ซิลิกาเจลดัดแปรทางเคมีด้วย 1,8-ไดอะมิโนแอนทราควิโนน สำหรับการเพิ่มความเข้มข้นของไอออนโลหะหนัก

นายอนุรักษ์ แย้มพลาย

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CHEMICALLY MODIFIED SILICA GEL WITH 1,8-DIAMINOANTHRAQUINONE FOR PRECONCENTRATION OF HEAVY METAL IONS

Mr. Anurak Yaemplai

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By	Mr. Anurak Yaemplai
Field of study	Chemistry
Thesis Advisor	Assistant Professor Wanlapa Aeungmaitrepirom, Ph.D.
Thesis Co-advisor	Passapol Ngamukot, Ph.D.

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

.... Dean of the Faculty of Science

(Professor Piamsak Menasveta, Ph.D.)

THESIS COMMITTEE

.......Sirirat Kokpol....... Chairman

(Associate Professor Sirirat Kokpol, Ph.D.)

.......Wanlapa Aeungmaitrepirom....... Thesis Advisor

(Assistant Professor Wanlapa Aeungmaitrepirom, Ph.D.)

.......Passapol Ngamukot....... Thesis Co-advisor

(Passapol Ngamukot, Ph.D.)

.... Member

(Assistant Professor Narong Praphairaksit, Ph.D.)

.......Withawat Mingvanish....... Member

(Withawat Mingvanish, Ph.D.)

อนุรักษ์ แย้มพลาย: ซิลิกาเจลดัดแปรทางเคมีด้วย 1,8-ไดอะมิโนแอนทราควิโนนสำหรับการเพิ่มความเข้มข้นของไอออนโลหะหนัก. (CHEMICALLY MODIFIED SILICA GEL WITH 1,8-DIAMINOANTHRAQUINONE FOR PRECONCENTRATION OF HEAVY METAL IONS) อ.ที่ปรึกษา: ผศ.ดร. วัลภา เอื้องไมตรีภิรมย์, อ.ที่ปรึกษาร่วม: ดร.ภัสสร์พล งามอุโฆษ, 125 หน้า.

การดัดแปรทางเคมีของซิลิกาเจลในขั้นแรกด้วย 3-อะมิโนโพรพิลไตรเอธอกซีไซเลนได้เป็น SiAP แล้วนำ SiAP ไปทำปฏิกิริยาต่อกับ 1,8-ไดอะมิโนแอนทราควิโนนได้เป็น SiDAAQ พิสูจน์ลักษณะของ SiDAAQ โดยการวิเคราะห์พื้นที่ผิวจำเพาะ อินฟราเรดสเปกโทรสโกปี อัลตราไวโอเลต-วิสิเบิลสเปกโทรโฟโตเมตรี การวิเคราะห์ธาตุ และคาร์บอนเอ็นเอ็มอาร์ ศึกษาสมบัติการดูดซับของ SiDAAQ โดยใช้ไอออนแคดเมียม โคบอลต์ ทองแดง นิกเกิล ตะกั่ว และสังกะสี ในสารละลายด้วยวิธีแบทช์และคอลัมน์ ตรวจสอบระบบการเพิ่มความเข้มข้นไอออนตะกั่วแบบออนไลน์ร่วมกับเทคนิคเฟลมอะตอมมิกแอบซอร์ชันสเปกโทรเมตรี พบว่าไอออนตะกั่วที่ถูกดูดซับจะถูกชะด้วยสารละลาย EDTA เข้มข้น 0.050 โมลต่อลิตร (พีเอช 5) ปริมาตร 174 ไมโครลิตร ที่อัตราการไหล 5.0 มิลลิลิตรต่อนาที โดยตรงไปยัง nebulizer ตรวจสอบประสิทธิภาพของระบบภายใต้พารามิเตอร์ทางเคมีและการไหลที่เหมาะสม โดยใช้สารละลายที่มีการเติมไอออนตะกั่วเข้มข้น 100 และ 300 ไมโครกรัมต่อลิตร พบว่าความเข้มข้นต่ำสุดที่ตรวจวัดได้ด้วยวิธีนี้เท่ากับ 20 ไมโครกรัมต่อลิตร enrichment factor เท่ากับ 27 และ sample throughput เท่ากับ 40 ตัวอย่างต่อชั่วโมง เวลาของการเพิ่มความเข้มข้นเท่ากับ 60 วินาที เปอร์เซ็นต์การกลับคืนเท่ากับ 100.8 และ %RSD เท่ากับ 10.8 เมื่อทำการทดลองซ้ำ 10 ครั้ง ระบบที่เสนอนี้ได้นำไปประยุกต์สำหรับการหาปริมาณของไอออนตะกั่วที่มีปริมาณน้อยในน้ำตัวอย่างได้

ภาควิชา............เคมี............. ลายมือชื่อนิสิต..
สาขาวิชา...........เคมี.............. ลายมือชื่ออาจารย์ที่ปรึกษา..
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ANURAK YAEMPLAI : CHEMICALLY MODIFIED SILICA GEL WITH 1,8-DIAMINOANTHRAQUINONE FOR PRECONCENTRATION OF HEAVY METAL IONS. THESIS ADVISOR: ASST.PROF.WANLAPA AEUNGMAITREPIROM, Ph.D., THESIS COADVISER: PASSAPOL NGAMUKOT, Ph.D., 125 pp.

Silica gel was chemically modified firstly with 3-aminopropyltriethoxysilane giving SiAP. The SiAP reacted subsequently with 1,8-diaminoanthraquinone obtaining SiDAAQ. The SiDAAQ was characterized by specific surface area analysis, IR-spectroscopy, UV-visible spectrophotometry, elemental analysis and ^{13}C-NMR. The adsorption properties of the SiDAAQ were studied using Cd(II), Co(II), Cu(II), Ni(II), Pb(II) and Zn(II) in aqueous solutions with batch and column modes. The on-line preconcentration system coupled with FAAS for Pb(II) was investigated. The sorbed lead ions were directly eluted by 174 µL of 0.050 mol L^{-1} EDTA solution (pH 5) at a flow rate of 5.0 mL min^{-1} to the nebulizer. The performance characteristics of the system were investigated under the optimum chemical and flow parameters using the solutions containing the spiked standard 100 and 300 µg L^{-1} Pb(II). The method detection limit of 20 µg L^{-1} with enrichment factor of 27 and the sample throughput of 40 h^{-1} at preconcentration time of 60 sec were obtained. The accuracy and precision evaluated as %recovery and %RSD (n=10) were 100.8 and 10.8, respectively. The proposed system was successfully applied for the determination of trace levels of Pb(II) in water sample.

Department……… Chemistry……. Student's signature Anurak Yaemplai
Field of study…..... Chemistry……. Advisor's signature W. Aeungmaitrepirom
Academic year……2007……….. Co-advisor's signature Passapol Ngamukot

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Finally, I am grateful to my family for their support, entirely care, encourage and love. The usefulness of this work, I dedicate to my father, my mother and all the teachers who have taught me since my childhood.

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Å	Angstrom
b	Langmuir constant related to energy of adsorption (L mol^{-1})
BET	Brunauuer Emmett Teller
br	Broad (^{1}H-NMR spectrum)
C	Concentration of metal ion solution at equilibrium (mol L^{-1})
°C	Degree Celsius
cm^{-1}	Per centimeter
^{13}C-NMR	Carbon nuclear magnetic resonance
dd	Doublet of doublet (^{1}H-NMR spectrum)
1,8-DAAQ	1,8-Diaminoanthraquinone
DMSO	Dimethylsulfoxide
DR-FT-IR	Diffuse reflectance fourier transforms infrared spectrometry
DR-UV-vis	Diffuse reflectance ultraviolet-visible spectrophotometry
EA	Elemental analysis
EDTA	Ethylenediaminetetraacetic acid
EF	Enrichment factor
ESI	Electrospray ionization
ETAAS	Electro thermal vaporization atomic absorption spectrometry
FAAS	Flame atomic absorption spectrometry
FI	Flow injection
FIA	Flow injection analysis
FT-IR	Fourier transforms infrared spectrometry
g	Gram
GFAAS	Graphite furnace atomic absorption spectrometry
h	Hour

^{1}H-NMR	Proton nuclear magnetic resonance
HCL	Hollow cathode lamp
Hz	Hertz
ICP-AES	Inductively coupled plasma atomic emission spectrometry
ICP-OES	Inductively coupled plasma optically emission spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
i.d	Internal diameter
IDL	Instrument detection limit
J	Coupling constant (^{1}H-NMR spectrum)
K	Kelvin
K	Freundlich constant related to adsorption capacity
K_d	Distribution coefficient
K_{eq}	Equilibrium constant
K_f	Formation constant
LOD	Limit of detection
LOQ	Limit of quantitation
m	Medium intensity (FT-IR spectrum)
mA	Milliamp
MDL	Method detection limit
mg	Milligram
$m^2\ g^{-1}$	Square meter per gram
$mg\ g^{-1}$	Milligram per gram
$mg\ L^{-1}$	Milligram per liter
min	Minute
mL	Milliliter
$mL\ min^{-1}$	Milliliter per minute
mm	Millimeter
mmol	Millimole

m/z	Mass-to-charge-ratio
n	Freundlich constant related to adsorption intensity
N_f	Amount of metal adsorbed per gram of sorbent (mol g^{-1})
N_f^s	Maximum amount of metal adsorbed per gram of sorbent (mol g^{-1})
nm	Nanometer
PE	Polyethylene
PEEK	Polyetheretherketone
PF	Preconcentration factor
pm	Picometer
ppm	Parts per million
ppb	Parts per billion
ppt	Parts per trillion
rpm	Round per minute
RSD	Relative standard deviation
s	Strong intensity (FT-IR spectrum)
sec	Second
SiAP	3-Aminopropyltriethoxysilane linked to silica gel
SiDAAQ	Silica gel chemically modified with 1,8-DAAQ
SPE	Solid-phase extraction
TGA	Thermogravimetric analysis
UV-vis	Ultraviolet-visible spectrophotometry
v/v	Volume by volume
w	Weak intensity (FT-IR spectrum)
WHO	World Health Organization
w/v	Weight by volume
$\overline{X}$	Mean value
µg	Microgram
µg g^{-1}	Microgram per gram

μg L^{-1}	Microgram per liter
μL	Microliter
λ	Wavelength
δ	Chemical shift

CHAPTER I

INTRODUCTION

1.1 Statement of the problem

Flame atomic absorption spectrometry (FAAS) is frequently used to determine the concentration of metals in samples since it offers many advantages, for example, low cost operation, high precision and specificity. However, the direct determination of any sample such as environmental samples, especially natural water that contain metals at trace level is very difficult and may affect the accuracy of the determination. Because these metals are usually present near or below the limit of detection (LOD) of the FAAS instrument. The preconcentration stage is frequently required in order to improve the FAAS sensitivity prior to the determination of trace metals by increasing the analyte concentrations as well as eliminating the potentially interferences matrix.

There are several methods used for preconcentration, one of which is the solid-phase extraction (SPE) as it offers a number of advantages, such as higher preconcentration factor, better removal of interfering species, lesser time-consuming, cost and labor and potential for automation analysis [1]. However, the conventional batch or off-line column preconcentration based on SPE method is usually tedious and time consuming, require large quantities of sample and reagents and risk to contamination and analyte losses. Thus, on-line flow injection (FI) preconcentration systems coupled with FAAS have been developed for trace metals determinations. This technique provides some favorable features such as simplcity and rapidity in the preconcentration step, lower matrix effects, low reagents and samples consumptions, avoid to contamination and analyte losses. Moreover, FI method offers higher sample throughput, lower LOD, better reproducibility and accuracy compared to off-line method [2].

The preparation of chemically modified silica gel with organic molecules containing nitrogen, sulfur, oxygen and the study of the adsorption of metal ions are of great interest [3]. Because the silica gel based sorbents present the advantages of mechanical, thermal and chemical stability under various conditions [4]. Moreover, the silica gel surface with large specific surface area is consisted of the siloxane group and several forms of silanol groups, making it easy to modify. In addition, the silica gel chemically modified with chelating agent provides the high selectivity [5-9]. Also, a large number of reactive sites exist on the silica gel surface, and so, the number of chelating agent chemically modified is high, resulting in good sorption capacity [10]. However, the fast kinetic properties for retaining and eluting of the analyte are special addition requirements for on-line column packings [11]. Thus, the packing materials have been developed and used in the various FI systems for the preconcentration and determination of trace metals.

In this work, 1,8-diaminoanthraquinone (1,8-DAAQ) is of interest as a chelating agent because it presents three donor atoms; two nitrogen and one oxygen and has the rigid and planar structure which suitably oriented to form metal-ligand complexes, and therefore, it is expected to act as tridentate ligand to form selective and stable complex with investigated metal ions. Thus, functionalized silica gel with 1,8-diaminoanthraquinone (SiDAAQ) are prepared and followed by studying its kinetic and thermodynamic of adsorption properties towards Cd(II), Co(II), Cu(II), Ni(II), Pb(II) and Zn(II) from aqueous solution. The optimization of the chemical and flow variables for an on-line preconcentration-FAAS system using a mini-column packed with SiDAAQ are reported. The application of this system for trace metal determination of water sample is also described.

1.2 Objectives and scope of the research

The objectives of this research are to synthesize and characterize the 1,8-diaminoanthraquinone (1,8-DAAQ) and the functionalized silica gel with 1,8-diaminoanthraquinone (SiDAAQ). The adsorption properties of the SiDAAQ were studied using Cd(II), Co(II), Cu(II), Ni(II), Pb(II) and Zn(II) in aqueous solutions with batch and column methods. The kinetics and thermodynamics results of the SiDAAQ were evaluated for on-line preconcentration system coupled with FAAS. The proposed system was validated and applied for the determination of trace levels of Pb(II) in water sample.

1.3 The benefits of this research

A novel chemically modified silica gel (SiDAAQ) for on-line preconcentration-FAAS system was obtained in order to apply for the determination of trace levels of Pb(II) in water sample.

CHAPTER II

THEORY AND RELATED RESEARCH

The determination of metals at low concentration levels is necessary in various fields, such as environmental analysis, biochemistry, pharmacy, electronic industry and food industry. In environmental analysis, the evaluation of the impact of industrial and technology developments on the environment is one of the main subjects. However, in many cases the sensitivity of analytical instrumentation is not sufficient for the analysis of such samples. Thus, the developments of accurate and precise methods for monitoring low level metals are of major importance.

2.1 Heavy metals in the environment

Heavy metals are one group of pollutants that has become the subject of analysis due to their toxic effects on the environment [12]. Some are toxic even in low quantities. The main pathways of pollution to humans are through inhalation (atmospheric pollution), ingestion (water, soil, plant, food pollution) and to a lesser degree dermal contact (e.g. occupational pollution). Thus pollution control involves the monitoring of metals in air, soil and water environments, with special attention to possible sources of entry of pollutants into the food chain.

Prevention of pollution thus involves the control of industrial production processes, the main source of heavy metals, through legislation and monitoring. Current international and governmental activities (in terms of legislation, promotion of scientific research, waste management etc.) aimed at making the environment safer can be obtained from Pollution Control Department, PCD (www.pcd.go.th), United

Nations Environmental Programme, UNEP (www.unep.org) or United States Environmental Protection Agency, USEPA (www.epa.gov.org) websites.

Water is a particularly important source as well as a sink for heavy metal pollution. The presence of even low concentration of some heavy metals in natural water systems can have toxic effects on humans. The WHO health based guideline limits for the concentration of metals in drinking water are given in Table 2.1.

Table 2.1 Guidelines and maximum permitted levels of heavy metals in drinking water [13]

Element	**Limit (mg L^{-1})**	**Element**	**Limit (mg L^{-1})**
Aluminium[a]	No limit set	Lead	0.01
Antimony	0.02	Manganese	0.4 (C)
Arsenic	0.01 (P)	Mercury[e]	0.001
Cadmium	0.003	Molybdenum	0.07
Chromium[b]	0.05 (P)	Nickel	0.02 (P)
Copper[c]	2	Uranium[f]	0.015 (P, T)
Iron[d]	No limit set	Zinc[g]	No limit set

[a] Owing to limitations in the animal data as a model for humans and the uncertainty surrounding the human data, a health-based guideline value cannot be derived; however, practicable levels based on optimization of the coagulation process in drinking-water plants using aluminium-based coagulants are derived: 0.1 mg L^{-1} or less in large water treatment facilities, and 0.2 mg L^{-1} or less in small facilitie

[b] For total chromium

[c] Staining of laundry and sanitary ware may occur below guideline value

[d] Not of health concern at concentrations normally observed in drinking-water, and taste and appearance of water are affected below the health-based value

[e] For total mercury (inorganic plus organic)

[f] Only chemical aspects of uranium addressed

[g] Not of health concern at concentrations normally observed in drinking-water, but may affect acceptability of water

P = provisional guideline value, as there is evidence of a hazard, but the available information on health effects is limited;

T = provisional guideline value because calculated guideline value is below the level that can be achieved through practical treatment methods, source protection, etc.;

C = concentrations of the substance at or below the health-based guideline value may affect the appearance, taste or odour of the water, leading to consumer complaints.

2.2 Determination of metals

Although there are several analytical techniques for elemental determination in various types of sample, the selection the proper technique is an important step in any laboratory. The most commonly used technique are atomic spectrometric techniques, for example, flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption spectrometry (GFAAS) or electro thermal vaporization atomic absorption spectrometry (ETAAS), inductively coupled plasma atomic emission spectrometry (ICP-AES) or inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS).

Proper selection requires a basic understanding of each technique and its capabilities and limitations of instrument. An important criterion is often considered from the concentration level of metals in samples, operator skill, sample throughput and cost. The comparison of atomic spectrometric techniques is summarized in Table 2.2.

The detection limits achievable for individual elements are most important in determining the usefulness of an analytical technique for a given analytical problem. The detection limits of typical metals for FAAS, GFAAS, ICP-AES and ICP-MS are listed in Table 2.3.

Table 2.2 Comparison of the various atomic spectrometric techniques [14]

Criterion	**FAAS**	**GFAAS**	**ICP-AES**	**ICP-MS**
Detection limits	high ppb	sub ppb	sub ppb-ppm	sub ppt
Analytical capability	single element	single element	multielement	multielement
Sample throughput	~ 3-10 sec/ element/ sample	~ 2-3 min/ element/ sample	~ 1-5 min/ sample	~ 1-4 min/ sample
Dynamic range	mid ppm range	low ppm range	high ppm range	mid ppm range
Precision				
Short-term	0.1-1%	0.5-5%	0.1-2%	~ 0.5-2%
Long-term	1-2%	1-10%	~ 1-5%	< 4% (4 h.)
Interferences				
Spectral	few	very few	some	few
Chemical	many	many	very few	some
Physical	some	very few	some	some
Dissolved solids handling	up to 5%	up to 10%	up to 20%	< 0.2%
Elements applicable	> 60	> 50	> 70	> 80
Sample volume required	4-8 mL min^{-1}	~ 0.2-1 mL min^{-1}	~ 1-2 mL min^{-1}	~ 0.02-2 mL min^{-1}
Semi-quantitative analysis	No	no	yes	yes
Isotopic analysis	No	no	no	yes
Ease-of-use	very easy	more difficult	easy	more difficult
Method development	easy, cookbooks	fairly easy, cookbooks	fairly easy	more difficult
Unattended operation	no, flammable gas	yes	yes	yes
Initial cost	Low	med	high	very high
Operating cost	Low	high	med	high
Cost per sample	Low	med	low	med

Table 2.3 Detection limits (μg L^{-1}) for some elements by atomic spectroscopy techniques [15]

Element	FAAS	GFAAS[a]	ICP-AES	ICP-MS
Ag	3	0.02	0.2	0.003
Al	30	0.2	0.2	0.06
Ba	20	0.5	0.01	0.002
Ca	1	0.5	0.0001	2
Cd	1	0.02	0.07	0.003
Cr	4	0.06	0.08	0.02
Cu	2	0.1	0.04	0.003
Fe	6	0.5	0.09	0.45
K	2	0.1	75	1
Mg	0.2	0.004	0.003	0.15
Mn	2	0.02	0.01	0.6
Mo	5	1	0.2	0.003
Na	0.2	0.04	0.1	0.05
Ni	3	1	0.2	0.005
Pb	5	0.2	1	0.007
Sn	15	10	1	0.02
V	25	2	8	0.005
Zn	1	0.01	0.1	0.008

[a] Based on a 10 μL of sample volume

2.3 Limitations of the FAAS sensitivity

FAAS is often used to determine many elements in samples since it offers many advantages, for example, rapid, low cost operation, high precision and specificity. Thus, it is used for routine determinations of analyte concentrations in the mg L^{-1} concentration region for most elements. However, the need for trace metal analyses at μg L^{-1} and even sub μg L^{-1} levels calls for a more sensitive technique as the detection limits of FAAS are relatively high for some elements.

Because the absorbance depends on the number of atoms in the optical path of the spectrometer at a given instant, in considering the FAAS process, a number of areas limiting the sensitivity of the technique can be found as follows: (1) the nebulization process, which draws sample solution into the burner chamber at approximately 3-8 mL min^{-1}, limits the sample introduction rate, and, therefore, the amount of sample available for transport to the flame; (2) the premix burner design, which has been universally adopted due to its many desirable characteristics, has the undesirable characteristic of being very wasteful of sample. Only a small fraction of the sample nebulized ever reaches the flame, with the remainder being directed to the drain; and (3) that 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 [16].

The sensitivity of FAAS can be improved by addressing the limitations of flame sampling efficiency in order to achieve a greater absorption. Moreover, this problem can also be solved by adding the preconcentration step before the determination of analyte. Also, the detection limits of FAAS can be enhanced by on-line coupling of a preconcentration step as described in many FI-FAAS analytical methods [17, 18].

2.4 Preconcentration of trace metals

Many elements occurring in an environment are present at very low levels, the term *trace* was coined to describe them. The definition of *trace element* in the IUPAC Compendium of Chemical Terminology, second edition is ''*Any element having an average concentration of less than about 100 parts-per-million atoms or less than 100* $\mu g\ g^{-1}$'' [19].

The analysis of heavy metals in environmental samples is usually carried out by various instrumental techniques. But, direct instrumental analysis of these samples is difficult because the concentration of heavy metals in environmental samples is generally extremely low, namely parts-per-billion or less, which are near or below the limit of detection of the instrument. On the other hand, alkali, alkaline earth metals and natural organic matter present at the higher concentration than heavy metals might interfere with the determination of trace heavy metals by FAAS, GFAAS, ICP-AES or ICP-MS. Therefore, the analytes are mostly required to be separated from matrix components and preconcentrated to eliminate the potential interferences and improve the sensitivity prior to the measurement. Various separation and preconcentration methods have so far been developed and reviewed in detail by Alfassi et al. [20]. Among these, co-precipitation, solvent extraction and solid-phase extraction have been widely used as a separation and preconcentration method of heavy metals, because these methods are simple, useful and versatile for various samples. In addition, the automation of analysis, including preconcentration steps, has been developed for trace heavy metals and extensively applied to routine analysis.

2.5 Solid-phase extraction (SPE)

Solid-phase extraction (SPE) is an important sample preparation technique that has been successfully used for the separation and preconcentration of component of interest from a sample matrix, instead of classical extraction procedures such as solvent extraction or liquid-liquid extraction (LLE). Because SPE can reduce the problems associated with LLE, such as incomplete phase separations, less-than-quantitative recoveries, less preconcentration efficiency, formation of the hard breaking emulsion, high consumption organic solvents, use of expensive, breakable specialty glassware, and required lab time, cost and labor. Moreover, it can provide the higher preconcentration factor and better removal of interfering species [1]. In addition, SPE can be easily interfaced on-line with analytical techniques, such as liquid chromatography or atomic absorption spectrometry. For SPE involved with the trace heavy metal analytes, an excellent publication regarding the solid-phase extraction of trace elements was reviewed by Camel in 2003 [21].

2.5.1 Theory of SPE

Solid-phase extraction involves distribution equilibrium that is the partition of solutes (analytes) between a liquid (sample matrix) and a solid (sorbent) phase based on their adsorption behaviors on a solid sorbent. When the partition reaches equilibrium, the ratio of the analytes remained in solution to those adsorbed on the solid phase will have the constant value that can be defined as the distribution coefficient, K_d [22], and expressed as follows:

$$K_d = \frac{\text{mole of analyte adsorbed on sorbent (mmol g}^{-1}\text{)}}{\text{mole of analyte remained in solution (mmol mL}^{-1}\text{)}} \qquad (2.1)$$

The values of K_d are usually determined by *batch method* and have been reported for analytes on various sorbents and in different solutions. If K_d is larger, analyte can be strongly adsorbed on solid sorbent. If it is smaller, on the other hand, analyte tends to remain in sample solution. Hence, we can preconcentrate analyte (larger K_d value) and remove matrix components (smaller K_d values) based on the difference of their K_d values (i.e. the difference of their adsorption behaviors) on a solid sorbent.

2.5.2 Basic principles

Solid-phase extraction refers to the nonequilibrium, exhaustive removal of analytes from a flowing liquid sample via retention on a contained solid sorbent and subsequent complete recovery of selected analytes by elution from the sorbent using the appropriate solvent [23]. Generally, SPE in *column method* consists of four steps: conditioning (column preparation or prewash), loading (sample retention or sorption), washing (column postwash or sample cleanup), and eluting (sample desorption) as illustrated in Figure 2.1.

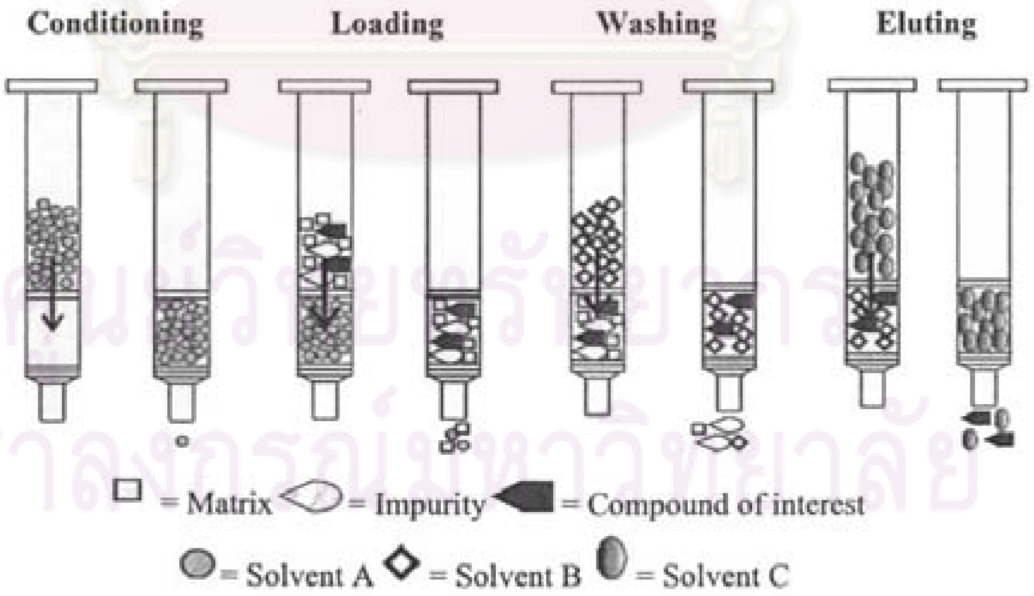


Figure 2.1 SPE operation steps [24].

Firstly, the conditioning step is performed to generate the wetting of the packing, solvate the functional groups of solid sorbent, remove the initial impurities contained in the packing and the air presented in the column and fill the void volume, using an appropriate solvent, followed by the same solvent as the sample solvent. Secondly, the sample is percolated through the solid sorbent with appropriate flow-rate which should be low enough to enable efficient retention of the analytes, and high enough to avoid excessive duration. The analytes are retained on the solid sorbent, while the matrix components and impurities are not retained. In addition, the appropriate sorbent which has a great affinity for the analytes, is selected to quantitatively obtain the analyte from sample. Thirdly, the solid sorbent is washed with an appropriate solvent which has a low elution strength to eliminate none retained species, without displacing the analytes. Moreover, the unbounded analytes are washed to ensure that in eluting step only the extracted analytes are eluted. Finally, the analytes are eluted with appropriate flow-rate to ensure efficient elution, using an appropriate solvent which should be carefully chosen to ensure efficient recovery of the retained analytes and be compatible with the analysis technique. In addition, the solvent volume should be low to obtain a high concentration of analytes but it should ensure the complete elution of the analytes.

2.5.3 Retention mechanism of elements on the sorbent

The mechanism of retention depends on the property of the sorbent and analyte. For the solid-phase extraction of trace elements, the main mechanism involve with a simple adsorption (by van der Waals forces or hydrophobic interaction), chelation (metal-ligand interaction) or ion-exchange (electrostatic or coulomibic interaction) [21].

Adsorption

The trace elements can be adsorbed on a solid sorbent through van der Waals forces or hydrophobic interaction, when it forms complex with chelating agent and transform to non-polar compound. Also, an ion-pair reagent can be added to the sorbent, the non-polar portion interacts with the non-polar sorbent, while the polar portion forms an ion-pair with the ionic species present in the solution.

Chelation

The chelating agent is a polydentate ligand that uses at least two donor sites to coordinate. Thus the chelation is the coordination (complex formation) of the chelating agent and metal ion. Several chelating organic molecules consist of functional groups which act as donor atoms, such as *nitrogen* presented in amines, imine, azo groups, nitriles, oxime, *oxygen* presented in amides, carboxylic, hydroxyl, phenolic, ether, carbonyl, phosphoryl groups and *sulfur* presented in thiols, thiocarbamates, thioethers. Chelating agents may be directly added to the sample to chelate with elements, the chelates being further retained on an appropriate sorbent. An alternative is to introduce the functional chelating group into the sorbent.

