

การแยกอาร์เซนไนต์ อาร์เซนเตและไดเมทิลอาร์เซนิกแอซิดในน้ำด้วยคอลัมน์ฐานซิลิกาสำหรับการ  
ตรวจวัดโดย ICP-OES

นายนวพงษ์ ปัทมจินตธำรง

จุฬาลงกรณ์มหาวิทยาลัย  
CHULALONGKORN UNIVERSITY

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต  
สาขาวิชาเคมี ภาควิชาเคมี

บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของวิทยานิพนธ์ตั้งแต่ปีการศึกษา 2554 ที่ให้บริการในคลังปัญญาจุฬาฯ (CUIR)

ปีการศึกษา 2557  
เป็นแฟ้มข้อมูลของนิสิตเจ้าของวิทยานิพนธ์ ที่ส่งผ่านทางบัณฑิตวิทยาลัย

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

The abstract and full text of theses from the academic year 2011 in Chulalongkorn University Intellectual Repository (CUIR)  
are the thesis authors' files submitted through the University Graduate School.

SEPARATION OF ARSENITE, ARSENATE AND DIMETHYLARSENIC ACID IN WATER BY  
SILICA-BASED COLUMNS FOR DETERMINATION BY ICP-OES

Mr. Nawapong Phattamajintatamrong



จุฬาลงกรณ์มหาวิทยาลัย  
CHULALONGKORN UNIVERSITY

A Thesis Submitted in Partial Fulfillment of the Requirements  
for the Degree of Master of Science Program in Chemistry

Department of Chemistry

Faculty of Science

Chulalongkorn University

Academic Year 2014

Copyright of Chulalongkorn University

Thesis Title	SEPARATION OF ARSENITE, ARSENATE AND DIMETHYLARSENIC ACID IN WATER BY SILICA-BASED COLUMNS FOR DETERMINATION BY ICP-OES
By	Mr. Nawapong Phattamajintatamrong
Field of Study	Chemistry
Thesis Advisor	Assistant Professor Apichat Imyim, Ph.D.
Thesis Co-Advisor	Mahitti Puanggam, Ph.D.

---

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

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

#### THESIS COMMITTEE

.....Chairman  
(Associate Professor Vudhichai Parasuk, Ph.D.)

.....Thesis Advisor  
(Assistant Professor Apichat Imyim, Ph.D.)

.....Thesis Co-Advisor  
(Mahitti Puanggam, Ph.D.)

.....Examiner  
(Assistant Professor Wanlapa Aeungmaitrepirom, Ph.D.)

.....External Examiner  
(Assistant Professor Rasamee Chaisuksant, Ph.D.)

นวพงษ์ ปัทมจินตธำรง : การแยกอาร์เซนไนต์ อาร์เซนเตตและไดเมทิลอาร์เซนิกแอซิดในน้ำ ด้วยคอลัมน์ฐานซิลิกาสำหรับการตรวจวัดโดย ICP-OES (SEPARATION OF ARSENITE, ARSENATE AND DIMETHYLARSENIC ACID IN WATER BY SILICA-BASED COLUMNS FOR DETERMINATION BY ICP-OES) อ.ที่ปริกษานิพนธ์หลัก: ผศ. ดร. อภิชาติ อิ่มยิ้ม, อ.ที่ปริกษานิพนธ์ร่วม: ดร.มัทธิธิ เพื่อนงาม, 59 หน้า.

