance characteristic with the previous literatures as shown in Table 4.17. The data from the literatures indicated that the synthesized silica in this work has good characteristics and comparable with other reports. The dominate point in this work is the use of very small amount of the packing material in the column which gave a relative high preconcentration factor. This benefits the economic advantages and can be commercially available in the future due to the low amount of sorbent used.

Table 4.17 Comparison of the performance characteristic of modified silica gel with the previous literatures

Modified ligands	Sample types	Metal ions	Capacity (μmol.g^{-1})	P.F.	Amount of adsorbent (mg)	Determination Technique	MDL (ng mL^{-1})	Ref.
p-dimethylaminobenzaldehde	River sediment, river water	Cr(III) Cu(II) Ni(II) Pb(II) Zn(II)	Cr(III) = 580 Cu(II) = 400 Ni(II) = 1150 Pb(II) = 450 Zn(II) = 560	125	50	ICP-OES	Cr(III) = 1.1 Cu(II) = 0.69 Ni(II) = 0.99 Pb(II) = 1.1 Zn(II) = 6.5	[73]
4-amino methyl pyridine	Water, soil	Co(II)	-	80	1000	UV-Vis	5.0	[74]
Alizarin violet	Water, soil	Pb(II)	16	500	500	FAAS	10	[75]
Gallic acid	River water	Pb(II) Cu(II) Cd(II) Ni(II)	Pb(II) = 60 Cd(II) = 50 Cu(II) = 240 Ni(II) = 80	200 Cd(II) = 100	1000	FAAS	Pb(II) = 0.58 Cu(II) = 0.86 Cd(II) = 0.65 Ni(II) = 0.92	[76]
8-hydroxyquinoline	River water, Cobalt in pharmaceutical	Cu(II) Pb(II) Ni(II) Fe(III) Cd(II) Zn(II) Co(II)	Cu(II) = 448 Pb(II) = 158 Ni(II) = 255 Fe(III) = 386 Cd(II) = 92 Zn(II) = 177 Co(II) = 205	Cu(II) = 300 Pb(II) = 250 Ni(II) = 200 Fe(III)= 300 Cd(II) = 150 Zn(II) = 200 Co(II)= 250	1000	FAAS	Cu(II) = 0.86 Pb(II) = 0.92 Ni(II) = 0.90 Fe(III) = 1.1 Cd(II) = 0.7 Zn(II) = 1.8 Co(II) = 0.45	[77]
Phase Q	Pond water, tap water, drinking water	Cu(II) Pb(II) Cd(II) Co(II) Ni(II)	Cu(II) = 300 Pb(II) = 560 Cd(II) = 70 Co(II) = 120 Ni(II) = 150	20 Ni(II) = 10	Cu(II)= 20 Pb(II) = 20 Cd(II) = 50 Co(II)= 60 Ni(II) = 60	FAAS	Cu(II)= 1.0 Pb(II) = 22.5 Cd(II) = 1.2 Co(II)= 0.95 Ni(II) = 3.0	This Work
Phase B	Pond water, tap water, drinking water	Cu(II) Pb(II)	Cu(II) = 141 Pb(II) = 212	20	Cu(II) = 20 Pb(II) = 70	FAAS	Cu(II) = 1.0 Pb(II) = 22.5	This Work
Phase A	Pond water, tap water, drinking water	Cu(II)	Cu(II) = 160	20	Cu(II) = 40	FAAS	Cu(II) = 1.0	This Work

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4.6 Naked Eye Sensor

If a methodology provides both quantitative and qualitative simultaneously, the method will be more useful than only one optional methodology. The anthraquinone moiety in phase Q structure can act as a chromogenic sensory unit. While concentrations of Co(II) and Cu(II) were increased and mixed with phase Q in the test tube, the color of phase Q changed from orange to dark blue and blue, respectively. Basically, the color of Co(II) in aqueous solution is pink. Changing color to blue may stem from the chelation between phase Q donor sites and Co(II). Phase Q, therefore, can possibly be used as an indicator for Co(II) in the analyte.

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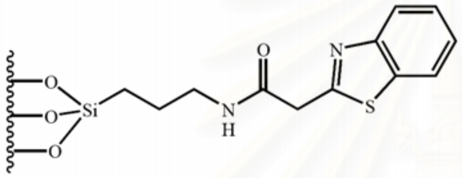
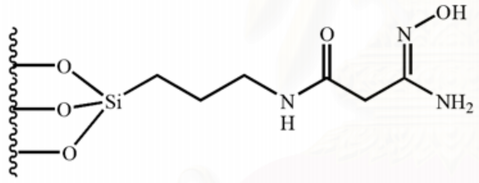
CHAPTER V

CONCLUSION

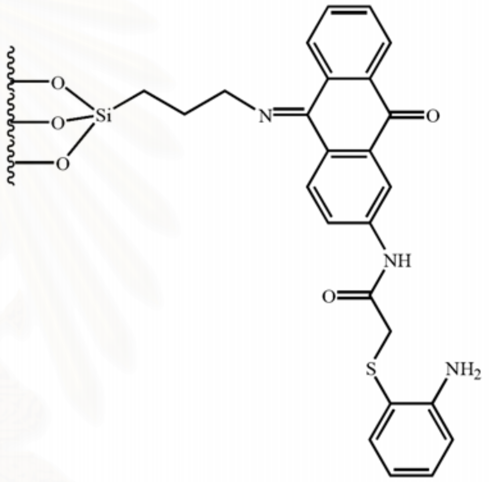
Three types of novel chemically modified silica, benzothiazoleamido silica (phase B), aminothioamido anthraquinone silica (phase Q) and amidoamidoxime silica (phase A) were synthesized. All of modified silicas were characterized by solid state ^{13}C-NMR, FT-IR, elemental analysis, nitrogen adsorption and TGA. The results from all techniques supported that the organic moieties were attached to the silica surface.



Phase B



Phase A



Phase Q

The parameters that may affect the extraction efficiency were evaluated in both batch and column method. The solution pH, contact time and adsorption isotherm were studied in both methods. For phase Q, all metal ions were quantitatively extracted at pH ≥ 4. Phase B could extract Cu(II) and Pb(II) at pH ≥ 4. However no significant extraction of Cd(II), Co(II) and Ni(II) was observed. Phase A showed highest selectivity toward Cu(II) and can quantitatively extract at pH ≥ 4. The effect of extraction time was evaluated. All of the modified silica showed moderately rapid extraction with the extraction time less than 20 minutes. Moreover, the metal ion adsorption behaviors on the solid-liquid interface were studied. The adsorption patterns were fit with Langmuir adsorption isotherm with high correlation coefficient (r ≥ 0.99). The selectivity orders were obtained from the maximum sorption capacity.

The selectivity orders for phase Q and phase B were Pb(II) > Cu(II) > Ni(II) > Co(II) > Cd(II) and Pb(II) > Cu(II), respectively. Moreover, adsorption of Cu(II) onto phase Q showed higher sorption capacity and binding constant than phase A.

The results from batch method were applied to column method optimization. Solution flow rates were evaluated. For phase Q, the results showed that Cu(II), Pb(II) and Cd(II) adsorption were not effected by the solution flow rate in the range of 0.25-6 mL min^{-1}. However, Ni(II) and Co(II) adsorption decreased while the flow rate was increased. Therefore, 60 and 40 mg of phase Q were used for Ni(II) and Co(II) extraction, respectively. This can improve the extraction efficiency and can be used at the solution flow rate of 0.25 - 5 mL min^{-1}. Consequently, nitric acid was chosen as the eluting solution. The concentration of acid was varied. The results showed that at 1-7%v/v of nitric acid resulted in insignificant differences in desorption efficiency. Moreover, the maximum sample volume of metal solution were used at 100 mL except 50 mL for Ni(II).

The practical method quantitation limit (PMQL) and linear range were evaluated. Phase Q showed PMQL at 5, 93, 2, 5, 18 ng mL^{-1} and linear range 5-100, 93-233, 2-40, 5-100 and 18-184 ng mL^{-1} for Cu(II), Pb(II), Cd(II), Co(II) and Ni(II), respectively. Phase B showed PMQL at 4 and 200 ng mL^{-1} with linear working range at 4-91 and 200-250 ng mL^{-1} for Cu(II) and Pb(II), respectively. Phase A showed the PMDL at 9 ng mL^{-1} with linear working range at 9-91 ng mL^{-1} for Cu(II). The effects of interfering ions were studied in the binary system. The results showed that no interferences from Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^- and SO_4^{2-} were observed at all 3 orders of the interfering concentration levels.