Ion-exchange

Ion-exchange sorbents are usually contained cationic or anionic functional groups that can exchange with the analyte using associated counter-ion. Ion exchangers are either cation exchangers that exchange positively charged ions (cations) or anion exchangers that exchange negatively charged ions (anions). Generally, the strong are referred to the sites which are always present as ion-exchange sites at any pH such as sulfonic acid groups (cation-exchange) and quaternary amines (anion-exchange), while weak sites are only ion-exchange sites at pH values greater or less than their pK_a such as carboxylic acid groups (cation-exchange) or primary, secondary and tertiary amines (anion-exchange).

2.5.4 Selection of solid sorbent

It is also important to consider the properties of sorbent in order to achieve excellent separation and preconcentration. In practice, the main requirements for a solid sorbent are: (1) the possibility to extract a large number of trace elements over a wide pH range (along with selectivity towards major ions); (2) the fast and quantitative sorption and elution; (3) high capacity; (4) regenerability, and (5) accessibility. In particular, sorbents that allow fast reaction rates are preferred to achieve faster extraction as well as higher loading capacity [21]. The main solid sorbents used for solid-phase extraction are shown in Figure 2.2 and 2.3.

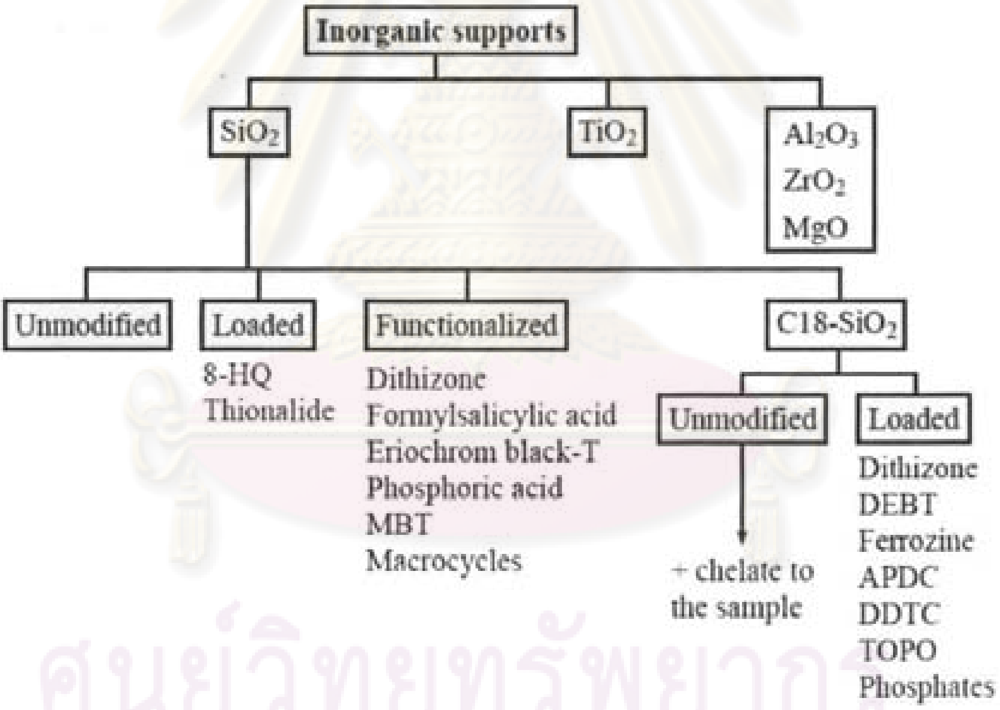


Figure 2.2 Sorbents based on inorganic supports [21].

APDC, Ammonium pyrrolidine dithiocarbamate; DDTC, Diethyldithiocarbamate; DEBT, N,N'-Diethyl-N'-benzoylthiourea; 8-HQ, 8-Quinolinol; MBT, 2-Mercaptobenzothiazole; TOPO, Tri-*n*-octylphosphine oxide.

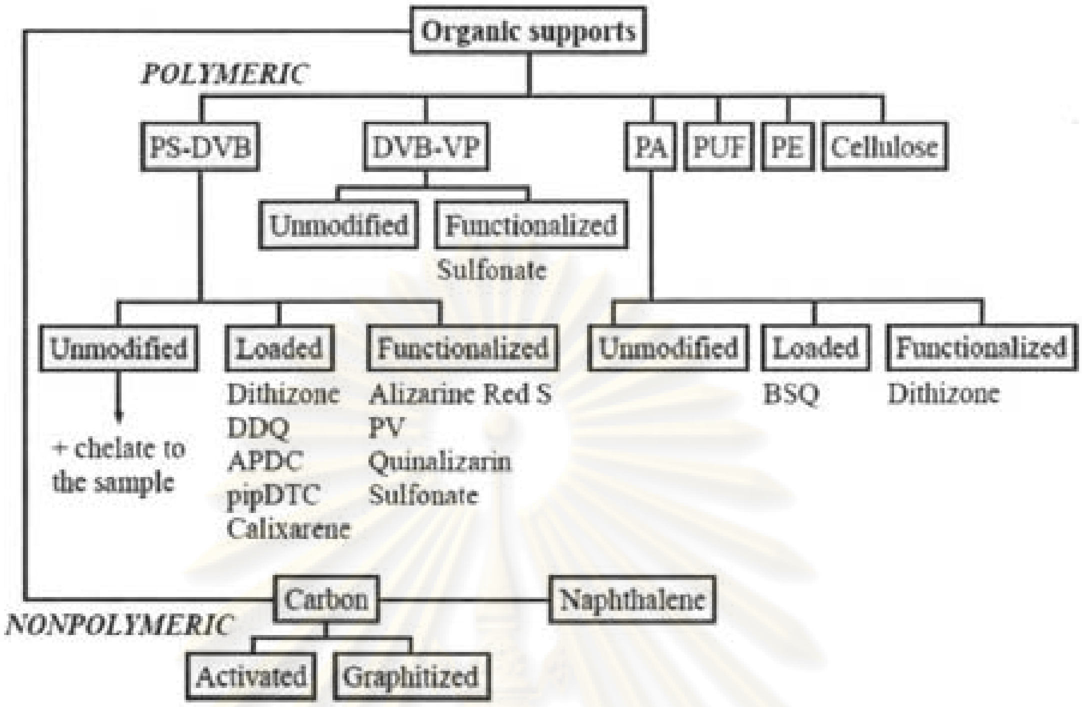


Figure 2.3 Sorbents based on organic supports [21].

APDC, Ammonium pyrrolidine dithiocarbamate; BSQ, 8-(Benzenesulfonamido)-quinoline; DDQ, 7-Dodecenyl-8-quinolinol; pipDTC, Piperidine dithiocarbamate; DVBVP, Divinylbenzene-vinylpyrrolidone; PA, Polyacrylate; PE, Polyethylene; PS-DVB, Polystyrene-divinylbenzene; PUF, Polyurethane foam; PV, Pyrocatechol violet.

The polymeric resin exhibit several disadvantages such as slow kinetics, irreversible adsorption of organic, swelling, sensitivity towards many chemical environments and loss of mechanical stability in modular operation. Thus the inorganic supports are suggested to use in place of polymeric resin due to it provide the more advantages as follows: (1) good selectivity; (2) no swelling; (3) rapid sorption of metal ions; and (4) good mechanical stability [3].

2.6 Chemically modified silica gel

Inorganic based sorbents are mainly made of silica gel even though other inorganic oxides may be used. The silanol groups on the silica gel surface are always aimed in order to modify the surface with such groups to extend the utilization of it. The development in chemically modified silica gel has been utilized in many applications, such as chemically bonded phase in chromatography [25], extraction of cations from aqueous [26] and non-aqueous [27] solvents, elimination of toxic heavy metals from waste water [28] and also in catalytic reaction [29]. One of the important properties widely used for quantitative determination in analytical methods is preconcentration of metal ions at trace level from aqueous sample.

2.6.1 Silica gel

Silica gel is a solid, amorphous, porous form of polysilicic acid. Silica gel based sorbents present the advantages of mechanical, thermal and chemical stability under various conditions. Moreover, the silica gel surface with large specific surface area is consisted of the siloxane group and the several forms of silanol groups, so it easy to modify.

Preparation of silica gel [30]

Silica gel is manufactured by releasing silicic acid from a strong solution of sodium silicate by hydrochloric acid.

$$Na_2SiO_3 + H_2O + 2HCl \rightarrow Si(OH)_4 + 2NaCl$$

And then the free acid quickly starts to condense itself with the elimination of water to form dimers, trimers and eventually polymeric silicic acid. The polymer grows, initially forming polymer aggregates and then polymer spheres, a few Angstrom in diameter. These polymeric spheres are called primary silica particles. These primary particles continue to grow until, at a particular size, the surface silanol groups on adjacent primary polymer particles, condense with the elimination of water. This condensation causes the primary particles to adhere to one another and at this stage the solution begins to gel. During this process, the primary particles of silica gel will have diameters ranging from a few Angstrom to many thousands of Angstrom depending on the conditions of formation.

Structure of silica gel

The matrix of the primary silica gel particle consists of a core of silicon atoms joined together with oxygen atoms by siloxane bonds (silicon-oxygen-silicon bonds). On the surface of each primary particle some residual, uncondensed hydroxyl groups from the original polymeric silicic acid remain. These residual hydroxyl groups confer upon silica gel its polar properties. The silica surface is quite complex and contains more than one type of hydroxyl group, strongly bound or chemically adsorbed water and loosely bound or physically adsorbed water. There are three types of hydroxyl group:

(1) the single hydroxyl groups called *free silanol* are a single hydroxyl group attached to a silicon atom which has three siloxane bonds joining it to the gel matrix;

(2) the twin hydroxyl groups called *geminal silanol* are one of two hydroxyl groups attached to the same silicon atom which, in turn, is joined to the matrix by only two siloxane bonds; and

(3) the twin hydroxyl groups called *vicinal silanol* are one of three hydroxyl groups attached to a silicon atom which is now only joined to the silica matrix by only a single siloxane bond.

An example of each type of hydroxyl group is shown in Figure 2.4.

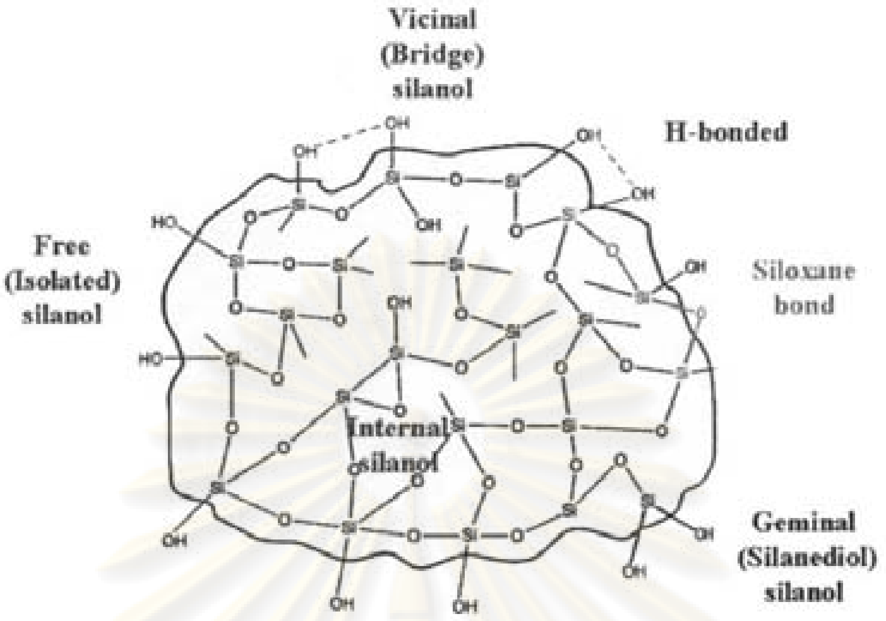


Figure 2.4 The surface of silica gel (Modified from ref [1]).

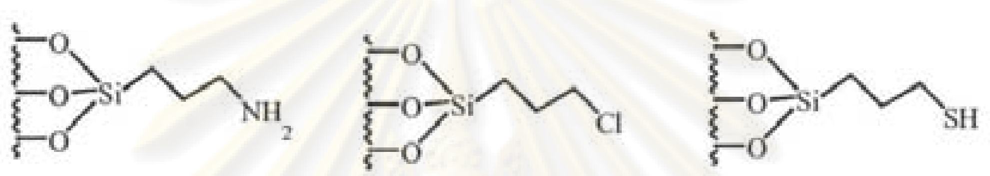
The surface of silica gel is characterized by the presence of silanol groups, which are known to be weak ion-exchangers (existed as Si–O$^-$ group) at pH higher than 4, causing low interaction, binding and extraction of ionic species [31]. In particular, silica gel presents high sorption capacity for metal ions, such as Cu, Ni, Co, Zn or Fe [32]. Retention is highly dependent on sample pH with quantitative retention requiring pH values over 7.5-8.0, as under acidic conditions silanol groups are protonated and the ion-exchange capacity of the silica gel is greatly reduced or even reduced to zero at low pHs. In addition, this sorbent has a very low selectivity, and is prone to hydrolysis at basic pH. Consequently, modification of the silica gel surface has been performed to obtain solid sorbents with greater selectivity.

2.6.2 Modification of the silica gel surface

Two different approaches are used for modification of the silica gel surface with specific organic compounds: (1) chemical immobilization or chemisorption, a chemical bond is formed between the silica gel surface groups and those of the organic compound (*functionalized* sorbent) and (2) physical adsorption or physisorption, the organic compound is directly adsorbed on the silanol groups of the silica gel surface (*impregnated, coated* or *loaded* sorbent), either by passing the reagent solution through a column packed with the adsorbent, or by soaking the adsorbent in the reagent solution [21]. However, the disadvantages of the later approach are the consumption of some active functional groups in the physical adsorption processes, diminishing the capability of these groups to interact and bind with the analyte of interest, as well as the possible leaching of the organic modifier in different solutions leading to very low metal capacity values for most metal ions [33].

The silica surface consists of two types of functional groups, siloxane (Si–O–Si) and silanol (Si–OH). Thus, the chemical modification of silica surface can occur via the reaction of a particular molecule with either the siloxane (nucleophilic substitution at the Si) or silanol (direct reaction with the hydroxyl group) functions. Although the former reaction is generally accepted, the reaction with the silanol function is constituted as the main modification pathway. There are three main methods in which organic molecules are covalently attached to the silica surface: (1) through reaction between organosilanes or organic molecules and silica surface functions; (2) chlorination of the silica surface followed by reaction of the Si–Cl with an appropriate functional molecule/reactant; and (3) incorporation of functional groups via sol-gel methodology followed by (where necessary) post-modification [34]. Modification through covalent attaching provides immobility, mechanical stability and water insolubility, thereby increases the efficiency, sensitivity and selectivity of the analytical application [35].

Some of the organic reagent or synthesized organic molecule containing the desired organic functional group that are directly attached to the silica surface produces steric hindrances at the silanol site and therefore restricts the number of incoming molecules. Thus, the surface silanol is conversed to a new silanized surface by the attachment of the organosilane linking group on silica surface via silanization process that acquires organophilic properties to act as precursors for further modification with the organic molecule, is better way [3]. The silylating reagents that widely used for initially modified the surface of silica gel are 3-aminopropyltriethoxysilane [7, 8, 33, 34, 36, 37], 3-chloropropyltrimethoxysilane [38-40] and 3-mercaptopropyltrimethoxysilane [6, 41]. The chemical linker attach to silica surface is shown in Figure 2.5.



Aminopropyl silica Chloropropyl silica Mercaptopropyl silica

Figure 2.5 The organosilane linking group on silica surface.

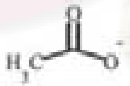
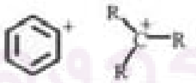
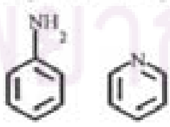
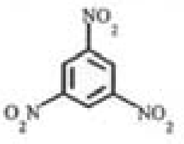
To achieve high efficiency in preconcentration of metal ions on silica surface following factors are to be investigated thoroughly: (a) surface characterization of modified silica; (b) ligating efficiency of the ligands anchored at silica surface; (c) kinetics of entrapment of metal ions; (d) effect of medium (change in pH, ionic strength, polarity, temperature and pressure) on the metal ion up take; (e) desorption of the metal ions from the solid matrix; and (f) reuse of the solid matrix in cyclic operation [3].

2.6.3 Organic function groups

The chelating organic molecules which have tendency to form metal complexes with various metal ions in solution, when it are chemically modified on a silica gel, present very important role in separation as well as preconcentration of metal analytes. Examples of organic reagents that chemically modified on silica gel for preconcentration of metal ions were reported by Jal et al. [3, 42]. Among them, the selection of suitable specific function groups makes them capable of reacting with metal species under certain favorable conditions to form the stable metal complexes.

Binding of metal ions to the chelate functional group is dependent on several factors: (1) nature, charge and size of the metal ion; (2) nature of the donor atoms present in the ligand; (3) buffering conditions which favor certain metal extraction and binding to active donor or groups; and (4) nature of the solid support (e.g. degree of cross-linkage for a polymer) [21]. Normally, the selectivity of the surface with the immobilized functional groups towards metal ions can be basically considered by the characteristics of hard-soft acid-base that was defined as follows: "*Hard (lewis) acids prefer to bind to hard (lewis) bases and soft (lewis) acids prefer to bind to soft (lewis) bases*" [43]. The Pearson hard-soft acid-base species are summarized in Table 2.4.

Table 2.4 The Pearson hard-soft acid-base species [43]

	Lewis acid	**Lewis base**
Hard	H^+ Na^+ K^+ Be^{2+} Mg^{2+} Ca^{2+} Mo^{3+} Mn^{2+} Al^{3+} Sc^{3+} In^{3+} Cr^{3+} Co^{3+} Fe^{3+} Ti^{4+} Zr^{4+} U^{4+} Ce^{3+} Sn^{4+} BF_3 $AlCl_3$ AlH_3 SO_3 NO_2^+ CO_2	H_2O OH^- F^- PO_4^{3-} SO_4^{2-} Cl^- CO_3^{2-} ClO_4^- NO_3^- NH_3 R–OH R–NH_2 H_2N–NH_2 R–O–R 
Boderline	Fe^{2+} Co^{2+} Ni^{2+} Cu^{2+} Zn^{2+} Pb^{2+} Sn^{2+} Sb^{3+} Bi^{3+} Ir^{3+} $B(CH_3)_3$ SO_2 Ru^{2+} 	$[N_3]^-$ Br^- SO_3^{2-} N_2 NO_2^- 
Soft	Cu^+ Ag^+ Au^+ Tl^+ Hg^{2+} Hg_2^{2+} Pd^{2+} Cd^{2+} Pt^{2+} Tl^{3+} BH_3 Br^+ I^+ Br_2 I_2  R–H^+ H–O^+	I^- SCN^- CN^- H^- R^- R–CN R–S–R R–SH R–S^- CO As–$(R)_3$ P–$(R)_3$ P–$(OCH_3)_3$ $H_2C{=}CH_2$ 

For soft metals, the following order of donor atom affinity is observed: O < N < S. A reversed order is observed for hard cations. For a bidentate ligand, affinity for a soft metal increases with the overall softness of the donor atoms: (O, O) < (O, N) < (N, N) < (N, S). The order is reversed for hard metals. In general, the competition for a given ligand essentially involves hard and boderline metals for O sites, and boderline and soft metals for N and S sites. The competition between hard and soft metals is weak [21].

2.7 Flow injection analysis (FIA)

The automation method of solution analysis has been attracting the attentions of various fields, because automated instruments can reduce the manpower cost of laboratory operations, enhance sample throughput, and improve the accuracy and precision of analytical results obtained. Additionally, they can be also used to minimize hazard in laboratory work. One of the techniques that is mostly used with widespread application in quantitative chemical analysis is flow injection analysis (FIA), a simple, rapid, and versatile technique. A present survey of chemical literature reveals that FIA is finding increased applications in analytical routine and research, monitoring of chemical processes, sensor testing and development, and enhancing the performance of various instrument. Moreover, on-line separation and preconcentration processes were also incorporated into FI systems which are attracting the development of FIA in the analytical laboratory.

2.7.1 Introduction to FIA

Flow injection analysis is apparent from the number of publication in scientific journals since 1975, and since then the scope of the method has grown at an unprecedented rate [44]. The designation of FIA was proposed in 1975 by Ruzicka and

Hansen [45]. They defined FIA as a technique for "*information gathering from a concentration gradient formed from an injection, well-defined zone of a fluid, dispersed into a continuous unsegmented stream of a carrier*". Furthermore, Fang [46] defines FIA as "*A non-chromatographic flow analysis technique for quantitative analysis, performed by reproducibly manipulating sample and reagent zones in a flow stream under thermodynamically non-equilibrated conditions*". Despite the broad scope covered by their author's definitions, the concept of FIA depends on a combination of three factors: reproducible sample injection volumes; controlled dispersion of the injected sample zone; and reproducible timing of the injected sample from the injection point through the flow system into the detector.

FIA offers several advantages in term of considerable decrease in sample (normally using 10 to 50 μL) and reagent consumption, high sample throughput (50 to 300 samples per hour), reduced residence times (reading time is about 3 to 40 sec), shorter reaction times (3 to 60 sec), easy switching from one analysis to another (manifolds are easily assembled and/or exchanged), reproducibility (usually less than 2% RSD), reliability, low carry over, high degree of flexibility, and ease of automation. Perhaps the most compelling advantage of the FIA technique is the great reproducibility in the results obtained by this technique that can be set up without excessive difficulties and at very low cost of investment and maintenance [44]. Moreover, except for detector warm-up, the system is ready for instant operation as soon as the sample is introduced. These advantages have led to an extraordinary development of FIA, unprecedented in comparison to any other technique.

2.7.2 Basic principles

For principle of FIA, the sample is injected into an unsegmented continuously flowing stream of reagent solution (carrier), dispersed, and transported to detector. The simplest flow analyzer consists of a pump, which is used to propel the carrier stream through a narrow tube, an injection valve, a microreactor in which the sample zone disperses and reacts with the components of the carrier stream, forming a species that is sensed by a flow-through detector and recorded. An example of one of the simplest FIA methods, the spectrophotometric determination of chloride in a single-channel system [47], is shown in Figure 2.6.

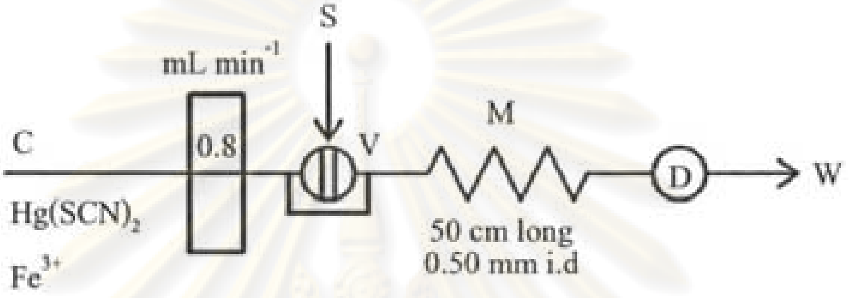


Figure 2.6 A schematic diagram of the basic FIA system for the spectrophotometric determination of chloride [47]. S, sample; C, carrier; P, pump; V, injector valve; M, mixing coil; D, flow through detector; and W, waste.

A typical recorder output has the form of a peak (Figure 2.7), the height H, width W, or area A of which is related to the concentration of the analyte. The time span between the sample injection S and the peak maximum, which yields the analytical readout as peak height, is the residence time T during which the chemical reaction take place. A well-designed FIA system has an extremely rapid response, because T is in the range of 5-20 sec. Therefore, a sampling cycle is less than 30 sec (roughly T + t_b), and thus, typically, two samples can be analyzed per minute [45].

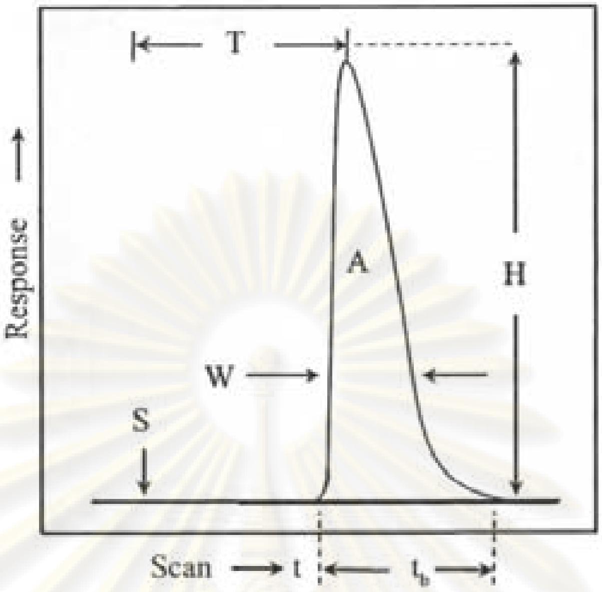


Figure 2.7 The peak output: S, starting record (time of injection t_o); H, peak height; W, peak width at a selected level; A, peak area; T, residence time corresponding to the peak height measurement; t_b, peak width at the baseline [45].

The injected sample volumes may be between 1 and 200 µL (typically 25 µL), which in turn requires no more than 0.5 mL of reagent per sampling cycle. This makes FIA a simple microchemical technique that is capable of having a high sampling rate and minimum sample and reagent consumption.

Moreover, the pump, valve, and detector may be computer controlled for completely automated operation.

2.7.3 Detection in the FIA

The usual location of a flow injection manifold is on-line and placed before a detector system in order that proper monitoring of the species is performed after it has been subjected to the step developed in a continuous fashion. While the interface between an flow injection system and a conventional detector is a commercial or laboratory-made flow cell (or simple aspiration to the flame in the case of atomic techniques assisted by this source), the interface to the high resolution detector has a decisive influence on the performance of the hyphenated system, as analytical quality parameters such as reproducibility, accuracy, sensitivity and selectivity are highly dependent on how the coupling is accomplished. The complexity of the interface is very different depending on whether the measurement is performed in solution, plasma, or vacuum.

The interfacing of FIA with atomic spectrometric detectors to extend the capabilities of atomic spectrometry techniques, particularly as far as matrix interferences and detection limits are concerned, is reviewed by Tyson [48].

2.7.4 Flow injection on-line separation and preconcentration

The most used sample preparation procedures for trace element analysis, such as separation and preconcentration, before determination with atomic spectrometry techniques such as FAAS, ETAAS, ICP-AES or ICP-MS are still performed manually and suffer from high risks of the contamination and losses of the analyte. Therefore, the elimination of human interaction in this tedious stage is obviously of great interest, particularly when large numbers of samples are involved. Thus, the FI on-line separation and preconcentration systems interfaced with atomic spectrometry techniques may play major roles in the delicate stages of separation and preconcentration of trace element.

According to Fang's definition in section 2.7.1 the general characteristics of FI method for separation and preconcentration are summarized as follows [11]:

(a) high sample throughput, 1-2 orders of magnitude higher than batch procedures, with short operation times typically in the range of 10-200 sec per determination (including separations);

(b) high enrichment efficiencies for preconcentration systems, typically a factor of 5-50 higher than batch procedures;

(c) low sample consumption, 1-2 orders of magnitude lower than batch procedures;

(d) low reagent consumption, 1-2 orders of magnitude lower than batch procedures;

(e) high reproducibility, typically in the range of 1-3% RSD;

(f) low contamination risks owing to closed and inert separation systems used, a feature particulary important for trace analysis;

(g) very limited laboratory bench space and utensils.

Moreover, the classifications of FI separation are mainly divided according to the type of interface across which mass transfer take place as follows: liquid-gas, liquid-liquid, and liquid-solid. Thus, some FIA systems are segmented by gas segments (for example, gas diffusion, hydride generation and cold vapour generation systems), segmented by liquid segments (for example, solvent extraction and dialysis systems), some are discontinuous (for example, preconcentration systems for GFAAS), and fluid zones containing the analyte are not always well-defined in the sense that they may not be encompassed by clear boundaries when introduced (for example, the concentrate zone formed during elution of an adsorbed analyte from an on-line column, coprecipitation-dissolution and electrodeposition-stripping systems).

The development of automated solution analysis by FI separation and preconcentration based on the on-line column solid-phase extraction method is a topic which has become one of the mostly interested researches. Because solid-phase extraction is inherently easier to operate than other methods, and the equipment is generally more robust. An additional benefit over other method is its extremely high versatility, owing to the availability of a broad range of choice for different sorbents, complexing systems, and eluents.

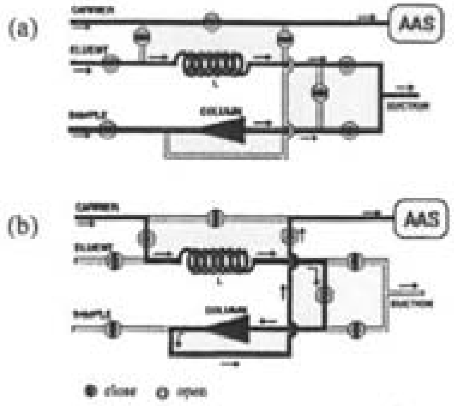
Because ETAAS, ICP-AES and ICP-MS have some disadvantages, the automated FI system coupled with FAAS is appropriate for the routine determination of most elements. Until recently, FI on-line preconcentration-FAAS systems have been developed for the determination of heavy metals in various applications.