ศึกษาการแยกอาร์เซนิก 3 สปีชีส์ ได้แก่ อาร์เซนิก(III) อาร์เซนิก(V) และไดเมทิลอาร์เซนิกแอซิด ในน้ำด้วยวิธีการสกัดด้วยเฟสของแข็งและวิเคราะห์เชิงปริมาณด้วยวิธีอินดักทีฟลิคฟเปลพลาสมาออฟติคัลอิมิสชันสเปกโทรเมตรี วิธีนี้เป็นวิธีที่ง่ายและราคาไม่แพง ทำการแยกอาร์เซนิกสปีชีส์โดยใช้คอลัมน์ 2 ชนิดคือ คอลัมน์ที่บรรจุตัวดูดซับแลกเปลี่ยนแอนไอออนแบบแก่ และคอลัมน์ที่บรรจุซิลิกาที่มีซิลเวอร์คลอไรด์เป็นองค์ประกอบ จากนั้นทำการศึกษาลักษณะที่เหมาะสมในการแยก ได้แก่ ค่าพีเอช อัตราการไหล ความเข้มข้นของตัวชะและความเข้มข้นของตัวอย่าง โดยที่พีเอช 9 อาร์เซนิกอนินทรีย์ (อาร์เซนิก(III) และอาร์เซนิก(V)) จะถูกดูดซับอยู่บนคอลัมน์ที่บรรจุซิลิกาที่มีซิลเวอร์คลอไรด์เป็นองค์ประกอบ ขณะที่ไดเมทิลอาร์เซนิกแอซิดจะไหลผ่านออกจากคอลัมน์ ซึ่งคอลัมน์ที่บรรจุตัวดูดซับแลกเปลี่ยนแอนไอออนจะสามารถดูดซับอาร์เซนิก(V) ได้ที่พีเอช 7 จากนั้นทำการชะอาร์เซนิก(V) ออกจากคอลัมน์ด้วยกรดไฮโดรคลอริกความเข้มข้น 1 โมลต่อลิตร สารละลายที่ได้จากการแยกทั้งหมดถูกนำมาวิเคราะห์หาความเข้มข้นของอาร์เซนิกด้วยวิธีอินดักทีฟลิคฟเปลพลาสมาออฟติคัลอิมิสชันสเปกโทรเมตรี ความเข้มข้นของอาร์เซนิก(V) และไดเมทิลอาร์เซนิกแอซิดหาได้จากการตรวจวัดได้โดยตรง ส่วนอาร์เซนิก(III) หาได้จากการคำนวณโดยการลบออกจากความเข้มข้นของอาร์เซนิกทั้งหมด จากการทดลองภายใต้ภาวะที่เหมาะสมพบว่า ขีดจำกัดการตรวจวัดของวิธี เปรอร์เซ็นต์การได้กลับคืนและค่าความเที่ยงในการวิเคราะห์ของอาร์เซนิก(III) อาร์เซนิก(V) และไดเมทิลอาร์เซนิกแอซิดอยู่ในช่วง 1.48-2.51 ไมโครกรัมต่อลิตร 93.3-101.1% และ 1.56-2.03% ตามลำดับ นอกจากนี้วิธีการนี้สามารถประยุกต์ใช้สำหรับการวิเคราะห์หาอาร์เซนิกแต่ละสปีชีส์ในตัวอย่างน้ำเสียจริงจากโรงงานอุตสาหกรรมซึ่งพบว่าเปอร์เซ็นต์การได้กลับคืนและค่าความเที่ยงในการวิเคราะห์ของอาร์เซนิก(III) อาร์เซนิก(V) และไดเมทิลอาร์เซนิกแอซิดอยู่ในช่วง 97.2-104.3% และ 0.72-2.56% ตามลำดับ

ภาควิชา เคมี

สาขาวิชา เคมี

ปีการศึกษา 2557

ลายมือชื่อนิสิต .....

ลายมือชื่อ อ.ที่ปริกษาหลัก .....

ลายมือชื่อ อ.ที่ปริกษาร่วม .....

# # 5472170623 : MAJOR CHEMISTRY

KEYWORDS: ARSENIC SPECIES / SEPARATION / SOLID PHASE EXTRACTION / ICP-OES

NAWAPONG PHATTAMAJINTATAMRONG: SEPARATION OF ARSENITE, ARSENATE AND DIMETHYLARSENIC ACID IN WATER BY SILICA-BASED COLUMNS FOR DETERMINATION BY ICP-OES. ADVISOR: ASST. PROF. APICHAT IMYIM, Ph.D., CO-ADVISOR: MAHITTI PUANNAM, Ph.D., 59 pp.

A simple and low cost method was proposed for the separation and determination of three arsenic species (As(III), As(V) and dimethyl arsenic acid (DMA)) in water by solid phase extraction (SPE) followed by inductively coupled plasma-optical emission spectrometry (ICP-OES). Two types of columns including a strong anion exchange column and a silver chloride-silica-based column were used for the separation of arsenic species. The pH of solution, flow rate, eluent concentration and sample concentration were studied for assessing optimal conditions. At pH 9, inorganic arsenic species (As(III) and As(V)) were retained on the silver chloride-silica-based column while DMA was pass through the column. The strong anion exchange could adsorb As(V) at pH 7 and afterwards the adsorbed As(V) was eluted by 1 mol/L HCl. All obtained solutions were analyzed for arsenic concentration by ICP-OES. The concentration of As(III/V/DMA) was determined directly or calculated from the subtraction from the total arsenic concentration. Under the optimal conditions, the method detection limit, percent recovery and percent relative standard deviation of As(III), As(V) and DMA were in the range of 1.48-2.51  $\mu\text{g/L}$ , 93.3-101.1 and 1.56-2.03, respectively. Moreover, the proposed method was successfully applied for the speciation of arsenic species in real industrial wastewater samples. The percent recovery and percent relative standard deviation of As(III), As(V) and DMA were in the range of 97.2-104.3 and 0.72-2.56, respectively.