Furthermore, the modified silicas were used for determination of trace metal ions in spiked pond water, tap water and drinking water. All the optimized parameters were applied in this task. The results showed that all phases can be used for such sample demonstrated here with acceptable %recovery and %RSD. According to these results it can be concluded that the particular proposed methods have high accuracy and high precision.

Potential Applications and suggestions for the future works:

The present method will be applied to other samples such as contaminated soil, food, or in non aqueous samples. These 3 types of modified silicas can be applied as an adsorbent in the chromatography, for example in the separation and determination of metals ion in pack column of the capillary electrophoresis technique.

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APPENDICES

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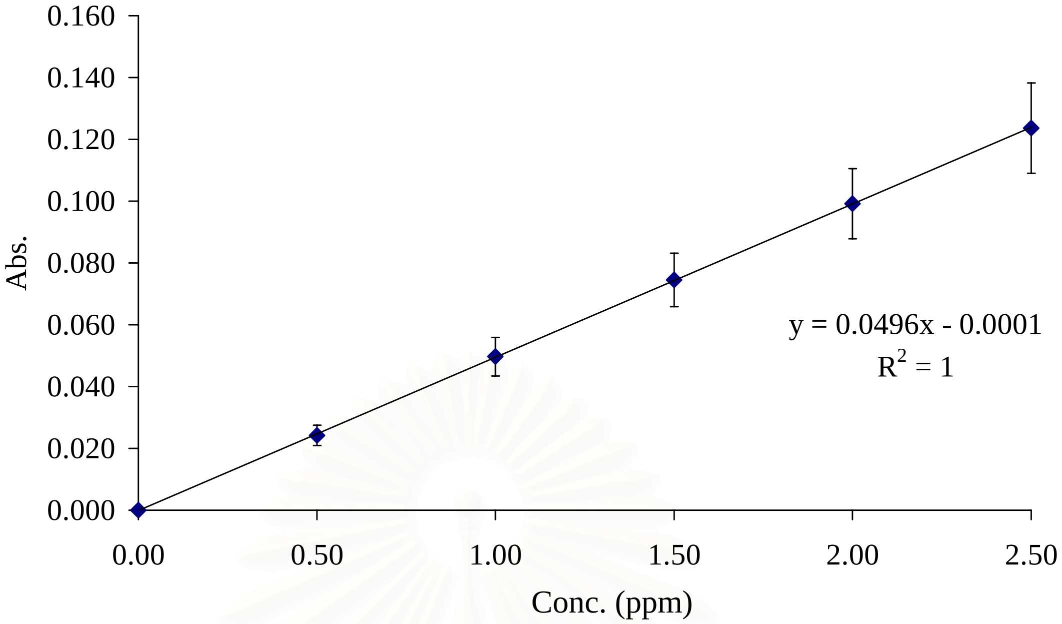

Figure A.1 Calibration curve of Cu used in this work (average from all experiment)

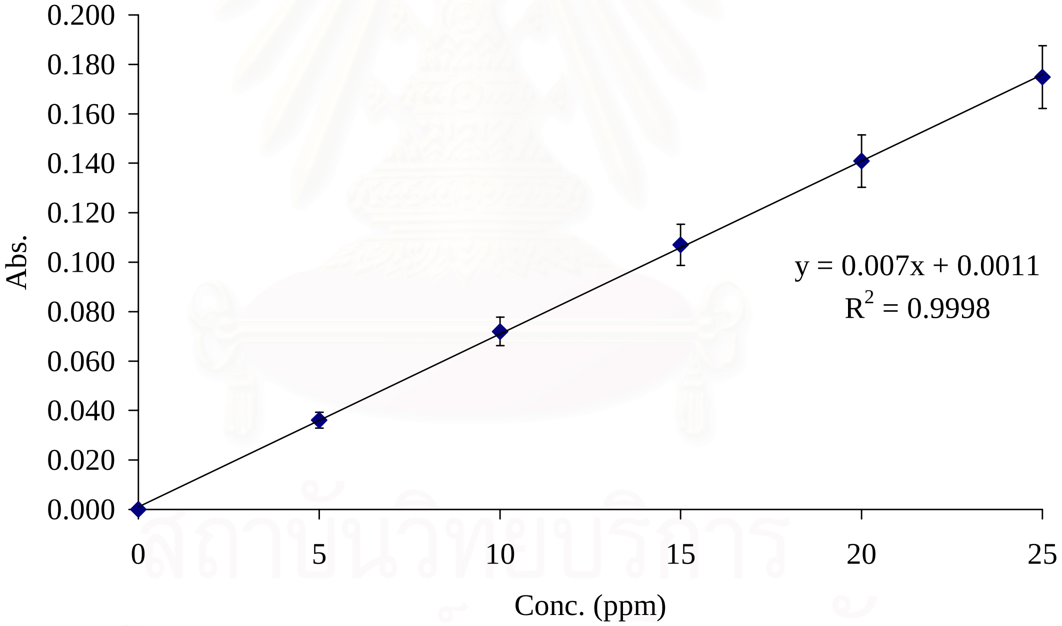

Figure A.2 Calibration curve of Pb used in this work (average from all experiment)

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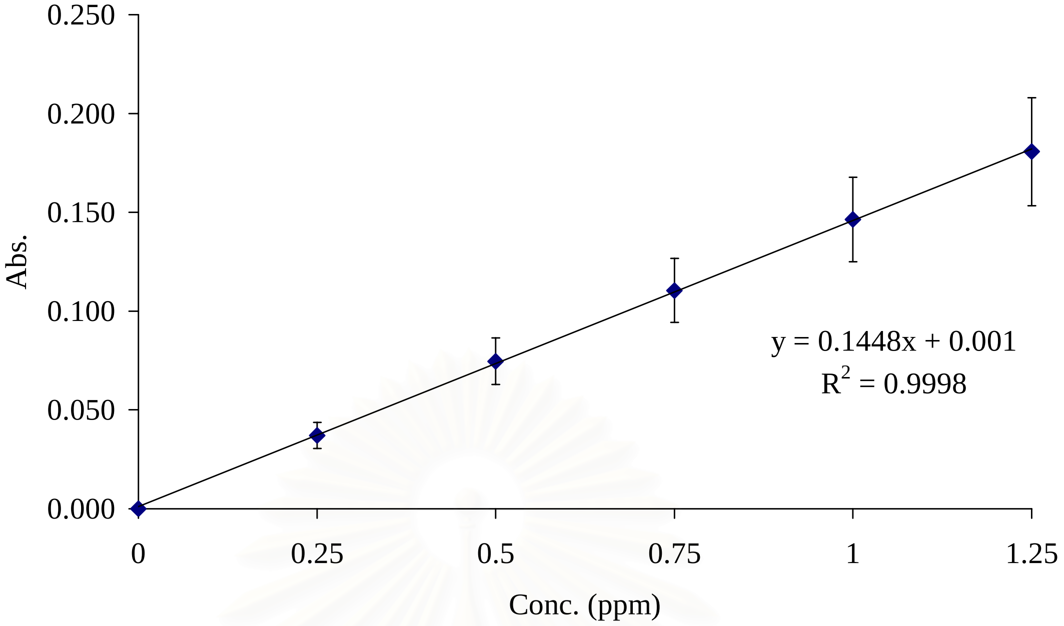

Figure A.3 Calibration curve of Cd used in this work (average from all experiment)