2.8 Related research

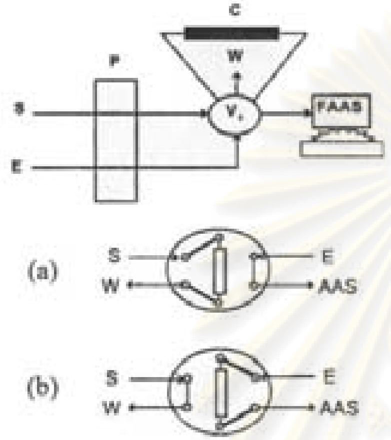
The determination of trace heavy metals in water sample by FAAS requires a preliminary preconcentration step to improve the detection limit. The preconcentration in the conventional approach may be performed either by static batch or by off-line column technique that show some disadvantages, such as inconvenience and inefficiency. While the flow injection (FI) on-line column preconcentration based on solid-phase extraction method are mainly used because it offers some very favorable features in relation to the conventional approaches, such as lower detection limit, higher sample throughput, better reproducibility, lower matrix effects, low reagent and samples consumptions, lesser risk of contamination, and simple automated operation. Therefore, the applications of FI on-line preconcentration (act as sample introduction into FAAS) coupled with FAAS (act as flow-through detector for FIA) systems have been used for enhancing the sensitivity of determination. The versatility of these systems has become one of the most active research fields in automated solution analysis in recently.

Sorbents used successfully as packing materials for batch column preconcentration purposes are not always adaptable to FI on-line column preconcentration systems. Thus, the packing materials have been developed and used in the various FI systems for the determination of heavy metals. The special requirements for on-line column packings may at least include the following: (a) The extent of swelling and shrinking should be negligible when being transformed from one form to another, or when solvent condition are changed; (b) The mechanical properties should be strong enough to withstand high linear flow-rates through the column, usually mechanically unsatisfactory; (c) The kinetic properties should be sufficiently favourable to allow easy retaining and elution of the analyte by an appropriate eluent [11]. Sorbents which retain an analyte so strongly that they can be recovered only by complete destruction of the sorbent obviously cannot be used in FI preconcentration systems.

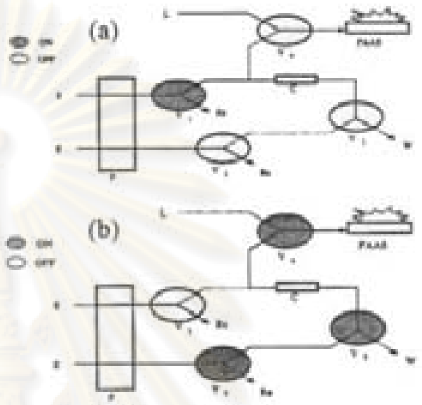
The related publications in FI on-line preconcentration coupled with FAAS offer various configurations of the system. The differences may be found mainly in the type of the analyte and sample matrix, the kind of the column packing and the system design, particularly position of column in the system. Moreover, the two modes for sample introduction which performing the solution flow through the column; the constant volume and constant time, can also be distinguished. According to the different column packings, the selected publications may be classified into two groups: based on polymer supports and silica gel supports as shown in Figure 2.8. Some parameters for these systems are summarized in Table 2.5.



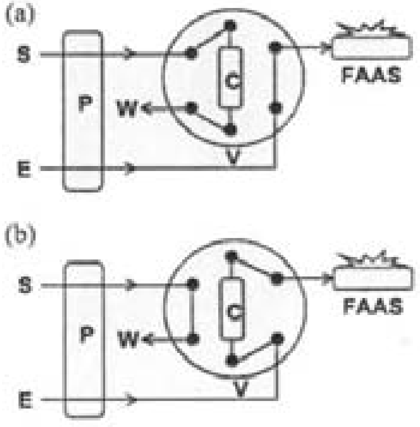
System 1[1] [49]



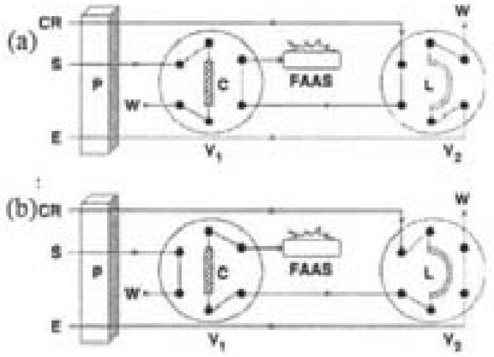
System 2[2] [50]



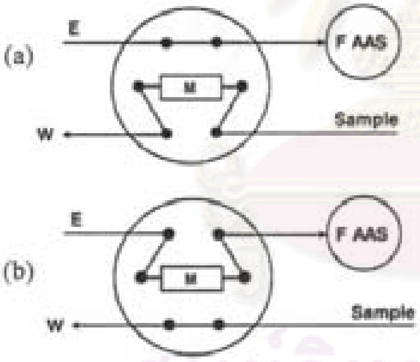
System 3[3] [51]



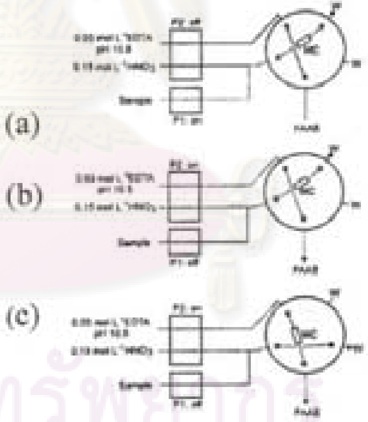
System 4[4] [52]



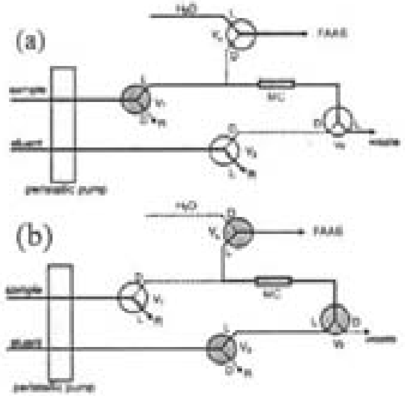
System 5[5] [53]



System 6[6] [54]



System 7[7] [55]



System 8[8] [56]

Figure 2.8 The differences in system design for selected FI on-line preconcentration-FAAS systems.

[1] The set-up and operating principle of the flow system. (a) Loading and (b) elution positions.

[2] Schematic diagram of the flow system used to preconcentration and determination of copper in seawaters. S, Sample; E, eluent; P, peristaltic pump; C, mini-column; V_1, four-way valve (electronically controlled in a time-based mode); FAAS, flame atomic absorption spectrometer (aspiration flow rate was 3.2 L min^{-1}) and W, waste. (a) four-way valve in the preconcentration step and (b) four-way valve in the elution position.

[3] Schematic diagram of the flow system used to pre-concentration and determination of lead by FAAS. S, sample; E, eluent; P, peristaltic pump; C, mini-column; V_1, V_2,V_3,V_4, three-way valves (electronically controlled in a time-based mode); Rs, sample back stream; Re, eluent back stream; L, water; FAAS, flame atomic absorption spectrometer (nebulizer flow rate was 5.3 mL min^{-1}) and W, waste. (a) System in the pre-concentration step and (b) system in the elution position.

[4] Schematic diagram of the flow system used to preconcentration and determination of lead by FAAS. S, sample; E, eluent; P, peristaltic pump; C, mini-column; V, four-way manual valve; FAAS, flame atomic absorption spectrometer (nebulizer flow rate was 5.0 mL min^{-1}) and W, waste. (a) four-way valve in the preconcentration step and (b) four-way valve in the elution positions.

[5] Schematic diagram of the online system used for cobalt determination and preconcentration by FAAS. S, sample; E, eluent; CR, carrier; P, peristaltic pump; C, minicolumn of NRS-XAD; V_1 and V_2, six-port valves; L, eluent loop; FAAS, flame atomic absorption spectrometer (nebulizer flow rate was 4.0 mL min^{-1}); W, waste. (a) six-port valves in preconcentration position, (a) six-port valves in elution position.

[6] System manifold for flow-injection preconcentration and determination of copper in water samples using functionalized resin as solid-phase: (a) preconcentration step and (b) elution step; S, sample; E, eluent solution; M, mini-column; F AAS, detector, atomic absorption spectrometer; W, waste.

[7] FI manifold and its operation sequence. P1 and P2, peristaltic pumps; MC, micro-column; W, waste; FAAS, flame atomic absorption spectrometer; dotted lines, active. (a) loading position, (b) washing the column, (c) elution position.

[8] On-line preconcentration system. (a) preconcentration stage and (b) elution stage. FAAS, flame atomic absorption spectrometer (aspiration rate of the spectrometer was 6 mL min^{-1}); V, three-way solenoid valve (controlled by a microcomputer); L, open way; D, closed way; MC, minicolumn containing adsorbent; R, sample or eluent back stream; hatched circle, valve on; and white circle, valve off.

Table 2.5 Some parameter for selected FI on-line preconcentration-FAAS systems

	System 1	System 2	System 3	System 4	System 5	System 6	System 7	System 8
Mini-column:								
Column dimension	200 µL (conical)	-	4.5 cm length 4.0 mm i.d	4.5 cm length 4.0 mm i.d	3.5 cm length 4.0 mm i.d	4.0 cm length 4.0 mm i.d	145 µL	5.8 cm length 3.0 mm i.d
Packing amount (mg)	25	-	100	100	100	35	-	130
Chemical variables:								
Sample pH	-	4.75	7.00 (buffer)	7.00 (buffer)	7.5 (buffer)	7.5 (buffer)	0. 5 M HNO_3	10 (buffer)
Eluent	5 mol L^{-1} HCl	1 mol L^{-1} HCl	0.1 mol L^{-1} HCl	0.1 mol L^{-1} HCl	1.0 mol L^{-1} HCl	2 mol L^{-1} HCl	0.03 mol L^{-1} EDTA (pH 10.5)	0.5 or 1 mol L^{-1} HNO_3
Hydrodynamic variables:								
Sample flow rate (mL min^{-1})	8	4.50	2.25	7.0	9.50	6.60	3.9	7.5
Sample volume (mL)	8	13.5	4.5	7	9.5 or 28.5	13.2 or 26.4	2	10
Eluent flow rate (mL min^{-1})	7	2.00	5.00	5.00	4.0	4.90	< 3.8 or > 3	5
Eluent volume (mL)	1	-	-	-	0.03	-	1.4	-
Preconcentration time (sec)	60	180	120	60	60 or 180	120 or 240	30	90
Peak measurements:	height	area	height	height	height	height	height, area	height, area

Burguera et al. [49] developed a fully automated on-line system with an electronically operated time-based injector and used a conical micro-column containing 25 mg of Dowex 50W-X8 (anion exchanger) resin to preconcentrate the copper in natural and drinking waters. A schematic diagram of this system is shown in Figure 2.8 (System 1). The advantages of this system were that time-based injector allow reproducible and sequential introduction of defined sample volumes into flowing systems, the sample flew from the narrower end to the broader of the column can minimize dispersion, and the sample and reagents were not consumed during the stoppage of the sample and eluent, thus their dead volumes were practically eliminated.

Ferreira et al. [50] used a mini-column packed with Amberlite XAD-2 resin loaded with calmagite reagent (1-(1-Hydroxy-4-methyl-2-phenylazo)-2-naphthol-4-sulfonic acid) for chemical sorption of copper in seawater and biological samples. A schematic diagram of this system is shown in Figure 2.8 (System 2). The higher preconcentration factors and lower detection limits can be achieved by increasing sample volumes. However, the more preconcentration time must be used resulting in the reduction of sample throughput.

Moreover, Ferreira et al. [51] also used a mini-column packed with Amberlite XAD-2 resin impregnated with 2-(2-benzothiazolylazo)-2-*p*-cresol (BTAC) to preconcentrate lead in biological samples. A schematic diagram of this system is shown in Figure 2.8 (System 3). This system shows a simple design, since requiring only one two-channel peristaltic pump. In addition, the same column can be used for at least 300 cycles without reconditioning at the end of each cycle, since the samples were buffered with a TRIS buffer solution before preconcentration. Nonetheless, according to the previous work, the mini-column must be reconditioned by percolating the acetate buffer solution for 10 sec.

Lemos et al. [52] developed an on-line preconcentration that used a mini-column packed with polyurethane foam loaded with 2-(2-benzothiazolylazo)-*p*-cresol

(BTAC) reagent for preconcentration of lead in wine samples. The samples were analyzed with digestion and without digestion to evaluate the matrix effect. The extremely low recovery resulted in the case of undigestion indicated that lead was strongly complexed with the neutral chelates, pectic polysaccharides, contained in wine. A schematic diagram of this system is shown in Figure 2.8 (System 4). This system can be used at higher flow rate in both preconcentration and elution steps because polyurethane foam shows low resistance to flow of solutions and does not show any overpressure.

The next publication of Lemos et al. [53] proposed a simpler apparatus with easy manipulation which involved only two steps; elution and preconcentration and used a mini-column packed with Amberlite XAD-2 functionalized with Nitroso R salt for preconcentration of cobalt in natural water samples. The main difference from the previous work was using two six-port valves which contain an accurate volume of eluent in the eluent loop before elution the sorbed cobalt into FAAS. Moreover, the distance between the end of mini-column and the nebulizer was as short as possible (about 5 cm) in order to reduce dispersion. A schematic diagram of this system is shown in Figure 2.8 (System 5). Peak height mode was used for the measurement of the absorbance because it yielded better precision than peak area. This system can significantly improve FAAS performance because it provided enrichment factor higher than many other literatures, including some procedures using detection by GFAAS.

Cassella et al. [54] used a mini-column packed with Styrene-divinylbenzene resin functionalized with (S)-2-[hydroxy-bis-(4-vinyl-phenyl)-methyl]-pyrrolidine-1-carboxylic acid ethyl ester to preconcentrate copper from water samples. A schematic diagram of this system is shown in Figure 2.8 (System 6). The salinity of water samples showed significant effect. When NaCl concentration was equal to or higher than 0.5 mol L^{-1}, decreases in absorbance were observed. So, for the analysis of a sea water (saline sample), the recovery was 79.5%.

Yan et al. [55] developed a simple, sensitive, and selective FI on-line preconcentration and separation FAAS method for routine determination of trace amounts of lead in biological and environmental samples. Based on the selective cavity of the macrocycle molecule, it was immobilized on silica gel and used as the column packing. A schematic diagram of this system is shown in Figure 2.8 (System 7). Three types of potential interferences: competitive preconcentration interferences (e.g., Ba(II), Sr(II), and K(I)) which have an ionic radius similar to Pb(II); competitive elution interferences (e.g., Fe(III), Ni(II), and Cu(II)) which can form stable complexes with EDTA; and interferences in the atomizer from residual matrix which can act as background absorption, were evaluated and the means for their elimination were proposed. The results showed that the last two kinds of interferences can efficiently be eliminated by adding the column washing step before elution, whereas the first one cannot.

Silva et al. [56] used flow injection on-line preconcentration with a mini-column packed with silica gel modified with 3-(1-imidazolyl)propyl groups for the determination of trace levels of copper(II) in an aqueous matrix. A schematic diagram of this system is shown in Figure 2.8 (System 8). The main difference of this system from the other works was in the elution step, the analyte was eluted in reverse direction to minimize its dispersion through the column. Moreover, the calibration graphs were obtained without preconcentration by using the sampling loop which replaced the column, filled with a concentrated solution of the analyte, and directly aspirated to the nebulizer. The measurements of the analytical signals were carried out as peak area and peak height, in order to verify which would be the most adequate mode of the absorption measurement for the proposed system. In the case of peak area, the analysis showed a good recovery. In the case of peak height was more subject to matrix interference, however, it provided the higher enrichment factors.

The more example of various FI on-line preconcentration coupled with FAAS systems are reported in Table 2.6.

Table 2.6 Comparison of performance for FI on-line preconcentration-FAAS systems

Analyte	Sorbent	Enrichment factor	LOD ($\mu g\ L^{-1}$)	Sample throughput (h^{-1})	Refernce
	Column packings based on polymer supports				
Cu	Dowex 50W-X8 (anion exchanger) resin	25-60	0.6-1.5	15-30	[49]
Cu	Amberlite XAD-2 resin loaded Calmagite	32	0.15	20	[50]
Pb	Amberlite XAD-2 impregnated 2(-2-benzothiazolylazo)-2-p-cresol (BTAC)	27	3.7	26	[51]
Pb	Polyurethane foam loaded 2(-2-benzothiazolylazo)-2-p-cresol (BTAC)	26	1	48	[52]
Co	Amberlite XAD-2 functionalized with Nitroso R salt	79 or 223	1.25 or 0.39	51 or 18	[53]
Cu	Styrene-divinylbenzene resin functionalized with (S)-2-[hydroxy-bis-(4-vinyl-phenyl)-methyl]-pyrrolidine-1-carboxylic acid ethyl ester	21	1.1 or 0.93	25 or 13	[54]
Cu	PTFE (chelating agent: ammonium pyrrolidine dithiocarbamate, APDC)	340	0.05	40	[57]
Pb	PTFE (chelating agent: ammonium pyrrolidine dithiocarbamate, APDC)	330	0.8	15	[58]
	Column packings based on silica gel supports				
Pb	Macrocycle molecule immobilized silica gel	52	5	63	[55]
Cu	Silica gel organofunctionalized with 3(1-imidazolyl)propyl	19.5-25.8 or 36.2-42.2	0.4 or 0.2	27	[56]
Cd	2-Aminothiazole modified silica gel	51	0.38	-	[59]
Ag, Au, Pd	Amidinothioureido-silica gel	-	1.1-17	-	[60]
Cu, Cd, Co	C_{18}-silica (chelating agent: 1,10-Phenantroline)	22-32	0.3-6.0	90	[61]
Cr(III), Cr(VI), Cr(total)	C_{18}-silica (chelating agent: chelating agent: diethyldithiocarbamate, DDTC)	90-500	20	30	[62]
Co	C_{18}-silica (chelating agent: 1-Nitroso-2-naphthol, NN)	17.2	3.2	90	[63]

From the literatures above, FI on-line preconcentration-FAAS systems were developed for the determination of trace heavy metals using mini-column for its preconcentration and separation from various samples. The proposed systems offer numerous advantages, especially improvement in the detection limit that affects the accuracy and precision of analysis. The manifold design can be quite simple, requiring only a pump and valve system which deliver the solutions into the nebulizer for detection. Since the detector measures continuously, the concentration of analyte in the detector stream could be monitored over time as peak signal. The column packing should have appropriate mechanical, physical and chemical properties, high selectivity, and good kinetics of analyte sorption and elution.

CHAPTER III

EXPERIMENTAL SECTIONS

3.1 Analytical instruments

The apparatus used in this work were supported by Environmental Analysis Research Unit (EARU), Department of Chemistry, The Scientific and Technological Research Equipment Centre, and The Petroleum and Petrochemical College, Chulalongkorn University.

Analytical instruments for characterization of 1,8-DAAQ

The chelating agent was characterized by ^{1}H-NMR spectroscopy (Varian Mercury Plus 400), FT-IR spectroscopy (Nicolet Impact 410) with KBr pellet technique in the range of 4000-400 cm^{-1}, mass spectrometry in ESI mode (Micromass Platform II HP 1050), UV-vis spectrophotometry (HP 8453, Hewllet Packard), and elemental analysis (PE 2400 Series II, Perkin-Elmer) in order to determine its molecular structure, functional groups, molecular weight, chromophore, and amounts of carbon, hydrogen and nitrogen, respectively.

Analytical instruments for characterization of SiAP and SiDAAQ

The specific surface area of modified silica gels were determined by the BET (Brunauuer, Emmett, Teller) method based on N_2 adsorption using a Quantachrome Autosorb-1. Amount of carbon, hydrogen, and nitrogen of functionalized silica gel were determined by a CHNS/O analyzer model PE 2400 Series II (Perkin-Elmer). Infrared spectra were obtained on a Nicolet Impact 410 and DR-FT-IR spectrometer model Nicolet Nexus 670. Raman spectra were recorded on a FT-raman spectrometer model Spectrum GX (Perkin-Elmer). Solid-state ^{13}C-NMR spectra were recorded on the DPX-300 Bruker Biospin. The functionalized silicas were also characterized by DR-UV-vis spectrophotometry (UV-2500PC, Shimadzu) and thermogravimetry (STA409, Netzsch).

Flame atomic absorption spectrometer

Metal concentrations were determined by a flame atomic absorption spectrometer model AAnalyst 100 (Perkin-Elmer). The instrumental parameters are listed in Table 3.1.

Table 3.1 FAAS conditions for determination of metals concentrations in solutions

Operating conditions	**Cd**	**Co**	**Cu**	**Ni**	**Pb**	**Zn**
Wavelength (nm)	228.8	240.7	324.8	232.0	283.3	213.9
Slit width (nm)	0.7	0.2	0.7	0.2	0.7	0.7
Lamp type	HCL	HCL	HCL	HCL	HCL	HCL
Lamp current (mA)	4	30	15	25	10	15
C_2H_2 flow-rate (mL min^{-1})	3	3	3	3	3	3
Air flow-rate (mL min^{-1})	10	10	10	10	10	10

On-line preconcentration system

An on-line preconcentration system was used for the preconcentration and determination of metal ions in sample solution. The main components in the part of a flow system were a peristaltic pump fitted with Tygon® tubes to deliver all solutions, two Rheodyne® low pressure switching six-port valves (0.8 mm bore size) to select the extraction and elution positions, a mini-column packed with SiDAAQ to preconcentrate the sample and an eluent loop. The manifold was built up with a

Teflon® tube with 0.5 mm internal diameter and directly connected to the nebulizer of a flame atomic absorption spectrometer. All Flange-Free Plus™ fitting (with stainless steel bushing and PEEK ferrule) and adapter (with 6.4 mm internal screw thread) were supplied from Ligand Scientific and Perkin Elmer. In the spectrometer part, the analytical signal was measured in peak height mode with read time of 15 sec using FAAS software. The nebulizer flow rate was adjusted at 5.0 mL min^{-1}.

3.2 Chemicals

All chemicals were of analytical grade and listed in Table 3.2. They were used without further purification unless otherwise noted.

Table 3.2 Chemicals list

Chemicals	**Supplier**
3-Aminopropyltriethoxysilane	MERCK
Calcium hydride	Fluka
Calcium nitrate tetrahydrate	Fluka
Copper nitrate trihydrate	Fluka
Copper powder, 300 mesh	Sigma-Aldrich
1,8-Dichloroanthraquinone	Aldrich
Dichloromethane	MERCK
Dimethyl sulfoxide-D6	MERCK
Ethanol	MERCK
Ether	MERCK
Ethylenediaminetetraacetic acid disodium salt dihydrate	Fluka
Hydrochloric acid 37%	MERCK
Magnecium nitrate hexahydrate	Fluka

Table 3.2 (continue)

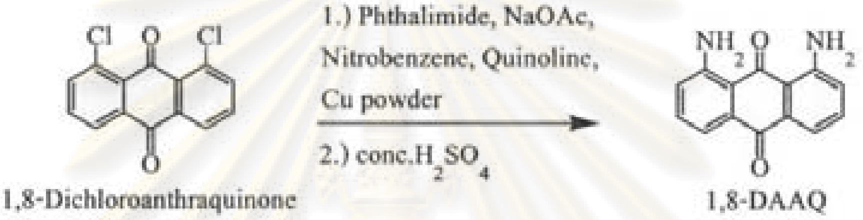
Nitric acid 65%	MERCK
Nitrobenzene	MERCK
Phthalimide	Fluka
Potassium nitrate	AJAX CHEMICALS
Quinoline	Fluka
Silica gel, 70-230 mesh, 60 Å pore diameter	MERCK
Sodium acetate anhydrous	Sigma-Aldrich
Sodium chloride	CARLO ERBR
Sodium hydroxide	MERCK
Sodium nitrate	CARLO ERBR
Sodium sulphate	CARLO ERBR
Sulfuric acid 95-97%	MERCK
Toluene[a]	Fisher Scientific
Cd standard solution (1000 mg L^{-1})	BDH SpectrosoL®
Co standard solution (1000 mg L^{-1})	BDH SpectrosoL®
Cu standard solution (1000 mg L^{-1})	BDH SpectrosoL®
Ni standard solution (1000 mg L^{-1})	BDH SpectrosoL®
Pb standard solution (1000 mg L^{-1})	BDH SpectrosoL®
Zn standard solution (1000 mg L^{-1})	BDH SpectrosoL®

[a] The elimination of the moisture in toluene was performed by drying over calcium hydride and freshly distilled under nitrogen atmosphere prior to use.

3.3 Immobilization of 1,8-diaminoanthraquinone onto silica gel

3.3.1 Preparation of 1,8-diaminoanthraquinone (1,8-DAAQ)

The 1,8-DAAQ was prepared as in dissertation of Keawwangchai S. [64]. The synthetic pathway of 1,8-DAAQ is shown in Scheme 3.1.



Scheme 3.1 Synthetic pathway of 1,8-DAAQ.

A mixture of 1,8-dichloroanthraquinone (20.1 g, 72.5 mmol), phthalimide (26.4 g, 179.4 mmol), sodium acetate anhydrous (14.8 g, 180.4 mmol) and nitrobenzene (3.9 mL) in a 250 mL round bottom flask was stirred and heated to 180 °C for 1 h on a sand bath. Quinoline (12.5 mL) and copper powder (0.5 g) were added, and the mixture was heated at 200 °C for 2 h on the sand bath. The reaction mixture was allowed to cool and left to stand overnight. The mixture was filtered off, and the solid was washed with nitrobenzene (3×50 mL), ethanol (3×50 mL), hot water (3×100 mL), ethanol (2×50 mL) and ether (2×50 mL) and dried. The solid as a pale-yellow/orange was obtained. The crude solid (28.0 g) was added to concentrated H_2SO_4 (200 mL) with stirring in a 250 mL round bottom flask connected to a condenser to prevent the outgoing acid vapor and the mixture was heated at 95 °C for 45 min on the sand bath. The reaction mixture was cooled to 5 °C in an ice bath and crushed ice (150 g) was slowly added into the flask. The mixture was poured onto ice/water beaker (1.5 L) with stirring, and the precipitate was obtained. The mixture was filtered using filter paper (glass microfibre filter for acid). The solid was washed with water until the pH of the filtrate become neutral and dried under vacuo. The product was purified by recrystallization in ethanol, the product as red/purple needles (13.5 g, 98%) was obtained. The product was kept in a desiccator.

Characterization data for 1,8-DAAQ

Melting point: 270-271 °C

^{1}H-NMR spectrum (DMSO-d_6, 400 MHz): δ (in ppm) 7.15 (dd, J = 8.5 and 1.4 Hz, 2H, H_a), 7.34 (dd, J = 7.4 and 1.4 Hz, 2H, H_c), 7.45 (dd, J = 8.5 and 7.4 Hz, 2H, H_b) and 7.86 (br, 4H, NH_2)

FT-IR spectrum (KBr pellet): wavenumber (in cm^{-1}) 3450 and 3500 (N–H stretching of primary amine), 3100 (C=C–H stretching of aromatic), 1650 (N–H bending of primary amine), 1610 and 1500 (C=C stretching of aromatic) and 1600 (C=O stretching) 1250 (C–N stretching of primary amine)

ESI mass spectrum for $C_{14}H_{10}N_2O_2 + H^+$

Anal calc: m/z = 239.082

Found: m/z = 239.007

UV-vis spectrum: λ (in nm) 279, 303 and 513

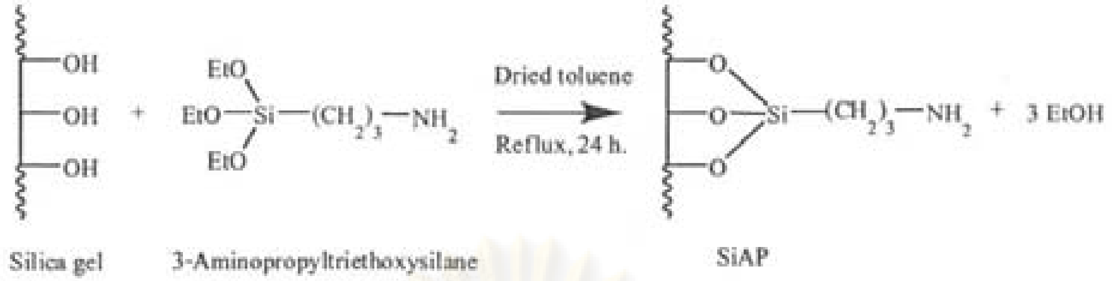
Elemental analysis for $C_{14}H_{10}N_2O_2$

Anal calc: %C 70.58, %H 4.23 and %N 11.76

Found: %C 66.68, %H 4.05 and %N 11.06

3.3.2 Preparation of 3-aminopropyltriethoxysilane linked to silica gel (SiAP)

The preparation of SiAP was carried out following the literature procedure [36]. The synthetic pathway of SiAP is shown in Scheme 3.2.



Scheme 3.2 Synthetic pathway of SiAP.

Silica gel (25.0 g) suspended in 200 mL of dry toluene in a 250 mL two-necked round bottom flask was refluxed and stirred under nitrogen atmosphere for 1 h on the sand bath. To this suspension, 10 mL of 3-aminopropyltriethoxysilane was slowly added dropwise. The mixture was refluxed and stirred under nitrogen atmosphere for 24 h. The solid was filtered, washed consecutively with ethanol and dichloromethane. This organofunctionalized silica gel, named SiAP, was dried in vacuum at room temperature and kept in the desiccator.