Department: Chemistry

Student's Signature .....

Field of Study: Chemistry

Advisor's Signature .....

Academic Year: 2014

Co-Advisor's Signature .....

## ACKNOWLEDGEMENTS

I would like to thank my advisor, Assistant Professor Dr. Apichat Imyim and Dr. Mahitti Puangam for suggestions, assistance, encouragement and inspiration during the time of thesis. Moreover, I am grateful to my committees Associate Professor Vudhichai Parasuk, Assistant Professor Wanlapa Aeungmaitrepirom and Assistant Professor Rasamee Chaisuksant for comments and offered suggestions for improvement.

This work cannot be completed without kindness and helps of many people. I would like to thank Assistant Professor Dr. Fuangfa Unob for their suggestions on solving some research problems. Next, I would like to thank all of many people in the Environmental Analysis Research Unit for their friendship and the great support. This thesis was financially supported by PTT Public Company Limited.

Finally, I am grateful to my family for love, support, care, understanding and encouragement throughout the entire education.

## CONTENTS

	Page
THAI ABSTRACT .....	iv
ENGLISH ABSTRACT .....	v
ACKNOWLEDGEMENTS .....	vi
CONTENTS .....	vii
LIST OF TABLES .....	xi
LIST OF FIGURES .....	xii
LIST OF ABBREVIATIONS .....	xiv
CHAPTER I INTRODUCTION .....	1
1.1 Statement of the problem.....	1
1.2 Objective.....	2
1.3 Scope of the research.....	2
1.4 Benefits of this research .....	3
CHAPTER II THEORY AND LITERATURE REVIEW .....	4
2.1 Arsenic .....	4
2.1.1 Properties of arsenic.....	4
2.1.2 Toxicity.....	6
2.1.3 Contamination of arsenic in the environment .....	7
2.2 Determination of arsenic .....	7
2.2.1 Spectrometric method .....	7
2.3 Speciation of arsenic .....	9
2.3.1 Chromatographic method.....	9
2.3.2 Non-chromatographic method.....	9

	Page
2.4 Solid phase extraction.....	10
2.4.1 Solid phase extraction procedure.....	10
2.4.1.1 Conditioning.....	11
2.4.1.2 Loading.....	12
2.4.1.3 Washing.....	12
2.4.1.4 Eluting.....	12
2.4.2 Mechanism of solid phase extraction process.....	13
2.4.3 Selection of solid sorbent.....	15
2.4.3.1 Silica and bonded silica sorbent.....	15
2.4.3.2 Polymeric sorbents.....	17
2.4.3.3 Graphitized or porous carbon.....	17
2.5 Literature Review.....	18
CHAPTER III EXPERIMENTAL.....	21
3.1 Apparatus.....	21
3.1.1 Inductively coupled plasma optical emission spectrometer (ICP-OES) ....	21
3.1.2 pH meter.....	21
3.2 Chemical.....	22
3.3 Solution preparation.....	22
3.4 Strong anion exchange and silver chloride-silica-based columns.....	23
3.5 Optimization of adsorption arsenic.....	25
3.6 Speciation of arsenic.....	26
3.6.1 Strong anion exchange column.....	26
3.6.2 Silver chloride-silica-based column.....	27