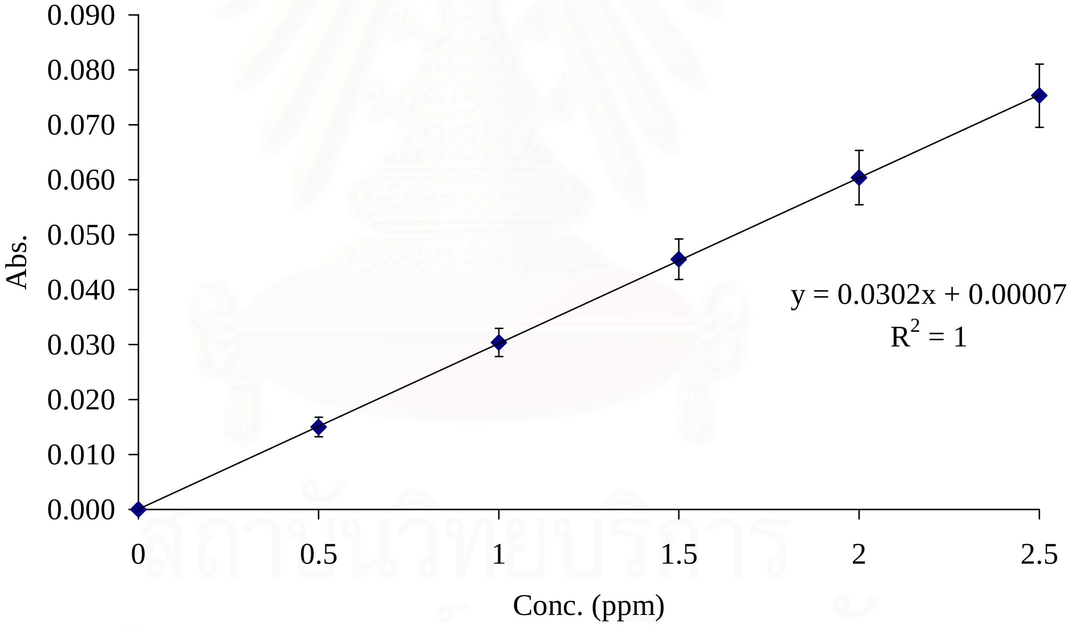

Figure A.4 Calibration curve of Co used in this work (average from all experiment)

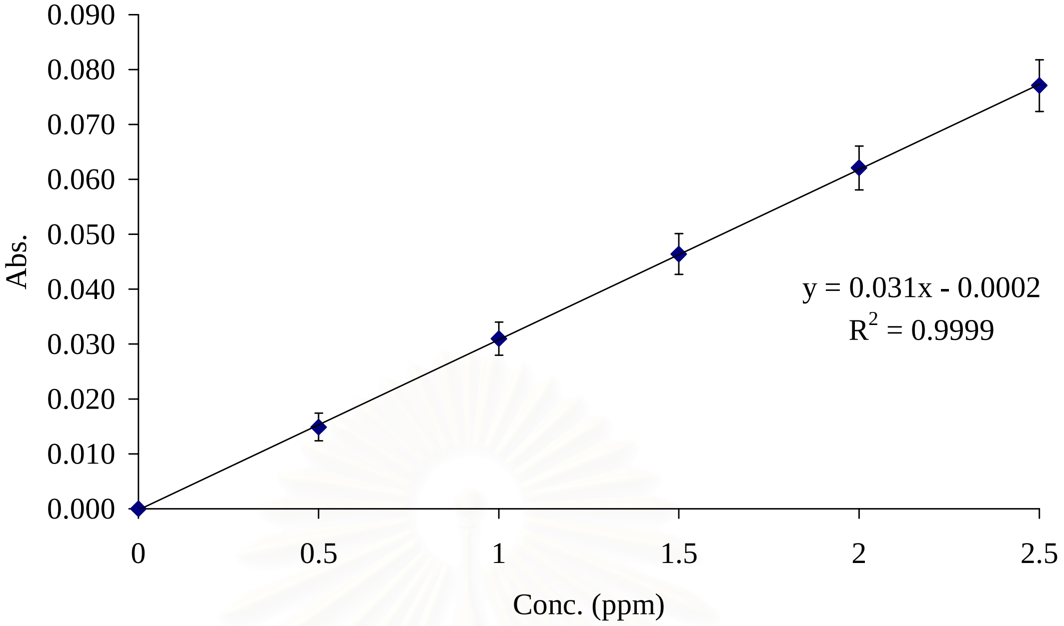

Figure A.5 Calibration curve of Ni used in this work (average from all experiment)

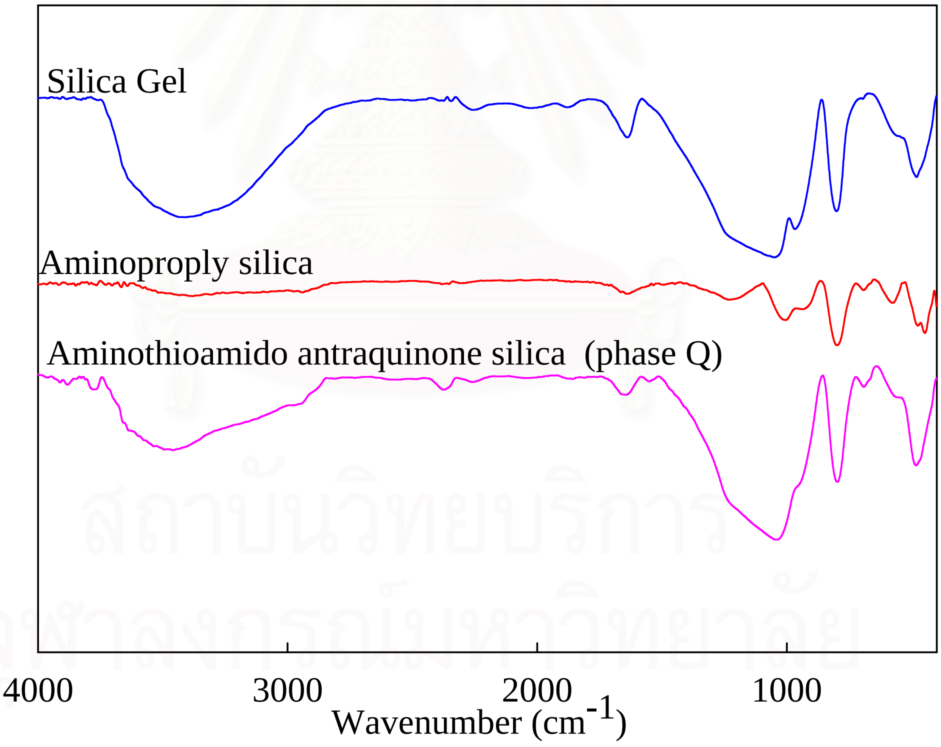

Figure A.6 FT-IR spectra of silica gel, aminopropyl silica and aminothioamido anthraquinone silica (phase Q)

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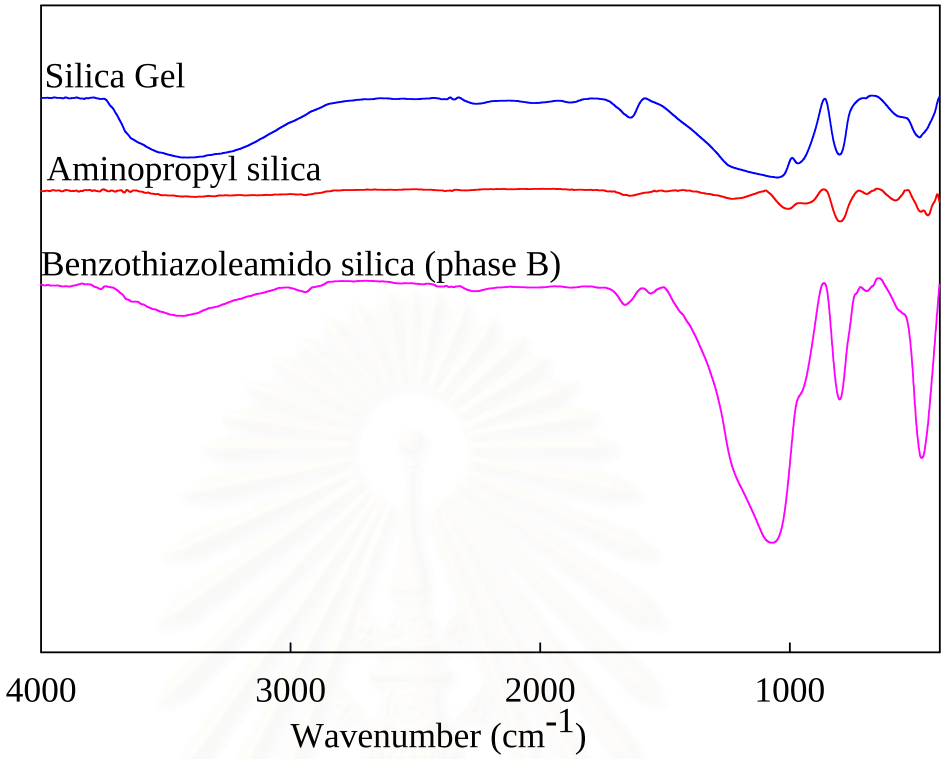