Characterization data for SiAP

FT-IR spectrum (KBr pellet): wavenumber (in cm^{-1}) 2950 (w, C–H stretching of alkane)

FT-Raman spectrum: wavenumber (in cm^{-1}) 2900 (s, C–H stretching of alkane) and 3300 (m, N–H stretching of primary amine)

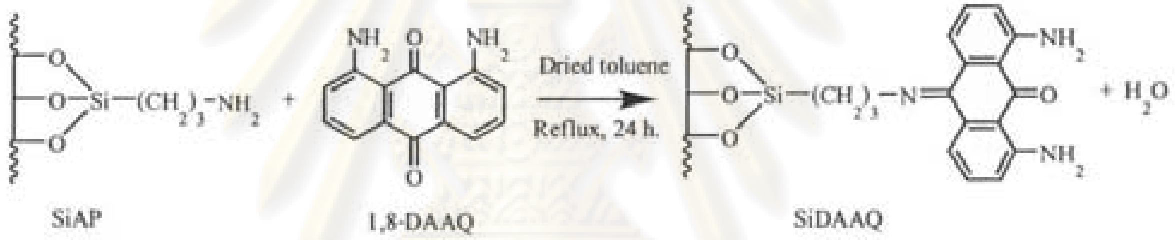
Elemental analysis: %C 5.57, %H 1.66 and %N 2.19

^{13}*C-NMR spectrum* (solid state): δ (in ppm) 10.39, 22.97 and 43.03

Specific surface area analysis: 272.25 m^2 g^{-1}

3.3.3 Preparation of silica gel chemically modified with 1,8-DAAQ (SiDAAQ) via Schiff's base bond

A mixture of SiAP (25.0 g) and 1,8-DAAQ (1.0 g) were suspended in 200 mL of dry toluene in a two-necked round bottom flask, refluxed and stirred under nitrogen atmosphere for 24 h on the sand bath. The solid was filtered and the excess chelating agent was eliminated by washing with ethanol and dichloromethane, consecutively. The silica gel surface containing the immobilized 1,8-DAAQ, named SiDAAQ, was dried in vacuum at room temperature and kept in the desiccator. The synthetic pathway of SiDAAQ is shown in Scheme 3.3.



Scheme 3.3 Synthetic pathway of SiDAAQ.

Characterization data for SiDAAQ

FT-IR spectrum (KBr pellet): wavenumber (in cm^{-1}) 2950 (w, C–H stretching of alkane)

DR-FT-IR spectrum: wavenumber (in cm^{-1}) 1545 (w, C=N stretching)

FT-Raman spectrum: wavenumber (in cm^{-1}) 2900 (s, C–H stretching of alkane) and 3300 (m, N–H stretching of primary amine)

Elemental analysis: %C 5.74, %H 1.32 and %N 2.53

DR-UV-vis spectrum: λ (in nm) 502

^{13}*C-NMR spectrum* (solid state): δ (in ppm) 9.95, 22.20 and 43.27

Specific surface area analysis: 292.52 m^2 g^{-1}

3.4 Preparation of solutions

All solutions were prepared by using deionized water and stored in PE bottle.

Metal solutions

The metal solutions of Cd(II), Co(II), Cu(II), Ni(II), Pb(II) and Zn(II) were prepared by stepwise dilution of 1000 mg L^{-1} stock standard solution to the required concentrations. The pH of solution was adjusted by nitric acid and sodium hydroxide solutions. The calibration curves were prepared in range of 0-2.5 mg L^{-1}, 0-5.0 mg L^{-1}, 0-5.0 mg L^{-1}, 0-5.0 mg L^{-1}, 0-25.0 mg L^{-1} and 0-2.5 mg L^{-1} for Cd(II), Co(II), Cu(II), Ni(II), Pb(II) and Zn(II), respectively.

Nitric acid solutions

Nitric acid solutions (1% and 5% v/v) were prepared daily by direct dilution from the concentrated solution.

Sodium hydroxide solutions

Sodium hydroxide solutions (1% and 5% w/v) were prepared daily by dissolving the appropriate amount of NaOH in deionized water.

Interfering ions solutions

The solution containing cations such as Na^+, K^+, Ca^{2+}, Mg^{2+} and Cu^{2+} and the anions such as Cl^-, NO_3^- and SO_4^{2-} at 10, 100 and 1000 mg L^{-1} were prepared by dissolving the appropriate amount of NaCl, $NaNO_3$, Na_2SO_4, KNO_3, $Ca(NO_3)_2{\cdot}4H_2O$, $Mg(NO_3)_2{\cdot}6H_2O$ and $Cu(NO_3)_2{\cdot}3H_2O$ in deionized water.

EDTA solutions

EDTA solutions were prepared by dissolving the appropriate amount of $C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$ in deionized water and were used without pH adjustment.

3.5 Adsorption of metal ions onto SiDAAQ

The properties of metal adsorption by SiDAAQ should be evaluated before on-line preconcentration study when the new sorbent was synthesized. Thus, the experiment was divided into two methods: batch and column methods. The batch method was used for the extraction study only, while the column method was used for the extraction, elution and preconcentration study.

3.5.1 Batch method

The effect of various parameters such as pH of solution, extraction time, sorption capacity and interfering ions on extraction efficiency was investigated by batch method, and all experiments were performed in triplicate. The experiment in batch method is illustrated in Figure 3.1.

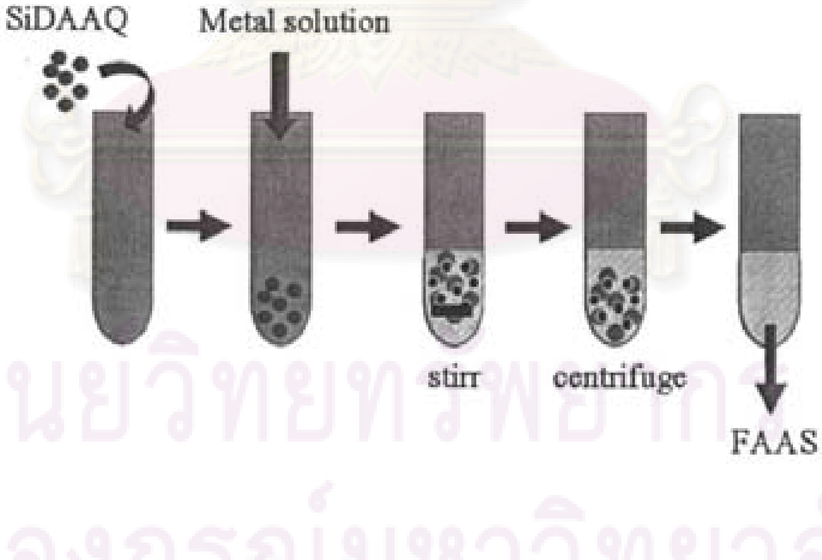


Figure 3.1 Batch method.

3.5.1.1 pH of solution

A suspension of 10 mg of SiDAAQ in 5.0 mL of metal solution at a concentration of 5.0 mg L^{-1} for Cd(II), 10.0 mg L^{-1} for Co(II), 10.0 mg L^{-1} for Cu(II), 10.0 mg L^{-1} for Ni(II), 15.0 mg L^{-1} for Pb(II) and 10.0 mg L^{-1} for Zn(II) under pH ranging from 2.0 to 7.0 in a test tube was stirred at room temperature for 30 min. The pH of solution was adjusted with various concentration of NaOH and HNO_3 solutions. The sorbent was separated by centrifugation at 3500 rpm for 5 min. Residual metal concentration of the supernatant was determined by FAAS.

3.5.1.2 Extraction time

A suspension of 10 mg of SiDAAQ in 5.0 mL of metal solution (after adjusting to optimum pH) at a concentration of 5.0 mg L^{-1} for Cd(II), 10.0 mg L^{-1} for Cu(II), 15.0 mg L^{-1} for Pb(II) and 10.0 mg L^{-1} for Zn(II) in a test tube was stirred at room temperature at different extraction time in the rang of 5-60 min. The sorbent was separated by centrifugation at 3500 rpm for 5 min. Residual metal concentration of the supernatant was determined by FAAS.

3.5.1.3 Sorption capacity

A suspension of 10 mg (or 5 mg for Pb(II)) of SiDAAQ in 5.0 mL of metal solution of which the concentration was varied between 5.0-100.0 mg L^{-1} under optimum pH in a test tube was stirred at room temperature for 30 min, and the temperature was controlled at 298 ± 1 K. The sorbent was separated by centrifugation at 3500 rpm for 5 min. Residual metal concentration of the supernatant was determined by FAAS.

3.5.1.4 Adsorption isotherm

Langmuir and Freundlich isotherms were used to analyze the adsorption data from section 3.5.1.3. The information that obtained from these isotherms was used to describe the adsorption phenomena on heterogeneous surface such as solid-liquid interface.

Langmuir adsorption isotherm

Langmuir isotherms is described by equation 3.1:

$$N_f = \frac{N_f^s bC}{1 + bC} \tag{3.1}$$

The Langmuir equation can be rearranged to linear form for the convenience of plotting and determining the wanted parameters, according to equation 3.2:

$$\frac{C}{N_f} = \frac{C}{N_f^s} + \frac{1}{bN_f^s} \tag{3.2}$$

where C = the concentration of metal ion solution at equilibrium (mol L^{-1})

N_f = the amount of metal adsorbed per gram of sorbent (mol g^{-1})

b = the Langmuir constant related to energy of adsorption (L mol^{-1})

N_f^s = the maximum amount of metal adsorbed per gram of sorbent (mol g^{-1})

When the $\frac{C}{N_f}$ as a function of C is plotted, the linear curve can be obtained. This curve yields the $slope = \frac{1}{N_f^s}$ and the $Y\text{-}intercept = \frac{1}{b \times N_f^s}$, thus, the maximum sorption capacity (N_f^s) and Langmuir constant ($b = \frac{K_{eq}}{a}$) are calculated by the $N_f^s = \frac{1}{slope}$ and the $b = \frac{1}{N_f^s \times Y - \text{int}\,ercept}$, respectively.

Freundlich adsorption isotherm

Freundlich isotherms is described by equation 3.3:

$$N_f = KC^{1/n} \tag{3.3}$$

The Freundlich equation can be linearized in logarithmic form for the convenience of plotting and determining the wanted parameters, according to equation 3.4:

$$\log N_f = \log K + \left(\frac{1}{n}\right)\log C \tag{3.4}$$

where C = the concentration of metal ion solution at equilibrium (mol L^{-1})

N_f = the amount of metal ion per gram of sorbent (mol g^{-1})

n = the Freundlich constant related to adsorption intensity

K = the Freundlich constant related to adsorption capacity

When the log N_f as a function of log C is plotted, the linear curve can be obtained. This curve yields the $slope = \frac{1}{n}$ and the $Y\text{-}intercept$ = log K thus, the Freundlich constant are calculated by the $n = \frac{1}{slope}$ and the $K = 10^{Y\text{-}intercept}$, respectively.

3.5.1.5 Effect of interfering ions

A suspension of 10 mg of SiDAAQ in 5.0 mL of Pb(II) at a concentration of 15.0 mg L^{-1} containing the interfering ions at 10, 100 or 1000 mg L^{-1} in a test tube was stirred at room temperature for 30 min. The sorbent was separated by centrifugation at 3500 rpm for 5 min. Residual metal concentration of the supernatant was determined by FAAS.

3.5.2 Column method

The column method was investigated to obtain the optimum conditions for extraction and elution of Pb(II). The effects of sample flow rate, eluent flow rate, concentration of eluent and sample volume were evaluated in triplicate. Moreover, the reuse of a mini-column was monitored in order to ensure that the sorbent still has the good extraction efficiency for several time. The process in column method consists of four steps: condition, extraction, washing and elution. In each step, the solutions were controlled by a peristaltic pump fitted with Tygon® tube. The experiment in column method is illustrated in Figure 3.2.

A laboratory-made mini-column (2.0 cm length and 5.0 mm i.d) was packed with 10 mg of SiDAAQ. Cotton was placed at both ends of the mini-column to prevent the sorbent losses. The mini-columns were used only one time, except the experiment in section 3.5.2.4.

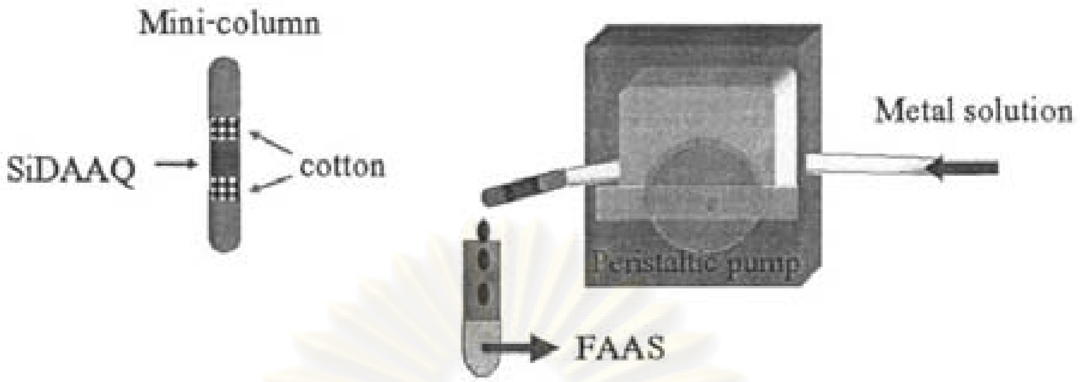


Figure 3.2 Column method.

3.5.2.1 Sample flow rate

5.0 mL of 5.0 mg L^{-1} Pb(II) solution at pH 6.0 was passed through a mini-column packed with SiDAAQ at different flow rates from 0.5-5.0 mL min^{-1} controlled with a peristaltic pump. The residual Pb(II) concentration of the effluent was determined by FAAS.

3.5.2.2 Effect of eluent

Eluent flow rate

5.0 mL of 5.0 mg L^{-1} Pb(II) solution at pH 6.0 was passed through a mini-column packed with SiDAAQ at a flow rate of 5.0 mL min^{-1} controlled with a peristaltic pump. The elution of sorbed Pb(II) from SiDAAQ was investigated using 5.0 mL of 0.100 mol L^{-1} EDTA passed through the mini-column at different flow rate in the range of 0.5-5.0 mL min^{-1}. The residual Pb(II) concentration of the effluent and the concentration of Pb(II) in the eluate were determined by FAAS.

Concentration of eluent

5.0 mL of 5.0 mg L^{-1} Pb(II) solution at pH 6.0 was passed through a mini-column packed with SiDAAQ at a flow rate of 5.0 mL min^{-1} controlled with a peristaltic pump. The sorbed Pb(II) was eluted by varying the concentration of EDTA in the range of 0.005-0.100 mol L^{-1} (5.0 mL) at a flow rate of 5.0 mL min^{-1}. The residual Pb(II) concentration of the effluent and the concentration of Pb(II) in the eluate were determined by FAAS.

3.5.2.3 Sample volume

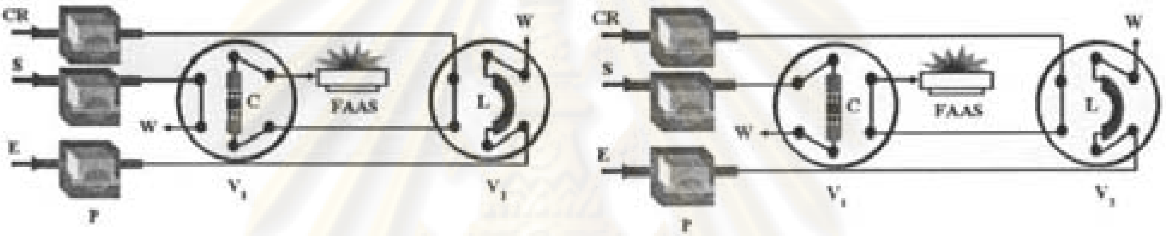
Sample solutions of different volumes (5-1000 mL) spiked with 250 µL of 80 mg L^{-1} Pb(II) solution were adjusted pH to 6.0 and then passed through a mini-column packed with SiDAAQ at a flow rate of 5.0 mL min^{-1} controlled with a peristaltic pump. The sorbed Pb(II) was eluted by 5.0 mL of 0.050 mol L^{-1} EDTA at a flow rate of 5.0 mL min^{-1}. The concentration of Pb(II) in the eluate was determined by FAAS.

3.5.2.4 Reuse of mini-column

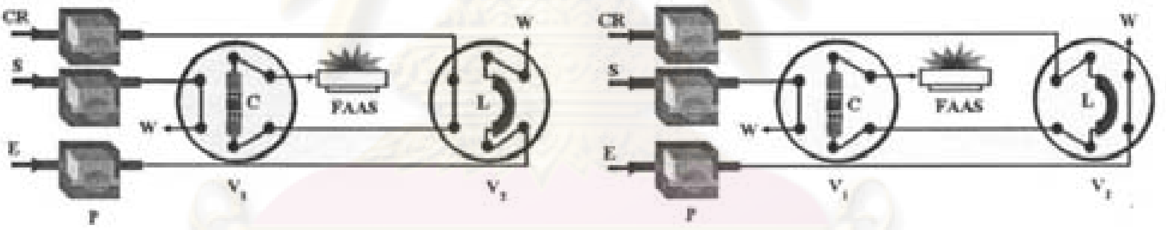
5.0 mL of 5.0 mg L^{-1} Pb(II) solution at pH 6.0 was passed through a mini-column packed with SiDAAQ at a flow rate of 5.0 mL min^{-1} controlled with a peristaltic pump. The sorbed Pb(II) was eluted by 5.0 mL of 0.050 mol L^{-1} EDTA at a flow rate of 5.0 mL min^{-1}. The same process was repeated for 25 cycles. The residual Pb(II) concentration of the effluent and the concentration of Pb(II) in the eluate were determined by FAAS.

3.6 On-line preconcentration study

The on-line preconcentration of the Pb(II) solution was investigated with on-line preconcentration-FAAS system. This system was composed of a flow system coupled with a flame atomic absorption spectrometer which has a function of preconcentration and determination, respectively. The diagram of the on-line preconcentration-FAAS system is shown schematically in Figure 3.3.


(a) six-port valves in *condition* position

(b) six-port valves in *extraction* position


(c) six-port valves in *washing* position

(d) six-port valves in *elution* position

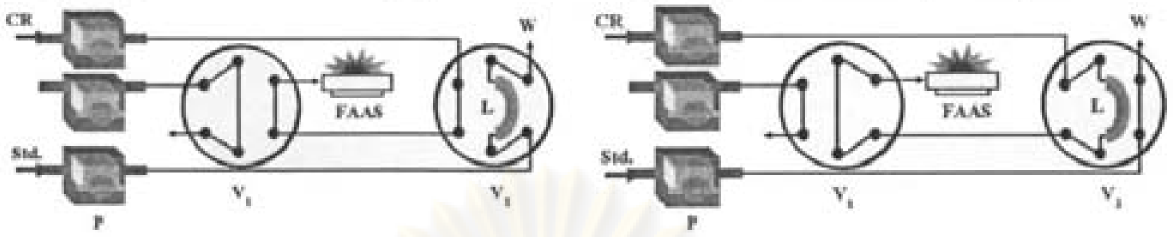
Figure 3.3 Schematic diagram of the on-line system used for Pb(II) preconcentration and determination (Redrawn from ref [53]). S = sample; E = eluent (EDTA solution); CR = carrier (deionized water); P = peristaltic pump; C = mini-column packed with SiDAAQ; V_1 and V_2 = six-port valves; L = eluent loop 174 µL; FAAS = flame atomic absorption spectrometer; W = waste.

The procedures in on-line preconcentration-FAAS system consist of four steps:

1) Condition: Turning and holding a V_1 about 15 sec, the carrier was passed through the mini-column. In this step, the surface of SiDAAQ was conditioned in order to activate to bind with Pb(II).
2) Extraction: A solution containing Pb(II) at optimum pH was continuously pumped through the mini-column. Pb(II) was extracted on the mini-column and the effluent flows towards waste. Carrier was taken directly to the nebulizer of the flame atomic absorption spectrometer while the eluent is filling eluent loop and flowing to waste.
3) Washing: Turning and holding a V_1 about 15 sec, the carrier was passed through the mini-column. In this step, the interfering species and the unbounded Pb(II) were washed in order to ensure that only the extracted Pb(II) was eluted and determined.
4) Elution: When turn two valves simultaneously, the carrier transports the eluent through the mini-column. The retained Pb(II) in the mini-column was eluted and the eluate was taken directly to the nebulizer of the flame atomic absorption spectrometer

The procedures for obtaining calibration curves without preconcentration are shown in Figure 3.4 and consist of two steps:

1) Loading: A standard Pb(II) solution was continuously pumped through the eluent loop and flowing to waste. While the carrier was taken directly to the nebulizer of the flame atomic absorption spectrometer.
2) Injecting: When turn two valves simultaneously, the carrier transports the standard Pb(II) solution directly to the nebulizer of the flame atomic absorption spectrometer



(a) six-port valves in *loading* position (b) six-port valves in *injection* position

Figure 3.4 Schematic diagram of the on-line system used for obtaining calibration curves without preconcentration. Std. = standard Pb(II) solution; CR = carrier (deionized water); P = peristaltic pump; V_1 and V_2 = six-port valves; L = eluent loop 174 μL; FAAS = flame atomic absorption spectrometer; W = waste.

In this part, the on-line preconcentration was studied by using spiked samples with standard Pb(II) and operated according to Figure 3.3. The effect of various parameters such as sample flow rate, eluent flow rate, concentration of eluent and sample volume on preconcentration capability was investigated. All experiments were performed in triplicate. Moreover, the lowest concentration level of Pb(II) that can be preconcentrated with this system was considered.

3.6.1 Sample flow rate

A solution (5.0 mL) containing 300 μg L^{-1} Pb(II) at pH 6.0 was passed through an on-line preconcentration system at different flow rates from 2.0-6.0 mL min^{-1}. The retained Pb(II) was eluted by 174 μL of 0.100 mol L^{-1} EDTA at a flow rate of 5.0 mL min^{-1}. The concentration of Pb(II) in the eluate was determined by FAAS. This experiment was performed in triplicate.

3.6.2 Effect of eluent

Eluent flow rate

A solution (5.0 mL) containing 300 μg L^{-1} Pb(II) at pH 6.0 was passed through an on-line preconcentration system at an optimum flow rate. The elution of retained Pb(II) from SiDAAQ was investigated using 174 μL of 0.100 mol L^{-1} EDTA at different flow rate in the range of 4.0-7.0 mL min^{-1}. The concentration of Pb(II) in the eluate was determined by FAAS. This experiment was performed in triplicate.

Concentration of eluent

A solution (5.0 mL) containing 300 μg L^{-1} Pb(II) at pH 6.0 was passed through a on-line preconcentration system at an optimum flow rate. The retained Pb(II) was eluted by varying the concentration of EDTA in the range of 0.010-0.250 mol L^{-1} (174 μL) at a flow rate of 5.0 mL min^{-1}. The concentration of Pb(II) in the eluate was determined by FAAS. This experiment was performed in triplicate.

3.6.3 Sample volume

A solution of 5.0 and 10.0 mL containing 300 μg L^{-1} Pb(II) at pH 6.0 was passed through an on-line preconcentration system at an optimum flow rate. The retained Pb(II) was eluted by 174 μL of 0.050 mol L^{-1} EDTA at a flow rate of 5.0 mL min^{-1}. The concentration of Pb(II) in the eluted solution was determined by FAAS. This experiment was performed in triplicate.

3.6.4 Initial concentration of Pb(II) in sample

A solution (5.0 mL) with varying concentration of Pb(II), 10-300 μg L^{-1}, at pH 6.0 was passed through an on-line preconcentration system at a flow rate of 5.0 mL min^{-1}. The retained Pb(II) was eluted by 174 µL of 0.050 mol L^{-1} EDTA at a flow rate of 5.0 mL min^{-1}. The concentration of Pb(II) in the eluted solution was determined by FAAS. This experiment was performed in triplicate.

3.7 Method validation

In order to demonstrate the validity of the proposed on-line preconcentration system, this system was validated under the optimum conditions by using the spiked sample solutions and repeating the same experiment. The validation data that showed the performance characteristics of the proposed system were accuracy, precision, limit of detection and limit of quantitation. Moreover, the method detection limit which was the ability of a measurement method to determine an analyte in a sample matrix was estimated.

In this part, the method validation was performed by using spiked samples with standard Pb(II) at two concentration levels (100 and 300 µg L^{-1}), repeating the same experiment in 10 replicates and operating according to Figure 3.3. The calibration curve was prepared using 0, 3, 6, 9, 12, 15, 18 and 21 mg L^{-1} standard Pb(II) solutions. The procedure is followed:

The sample solutions (5.0 mL) containing deionized water spiked with 50 and 150 µL of 10 mg L^{-1} Pb(II) solution were adjusted pH to 6.0 and then passed through an on-line preconcentration system at a flow rate of 5.0 mL min^{-1}. The retained Pb(II) was eluted by 174 µL of 0.050 mol L^{-1} EDTA at a flow rate of 5.0 mL min^{-1}. The concentration of Pb(II) in the eluted solution was determined by FAAS. The accuracy and precision were calculated as recovery (%) and relative standard deviation (%), respectively. The limit of detection and limit of quantitation were calculated from standard deviation of 10 measurements of a reagent blank (0.050 mol L^{-1} EDTA) using the on-line system without preconcentration.

3.8 Real sample analysis

In order to demonstrate the applicability of the proposed system for determination of Pb(II) in real samples, this system was evaluated by using spiked real samples with standard Pb(II) at two concentration levels (100 and 300 µg L^{-1}), and operating according to Figure 3.3. The calibration curve was prepared at 0, 3, 6, 9, 12, 15, 18 and 21 mg L^{-1}.

3.8.1 Drinking water

The sample solutions (5.0 mL) containing the drinking water spiked 0.45 and 1.35 µg of Pb(II) were adjusted pH to 6.0 and then passed through an on-line preconcentration system at a flow rate of 5.0 mL min^{-1}. The retained Pb(II) was eluted by 174 µL of 0.050 mol L^{-1} EDTA at a flow rate of 5.0 mL min^{-1}. The concentration of Pb(II) in the eluted solution was determined by FAAS. This experiment was performed in 10 replicates. The accuracy and precision were calculated as recovery (%) and relative standard deviation (%), respectively.

3.8.2 Tap water

The experiment was performed in the same manner using the sample solutions (5.0 mL) containing the tap water spiked 0.50 and 1.50 µg of Pb(II).

CHAPTER IV

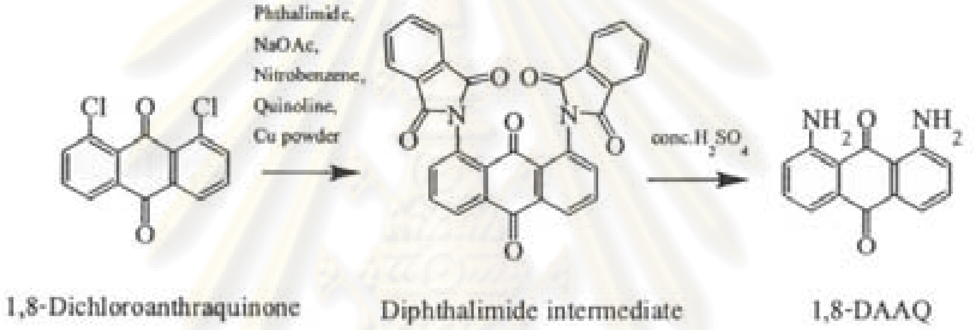
RESULTS AND DISCUSSION

4.1 Synthesis and characterization of 1,8-DAAQ

Diaminoanthraquinone (DAAQ) is an important group of anthraquinone derivatives. They have been used in various applications due to their electrical and optical properties. For example, 1,4-diaminoanthraquinone was used as electron acceptor for bulk heterojunctions photovoltaic devices of organic solar cells [65]. 1,2-Diaminoanthraquinone was used as colorimetric anion sensors that showed dramatic spectral changes when exposed to fluoride, chloride, bromide, iodide, phosphate, and sulfate ions [66]. 1,4,5,8-Tetraaminoanthraquinone was used as fluoride ion chemosensors that showed a high selectivity with fluoride and changed the colour that can be detected by naked eye [67]. However, the research involved with the extraction of metals by those diaminoanthraquinones has been rarely existed. In this work, 1,8-DAAQ was chosen as chelating organic molecule in order to extract the metal ions in solution. This molecule is interesting due to its presence of three donor atoms: two nitrogen and one oxygen and it has the rigid and planar structure which suitably orientes to form metal-ligand complexes. Thus, 1,8-DAAQ is expected to act as tridentate ligand to form selective and stable complex with various metals. Moreover, there has not been any report on the use of silica gel functionalized with 1,8-DAAQ for preconcentration of the metal ions from the sample matrix.

Synthesis

The synthesis of 1,8-DAAQ was carried out following the dissertation procedure [64] via Gabriel synthesis. The reaction was succeeded in two steps. Firstly, the nucleophilic acyl substitution reaction of 1,8-dichloroanthraquinone with phthalimide in the presence of sodium acetate anhydrous, nitrobenzene, quinoline and copper powder was carried out to generate the diphthalimide intermediate. Secondly, the diphthalimide intermediate was reduced to diamino by concentrated H_2SO_4. The proposed mechanism of 1,8-DAAQ is shown in Scheme 4.1.



Scheme 4.1 Proposed mechanism for 1,8-DAAQ [57].

Characterization

The characterization of 1,8-DAAQ was carried out by ^{1}H-NMR spectroscopy (Figure 4.1), FT-IR spectroscopy (Figure 4.2), ESI mass spectrometry (Figure 4.3), UV-vis spectrophotometry (Figure 4.4) and elemental analysis.