	Page
3.6.2.1 Effect of the addition of $\text{AgNO}_3$ .....	27
3.7 Method validation.....	28
3.7.1 Accuracy and precision.....	28
3.7.2 Instrument detection limit (IDL) and method detection limit (MDL).....	28
3.8 Influence of coexisting ion interference.....	29
3.9 Speciation of arsenic species in industrial waste water.....	29
CHAPTER IV RESULTS AND DISCUSSION.....	30
4.1 Preparation of silver chloride-silica-based sorbent.....	30
4.2 Optimization of arsenic adsorption.....	31
4.2.1 Effect of pH.....	32
4.2.1.1 Strong anion exchange column.....	32
4.2.1.2 Silver chloride-silica-based column.....	36
4.2.1.3 Effect of $\text{AgNO}_3$ addition.....	37
4.2.2 Effect of flow rate.....	39
4.2.3 Effect of eluent concentration.....	40
4.2.4 Effect of sample concentration.....	41
4.3 Speciation of arsenic.....	42
4.3.1 Strong anion exchange column.....	42
4.3.2 Silver chloride-silica-based column.....	44
4.3.3 Calculating the concentration of each arsenic species.....	45
4.4 Method validation.....	46
4.4.1 Accuracy and precision.....	46
4.4.2 Instrument detection limit (IDL) and method detection limit (MDL).....	47

	Page
4.5 Influence of coexisting ion interference.....	48
4.6 Speciation analysis of arsenic species in industrial wastewater.....	51
CHAPTER V CONCLUSION.....	52
5.1 Conclusion.....	52
5.2 Suggestions for future work.....	53
REFERENCES.....	54
VITA.....	59



จุฬาลงกรณ์มหาวิทยาลัย  
CHULALONGKORN UNIVERSITY

## LIST OF TABLES

Table	Page
2.1	Physical and chemical properties of organic arsenic compound ..... 4
2.2	Physical and chemical properties of inorganic arsenic compound..... 5
2.3	LD <sub>50</sub> of some arsenic species..... 6
2.4	Spectrometric methods for determination of arsenic..... 8
2.5	Sorbents for solid phase extraction and separation mechanisms for solid phase separations..... 16
3.1	ICP-OES conditions for determination of arsenic..... 21
3.2	Chemicals lists ..... 22
3.3	Specifications of the strong anion exchange columns..... 24
4.1	Accuracy and precision of the proposed method (n=3)..... 47
4.2	Concentration at instrument detection limit and method detection limit of the proposed method..... 48
4.3	Speciation of As(III), As(V) and DMA in industrial wastewater sample and method validation results in term of accuracy and precision (n=3) ..... 51
5.1	The detail of extraction with strong anion exchange column and silver chloride-based column..... 52

## LIST OF FIGURES

Figure	Page
2.1 Solid phase extraction steps [27].....	11
2.2 Choice of SPE procedure [28].....	14
2.3 The ternary complex of As(III)–Pyronine B–SDS [19].....	19
3.1 Photograph of vacuum manifold system.....	26
4.1 Preparation of silver chloride-silica-based sorbent.....	30
4.2 Pictures of columns (A) Si-Cl, (B) Si-AgCl.....	31
4.3 Effect of pH on the adsorption percentage of As(III) and As(V) on strong anion exchange column, concentration of As(III and V): 1.0 mg/L.....	33
4.4 The distribution of As(III) and As(V) at various pH in the solution [32]. .....	35
4.5 Effect of pH on the adsorption percentage of As(III), As(V) and DMA .....	36
4.6 Effect of the addition of AgNO <sub>3</sub> on adsorption of As(III) on the silver chloride-silica-based column, concentration of As(III) = 1.0 mg/L.....	37
4.7 Adsorption percentage of arsenic species on the silver chloride-silica-based column, concentration of As(III/V/DMA): 1.0 mg/L.....	38
4.8 Effect of flow rate on the adsorption percentage on strong anion exchange column, concentration of As(V) = 0.3 mg/L.....	39
4.9 Effect of eluent concentration on percent elution using the strong anion exchange column, concentration of As(V) = 0.3 mg/L.....	40
4.10 Effect of sample concentration on percent adsorption on the strong anion exchange, concentration of As(V) in the range of 0.3-200 mg/L.....	41
4.11 Arsenic concentration in the obtained solutions from the SAX column: concentrations of As(III/V/DMA) = 0.3, 0.3 and 0.3 mg/L, flow rate = 2.0 mL/min.....	43

<b>4.12</b>	Arsenic concentration of the obtained solution from the silver chloride-silica-based column: concentrations of As(III/V/DMA) = 0.3, 0.3 and 0.3 mg/L, flow rate = 2.0 mL/min. ....	44
<b>4.13</b>	Scheme of the separation of arsenic species using (A) strong anion exchange column and (B) silver chloride-silica-based column. ....	45
<b>4.14</b>	Interfering effect of $\text{PO}_4^{3-}$ in the separation of arsenic species: (left side) using strong anion exchange column, (right side) using silver chloride-silica-based column. ....	49
<b>4.15</b>	Interfering effect of $\text{SO}_4^{2-}$ in the separation of arsenic species: (left side) using strong anion exchange column, (right side) using silver chloride-silica-based column. ....	50