Figure A.7 FT-IR spectra of silica gel, aminopropyl silica and benzothiazoleamido silica (phase B)

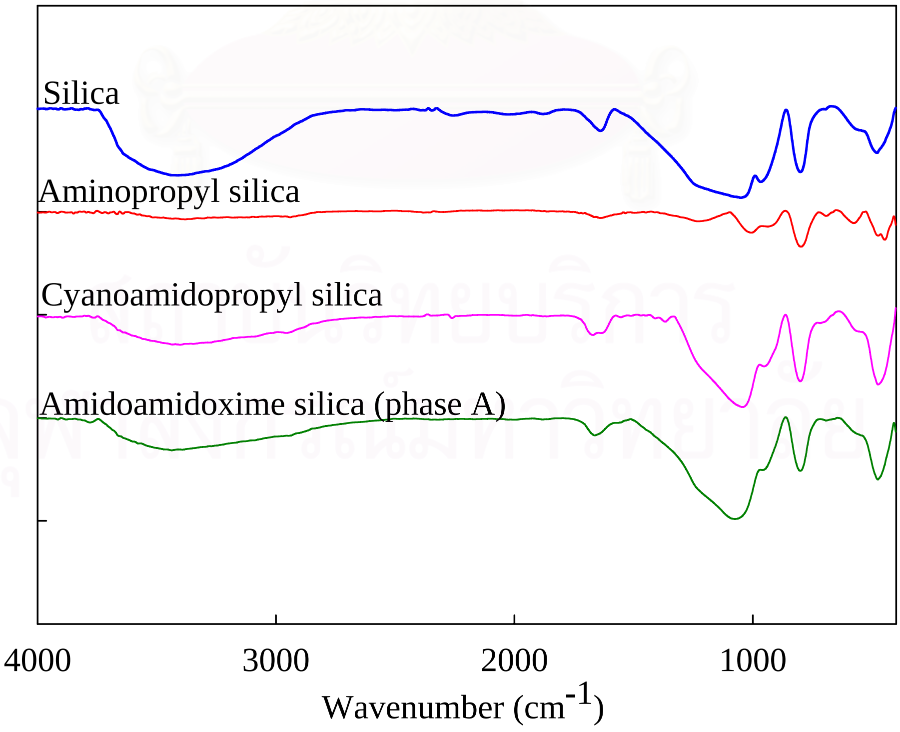

Figure A.8 FT-IR spectra of silica gel, aminopropyl silica and amidoamidoxime silica (phase A)

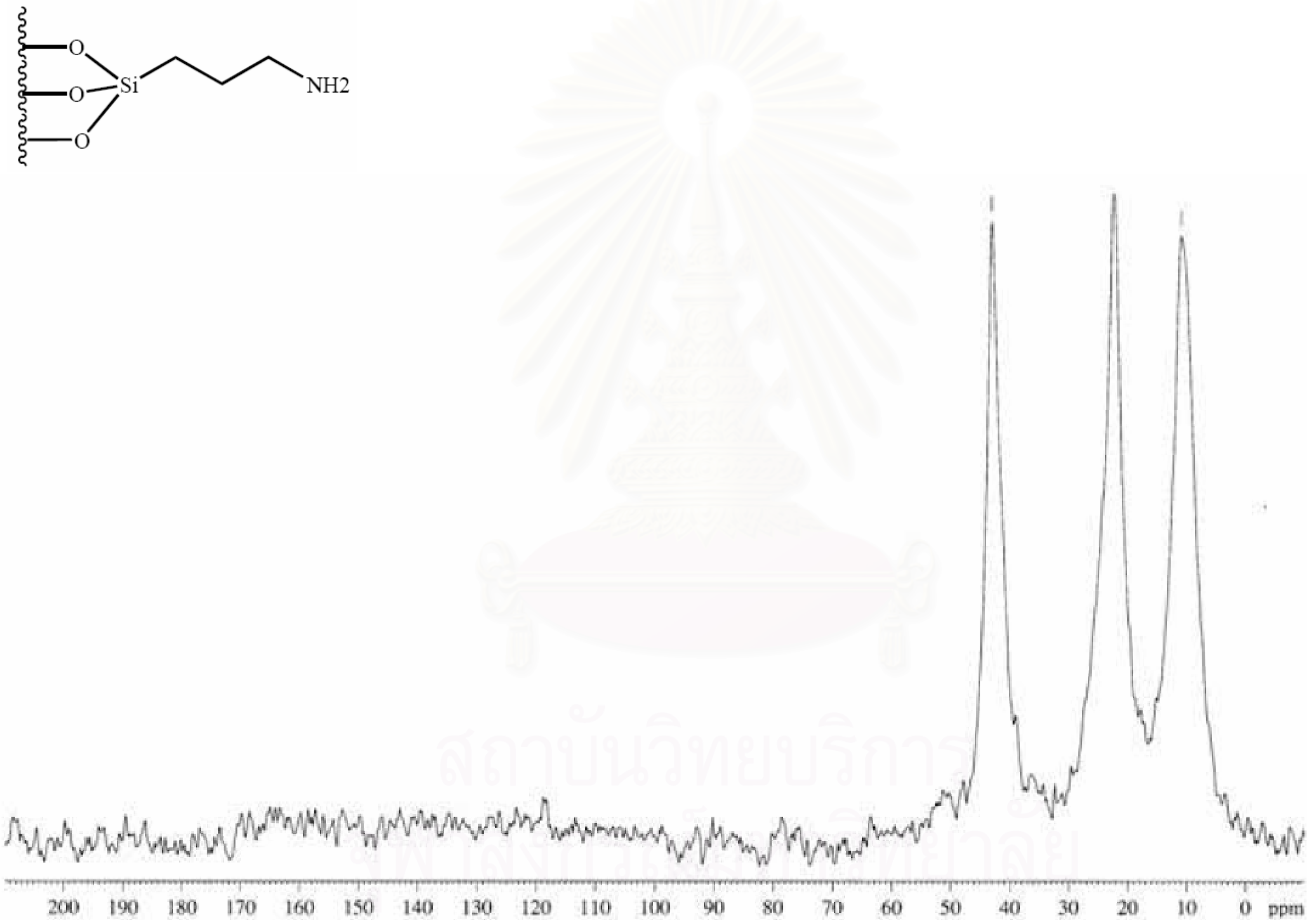

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Figure A.9 13C-NMR spectrum (solid state) of aminopropyl silica