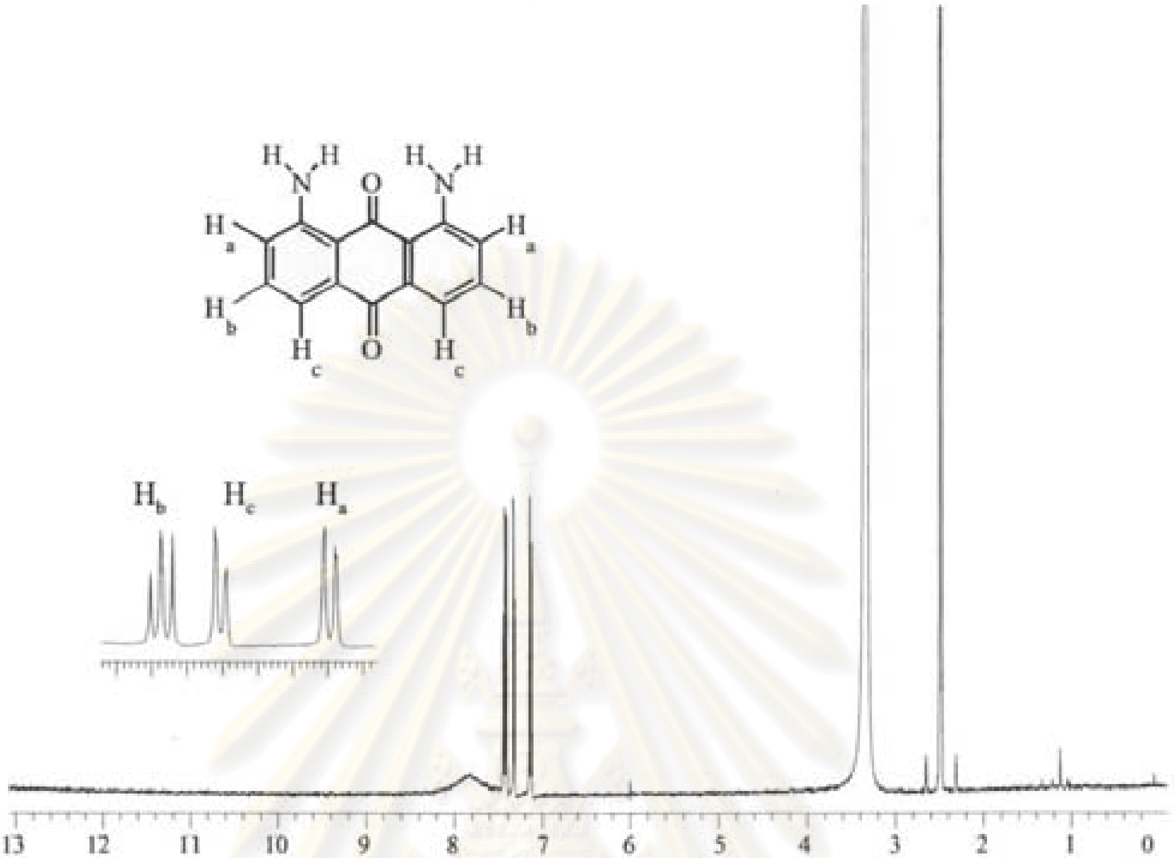


Figure 4.1 ^{1}H-NMR spectrum (DMSO-d_6, 400 MHz) of 1,8-diaminoantharaquinone.

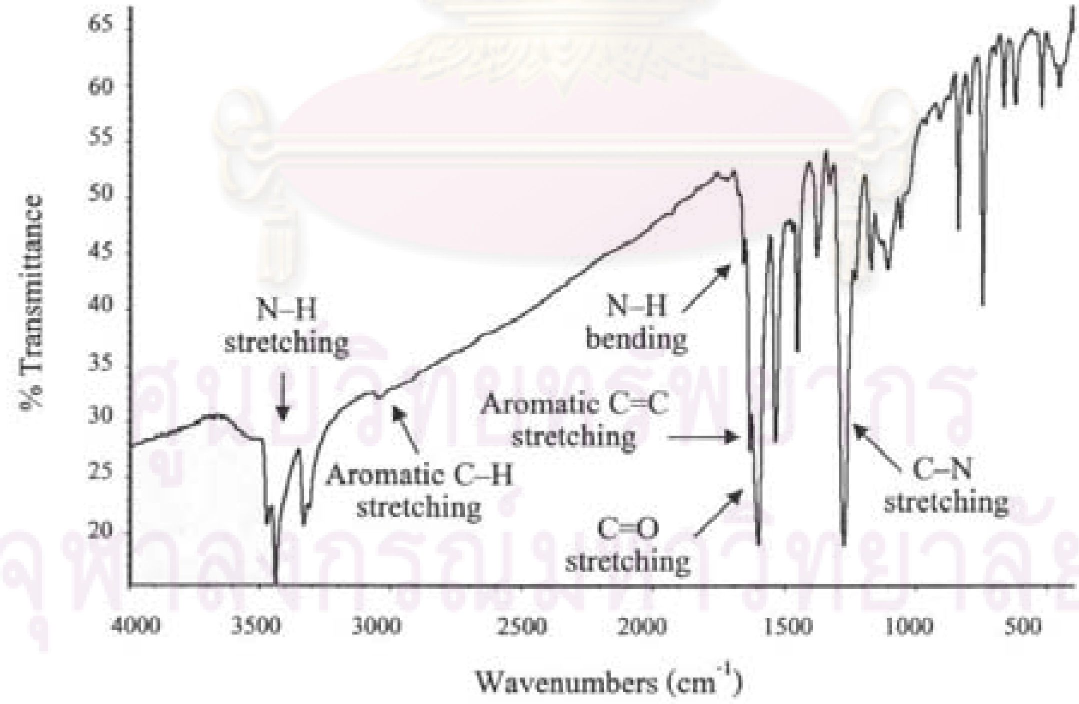


Figure 4.2 FT-IR spectrum (KBr pellet) of 1,8-diaminoantharaquinone.

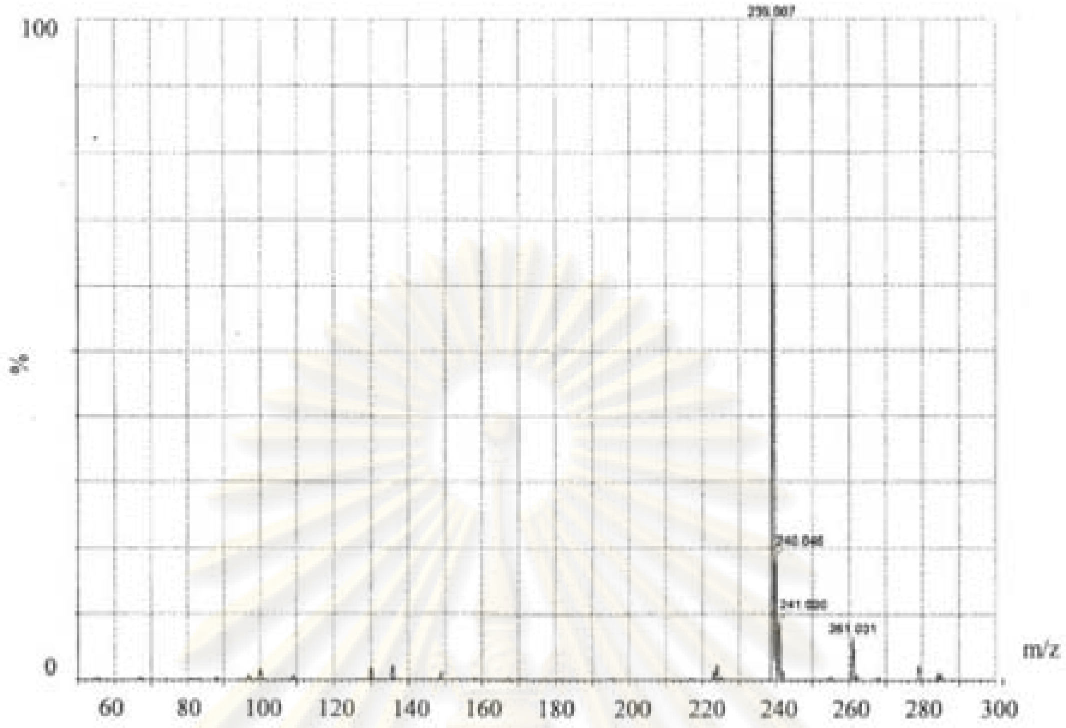


Figure 4.3 ESI mass spectrum of 1,8-diaminoantharaquinone.

(Sample was disloved in ethanol and determined by Micromass Platform quadrupole mass analyzer with an electrospray ion source using acetonitrile as mobile phase.)

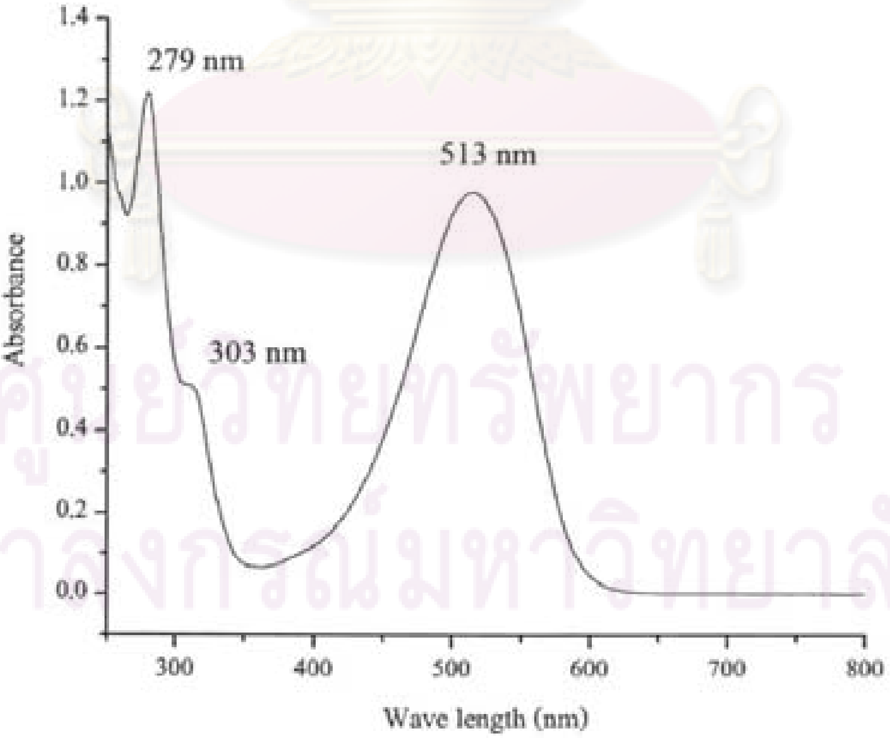
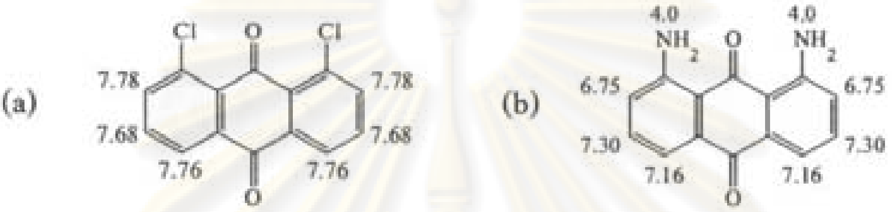


Figure 4.4 UV-vis spectrum (DMSO) of 1,8-diaminoantharaquinone.

The ^{1}H-NMR spectrum of 1,8-diaminoantharaquinone shows the positions of the signals that clearly differs from 1,8-dichloroanthraquinone. Since $-NH_2$ group acts as the electron donating group and –Cl group acts as the electron withdrawing group, the *-ortho* and *-para* protrons of aromatic rings are shielded and deshielded, respectively. Thus the chemical shift of 1,8-dichloroanthraquinone appeared at the higher position than those of 1,8-diaminoantharaquinone.



The chemical shift predicated for (a) 1,8-dichloroanthraquinone and (b) 1,8-diaminoantharaquinone.

Moreover, the $-NH_2$ functional group signal appearing in FT-IR spectrum and UV-vis spectrum provide the supporting information. To identify the molecular weight and C, H and N amounts, ESI mass spectrum and elemental analysis were employed.

The characterization data above indicated that 1,8-DAAQ was successfully synthesized.

4.2 Synthesis and characterization of SiDAAQ

The chemical modification method is generally used in the process of binding the surface of silica gel with a chelating agent. However, with this method, the surface of silica gel is initially modified with a silylating reagent (3-aminopropyltriethoxysilane) in order to generate amino groups which can further participate in chemical reaction with ketone functional group of the chelating agent to yield the Schiff's base (imine) compound. Thus, the immobilization of 1,8-DAAQ onto silica gel was carried out in two steps: silylation [68, 69] and Schiff's base formation [33].

Synthesis

Firstly, the silanol groups (act as nucleophile) on silica gel surface reacted with ethoxy groups (act as leaving group) of 3-aminopropyltrimethoxysilane by silanization or nucleophilic substitution reaction to give the SiAP product. The reaction was prevented the hydrolysis of ethoxy groups of 3-aminopropyltriethoxysilane by using anhydrous condition. Secondly, the synthesis of SiDAAQ was accomplished via Schiff's base formation between amino groups (act as nucleophile) of SiAP and the carbonyl carbon atom (act as electrophile) at 10-position of the anthraquinone ring by addition and condensation reaction, respectively, to give the desired product. Both reactions were performed under nitrogen atmosphere and at the reflux temperature of 110 °C to completely react.

Characterization

The characterization of modified silica gel was carried out by ^{13}C-NMR solid state (Figure 4.5), FT-IR spectroscopy (Figure 4.6), DR-FT-IR spectroscopy (Figure 4.7), FT-Raman spectroscopy (Figure 4.8), elemental analysis (Table 4.2), DR-UV-vis spectroscopy (Figure 4.9), TGA (Figure 4.10) and specific surface area analysis (Table 4.4).

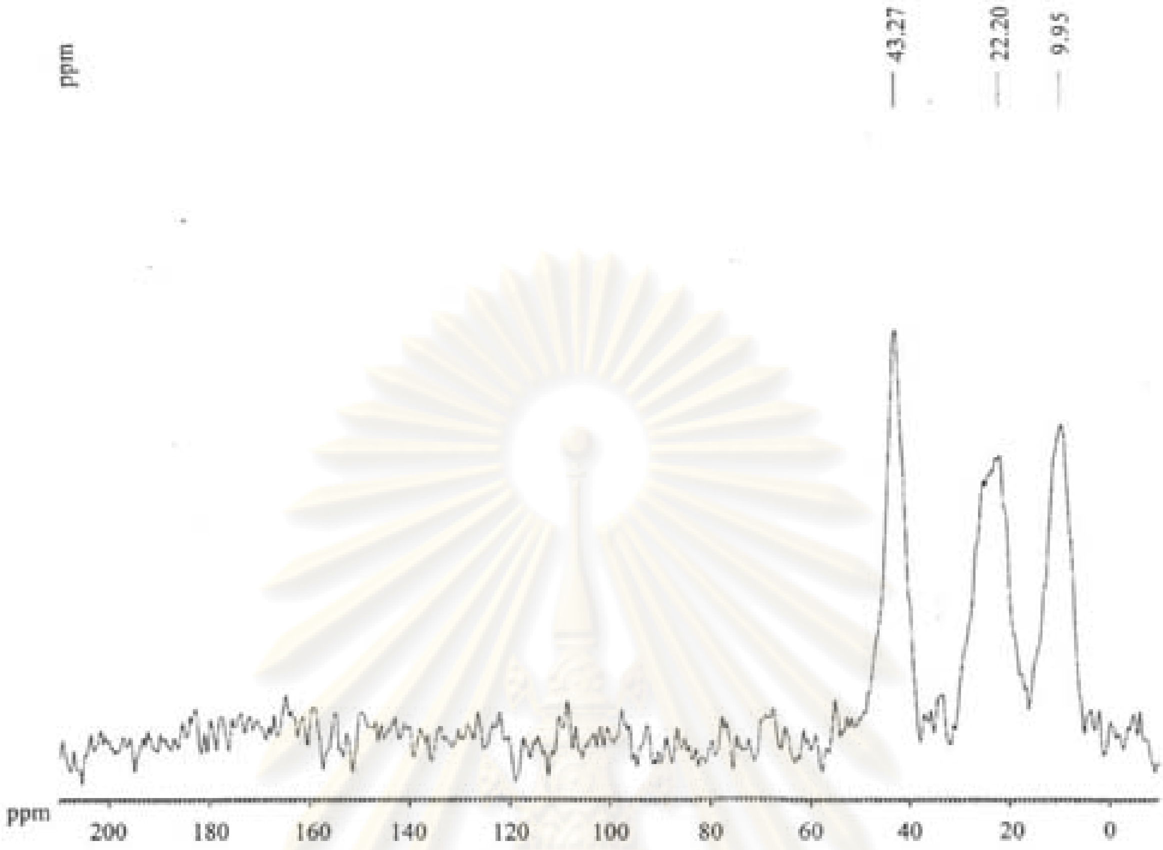


Figure 4.5 ^{13}C-NMR spectrum of SiDAAQ.

The signals at 9.95, 22.20 and 43.27 ppm were observed in ^{13}C-NMR spectrum of SiDAAQ which can be assigned to the propyl carbon: Si–CH_2, –CH_2 and N–CH_2, respectively [7]. The signals of aromatic carbons at the range of 100-140 ppm were not found due to its low intensity in comparison with propyl carbons.

FT-IR spectra of silica gel, SiAP and SiDAAQ (Figure 4.6) show only small difference which was similar to that of Roldan et al. [70]. Because the organic molecules are presented on silca gel surface less than the silica gel functional groups, the absorption bands were mainly occurred from silica gel structure. Characteristic absorption bands found in silica gel are summarized in Table 4.1. However any information in FT-IR spectra below could be obtained. For example, the decreasing in O–H and Si–OH intensities was observed when the silica gel was modified [71].

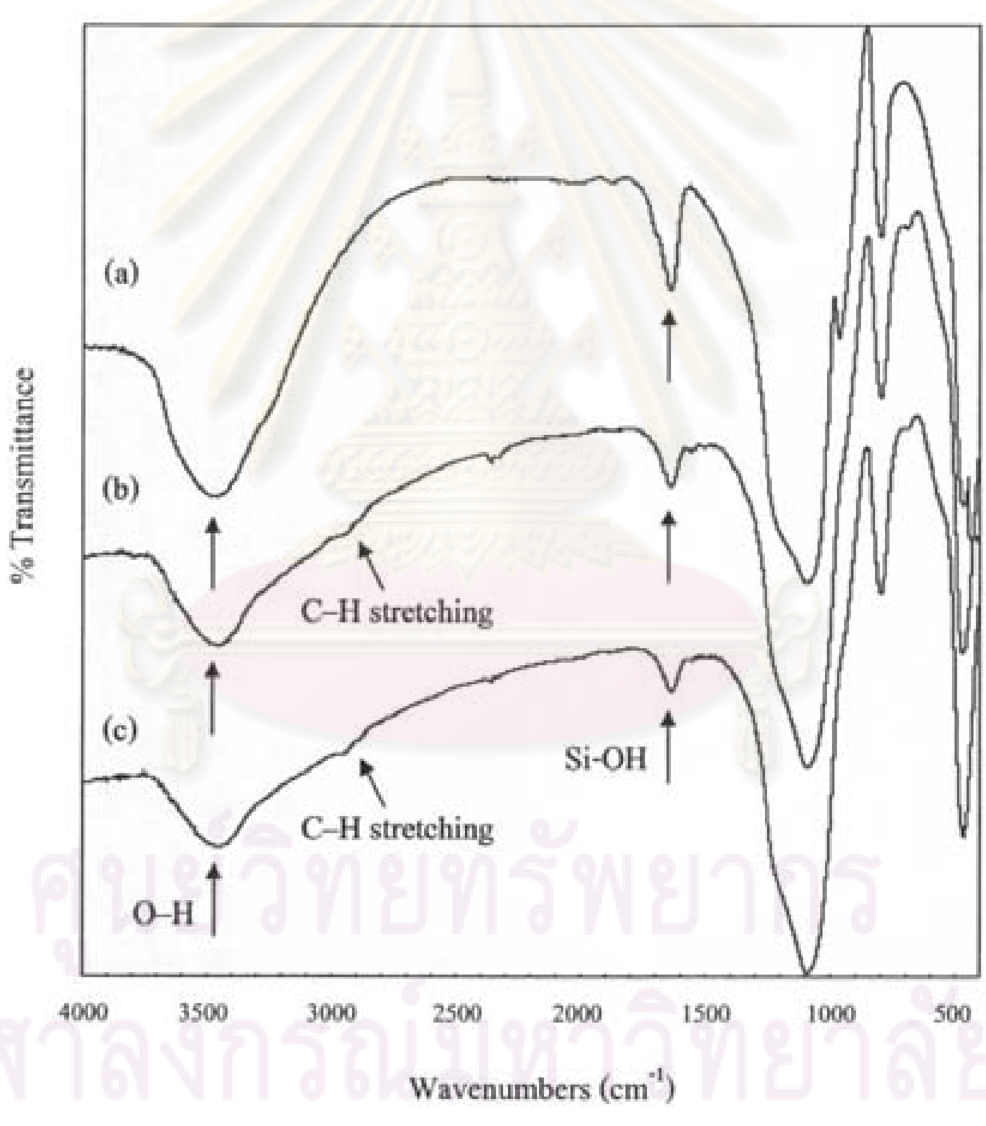


Figure 4.6 FT-IR spectra of (a) silica gel, (b) SiAP and (c) SiDAAQ.

Table 4.1 Characteristic of IR spectrum for silica gel [70]

Absorption band (cm^{-1})	Intensity	Assignment
1630	strong	Si–OH
2500-3800	broad	O–H stretching vibrations of the surface silanols (Si–OH) groups perturbed by hydrogen bonding either intramolecularly or with adsorbed water
1100	broad and intense	Si–O–Si anti-symmetric stretching vibration
900	less intense	Si–O–Si symmetric stretching vibrations

The presence of the absorption band in DR-FT-IR spectrum (Figure 4.7) at 1545 cm^{-1} was attributed to C=N azomethine stretching vibration. This indicated that 1,8-diaminoanthraquinone was chemically bonded with silica gel surface through Schiff's base bond formation [5]. In addition, the absorption bands at 1653 and 3379 cm^{-1} were assigned as N–H bending and N–H stretching, respectively [72].

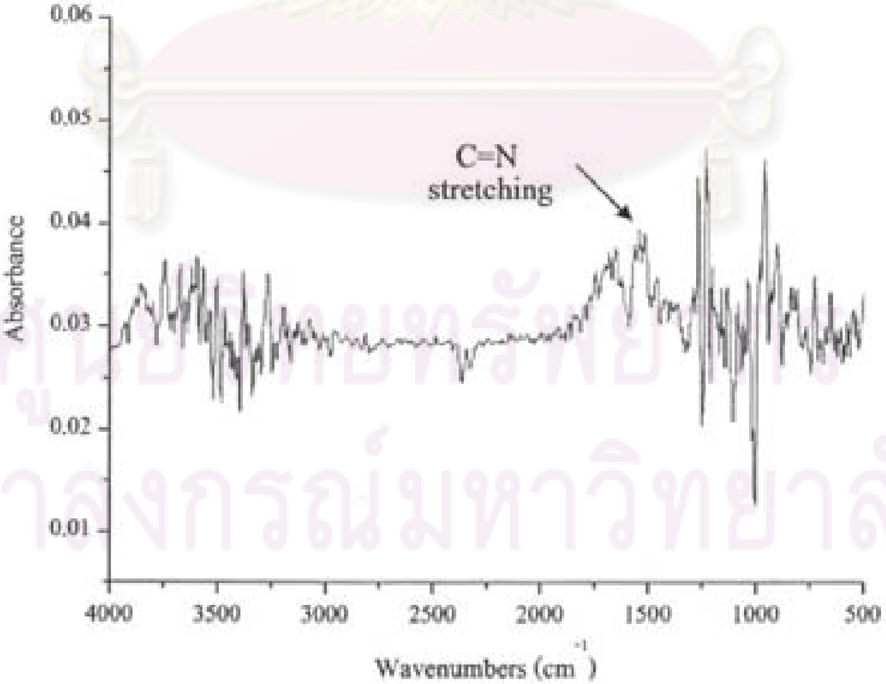


Figure 4.7 DR-FT-IR spectrum of SiDAAQ.

The O–H stretching vibration intensity is very strong in IR spectrum, but very weak in Raman spectrum. Thus FT-Raman spectra (Figure 4.8) can provide additional information in the range of 2500-3800 cm^{-1}. For example, the C–H stretching of aliphatic and N–H stretching of primary amine was clearly observed at 2900 and 3300 cm^{-1}, respectively.

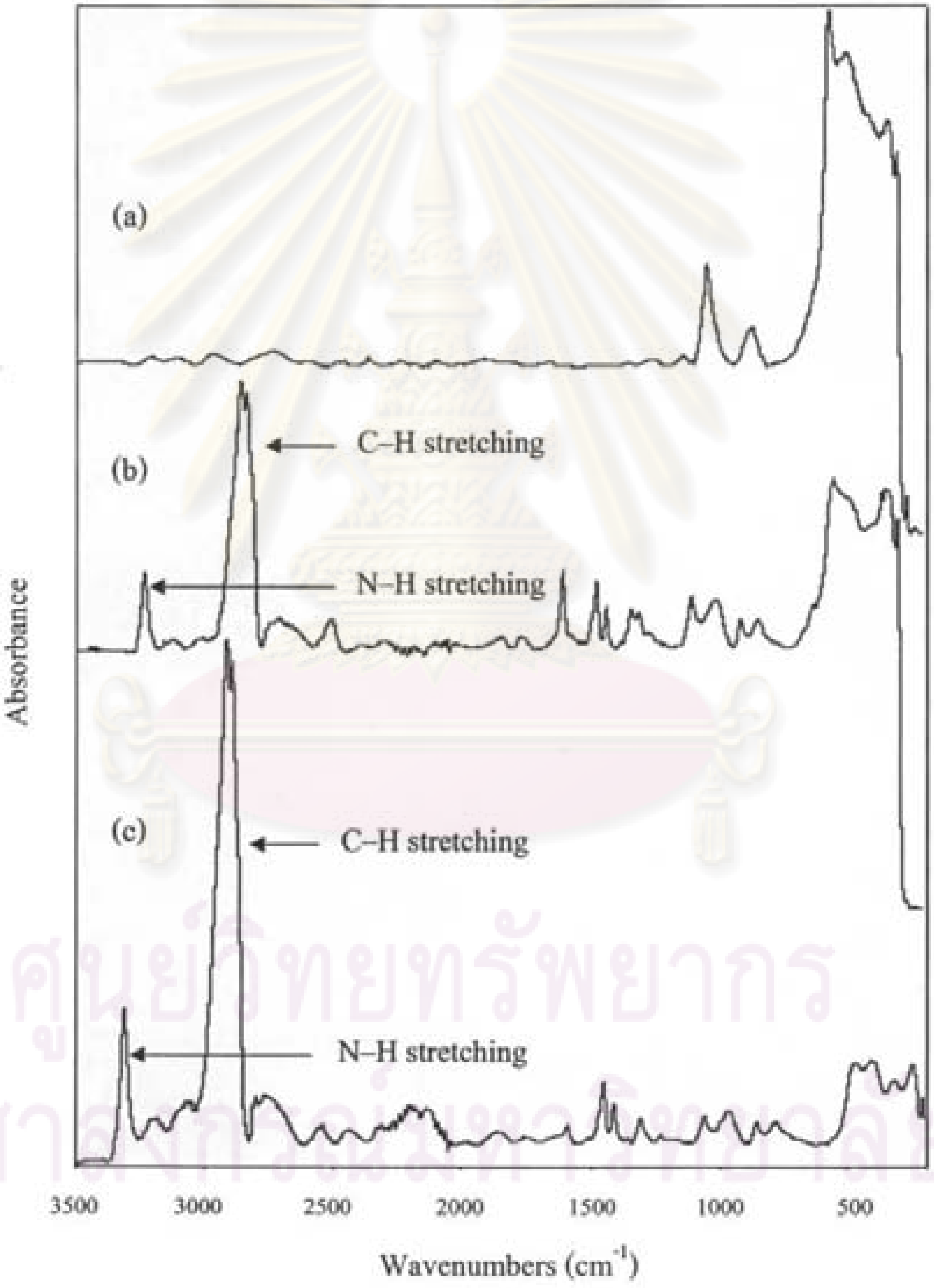


Figure 4.8 FT-Raman spectra of (a) silica gel, (b) SiAP and (c) SiDAAQ.

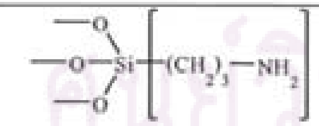
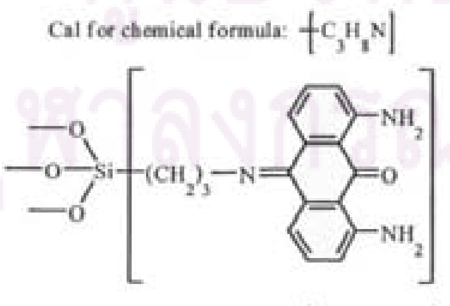
Percentages of C, H and N in SiAP and SiDAAQ listed in Table 4.2 are different from those in pure silica gel; it indicated that chemically modified silica with organic molecule were obtained. Percentages of C, H and N calculated from one molecule presented per repeat unit of silica gel are shown in Table 4.3. The theoretical ratio of N/C for SiDAAQ was less than that of in experimental result indicating that amino groups of SiAP may not be completely reacted with 1,8-DAAQ.

Table 4.2 Elemental analysis results of sorbents[a]

Sorbent	C (%)	H (%)	N (%)	N/C
Silica gel	0.053	0.011	0.057	-
SiAP	5.566	1.662	2.192	0.399
SiDAAQ	5.743	1.319	2.525	0.461

[a]Gaseous products were freed by pyrolysis in high-purity oxygen and were chromatographically separated by frontal analysis and quantitatively detected by thermal conductivity detector.