## LIST OF ABBREVIATIONS

ICP-OES	=	inductively coupled plasma optical emission spectrometry
°C	=	degree Celsius
W	=	watt
M	=	molar
mol	=	mole
nm	=	nanometer
g	=	gram
kg	=	kilogram
mg	=	milligram
µg	=	microgram
ng	=	nanogram
L	=	litre
mL	=	millilitre
µL	=	microgram
m <sup>2</sup> /g	=	square metre per gram
g/cm <sup>3</sup>	=	gram per cubic centimetre
min	=	minute
w/v	=	weight by volume
v/v	=	volume by volume
Rpm	=	revolutions per minute
IDL	=	instrument detection limit
MDL	=	method detection limit

## CHAPTER I

### INTRODUCTION

#### 1.1 Statement of the problem

Arsenic is widely known as a toxic element and can cause adverse health effects, including hyperpigmentation, respiratory, cardiovascular, respiratory, gastrointestinal and carcinogenic effects [1]. Arsenic is a semi-metallic element that naturally occurs in the earth's crust in four oxidation states: As(V), As (III), As (0) and As (-III). Arsenic is leached from natural decay of soil and rock into natural water at low concentration of arsenic [2]. Nevertheless, industrial and agriculture activities such as mining, smelting and using pesticides, herbicides and insecticides distribute higher concentrations of arsenic to the environment [3]. In many countries arsenic is found in drinking water at levels exceeding the World Health Organization's arsenic guideline value of 10 µg/L [4].

Arsenic species in natural water exist in inorganic species as arsenite or As(III) and arsenate or As(V) and organic arsenic species as monomethylarsenic acid (MMA) and dimethylarsenic acid (DMA). The toxicity of arsenite is higher than that of arsenate, while inorganic species of arsenic are more toxic than its organic forms [5]. Determination of total arsenic does not indicate the level of their actual hazard. Therefore, speciation analysis of arsenic is more significant than the determination of total arsenic concentration.

Determination methods of arsenic are mainly based on spectrometric methods including atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS) and inductively coupled plasma optical emission spectrometry (ICP-OES). However, direct determination of arsenic by spectrometric methods cannot provide the speciation of arsenic species. Therefore, preliminary separation of arsenic is required prior to determination.

Chromatographic methods such as high performance liquid chromatography, capillary electrophoresis and ion-chromatography have been developed for speciation of arsenic species by being combined with spectrometric determination but they have high operation cost and need expensive instrument and complicated process. However, non-chromatographic separation methods such as cloud point extraction (CPE), co-precipitation and solid phase extraction (SPE) have been employed for solving the problem due to low cost and simple operation.

Solid phase extraction (SPE) is an interesting method that can separate each arsenic species before the determination step, because of its time and cost saving, simple operation, reduced solvent using and high enrichment factor. In this work, we propose the speciation of arsenic species (arsenite, arsenate and DMA) by using a solid phase extraction with selective adsorption of a strong anion exchange column and a silver chloride-silica-based column.

## 1.2 Objective

This research aimed at (1) development of solid phase extraction for separation of arsenite, arsenate and DMA in water (2) speciation of arsenic species in real industrial wastewater by using the developed method.

## 1.3 Scope of the research

The scope of this research firstly involved the preparation of silver chloride-silica-based column. Selective separation of arsenite, arsenate and DMA on a strong anion exchange column and a silver chloride-silica-based column at various pH values were investigated. Then, effects of pH, flow rate and concentration of eluent were evaluated to find out the optimal conditions for the separation of arsenic species. After that, under optimal conditions, a ternary mixture of arsenic (As(III), As(V) and DMA) was used and evaluated for interfering ions. Finally, the developed method was validated and employed to perform arsenic speciation in the real industrial wastewater samples.



#### 1.4 Benefits of this research

The strong anion exchange column and silver chloride-silica-based column was available for the separation of arsenite, arsenate and DMA in speciation of arsenic species.