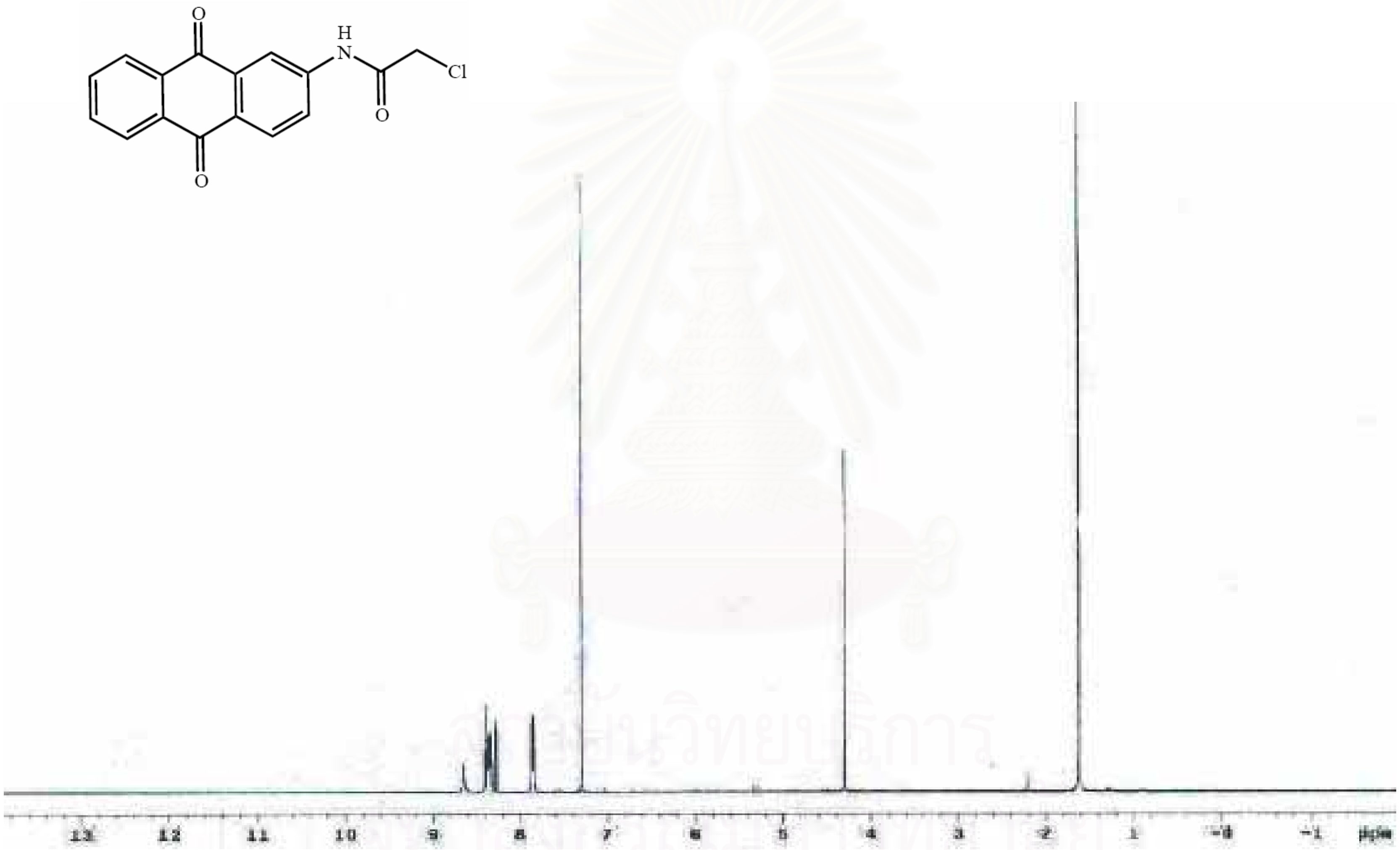

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Figure A.10 1H-NMR spectrum (400 MHz, $CDCl_3$) of chloroamido anthraquinone