Table 4.3 Elemental analysis calculated from one molecule presented per repeat unit of silica gel

Sorbent	C (%)	H (%)	N (%)	N/C
 Cal for chemical formula: $\left[C_3H_8N\right]$	62.01	13.88	24.11	0.39
 Cal for chemical formula: $\left[C_{17}H_{16}N_3O\right]$	73.36	5.79	15.10	0.21

In addition, DR-UV-vis spectra as shown in Figure 4.9 showed additional band at 502 nm which is the same position of pure 1,8-DAAQ, indicating that 1,8-DAAQ was immobilized onto SiAP.

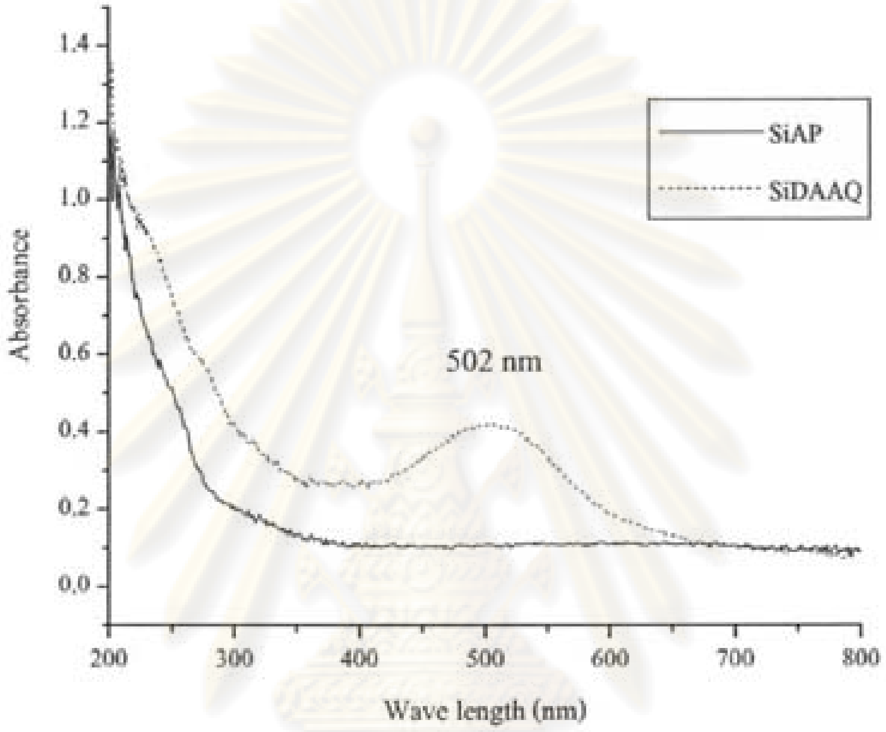


Figure 4.9 DR-UV-vis spectra of SiAP and SiDAAQ.

The TGA of SiAP and SiDAAQ further supported the results of elemental analysis. To identify the decomposition of different species, derivative thermograms can also be plotted (Figure 4.10b). For silica gel, the derivative thermogram was seen to have two distinctly different decomposition processes [30]. The first step loss of water between 50 °C and 200 °C can be attributed to physically adsorbed water. The second step that was lost between 200 °C and 400 °C appears to be strongly held (probably hydrogen bonded) water. For SiAP and SiDAAQ, the thermogram show the weight loss in the range of 200-600 °C more than silica gel, which confirms that the organic moiety are present on silca gel surface.

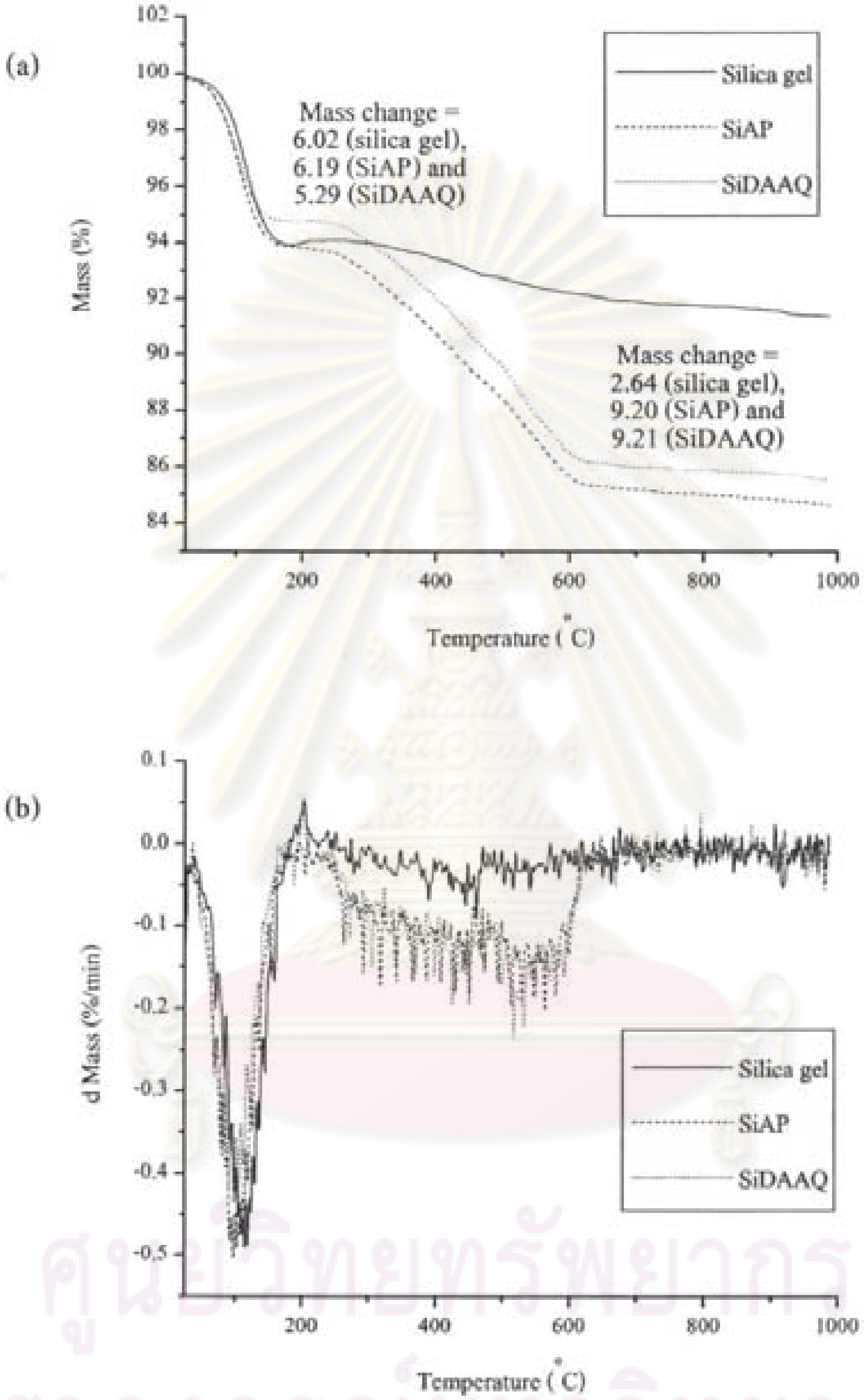


Figure 4.10 Thermogram of silica gel, SiAP and SiDAAQ at a heating rate of 10 °C min^{-1} under nitrogen atmosphere: (a) the weight loss of the sample as a function of temperature, (b) differential form (the change of sample weight with time) as a function of temperature.

The specific surface area of SiDAAQ as shown in Table 4.4 was clearly reduced as compared to that for silica gel. Because the organic modifier may be covered on the silica gel surface or crosslinked at the bottle-necking of silica gel pore, confirming that 1,8-diaminoanthraquinone was covalently bound into the silica gel surface [70].

Table 4.4 Specific surface area analysis results of sorbents

Sorbent[a]	**Multipoint BET (m^2 g^{-1})**
Silica gel	421.10
SiAP	272.25
SiDAAQ	292.52

[a] The temperature to remove the adsorbed gas in silica gel, SiAP and SiDAAQ is 250 °C (6 h), 150 °C (16.5 h) and 150 °C (10 h), repectively.

All characterization data above supported that the 1,8-diaminoanthraquinone was immobilized onto the silica gel surface through $-O_3Si-(CH_2)_3-$ spacer and Schiff's base chemical bonding.

4.3 Adsorption of metal ions onto SiDAAQ

The metal extraction efficiency, elution efficiency and preconcentration capability were reported in the term of distribution coefficient, extraction (%), sorption capacity, elution (%) and recovery (%) which can be calculated by equation 4.1, 4.2, 4.3, 4.4 and 4.5, respectively.

$$distribution\ coefficient = \frac{C_i - C_f}{C_f} \tag{4.1}$$

$$extraction\ (\%) = \frac{C_i - C_f}{C_i} \times 100 \tag{4.2}$$

where C_i = the initial concentration of metal (mg L^{-1})

C_f = the residual concentration of metal in supernatant (mg L^{-1})

$$sorption\ capacity = \frac{n_i - n_f}{m} \tag{4.3}$$

where n_i = the initial amount of metal (mg)

n_f = the residual amount of metal in supernatant (mg)

m = the amount of sorbent (g)

$$elution\ (\%) = \frac{C_d}{C_i - C_f} \times 100 \tag{4.4}$$

where C_i = the initial concentration of metal (mg L^{-1})

C_f = the residual concentration of metal in supernatant (mg L^{-1})

C_d = the concentration of metal eluted from sorbent (mg L^{-1})

$$recovery\ (\%) = \frac{n_d}{n_i} \times 100 \tag{4.5}$$

where n_i = the initial amount of metal (mg)

n_d = the amount of metal eluted from the sorbent (mg)

4.3.1 Batch method (static extraction)

In batch experiment, the effect of various parameters influencing the extraction efficiency of Cd(II), Co(II), Cu(II), Ni(II), Pb(II) and Zn(II) was investigated. After that, the optimization conditions from batch experiment were used for their elution and preconcentration in column method.

4.3.1.1 Effect of solution pH

The pH of solution is an important parameter which has affected on the efficiency of metal ion extraction. Because the solid-phase extraction based on chelation mechanism normally involves the acid-base equilibrium of ligand and the complex formation equilibrium between metal and ligand. The pH affects to the donor sites of ligands and the metal species in solution. In acidic medium, the competitive equilibriums between metal ions and hydrogen ions with ligand can be presented in order to coordinate and protonate, respectively. In basic medium the competitive equilibriums between ligands and hydroxide ions with metal ions can be presented in order to coordinate and precipitate, respectively. Thus, the optimum pH for metal ions extraction must prevent the protonation of ligand, precipitation of metal ion, or formation of none extractable metal species.

For SiDAAQ, it was found that amine nitrogen atoms can act as electron donor to form complex with various metal ions and can also be protonated giving the positive charge of nitrogen. Thus, the pH effect on sorption capacity was studied at pH ranging from 2.0-7.0. The experiments pH > 7.0 were not performed because the precipitation of metal hydroxide occurred, respectively. Moreover, the silica gel structure can be dissolved at pH higher than 7.5 [24]. The extraction results of each metal are shown in Figure 4.11.

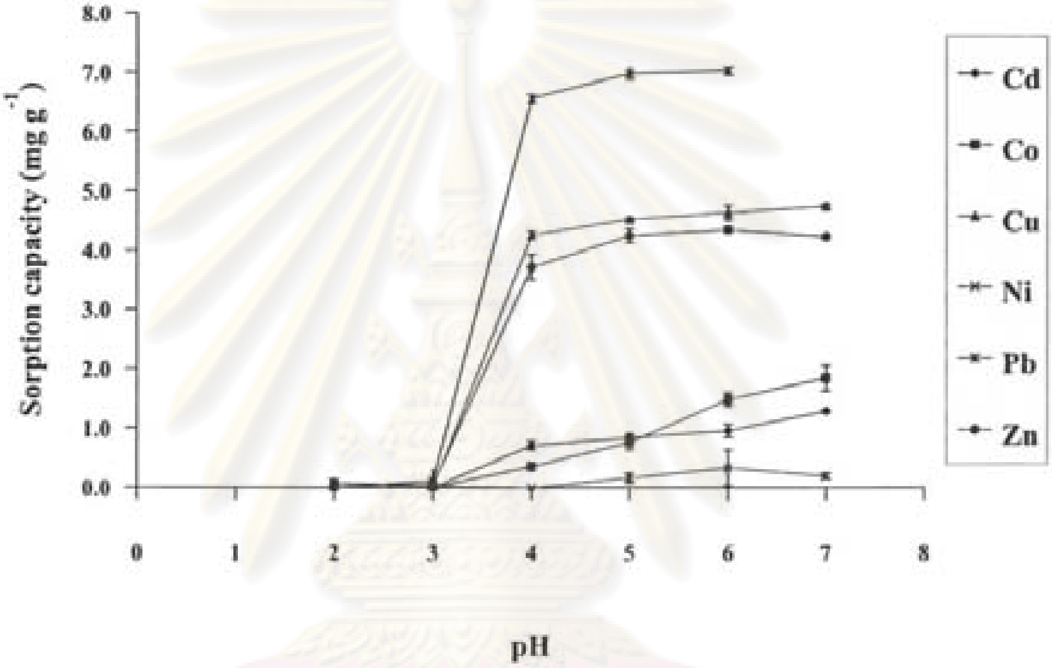


Figure 4.11 Effect of solution pH on metal extraction.

(Conditions: SiDAAQ = 10 mg, metal solution volume = 5.0 mL, extraction time = 30 min, [Cd] = 5.0 mg L^{-1}, [Co] = 10 mg L^{-1}, [Cu] = 10 mg L^{-1}, [Ni] = 10 mg L^{-1}, [Pb] = 15 mg L^{-1}, [Zn] = 10 mg L^{-1})

At pH ≤ 3.0, the extraction was inhibited for all metal ions, which were attributed to the protonation of the donor sites of SiDAAQ. Therefore the sorption capacity was very low. However, the metal ion extractions were enhanced in increase of pH solution and reached their higher sorption capacities at pH ~ 4.0-7.0 due to the occurrence of complex formation between metal ions and amine nitrogen atoms of SiDAAQ. For lead ion, the maximum sorption capacity was obtained at pH ~ 5.0-6.0

and the experiments at pH > 6.0 were not performed because the anionic lead species such as PbO_2^{2-} [34] or the precipitation of $Pb(OH)_2$ [73], could be formed. Thus, the optimum pH for the extraction of Cd(II), Co(II), Cu(II), Ni(II), Pb(II) and Zn(II) are at 6.0, 7.0, 6.0, 7.0, 6.0 and 6.0, respectively.

The extractions of cobalt and nickel ions were not further studied because their sorption capacities were very low. Thus the extraction efficiency of Cd(II), Cu(II), Pb(II) and Zn(II) was only investigated in the next experiments.

4.3.1.2 Effect of extraction time

The adsorption phenomena of solute on solid particles can be described by the boundary layer model that has the overall mechanism are achieved according to the following steps: (1) solute transport from the bulk solution to the boundary film, (2) solute transfer from the boundary film to the surface of the adsorbent, (3) transfer from the surface to the intraparticle active sites, and (4) uptake on the active sites, via complexation, sorption or precipitation. However the adsorption and complexation occurs rapidly, so the fourth step is also unimportant in the overall control of sorption kinetics [74]. Thus adsorption phenomena are mainly controlled by the external diffusion and intraparticle diffusion which their kinetics of mass transport are dependent of the time.

The extraction time must be strictly considered, especially in the case of slow kinetics of equilibrium. Because by using the extraction time less than the time required for reaching equilibrium, the efficiency of metal ion extraction was reduced. Moreover, the application to flow system which involves the dynamic equilibrium, normally the sorbent which offer fast kinetics of equilibrium will be used. The results of each metal extraction as a function of time are shown in Figure 4.12.

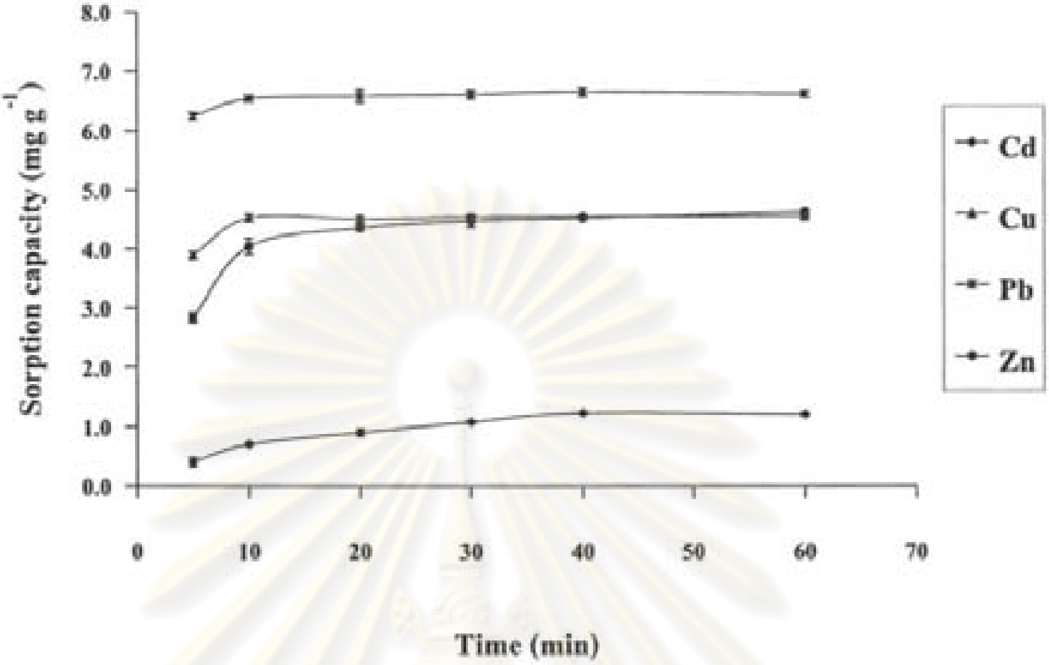


Figure 4.12 Effect of extraction time on metal extraction.

(Conditions: SiDAAQ = 10 mg, metal solution volume = 5.0 mL, [Cd] = 5.0 mg L^{-1} (pH = 6), [Cu] = 10 mg L^{-1} (pH = 6), [Pb] = 15 mg L^{-1} (pH = 6), [Zn] = 10 mg L^{-1} (pH = 6))

The extraction that provided the maximum sorption capacity increased with time and it was found to be constant after it reached equilibrium. Increase of the extraction time after its equilibrium will not affect the sorption capacity. Pb(II) and Cu(II) required the time for reaching equilibrium for 10 min, while Zn(II) and Cd(II) required more time for reached equilibrium after 30 and 40 min, respectively. Pb(II) and Cu(II) showed fast kinetics of extraction so they were suitable for the application with the flow system.

To ensure that the functionalized silica gel can raise the extraction efficiency, the comparison test between silica gel and SiDAAQ under the optimum conditions (pH and time) for each metal ion was performed using the following procedure:

A suspension of 10 mg of SiDAAQ in 5.0 mL of Cd(II) solution at 3 concentration levels, i.e. 5, 20 and 30 mg L^{-1}, in a test tube was stirred at room temperature for 40 min. The sorbent was separated by centrifugation at 3500 rpm for 5 min. Residual metal concentration in the supernatant was determined by FAAS. The same manner was performed with Co(II), Cu(II), Ni(II), Pb(II) and Zn(II).

The results are shown in Figure 4.13.

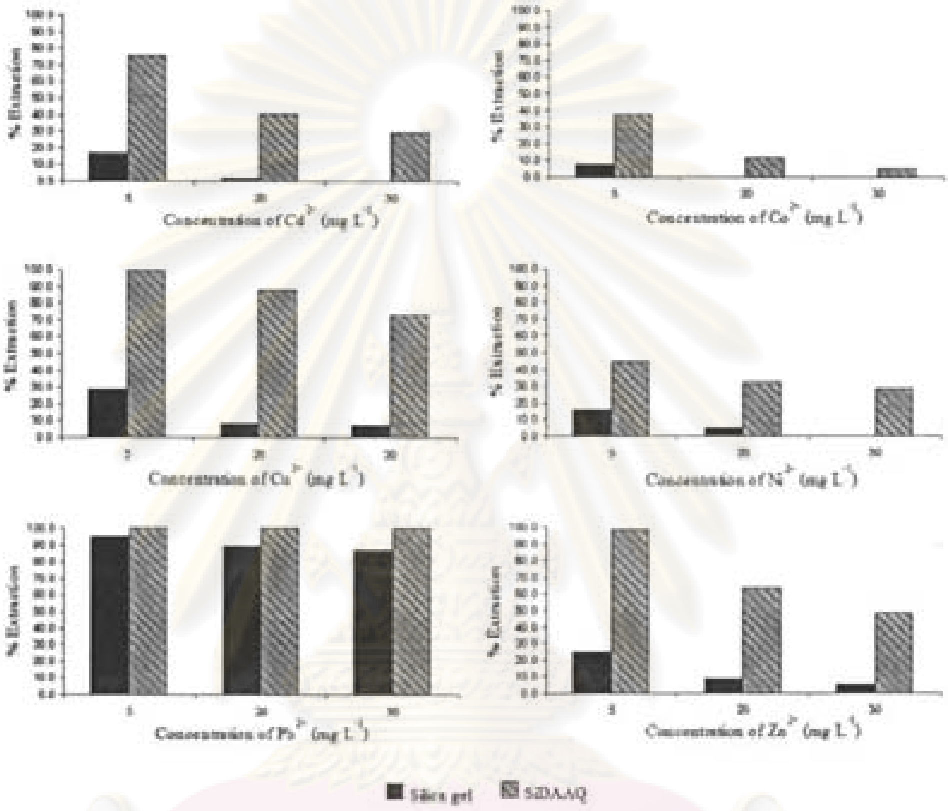


Figure 4.13 Comparison test of silica gel and SiDAAQ on metal extraction. (Conditions: SiDAAQ or silica gel = 10 mg, metal solution volume = 5.0 mL, extraction time = 30 min, pH = 6 (Cd(II), Cu(II), Pb(II), Zn(II), pH = 7 (Co(II), Ni(II))

It was found that silica gel itself can extract the metal ions due to the presence of silanol groups, which are known to be weak cation-exchangers (existed as Si–O$^-$ group) at pH higher than 4, causing the extraction of cationic species [31]. However, when the concentration of metal ions was increased, the percentage extraction was reduced. In case of SiDAAQ, the results showed the better extraction efficiency than silica gel, indicating that the functionalized silica gel enhanced the extraction efficiency.

4.3.1.3 Effect of initial concentration on sorption capacity

The extraction capacity of sorbent should be estimated prior to its application with real samples in order to protect the adverse effect on the determination. If the amount of analyte is larger than the maximum sorption capacity of sorbent, phenomena called sample breakthrough [23] may be occurring and then the negative error of analysis is obtained. The estimation can be performed by increasing the metal concentrations until the sorbent provide the constant value of sorption capacity. The results of each metal extraction are shown in Figure 4.14.

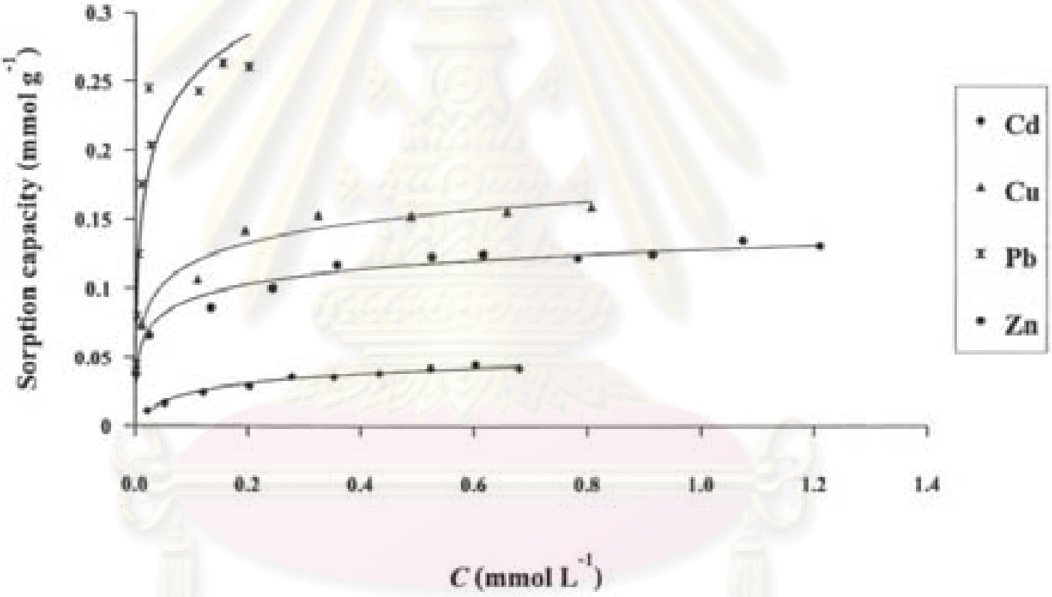


Figure 4.14 Effect of metal concentrations on metal extraction.
(Conditions: SiDAAQ = 10 mg (or 5 mg for Pb(II)), metal solution volume = 5.0 mL, Cd(II) (pH = 6, 40 min), Cu(II) (pH = 6, 30 min), Pb(II) (pH = 6, 30 min), Zn(II) (pH = 6, 30 min))

The results showed that when metal concentrations increased, the sorption capacity increased. The estimated maximum sorption capacity values of 0.26, 0.17, 0.13 and 0.04 mmol g^{-1} were found for Pb(II), Cu(II), Zn(II) and Cd(II), respectively.

4.3.1.4 Adsorption isotherm

Langmuir and Freundlich isotherms are the two most commonly used for adsorption isotherm equations [75]. Both isotherm equations can be transformed to a linear form and so their two adjustable parameters are easily estimated either by graphical means or by linear regression. The adjustable parameters in the Langmuir and Freundlich isotherms are normally estimated by linear regression. Thus, both isotherms were used to describe the adsorption phenomena of investigated metal ions on SiDAAQ.

Langmuir adsorption isotherm

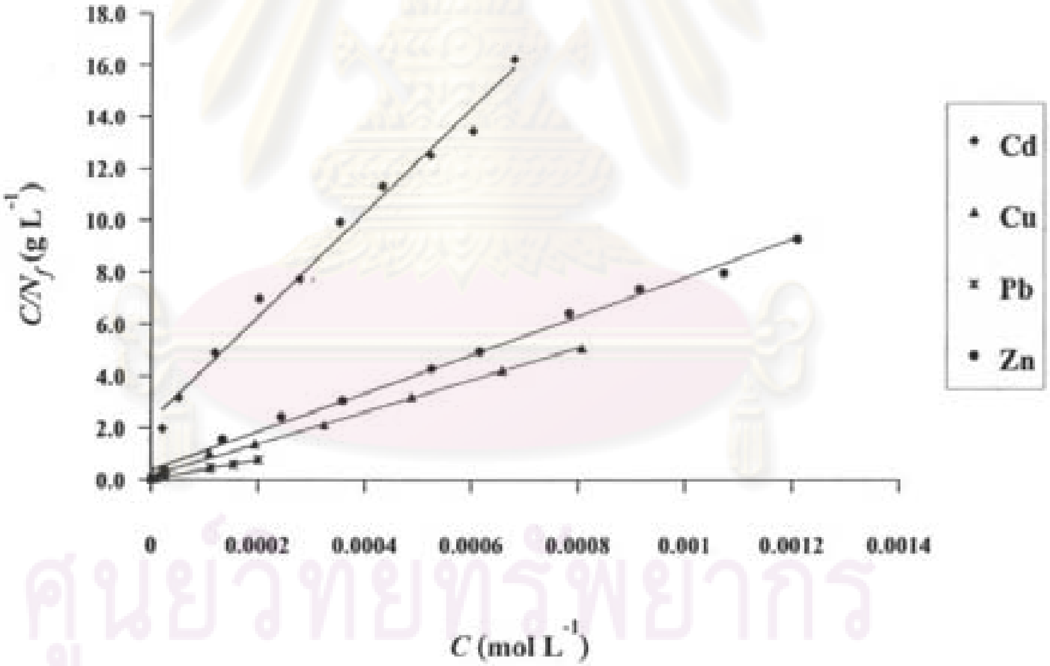


Figure 4.15 Langmuir plot for Cd(II), Cu(II), Pb(II) and Zn(II) adsorptions.

The maximum sorption capacity (N_f^s) and Langmuir constant ($b = \frac{K_{eq}}{a}$) determined from the Langmuir plot are given in Table 4.5.