## CHAPTER II

### THEORY AND LITERATURE REVIEW

#### 2.1 Arsenic

##### 2.1.1 Properties of arsenic

Arsenic is a metalloid, having both properties of a metal and non-metal. Arsenic exists in four oxidation state: 0, +3, +5 and -3. Elemental arsenic or metallic arsenic occurs in the  $\alpha$ - and  $\beta$ -forms. The  $\alpha$ -form is brittle crystalline solid and steel gray color. The  $\beta$ -form is an amorphous solid and dark grey color. In addition, arsenic compounds exist in both inorganic and organic forms. In inorganic arsenic, arsenic combines with oxygen, chlorine and sulfur. In organic arsenic, arsenic combines with an organic moiety through an arsenic-carbon bond (As-C). Physical and chemical properties of organic arsenic and inorganic arsenic are shown in Table 2.1-2.2 [1].

**Table 2.1** Physical and chemical properties of organic arsenic compound

Name	Molecular weight	Color	Physical state	Melting point	Boiling point	Density
Monomethyl arsenic acid	139.97	white	solid	160.5 °C	No data	2.2 g/cm <sup>3</sup>
Dimethyl arsenic acid	138.00	colorless	solid	195 °C	>200 °C	No data
Arsenobetaine	196.1	No data	solid	203-210 °C	No data	No data

**Table 2.2** Physical and chemical properties of inorganic arsenic compound

Name	Molecular weight	Color	Physical state	Melting point	Boiling point	Density
Arsenic acid	141.94	white	solid	35 °C	160 °C	2.2 g/cm <sup>3</sup>
Arsenic trioxide	197.84	white	solid	274 °C	460 °C	3.86 g/cm <sup>3</sup>
Arsenic pentaoxide	229.84	white	solid	315 °C	No data	4.32 g/cm <sup>3</sup>
Sodium arsenite	130.92	Gray-white	solid	No data	No data	1.87 g/cm <sup>3</sup>
Disodium arsenate	185.91	colorless	solid	57 °C	No data	1.87 g/cm <sup>3</sup>
Calcium arsenate	398.07	colorless	solid	Decomposes on heating	No data	3.62 g/cm <sup>3</sup>



### 2.1.2 Toxicity

Arsenic is widely known as a toxic element. The toxicity of arsenic can differ greatly due to effects of inorganic or organic forms, valence state, solubility and physical state. Generally, inorganic species of arsenic are more toxic than its organic forms and the toxicity of arsenite is higher than that of arsenate. LD<sub>50</sub> of arsenic is shown in Table 2.3 [5]. Arsenic in gas form or arsine gas (AsH<sub>3</sub>) is the highest toxic of arsenic species.

**Table 2.3** LD<sub>50</sub> of some arsenic species

Species	Formula	LD <sub>50</sub> (mg/kg)
Arsenite	As(OH) <sub>3</sub>	4.5
Arsenate	AsO(OH) <sub>3</sub>	14-18
Monomethylarsenic acid	CH <sub>3</sub> AsO(OH) <sub>2</sub>	700-1800
Dimethylarsenic acid	(CH <sub>3</sub> ) <sub>2</sub> AsOOH	700-2600
Tetramethyl arsonium ion	(CH <sub>3</sub> ) <sub>4</sub> As <sup>+</sup>	900
Arsenocholine	(CH <sub>3</sub> ) <sub>3</sub> As <sup>+</sup> CH <sub>2</sub> CH <sub>2</sub> OH	6500
Arsenobetaine	(CH <sub>3</sub> ) <sub>3</sub> As <sup>+</sup> CH <sub>2</sub> COO <sup>-</sup>	>10000
Trimethylarsine oxide	(CH <sub>3</sub> ) <sub>3</sub> AsO	10600

Arsenic poisoning can destroy the liver and cause the risk of lung and skin cancers and other cancer. Arsenic can be excreted from the body by urine if the body receives arsenic at low level and short time. The toxicity of arsenic has chronic toxicity and acute toxicity. Arsenic has a fetal human dose of 0.6 mg/kg (arsenic trioxide).

Chronic toxicity: Chronic arsenic poisoning is associated with receiving arsenic for long time and continually. The symptoms of chronic arsenic poisoning including hyperpigmentation, numbness in hands and feet, peripheral neuropathy, weakness muscle in arms and legs, lung cancer and skin cancer.

Acute toxicity: The symptoms of acute arsenic poisoning including nausea, vomiting, abdominal pain, diarrhea. In extreme cases, patients may have symptoms of muscle cramping, numbness in hands and feet and death due to cardiovascular collapse [6].