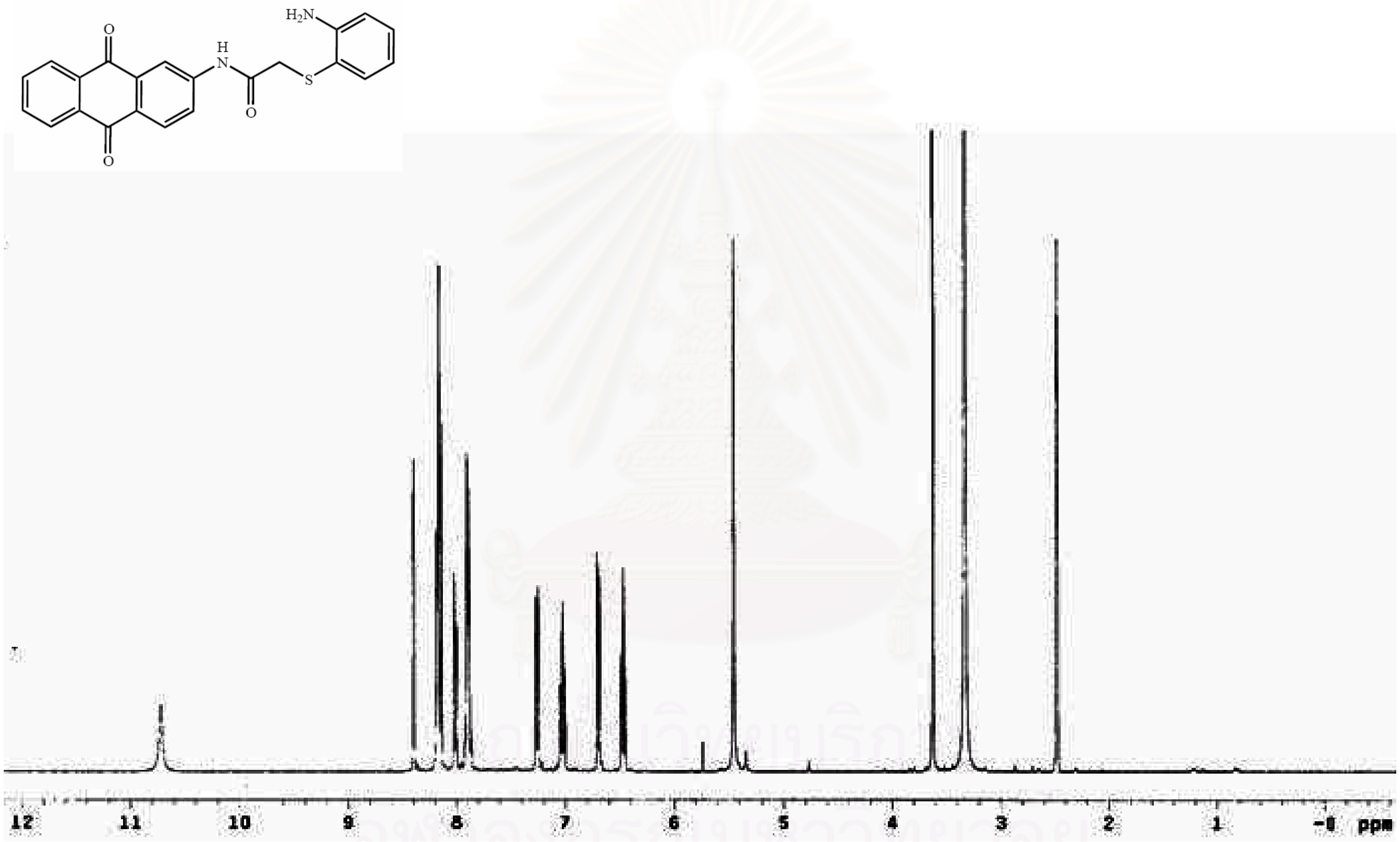

Figure A.11 1H-NMR spectrum (400 MHz, DMSO-d_6) of aminothioamido anthraquinone

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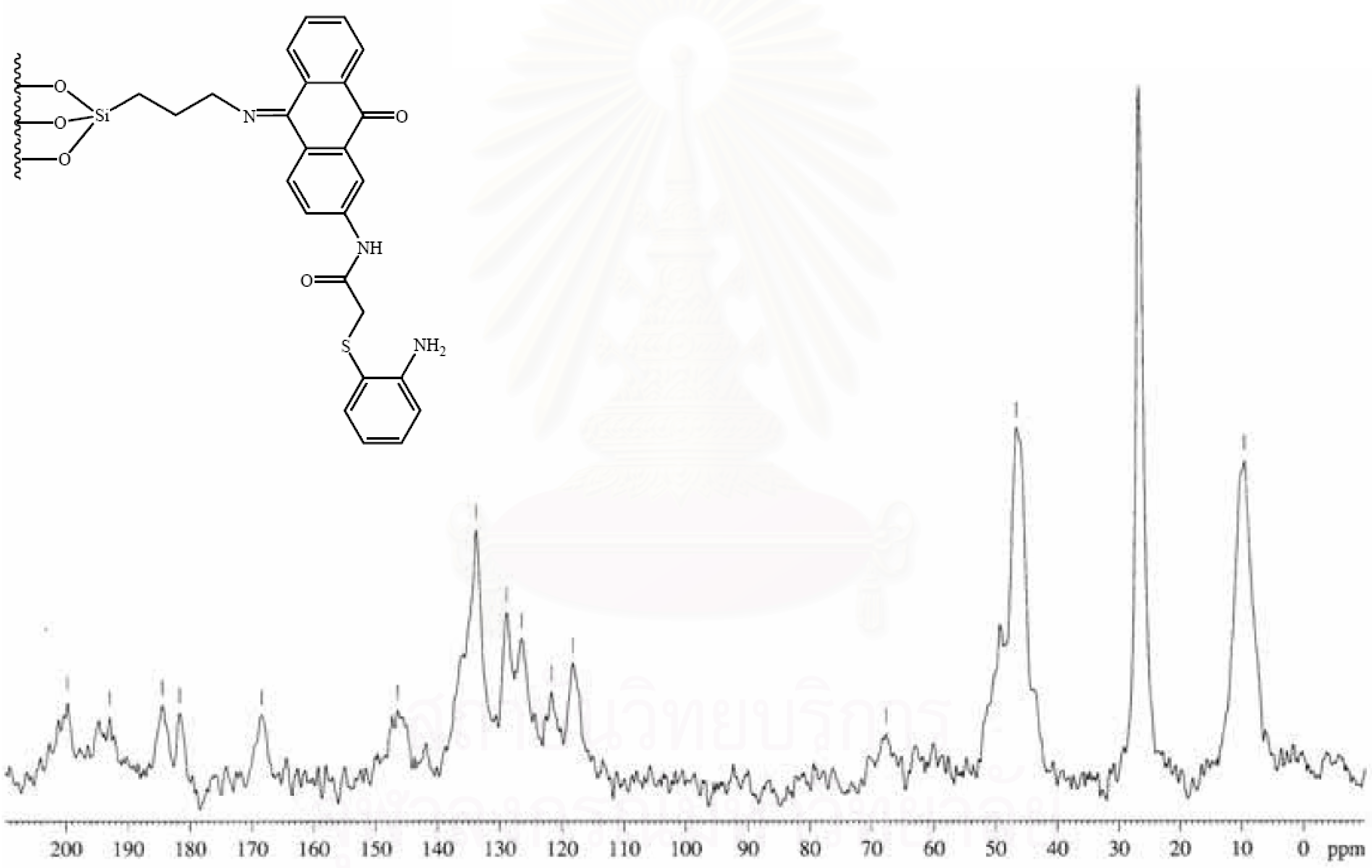

Figure A.12 13C-NMR spectrum (solid state) of phase Q

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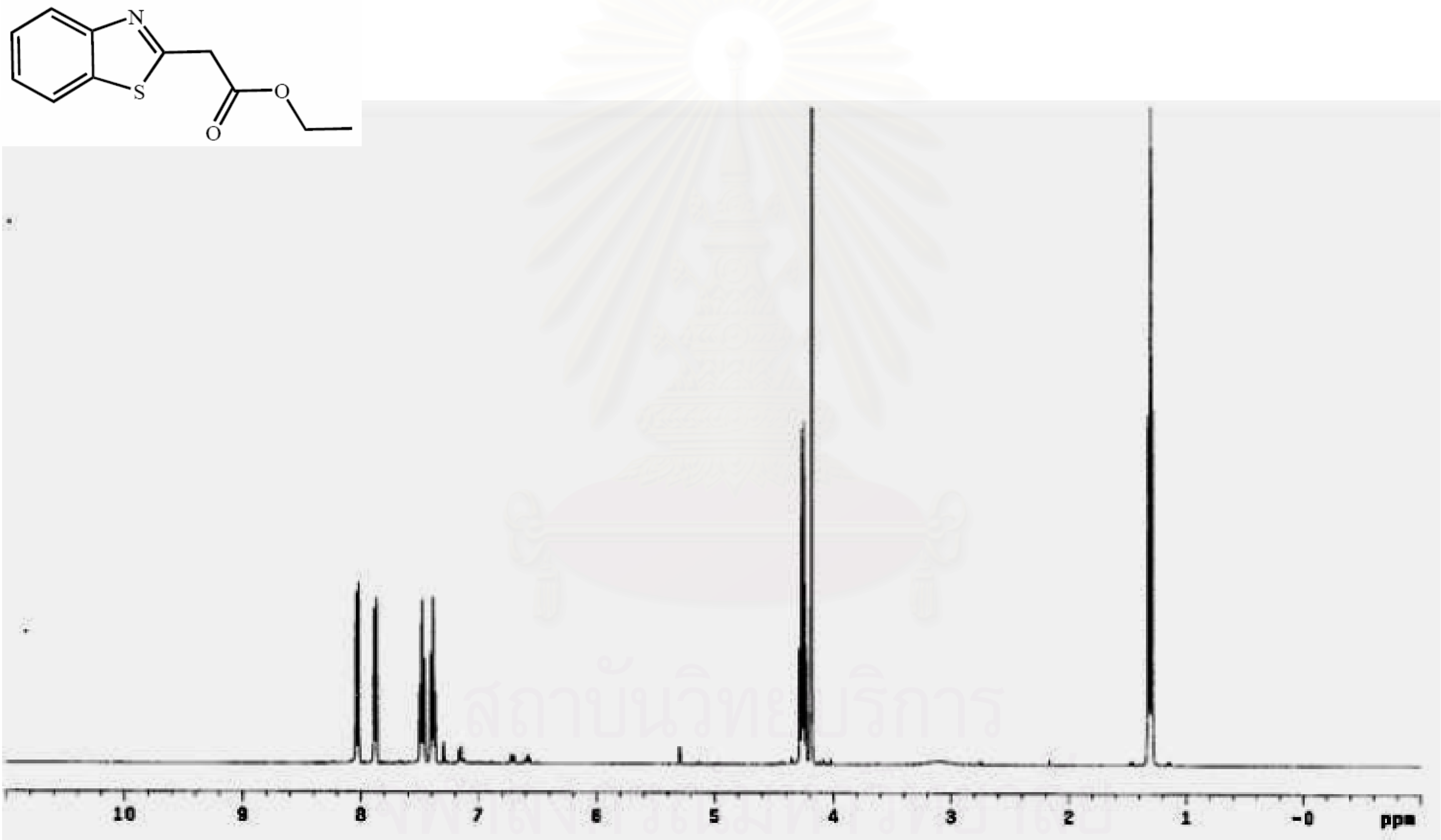

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Figure A.13 1H-NMR spectrum (400 MHz, $CDCl_3$) of benzothiazole ethyl ester