Table 4.5 Langmuir parameters at 298 ± 1 K

Metal ions	**Equation** ($C/N_f = f(C)$)	R^2	$b \times 10^4$ (L mol^{-1})	$N_f^s \times 10^{-4}$ (mol g^{-1})	N_f^s (mg g^{-1})
Cd(II)	y = 20079x + 2.259	0.9874	0.89	0.50	5.62
Cu(II)	y = 6189.4x + 0.1448	0.9969	4.37	1.62	10.30
Pb(II)	y = 3690.1x + 0.0293	0.9983	12.59	2.71	56.15
Zn(II)	y = 7417.6x + 0.3728	0.9951	1.99	1.35	8.83

Freundlich adsorption isotherm

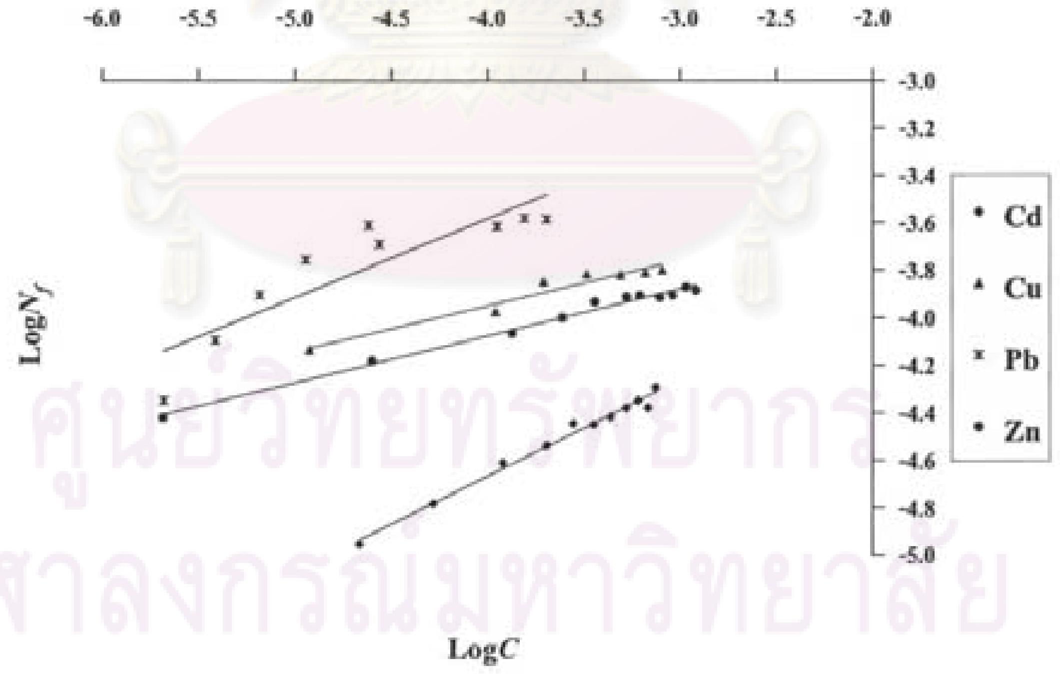


Figure 4.16 Freundlich plot for Cd(II), Cu(II), Pb(II) and Zn(II) adsorptions.

The Freundlich constant (*K* and *n*) determined from the Freundlich plot are given in Table 4.6.

Table 4.6 Freundlich parameters at 298 ± 1 K

Metal ions	**Equation** **($\log K = f(\log C)$)**	R^2	$K \times 10^{-4}$	*1/n*	*n*
Cd(II)	y = 0.4068x - 3.0394	0.9861	9.13	0.407	2.458
Cu(II)	y = 0.1930x - 3.1778	0.9443	6.64	0.193	5.181
Pb(II)	y = 0.3326x - 2.2525	0.7845	-	-	-
Zn(II)	y = 0.1980x - 3.2840	0.9875	5.20	0.198	5.051

The results presented in Table 4.5 and Table 4.6 with satisfying correlation coefficient (R^2) indicated that the adsorption of all metal ions onto SiDAAQ fits well the Langmuir and Freundlich model [73], except Pb(II) that showed unfavorable correlation coefficient in Freundlich adsorption isotherm.

According to the Langmuir model, it can be assumed that (1) the surface is homogeneous and all binding sites are equivalent, (2) solute uptake occurs by a monolayer sorption at definite and localized sites, and other mechanisms such as adsorbate- adsorbate interactions or second adsorption on the first layer not occur, and (3) each site can bind only one metal ion [75]. Moreover, Langmuir adsorption isotherm allows to estimate the maximum sorption capacity of sorbent as follows: Pb(II) > Cu(II) > Zn(II) > Cd(II).

The ability of adsorption of metal ion to form a stable complex can be considered from Langmuir constant (*b*) that has the ration $b = \frac{K_{eq}}{a}$, where *a* represents the activity of the solvent in solution. Thus the stability order of Pb(II) > Cu(II) > Zn(II) > Cd(II) was observed. Moreover, the *b* values obtained are high for all metal ion indicating that the sorption of metal ions onto SiDAAQ are directly coordinated with donor sites [40].

The degree of favorability of adsorption can be considered from Freundlich constant (n). Normally the n value in the range of 1 to 10 is indicated as favorable adsorption [75]. Moreover, the small value of $\frac{1}{n}$ indicates that the bond between sorbate and sorbent is strong. The Freundlich parameters of Cd(II), Cu(II) and Zn(II) showed small values of $\frac{1}{n}$ and the n value between 2.458 to 5.181, indicated the favorable adsorption of these metal ions onto SiDAAQ. The favorability of adsorption onto SiDAAQ decreased in the following order: Cu(II) > Zn(II) > Cd(II).

The principles of the complex formation between metal and ligand, such as the hard-soft acid-base principle [76, 77] and the size of the metal ion [78], were referred in order to support the information obtained from Langmuir and Freundlich isotherm. According to the hard-soft acid-base principle, the investigated metal ions are classified into two groups which are listed with their ionic radius in Table 4.7.

Table 4.7 The types of Lewis acid and ionic radius of the investigated metal ions

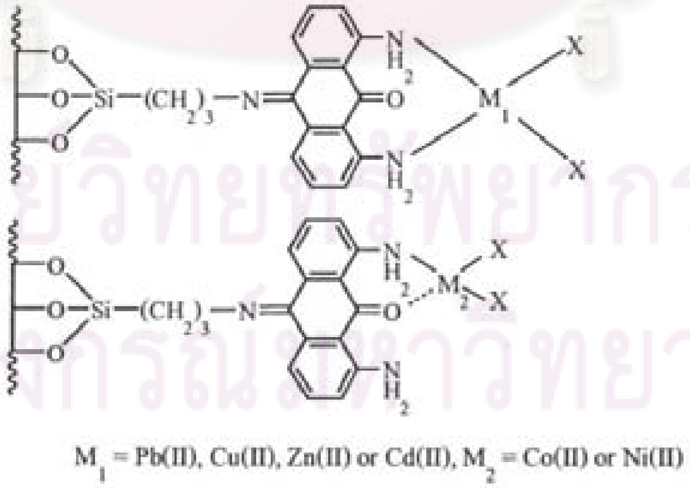
Lewis acid	**Metal ions**	**Ionic radius (pm)**	
		6 coordinated sites	4 coordinated sites
Boderline	Co(II)	79	72
	Ni(II)	83	63
	Cu(II)	87	71
	Zn(II)	88	74
	Pb(II)	133	-
Soft	Cd(II)	109	92

From the experimental results in batch experiment, the order of sorption capacity value was Pb(II) > Cu(II), Zn(II) > Cd(II) > Co(II), Ni(II). Thus, the investigated metal ions can be classified according to their sorption capacity values as two groups: (1) Pb(II), Cu(II), Zn(II), Cd(II) and (2) Co(II), Ni(II).

In the consideration of the first group, it is found that the two amine nitrogen atoms of SiDAAQ are boderline lewis base, while Pb(II), Cu(II) and Zn(II) are boderline lewis acid but Cd(II) is soft lewis acid. Thus SiDAAQ provided the lowest maximum sorption capacity for Cd(II) due to the unfavorable coordination with Cd(II). In addition, the rearrangement of donor sites of SiDAAQ provided the large cavity site, so the most suitable metal ion was Pb(II) that had the biggest ionic radius. As a result, SiDAAQ provided the highest maximum sorption capacity for Pb(II). In case of Cu(II) and Zn(II), they had nearly the ionic radius, and their maximum sorption capacities were thus similar.

For the second group, although Co(II) and Ni(II) act as the boderline lewis acid, the sorption capacities were very low. Because Co(II) and Ni(II) have too small ionic radius for the cavity site of SiDAAQ.

The proposed modes of chelation of SiDAAQ with the investigated metal ions are shown in Scheme 4.2.



M_1 = Pb(II), Cu(II), Zn(II) or Cd(II), M_2 = Co(II) or Ni(II)

X = H_2O or $-NH_2$

Scheme 4.2 Proposed modes of chelation of SiDAAQ with the investigated metal ions.

4.3.1.5 Effect of interfering ions

The cations such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Cu^{2+} and the anions such as Cl^-, NO_3^-, SO_4^{2-} can be found in natural water samples and may have the adverse effect on the determination of investigated metal ions. Because FAAS provides the high specificity for the detection of interested element in sample, the interfering ions in sample can influence the preconcentration step only. The cations can show the competitive extraction with the analyte metal ions, while the anions can form complex with the analyte metal ions or generate the unextractable metal species. Pb(II) was selected to study the effect of interfering ions and studied with natural water samples, because the previous experiment showed that SiDAAQ had the best extraction properties for Pb(II). The effect of interfering ions on the Pb(II) extraction is shown in Table 4.8.

Table 4.8 Effect of interfering ions on the Pb(II) extraction

Interfering ions	**Pb(II) extraction (%)**		
	10 mg L^{-1}	**100 mg L^{-1}**	**1000 mg L^{-1}**
Na^+	100	100	100
K^+	100	100	100
Ca^{2+}	100	100	100
Mg^{2+}	100	100	100
Cu^{2+}	70	4	0
Cl^-	100	> 99	> 99
NO_3^-	100	100	100
SO_4^{2-}	100	100	100

(Conditions: SiDAAQ = 10 mg, metal solution volume = 5.0 mL, extraction time = 30 min, [Pb] = 15 mg L^{-1} (pH = 6))

The presence of Cu^{2+} in lead solution showed an interfering effect on the Pb(II) extraction. Because Cu^{2+} can show the competitive complex formation with Pb(II) on the SiDAAQ, the sample breakthrough may be occurred. Increasing the weight of sorbent may be used to solve this problem. Moreover, the presence of Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, NO_3^- and SO_4^{2-} ions did not affect the Pb(II) extraction, indicating that the main retention mechanism of Pb(II) on the SiDAAQ is chelation mechanism.

4.3.2 Column method (dynamic or flow through extraction)

In column experiment, the effect of various parameters influencing the extraction and elution efficiency of Pb(II) were investigated. After that, the information from column method was evaluated for the application to the on-line preconcentration of Pb(II).

4.3.2.1 Effect of sample flow rate

In static extraction, the sorbent is contacted with the solution for long enough period of time until the equilibrium conditions are reached. However, the equilibrium is achieved once in batch extraction. In dynamic extraction on the other hand, the solution containing analyte is being extracted to the sorbent as it is passed through the new sorbent, causing the multiple equilibria of extraction may be occurred. Thus the dynamic extraction can provide the extraction more than the static extraction [1]. However the kinetics of mass transport is depended on the time, so the flow rate of solution are considered. At high flow rate, the solution passes through the column too quickly, so the contact time between the phases may not be sufficient for complete retention. At low flow rate, the solution passes through the column as long time, so the analysis time is increased.

The effect of sample flow rate on extraction efficiency was observed at the flow rate ranging from 0.5 and 5.0 mL min^{-1}. The flow rate lower than 0.5 mL min^{-1} cannot perform due to the in-use limit of a peristaltic pump. While the flow rate higher than 5.0 mL min^{-1} cannot be operated because the back pressure in mini-column can occur and cause a leak at the junction of column. The results of Pb(II) extraction is shown in Figure 4.17.

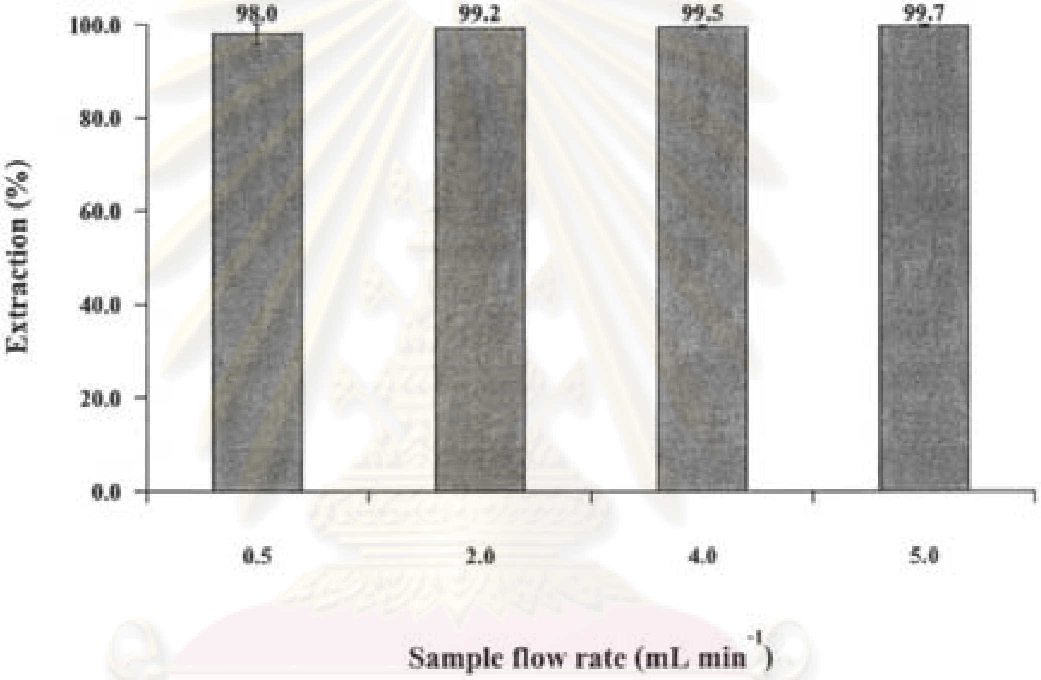


Figure 4.17 Effect of sample flow rate on Pb(II) extraction.

(Conditions: SiDAAQ = 10 mg, metal solution volume = 5.0 mL, [Pb] = 15 mg L^{-1} (pH = 6))

The flow rate in the range of 0.5 and 5.0 mL min^{-1}for loading the Pb(II) was not affected on extraction efficiency. Moreover, in this range, the maximum of extraction efficiency can be obtained. Thus, the flow rate of 5.0 mL min^{-1} was chosen due to the lowest of analysis time.

4.3.2.2 Effect of eluent

The proper eluent should be completely recovering the extracted analyte from the sorbent. The reuse of the sorbent can be achieved when the complete elution is obtained because the cross contamination between each sample is prevented. Moreover, the elution using a volume of eluent is very smaller than a volume of sample solution and so the higher preconcentration can be obtained.

The selection of eluent may be considered from the retention mechanism of analyte on the sorbent. For the chelation mechanism, the elution is based on using an acid to reduce the capability in complexation of the functional groups which act as the electron donor, or using a stronger chelating reagent such as EDTA that forms a more stable complex with the analyte. In the former approach, the residual acid in the column must be completely washed. To evaluate the reuse of the sorbent, the experiment was performed with both HNO_3 and EDTA as eluent. The sorbed Pb(II) was eluted by 5.0 mL of 10% HNO_3 and 0.100 mol L^{-1} EDTA at a flow rate of 5.0 mL min^{-1}. After that, the column was washed again using 5.0 mL of deionized water before reuse.

The results are shown in Table 4.9.

Table 4.9 The effect of the type of eluent on Pb(II) extraction efficiency

Eluent	**Repeated cycles**	**Extraction (%)**[a]
10% HNO_3	1	100.0 ± 0.1
	2	66.7 ± 4.9
0.100 mol L^{-1} EDTA	1	100.0 ± 0.1
	2	100.0 ± 0.1

[a] Mean value ± SD, (n = 3)

The results showed no reusability of sorbent when 10% HNO_3 was used as eluent due to the protonation of hydrogen ions at donor site of SiDAAQ resulting in decrease of extraction efficiency in the second repeated cycle. Thus EDTA was chosen as eluent. In addition, EDTA is a hexaprotic acid commonly used as a chelator because it can form the strong complexes with most metal ions with the ratio of EDTA:metal ion as 1:1, binding through 4 oxygen and 2 nitrogen atoms. Moreover, the log K_f of EDTA and Pb(II) is 18.04 [79], therefore it can form a stable complex with Pb(II), suggesting that EDTA is an appropriate eluent for SiDAAQ. Also, the minimum pH of EDTA for the effective complex formation with Pb(II) is about 4 [79], so the EDTA disodium salt form used in this work can be used without the pH adjustment because the pH of EDTA solution is also around 4.

Eluent flow rate

The results of Pb(II) elution is shown in Figure 4.18.

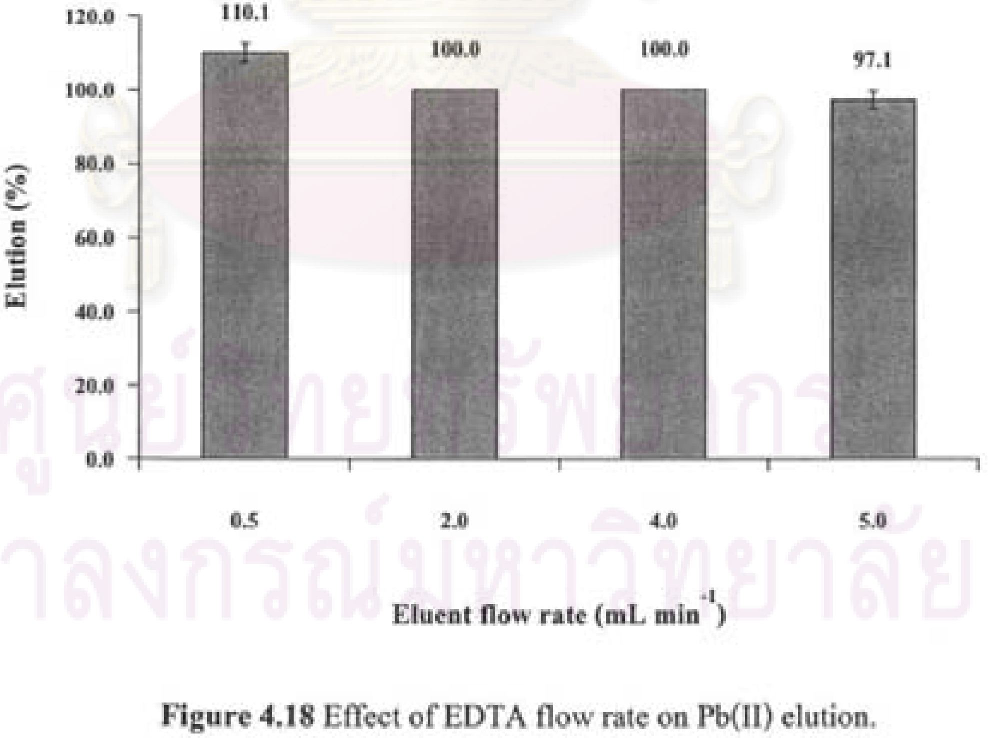


Figure 4.18 Effect of EDTA flow rate on Pb(II) elution.

(Conditions: SiDAAQ = 10 mg, metal solution volume = 5.0 mL, [Pb] = 5 mg L^{-1} (pH = 6), sample flow rate = 5.0 mL min^{-1}, eluent volume = 5.0 mL, [EDTA] = 0.100 mol L^{-1})

The flow rate in the range of 0.5 and 5.0 mL min^{-1} for eluting the Pb(II) from SiDAAQ was not affected on elution efficiency. Moreover, in this range, the optimum of elution efficiency can be obtained. Thus, the flow rate of 5.0 mL min^{-1} was chosen because it provided the lowest of analysis time.

Concentration of eluent

The higher concentration of EDTA provides the stronger elution strength. The complete elution and the higher preconcentration efficiency will be obtained. However, the presence of high concentration of EDTA in solutions can affect to the sensitivity of FAAS because of its precipitation in the nebulizer system. Thus, the appropriate concentration of EDTA was investigated. The results of Pb(II) elution are shown in Figure 4.19.

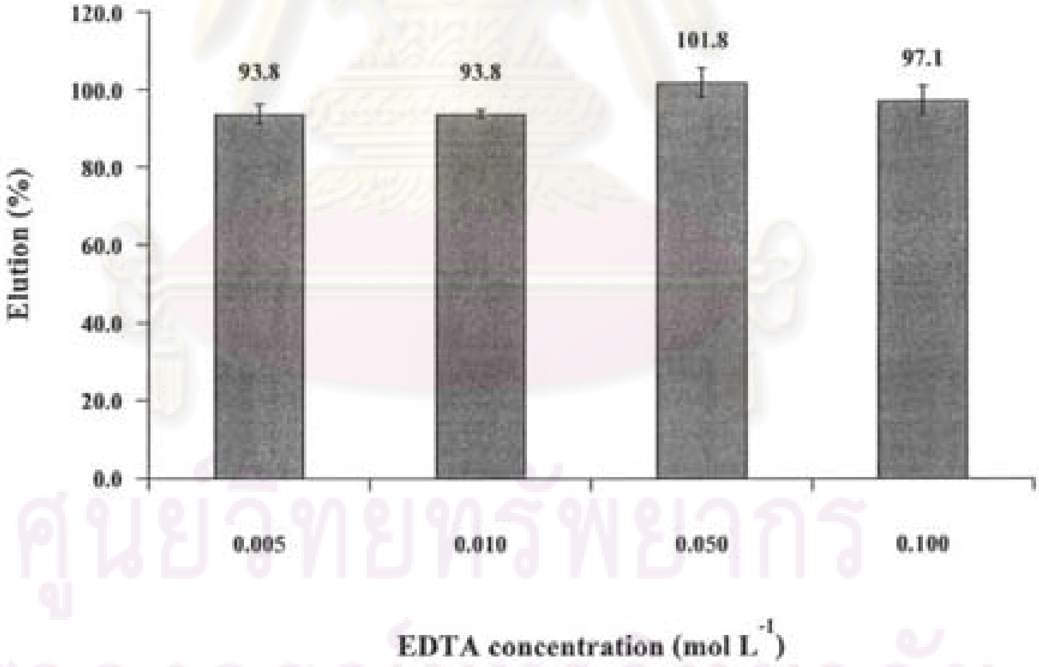


Figure 4.19 Effect of EDTA concentration on Pb(II) elution.

(Conditions: SiDAAQ = 10 mg, metal solution volume = 5.0 mL, [Pb] = 5 mg L^{-1} (pH = 6), sample flow rate = 5.0 mL min^{-1}, eluent volume = 5.0 mL, eluent flow rate = 5.0 mL min^{-1})

The concentration of 0.005 and 0.010 mol L^{-1} EDTA showed incomplete elution, which may be attributed to the weak elution strength. While the concentration of 0.050 and 0.100 mol L^{-1} EDTA provided the optimum elution efficiency. However, to prevent the precipitation of EDTA salts in the nebulizer system, 0.100 mol L^{-1} EDTA was not chosen. Thus, an appropriate concentration of EDTA was 0.050 mol L^{-1}.

4.3.2.3 Effect of sample volume

The column mode is a general procedure for preconcentration of the trace metal ions from a large sample volume. Thus, the effect of sample volume on the preconcentration capability of the mini-column containing SiDAAQ was investigated. It was performed by increasing volume of Pb(II) solution, while keeping the total amount of spiked Pb(II) constant [7].

The acceptable recovery and RSD of analyte at different concentrations are summarized in Table 4.10.

Table 4.10 Analyte recovery and precision at different concentrations [80]

Analyte, %	Unit	Mean recovery, %	RSD, %
100	100%	98-102	1.3
10	10%	98-102	2.8
1	1%	97-103	2.7
0.1	0.1%	95-105	3.7
0.01	100 ppm	90-107	5.3
0.001	10 ppm	80-110	7.3
0.0001	1 ppm	80-110	11
0.00001	100 ppb	80-110	15
0.000001	10 ppb	60-115	21
0.0000001	1 ppb	40-120	30

The results of Pb(II) preconcentration is shown in Figure 4.20.

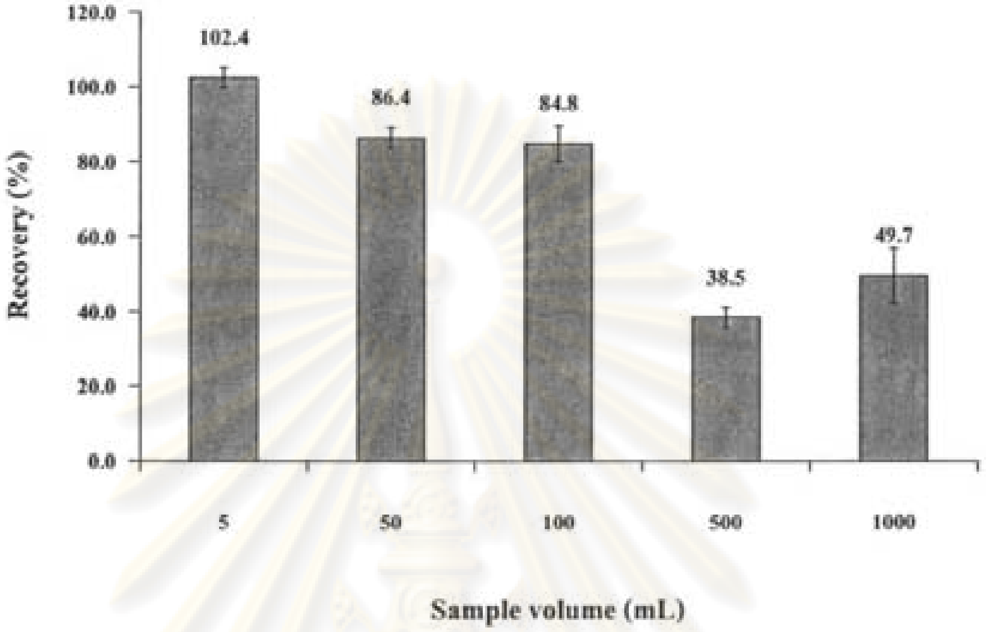


Figure 4.20 Effect of sample volume on Pb(II) preconcentration.

(Conditions: SiDAAQ=10 mg, sample spiked with 20 mg Pb(II) (pH = 6), sample flow rate = 5.0 mL min^{-1}, eluent flow rate = 5.0 mL min^{-1}, eluent volume = 5.0 mL, [EDTA] = 0.050 mol L^{-1})

The sample volumes of 500 and 1000 mL showed the phenomena that called breakthrough volume, resulting in unacceptable recoveries. This phenomena suggested that the extracted Pb(II) was desorbed to the sample solution again by substitution or solvent exchange mechanism which were the competition of solvent and donor sites of SiDAAQ. In addition, this mechanism may be supported by the continuous flowing of solvent. These results indicated that Pb(II) preconcentration using column mode cannot be performed when those sample volumes were used. This problem may be solved by increasing the weight of sorbent or reducing the flow rate to improve the retention capability of Pb(II). However, the adverse effect such as the back pressure or time consuming may also arise. Thus, the maximum sample volume that can be percolated through the column and provide the acceptable recovery was 5.0, 50.0 and 100.0 mL.

4.3.2.4 Reuse of mini-column

The sorption capacity of SiDAAQ after several extraction and elution of Pb(II) repeated which referred to reusability of mini-column are shown in Table 4.11.

Table 4.11 The performance of reused mini-column

Column no.	**Sorption capacity (mg g^{-1})^a**	**Changed sorption capacity in comparision with the first cycle (%)**
1	2.17 ± 0.04	< 4
2	2.12 ± 0.04	< 5
3	2.35 ± 0.04	< 7

^{a}Mean value ± SD, (n = 25)

(Conditions: SiDAAQ = 10 mg, metal solution volume = 5.0 mL, [Pb] = 5 mg L^{-1} (pH = 6), sample flow rate = 5.0 mL min^{-1}, eluent flow rate = 5.0 mL min^{-1}, eluent volume = 5.0 mL, [EDTA] = 0.050 mol L^{-1})

It was found that the sorption capacities of SiDAAQ in each column were not significantly changed after 25 cycles of loading and elution. Moreover, the sorption capacities of SiDAAQ of three columns were not significantly different. This indicated that the laboratory-made mini-column showed the reproducibility in both within and between column.

4.4 On-line preconcentration study

The information obtained from the adsorption study showed that SiDAAQ was an appropriate sorbent for on-line preconcentration system because it provided the fast kinetic of extraction and elution, the high sorption capacity and the high selectivity for Pb(II). Thus, it was applied to on-line preconcentration-FAAS system using the System 5 (see Figure 2.8). This system was selected because it can collect the constant volume of eluent in the eluent loop before elution, resulting in more accuracy in comparision with other systems. However, this system was also modified by adding the washing step to ensure that only extracted Pb(II) was eluted and determined, resulting in more precision.

In order to achieve the optimum parameters for the on-line preconcentration-FAAS system, the influence of the sample flow rate, eluent flow rate, concentration of eluent and sample volume was studied by considering the recovery (%) of the spiked sample and using the procedures according to the Figure 3.3 and Figure 3.4.

4.4.1 Effect of sample flow rate

The results of Pb(II) on-line preconcentration are shown in Figure 4.21.