### **2.1.3 Contamination of arsenic in the environment**

Arsenic is a naturally occurring element and ubiquitous in the earth's crust. However, the contamination of arsenic in the environment is increasing nowadays. This contamination is a result from a natural disaster such as volcanic effect, corrosion of arsenic containing rock. In addition, arsenic is widely used in industries and agricultural activities such as pesticides, herbicides. Arsenic contaminated industrial wastewater is drained to the environment and arsenic is leached from mine tailing waste into surface water and ground water. Thus arsenic can accumulate in water, soil and enters in a food chain. As very well known that arsenic is very toxic to humans and living organisms, the determination of arsenic becomes increasingly necessary.

## **2.2 Determination of arsenic**

Several techniques for the determination of arsenic are shown below:

### **2.2.1 Spectrometric method**

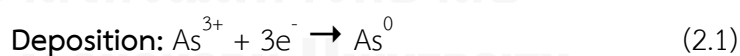
Spectrometric methods such as atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS) and inductively coupled plasma based techniques are popular techniques for the determination of arsenic due to high accuracy and precision, sensitivity and selectivity, easy operation. Some techniques for determination of arsenic are shown in Table 2.4.

**Table 2.4** Spectrometric methods for determination of arsenic

Techniques	Abbreviation	Ref
Hydride generation atomic absorption spectrometry	HG-AAS	[7], [8]
Hydride generation atomic fluorescence spectrometry	HG-AFS	[9]
Graphite furnace atomic absorption spectrometry	GFAAS	[10]
Inductively coupled plasma optical emission spectrometry	ICP-OES	[11]
Inductively coupled plasma mass spectrometry	ICP-MS	[12]
UV-vis spectrometry	UV-vis	[13]
Fluorescence spectrometry	-	

### 2.2.2 Electrochemical method

Electrochemical methods are one of alternative choices for determination of arsenic. This technique shows high sensitivity, low cost and portability. Electrochemical methods such as anodic stripping voltammetry (ASV) [14] have been employed for determination of arsenic. Arsenite can electroplate on the working electrode during a deposition step and oxidized during stripping step (Equations 2.1-2.2). The working electrodes for determination of arsenic have been reported such as Au, Au-coated carbon electrode and glassy carbon.



## 2.3 Speciation of arsenic

Speciation of arsenic species is important because the toxicity of each arsenic species is different. A direct determination of arsenic by spectrometric methods cannot provide speciation of arsenic species. An additional step has to be performed in order to separate arsenic species before being analyzed by such spectrometric methods for example atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), atomic emission spectrometry (AES) and inductively coupled plasma optical emission spectrometry (ICP-OES) which are the most commonly employed detection methods. An off-line sample preparation seems to be suitable for many laboratories due to the ease of operation. However, an on-line separation and determination for arsenic has great attention. The combination of a chromatographic method with elemental detection methods and/or mass spectrometric detection has also attracted great interest.

### 2.3.1 Chromatographic method

A combination of chromatographic method and elemental or mass detection methods is called “hyphenated techniques”. These techniques can be used in various modes with several sensitive spectroscopic detection methods, for example HPLC-ICP-MS [15, 16], HPLC-ICP-OES [17]. The main advantages of these hyphenated techniques are full automation, good reproducibility and high throughput. However, a competent and specialized operator is required and the cost of the instrument is relatively high compared to the sole spectroscopic instruments [18].

### 2.3.2 Non-chromatographic method

Non-chromatographic methods based on chemical speciation are evolutionally growing because a simple operation and inexpensive instruments are advantageous. Non-chromatography methods such as cloud point extraction (CPE) [19, 20], co-precipitation [21, 22] and solid phase extraction (SPE) [23, 24] have been employed for speciation of arsenic. In this research the SPE technique was used to

separate arsenic species due to its extreme simplicity, low cost, high selectivity and recovery [25].

## 2.4 Solid phase extraction

SPE was initially a complementary technique for liquid-liquid extraction (LLE). SPE principle is based on the partitioning of solutes between two phases (solid and liquid). The liquid phase is an analyte containing phase, being generally an aqueous solution (sample solution), while the solid phase is a solid sorbent which has affinity towards analytes. The SPE technique is commonly used in sample preparation for a separation of analytes from sample matrices or for an elimination of matrix interferences. The analytes are favorably partitioned onto the solid sorbent surface; on the other hand, the interferences presented in the sample matrix are transferred to the solid phase. In the former case the analytes are eluted from the solid phase by using an appropriate eluting solution. SPE is one of the separation methods which is very popular for sample preparation due to process rapidity, high selectivity, easy automation, high preconcentration factor and ability to clean up samples before the detection step. Various types of samples in chemical analysis, such as biological fluid samples, water, beverages, and soil can be undergone the SPE.