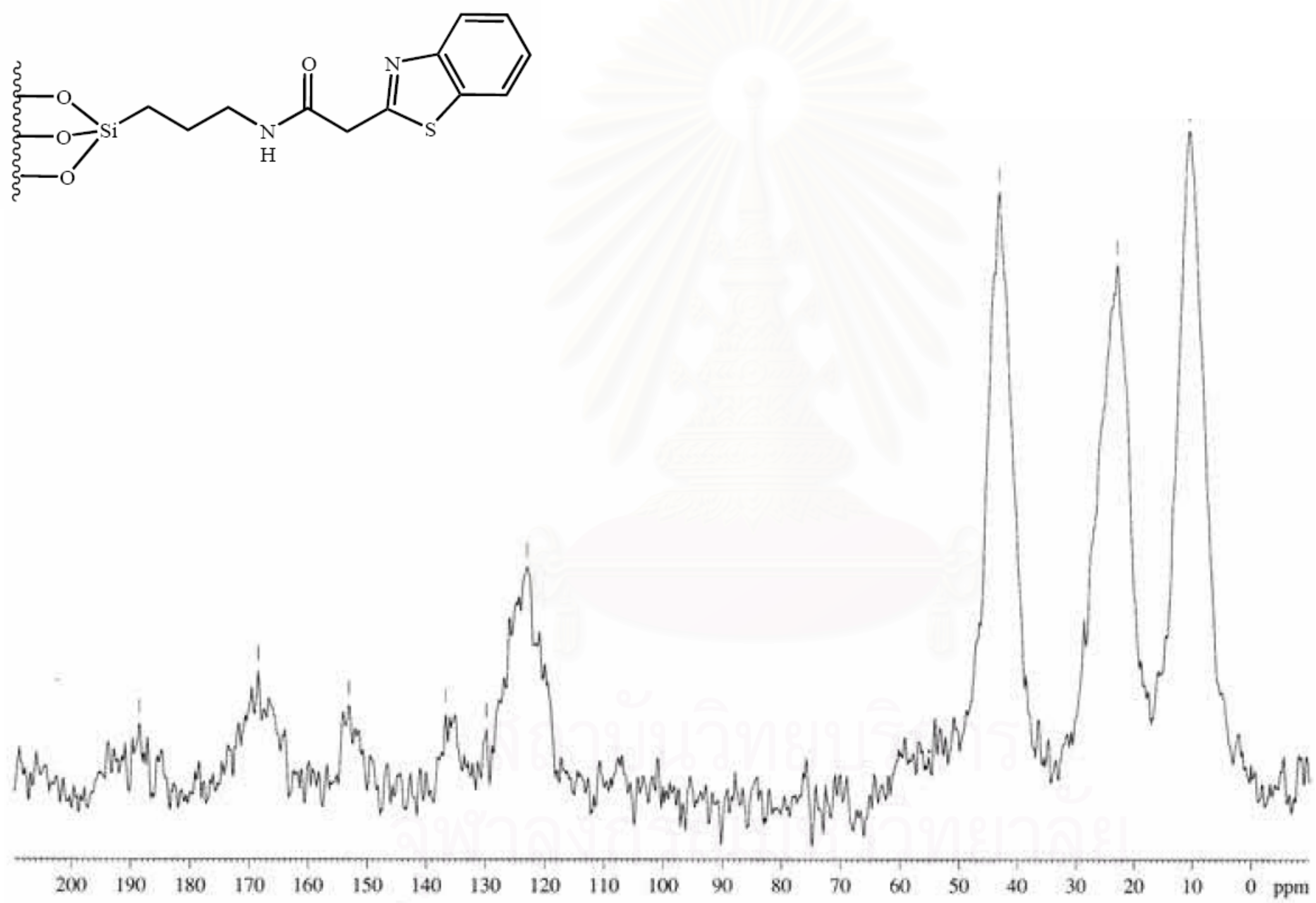

Figure A.14 13C-NMR spectrum (solid state) of phase B

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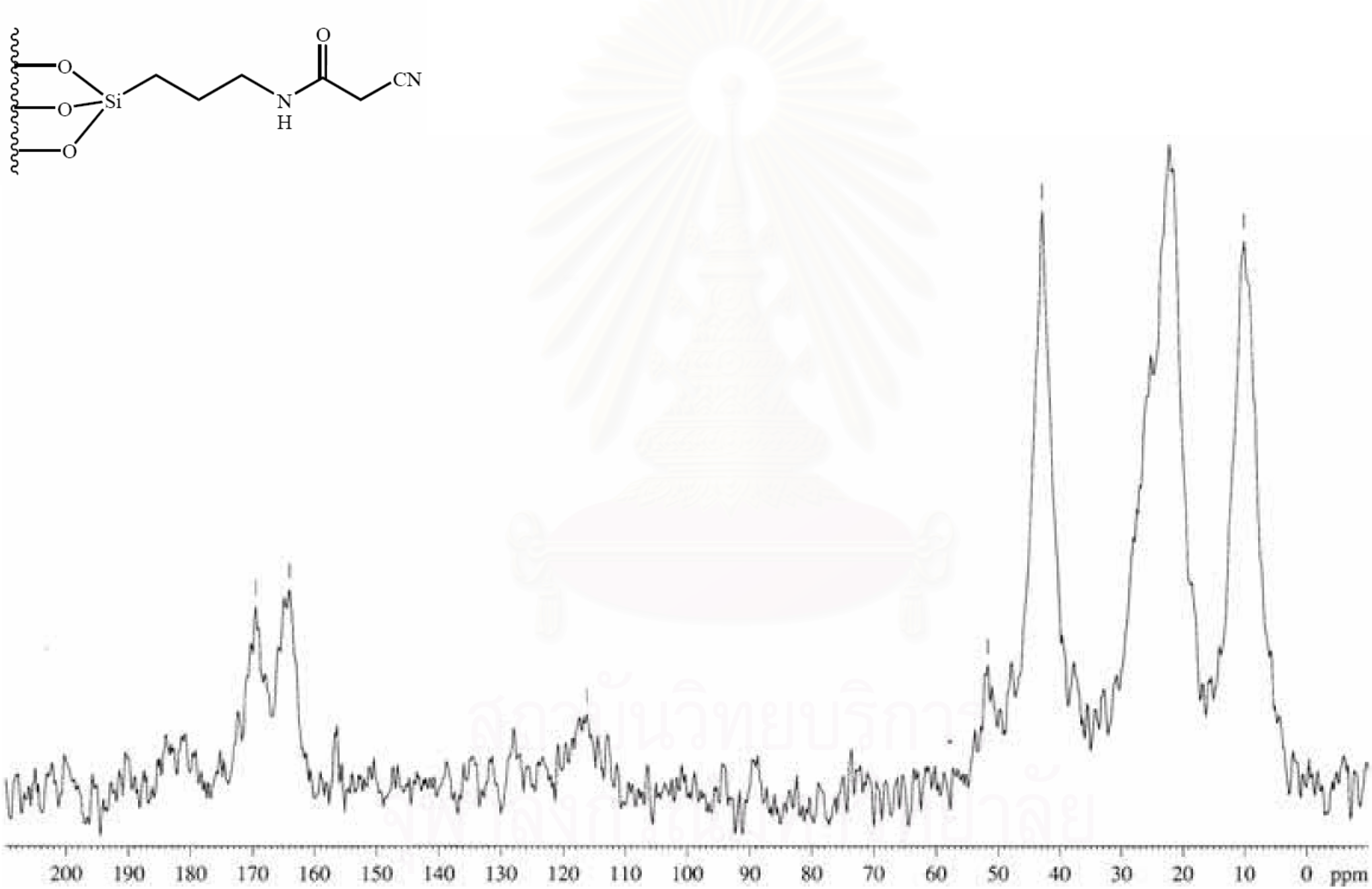

Figure A.15 13C-NMR spectrum (solid state) of cyanoamidopropyl silica

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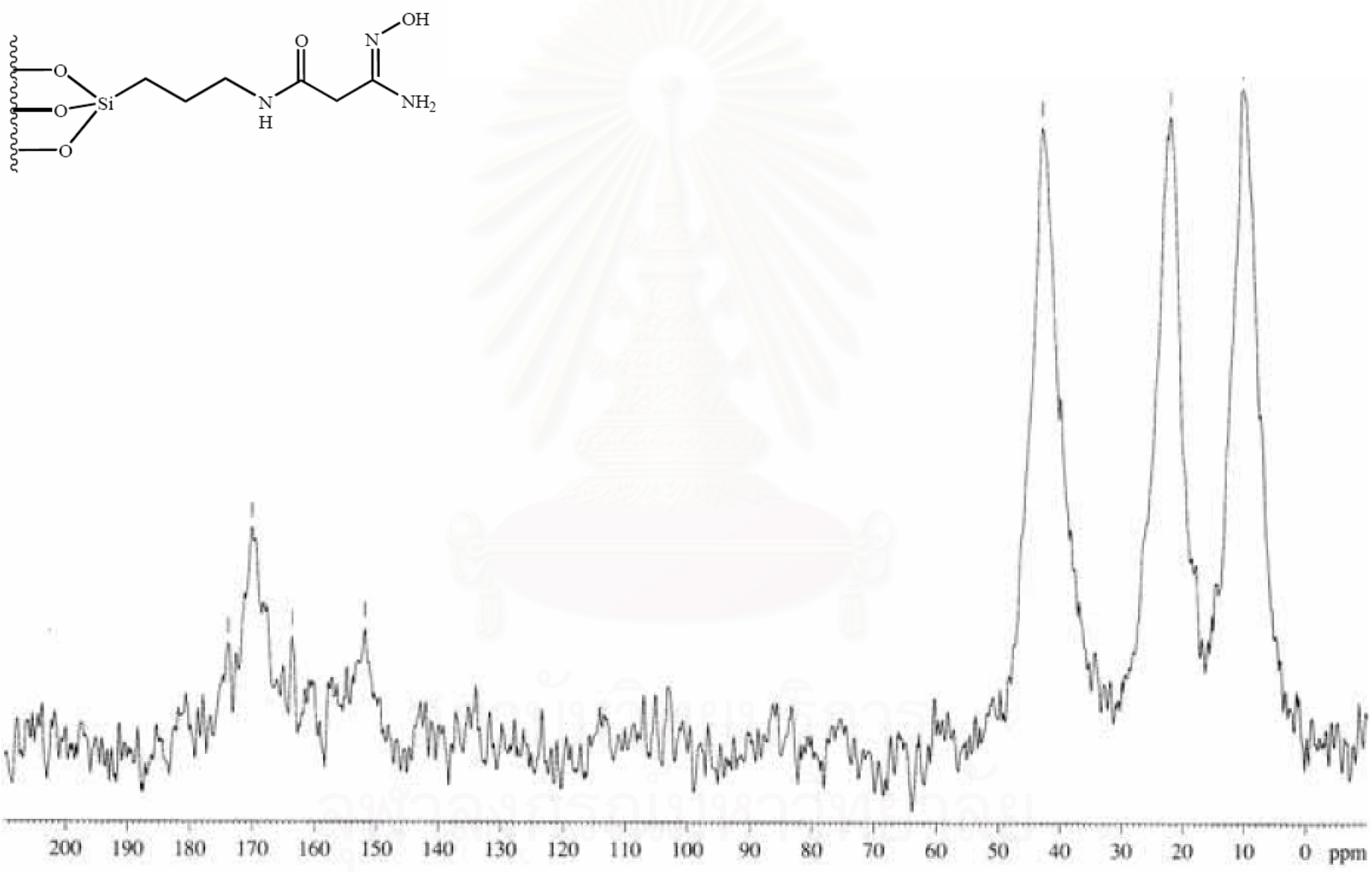