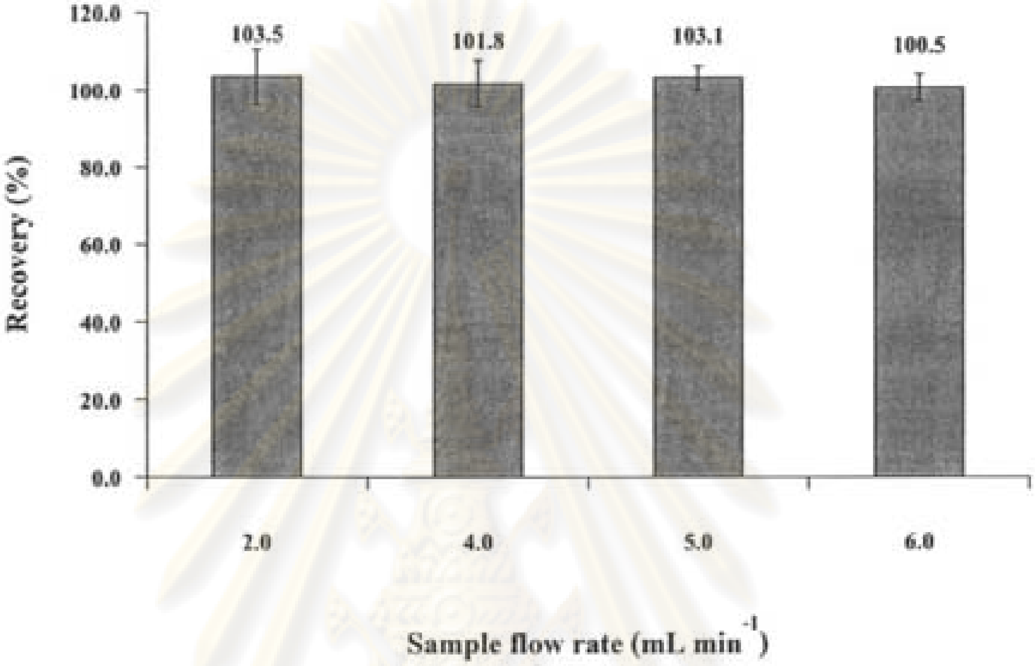


Figure 4.21 Effect of sample flow rate on Pb(II) on-line preconcentration-FAAS. (Conditions: SiDAAQ = 10 mg, metal solution volume = 5.0 mL, [Pb] = 300 µg L^{-1} (pH = 6), eluent flow rate = 5.0 mL min^{-1}, eluent volume = 174 µL, [EDTA] = 0.100 mol L^{-1})

It was found that all flow rates provided acceptable recoveries. This indicated that SiDAAQ showed the fast kinetics of Pb(II) extraction and the extractability of SiDAAQ was independent on the sample flow rate. In addition, the phase transfer factor, *P*, which defined as the ratio between analyte mass in the concentrate after elution, m_e, and in the original sample solution, m_s, [11] obtained from a flow rate of 5.0 mL min^{-1} was 1.0. This indicated that Pb(II) was completely transferred from the sample phase into the SiDAAQ. So, the flow rate of 5.0 mL min^{-1} was chosen for further experiments.

4.4.2 Effect of eluent

For on-line preconcentration-FAAS system, the additional requirements for increasing of elution efficiency from the column are the eluent volume, which should provide the shape of analytical signal as peak and the eluent flow rate which must be compatible with the nebulizer flow rate.

To evaluate the effect of the eluent volume, the Pb(II) standard solution at a concentrations of 25.0 mg L^{-1} was passed directly to the nebulizer at different volume of 174, 274 and 574 µL. The results are shown in Figure 4.22.

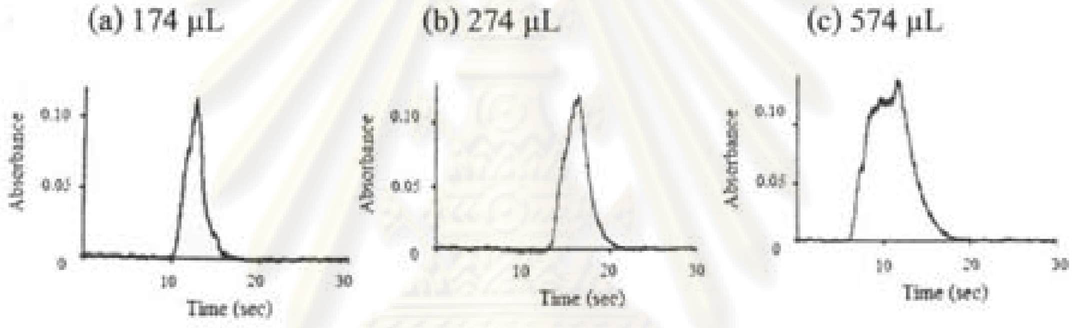

(a) 174 µL (b) 274 µL (c) 574 µL

Figure 4.22 The effect of eluent volume on the analytical signal.

Although 274 µL can provide the shape of analytical signal as peak like 174 µL, the higher preconcentration factor, which obtained from 174 µL.

To evaluate the effect of the incompatibility between the elution and nebulization flow rates, the experiment set up was performed by constructing the calibration curve at different eluent flow rate as follows:

174 µL of Pb(II) standard solution at the concentrations of 0, 5, 10, 15, 20, 25 mg L^{-1} was passed directly to the nebulizer at different flow rates in the range of 4.0-7.0 mL min^{-1} without a mini-column according to the Figure 3.4. The analytical signal was measured in peak height mode. The nebulizer flow rate was adjusted at 5.0 mL min^{-1}.

The slope of calibration curve called calibration sensitivity is shown in Table 4.12.

Table 4.12 The effect of eluent flow rate on the analytical signal

Eluent flow rate (mL min^{-1})	Calibration sensitivity
4.0	0.0052
5.0	0.0053
6.0	0.0047
7.0	0.0046

The calibration sensitivity is the slope of calibration curve which is used to demonstrate the sensitivity of detection. The higher sensitivity was observed at the flow rate of 4.0 and 5.0 mL min^{-1}. However, the broader peaks were obtained at a flow rate of 4.0 mL min^{-1} probably due to smaller flow rate than the nebulizer flow rate. So, the flow rate of 5.0 mL min^{-1} was chosen because of the compatible with the nebulizer flow rate. The incomplete nebulization process may be obtained at a flow rate of 6.0-7.0 mL min^{-1} which were larger than the nebulizer flow rate, so a lot of the sample solution were drained as a waste. As a result, the analytical signal was decreased.

Eluent flow rate

The results of Pb(II) on-line preconcentration are shown in Figure 4.23.

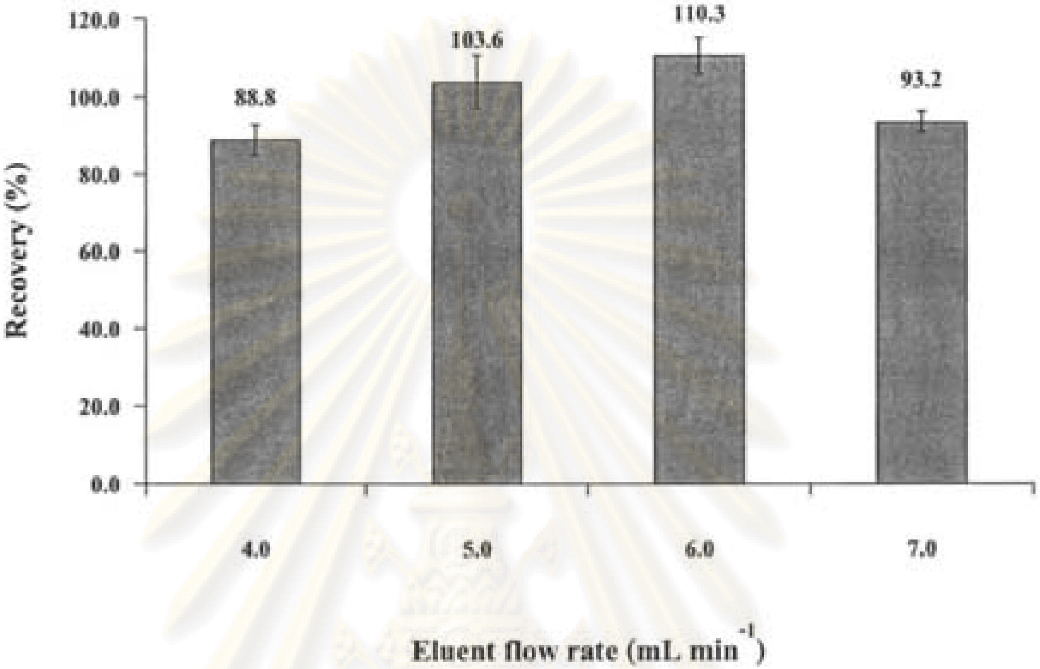


Figure 4.23 Effect of EDTA flow rates on Pb(II) on-line preconcentration-FAAS. (Conditions: SiDAAQ = 10 mg, metal solution volume = 5.0 mL, [Pb] = 300 µg L^{-1} (pH = 6), sample flow rate = 5.0 mL min^{-1}, eluent volume = 174 µL, [EDTA] = 0.100 mol L^{-1})

Although all flow rates provided the acceptable recoveries, the best recovery was obtained at a flow rate 5.0 mL min^{-1} which was compatible with the nebulizer flow rate. Moreover, this flow rate was the same value with the selected sample flow rate, thus, the simple system can be designed using only one peristaltic pump.

Concentration of eluent

The results of Pb(II) on-line preconcentration are shown in Figure 4.24.

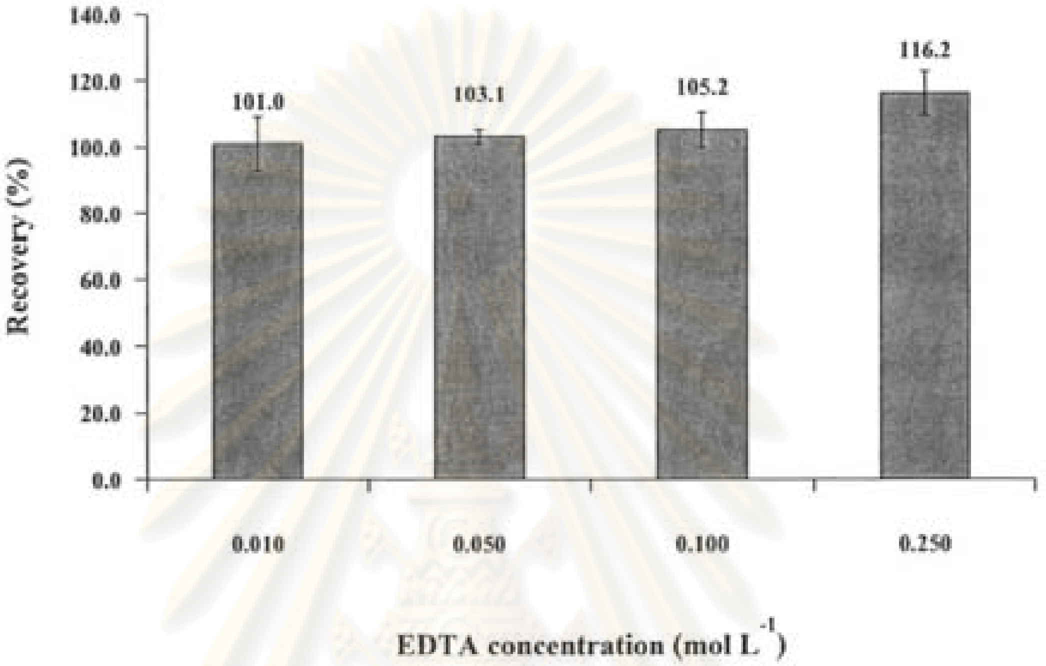


Figure 4.24 Effect of EDTA concentrations on Pb(II) on-line preconcentration-FAAS. (Conditions: SiDAAQ = 10 mg, metal solution volume = 5.0 mL, [Pb] = 300 μg L^{-1} (pH = 6), sample flow rate = 5.0 mL min^{-1}, eluent flow rate = 5.0 mL min^{-1}, eluent volume = 174 μL)

The unacceptable recovery was found when 0.250 mol L^{-1} EDTA was used as eluent, probably due to too high concentration of eluent which affected the detection of instrument. While 0.010, 0.050 and 0.100 mol L^{-1} EDTA provided the acceptable recoveries. However, the EDTA solution with the concentration of 0.050 mol L^{-1} was chosen to ensure the efficient elution and to prevent the precipitation of EDTA salts.

4.4.3 Effect of sample volume

In order to achieve the higher preconcentration factor, the Pb(II) sample volume of 10.0 mL at the concentration of 300 μg L^{-1} was tested under the optimum conditions. The results are shown in Table 4.13.

Table 4.13 Effect of sample volume on Pb(II) on-line preconcentration

Sample volume (mL)	Recovery (%)[a]
5.0	97.5 ± 7.6
10.0	89.9 ± 1.3

[a] Mean value ± SD, (n = 3)

(Conditions: SiDAAQ = 10 mg, [Pb] = 300 μg L^{-1} (pH = 6), sample flow rate = 5.0 mL min^{-1}, eluent flow rate = 5.0 mL min^{-1}, eluent volume = 174 μL, [EDTA] = 0.050 mol L^{-1})

It was found that both 5.0 and 10.0 mL of sample volume yielded acceptable recoveries. The preconcentration factor which is the ratio of the initial volume of sample and the final volume were 29 and 58 for 5.0 and 10.0 mL of sample volume, respectively. However, the preconcentration time of 120 sec was required for 10.0 mL of sample volume.

4.4.4 Initial concentration of Pb(II) in sample

In order to investigate the lowest concentration level of Pb(II) that can be preconcentrated by this proposed system, the sample concentration was reduced from 300 μg L^{-1} to 10 μg L^{-1}. The results of Pb(II) on-line preconcentration are shown in Figure 4.25.

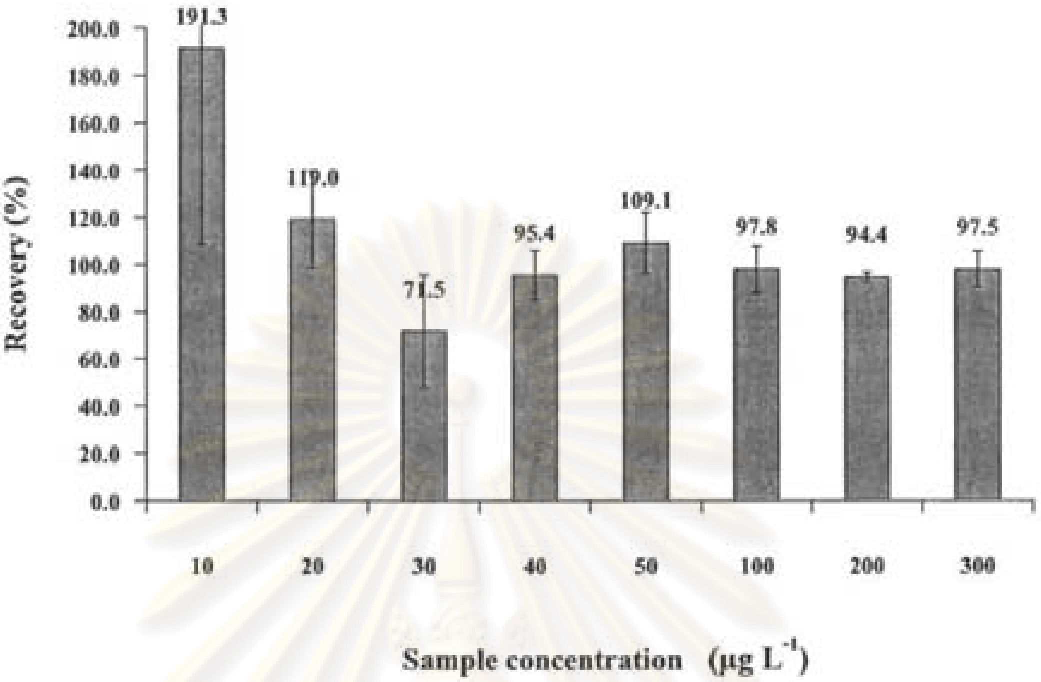


Figure 4.25 Effect of sample concentration on Pb(II) on-line preconcentration-FAAS. (Conditions: SiDAAQ = 10 mg, metal solution volume = 5.0 mL, Pb(II) (pH = 6), sample flow rate = 5.0 mL min^{-1}, eluent flow rate = 5.0 mL min^{-1}, eluent volume = 174 μL, [EDTA] = 0.050 mol L^{-1})

The sample concentration ranging from 10 to 30 μg L^{-1} provided the unacceptable recoveries, while the acceptable recovery can be obtained at 40 to 300 μg L^{-1}. However, the calculated concentration after preconcentration of solution with the concentration of 40 and 50 μg L^{-1} were 1.0 and 1.6 mg L^{-1}, respectively, which were lower than instrument detection limit, IDL (2.0 mg L^{-1}, see section 4.5). Thus, the lowest concentration level of Pb(II) that can be preconcentrated with this system was 100 μg L^{-1}. This means that around 100 μg L^{-1} is the minimum limit of initial concentration of Pb(II) in sample for this system.

The optimum conditions obtained from the on-line preconcentration study above are summarized in Table 4.14.

Table 4.14 Optimum conditions for the on-line preconcentration of Pb(II)

System	Parameter	Condition
FAAS spectrometer	Wavelength	283.3 nm
	Slit width	0.7 nm
	Lamp current	10 mA
	C_2H_2 flow-rate	3.0 mL min^{-1}
	Air flow-rate	10.0 mL min^{-1}
	Nebulizer flow rate	5.0 mL min^{-1}
	Signal measurement	Peak height with read time 15 sec
Mini-column	Column dimension	2.0 cm length and 5.0 mm i.d
	Column packing	10.0 mg of SiDAAQ
Chemical	Standard calibration curve	0-25.0 mg L^{-1}
	Sample solution	5.0 mL of 100 and 300 mg L^{-1} (pH 6.0)
	Elution solution	174 µL of 0.05 mol L^{-1} EDTA (pH 5.0)
	Conditioning solution	Deionized water
	Washing solution	Deionized water
Flow injection	Sample flow rate	5.0 mL min^{-1}
	Eluent flow rate	5.0 mL min^{-1}
	Preconcentration time	60 sec
	Elution time	15 sec
	Washing time	15 sec
	Sample throughput	40 h^{-1}
	Preconcentration factor	29
	Enrichment factor^{a}	27

[a] The approximation of EF was calculated as the ratio of the slopes of the calibration graphs obtained with and without preconcentration [56]. The graph with and without preconcentration were given as following equations, respectively:

$A = 0.0001478C - 0.0008007$, for the interval concentration of 40-300 μg L^{-1},

$A = 0.0000055C - 0.0029000$, for the interval concentration of 5000-25000 μg L^{-1},

where A is the absorbance measured as peak height and C is the concentration in μg L^{-1}.

4.5 Method validation

Method validation is the process of defining an analytical requirement, and confirming that the method under consideration has performance capabilities consistent with what the application requires [81]. It is used to validate the new method developed.

Thus, the proposed on-line preconcentration system was validated by demonstrating the performance characteristics that related with accuracy, precision, required qualitation or quantitation, which were reported in the term of recovery (%), relative standard deviation (%), LOD and LOQ, respectively.

Accuracy: The closeness of agreement between a test result and the accepted reference value. Accuracy is usually determined by spiking the known concentration of analyte in sample matrix (spiked sample) and its percentage recovery values is calculated by equation 4.6.

$$Recovery\,(\%) = \frac{n_{bs} - n_b}{n_s} \times 100 \tag{4.6}$$

where n_s = the amount of metal spiked in a sample (mg)

n_{bs} = the amount of metal determined in a spiked sample (mg)

n_b = the amount of metal determined in a matrix blank or unspiked sample (mg)

Precision: The closeness of agreement between independent test results obtained under stipulated conditions. The measure of precision is usually expressed in terms of the relative standard deviation (RSD) of the test results which calculated by equation 4.7.

$$RSD\ (\%) = \frac{SD}{\overline{X}} \times 100 \tag{4.7}$$

where SD = the standard deviation

$\overline{X}$ = the mean value

Limit of detection: The lowest concentration of analyte in a sample that can be detected with reasonable statistical certainty, but not necessarily quantitated under the stated conditions of the test. LOD is estimated by equation 4.8.

$$LOD_{(mgL^{-1})} = y_{(mgL^{-1})} + 3SD_{(mgL^{-1})} \tag{4.8}$$

where $y_{(mgL^{-1})}$ = the mean value of the concentration of reagent blank

$SD_{(mgL^{-1})}$ = the standard deviation of the concentration of the reagent blank

Limit of quantitation: The lowest concentration of an analyte that can be determined quantitatively with acceptable precision (repeatability) and accuracy under the stated conditions of the test. LOQ is estimated by equation 4.9.

$$LOQ_{(mgL^{-1})} = y_{(mgL^{-1})} + 10SD_{(mgL^{-1})} \tag{4.9}$$

where $y_{(mgL^{-1})}$ = the mean value of the concentration of reagent blank

$SD_{(mgL^{-1})}$ = the standard deviation of the concentration of the reagent blank

Method detection limit: The ability of a measurement method to determine an analyte in a sample matrix. MDL is estimated by equation 4.10.

$$MDL_{(mgL^{-1})} = \frac{LOD}{PF} \tag{4.10}$$

where PF = the preconcentration factor which is the ratio of the initial volume of sample used and the final volume

LOD = the limit of detection (mg L^{-1})

The accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ) and method detection limits (MDL) of spiked synthetic sample are shown in Table 4.15.

Table 4.15 Accuracy, precision, limit of detection, limit of quantitation and method detection limit of the proposed system using spiked synthetic sample (n = 10)

Pb(II) concentration (μg L^{-1})	**Recovery [a] (%)**	**RSD (%)**	**LOD (mg L^{-1})**	**LOQ (mg L^{-1})**	**MDL (mg L^{-1})**
100	100.8	10.8	0.6	1.8	0.02
300	95.3	8.5			

[a] Mean value

The results showed the acceptable recovery and RSD at both concentration levels, indicating that this system provided the accuracy and the precision. Moreover, the lowest concentration of Pb(II) in a sample that can be detected and quantified are 0.6 and 1.8 mg L^{-1}, respectively. Also, the lowest concentration of Pb(II) in a sample that can be detected by this on-line preconcentration system is 0.02 mg L^{-1}.

In addition, instrument detection limit (IDL) for Pb(II) reported in the cookbook of FAAS spectrometer which is used in this work is 15 μg L^{-1}. However, this value may be changed due to decreasing the performance of spectrometer, so it was estimated by reducing the Pb(II) concentration from 4.0 mg L^{-1} until the spectrometer cannot report the correct Pb(II) concentration. The results are shown in Table 4.16.

Table 4.16 The estimation of IDL for FAAS spectrometer

Concentration (mg L^{-1})	**Peak height**	**Calculated concentration**[a] (mg L^{-1})	**Concentration error** (%)
4.0	0.020	3.96	0.93
3.0	0.015	3.04	1.23
2.0	0.009	1.93	3.70
1.0	0.003	0.82	18.52
0.9	0.004	1.00	11.11
0.8	0.003	0.82	1.85
0.7	0.002	0.63	10.05
0.6	0.002	0.63	4.94
0.5	0.001	0.44	11.11
0.4	0.000	0.26	35.19
0.3	0.000	0.26	13.58
0.2	-0.001	0.07	62.96
0.1	0.000	0.26	159.26

[a] Pb(II) concentrations were calculated from peak height using the calibration curve which was prepared in range of 0-25.0 mg L^{-1}.

The results showed that FAAS spectrometer can correctly report the lowest Pb(II) concentration at about 2.0 mg L^{-1}. While the smaller Pb(II) concentration than this value was not reliable.

4.6 Real sample analysis

The interested real samples for this work were drinking and tap water because eventhough there is very low concentration of Pb(II) in samples can have significantly toxic effects on human health. However, the developed on-line preconcentration system must be tested by recovery of spiked analytes in a matrix similar to that of the real samples before applying.

4.6.1 Drinking water

The accuracy and precision are shown in Table 4.17.

Table 4.17 Accuracy and precision of the proposed on-line preconcentration system for determination of Pb(II) in drinking water (n = 10)

Spiked Pb(II) concentration (μg L^{-1})	Added (μg)	Found [a] (μg)	Recovery (%)	RSD (%)
-	0	nd	-	-
90	0.45	0.44 ± 0.02	98.36	7.59
270	1.35	1.31 ± 0.04	97.01	4.69

[a] Mean value $\pm \frac{t_{0.05}s}{\sqrt{n}}$

nd = not detecable (< LOD)

The results showed the acceptable recovery and RSD at both concentration levels (90 and 270 μg L^{-1}), indicating that this system can be applied for determination of Pb(II) in drinking water.

4.6.2 Tap water

The accuracy and precision are shown in Table 4.18.

Table 4.18 Accuracy and precision of the proposed on-line preconcentration system for determination of Pb(II) in tap water (n = 10)

Spiked Pb(II) concentration (μg L^{-1})	Added (μg)	Found [a] (μg)	Recovery (%)	RSD (%)
-	0	nd	-	-
99	0.50	0.36 ± 0.03	71.46	10.58
297	1.50	0.83 ± 0.02	55.68	2.60

[a] Mean value $\pm \frac{t_{0.05}s}{\sqrt{n}}$

nd = not detecable (< LOD)

It was found that the unacceptable recovery and RSD at both concentration levels (99 and 297 μg L^{-1}) were obtained, probably due to incompleteness of the extraction. The matrix interferences in tap water may be expected in three groups: (1) transition metal ions, (2) anions which may form complexes with Pb(II) at the low K_{sp} and (3) chelating organic species. The effect of interferences in the first group was from the competitive extraction with Pb(II), occurring the sample breakthrough. The effect of interferences in the second and third groups was from the complex formation with Pb(II), generating the none extractable species.

CHAPTER V

CONCLUSION

The chelating agent, 1,8-diaminoanthraquinone (1,8-DAAQ), was synthesized in two steps: (1) nucleophilic substitution of 1,8-dichloroanthraquinone with phthalimide and (2) reduction of diphthalimide intermediate to diamino. The silica gel functionalized with 1,8-DAAQ (SiDAAQ) was prepared in two steps: (1) silylation of 3-aminopropyltriethoxysilane with silanol group and (2) Schiff's base formation between amino group and carbonyl carbon of 1,8-DAAQ. The results of spectrometric, elemental analysis and specific surface area analysis characterization indicated that 1,8-DAAQ and SiDAAQ were successfully synthesized.

The adsorption results of Cd(II), Co(II), Cu(II), Ni(II), Pb(II) and Zn(II) onto the SiDAAQ by batch experiment gave the optimum pH at 6.0, 7.0, 6.0, 7.0, 6.0 and 6.0, respectively. The extractions of Co(II) and Ni(II) are not further studied because their sorption capacities were very low. The extraction times for reaching equilibrium were 40, 10, 10 and 20 min for Cd(II), Cu(II), Pb(II) and Zn(II), respectively. The Langmuir adsorption isotherms at 298 ± 1 K under the optimum conditions gave the maximum sorption capacity of 5.62, 10.30, 56.15 and 8.83 mg g^{-1} for Pb(II), Cu(II), Zn(II) and Cd(II), respectively. In addition, the maximum sorption capacities for the extraction of Pb(II), Cu(II), Zn(II) and Cd(II) have been also compared with other modified silica gel as tabulated in Table 5.1. Pb(II) was selected to study the effect of interfering ions and studied with real samples, because the previous experiment results showed the best extraction properties for Pb(II). The presence of Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, NO_3^- and SO_4^{2-} ions did not affected the Pb(II) extraction, which indicated that SiDAAQ has the selectivity in the extraction of Pb(II) over those ions.

Table 5.1 Comparison of maximum sorption capacity (μmol g^{-1}) and sorbent weight used (mg) for the extractions of Cd(II), Cu(II), Pb(II) and Zn(II) using modified silica gel

Chelating agent	**Weight (mg)**	**Maximum sorption capacity (μmol g^{-1})**			
		Cd(II)	Cu(II)	Pb(II)	Zn(II)
1,8-Dihydroxyanthraquinone [7]	100	70	-	76	180
8-Hydroxyquinoline [8]	100	79	400	122	145
1-Aminoanthraquinone [33]	30	167	463	183	167
Aminothioamido-anthraquinone [82]	20	70	300	560	-
1,8-Diaminoanthraquinone [This work]	10	50	162	271	135

The adsorption of Pb(II) onto SiDAAQ by column experiment showed the fast kinetics of extraction and elution. The sorption capacities of mini-column packed with SiDAAQ were not significantly changed after 25 cycles of extractions and elution; i.e. SiDAAQ exhibited the reusability.

The information obtained from the batch and column experiments showed the excellent Pb(II) adsorption properties of SiDAAQ which were suitably used in the proposed on-line flow injection (FI) preconcentration systems coupled with FAAS.

Steps for the evaluation of the efficiency of SiDAAQ for the on-line preconcentration-FAAS system are shown schematically in Figure 3.3, i.e. condition, extraction, washing and elution. The performance characteristics of the system were investigated under the optimum chemical and flow parameters (Table 4.14) using the solutions containing the spiked standard 100 and 300 μg L^{-1} Pb(II) and reported in the term of recovery (%), RSD (%), LOD, LOQ and MDL (Table 4.15). In addition, some parameters for the Pb(II) on-line preconcentration-FAAS system were compared with other systems as shown in Table 5.2. The proposed system was successfully applied for the determination of trace levels of Pb(II) in drinking water sample.

Table 5.2 Comparison of some parameters for the Pb(II) on-line preconcentration-FAAS system

Sorbent	**EF**	**LOD (μg L^{-1})**	**Sample throughput (h^{-1})**
Amberlite XAD-2 impregnated BTAC [51]	27	3.7	26
Polyurethane foam loaded BTAC [52]	26	1	48
Polytetrafluoroethylene (chelating agent: APDC) [58]	330	0.8	15
Macrocycle molecule immobilized silica gel [55]	52	5	63
Chemically modified silica gel with 1,8-diaminoanthraquinone [This work]	27	20	40

Suggestions for future work

The main problem in this work that should be studied further is the matrix compositions of tap water that affect to the accuracy of method. Moreover, the enrichment factor should be improved in order to obtain the lower limit of detection by studying the effect of breakthrough volume. In order to obtain a more effective and comfortable system, the on-line preconcentration-FAAS system used in this work may be developed to the fully automated on-line system using computer software for electronically operation.

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VITA

Mr. Anurak Yaemplai was born on April 25, 1982 in Bangkok, Thailand. He received his Bachelor degree of Science in Chemistry from Chulalongkorn University in 2005. After that, he has been a graduate student at the Department of Chemistry Chulalongkorn University and a member of Environmental Analysis Research Unit under the supervision of Assistant Professor Dr. Wanlapa Aeungmaitrepirom. He finished his postgraduate study with the Master degree of Science in September, 2007.