Figure A.16 13C-NMR spectrum (solid state) of phase A

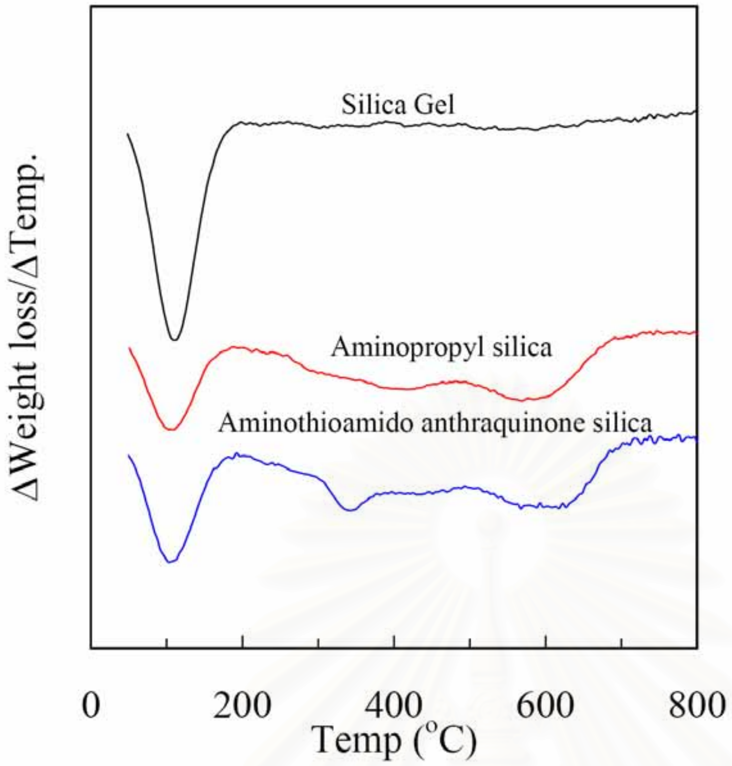

Figure A.17 Comparison of the thermogram of silica gel, aminopropyl silica and aminothioamido anthraquinone silica

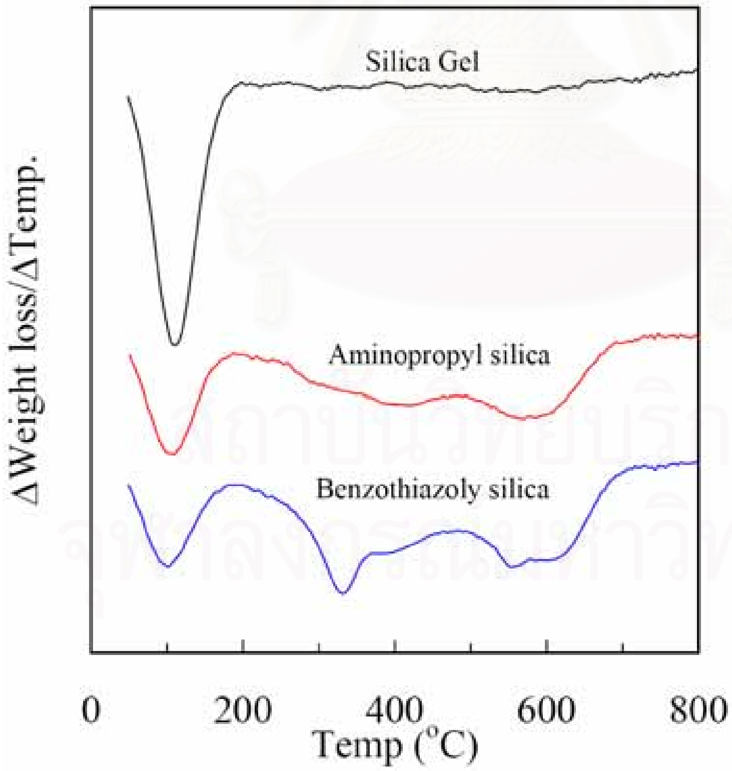

Figure A.18 Comparison of the thermogram of silica gel, aminopropyl silica and benzothiazole silica

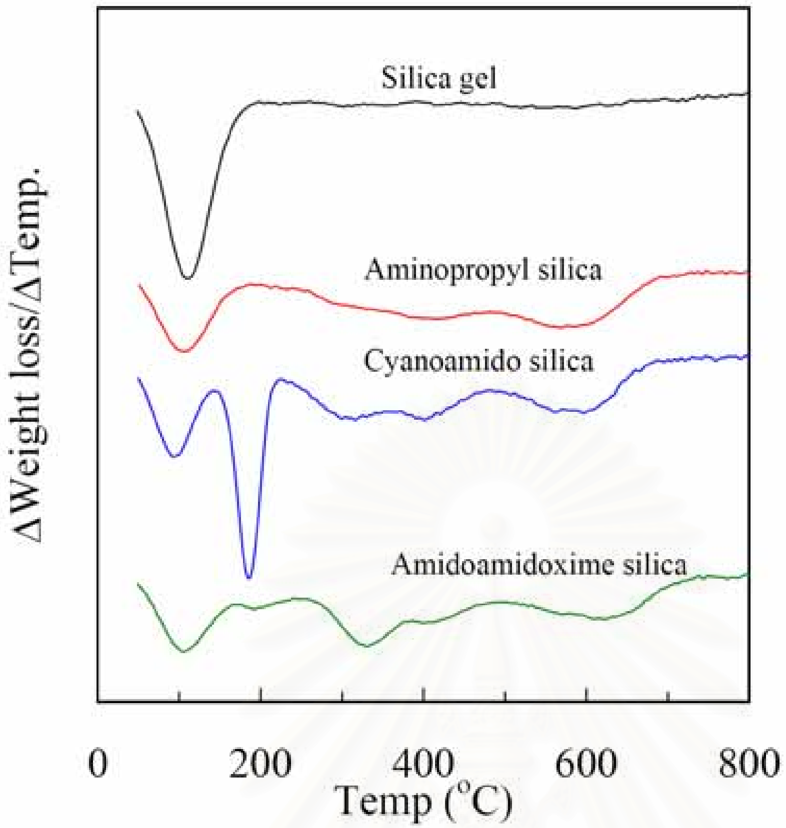

Figure A.18 Comparison of the thermogram of silica gel, aminopropyl silica, cyanoamido silica and amidoamidoxime silica

VITA

Mr. Wittaya Ngeontae was born on December 21, 1980 in Chiangrai, Thailand. Since 2000, he has been founded from the Development and Promotion of Science and Technology Talent Project Scholarship (DPST). He received his bachelor degree of science in chemistry from Chulalongkorn University in 2003. Since 2004, he has been a graduate student at the Department of Chemistry, Chulalongkorn University and become a member of Environmental Analysis Research Unit and Supramolecular Chemistry Research Unit under the supervision of Assistant Professor Dr. Wanlapa Aeungmaitrepirom and Associate Professor Dr. Thawatchai Tuntulani. He finished his Doctor of Philosophy in the academic year 2007.

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