### 2.4.1 Solid phase extraction procedure

Four steps in SPE (Figure 2.1) are traditionally performed including (i) conditioning of solid sorbent, (ii) loading of samples onto the SPE column or cartridge, (iii) rinsing of unwanted compounds/ions from the SPE sorbent, and (iv) elution of the interested analytes for determination [26].



### 2.4.1.1 Conditioning

Firstly, an appropriate solvent is passed through a SPE column to condition the SPE sorbent. In general, the conditioning solvent is similar to the sample solvent. This step is essential as it enables the wettability of the SPE sorbent and the solvation of active sites of the sorbent. In addition, impurities initially contained in the sorbent, and air voids can be removed from the SPE column. The dryness of the SPE sorbent should be avoided in this step, otherwise the efficiency of the SPE column becomes poorer.

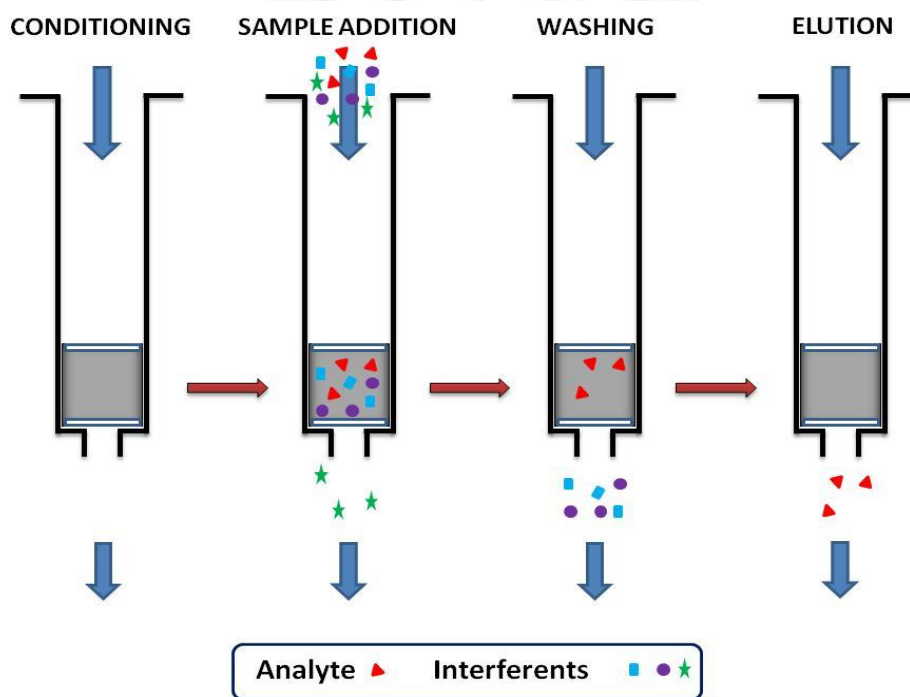


Figure 2.1 Solid phase extraction steps [27]

#### **2.4.1.2 Loading**

The second step involves the passing of liquid samples through the SPE column. The volume of the sample varies depending on the designed SPE system, generally ranging from 1 to 1000 mL. The sample flow or percolation can be spontaneous by gravity or with the aid of a pump that allows controlling a flow rate. The sample flow rate through the SPE sorbent should be low enough to ensure the retention of the analytes onto the SPE sorbent and high enough to avoid long operation duration. During this step, the interested analytes are attached to the SPE sorbent.

#### **2.4.1.3 Washing**

This step involves rinsing or washing the SPE column. It is optional. An appropriate solvent is passed through the SPE column to remove matrix interferences that have previously retained on the solid sorbent. The washing solvent should not flush the retained analytes. If water is unwanted in the determination step, a drying process should be done in this step.

#### **2.4.1.4 Eluting**

Finally, the analytes is eluted from the SPE sorbent by using an appropriate eluting solution without eluting retained matrix interferences. The volume of eluting solution should be optimized to quantitatively elute all analytes with the lowest dilution of analytes. The elution flow rate should be correctly considered to ensure the elution efficiency. Two elution steps of elution are always recommended. A small aliquot of eluting solution is initially applied to soak the SPE sorbent surface, followed by the passing of the actual eluting solution.

### 2.4.2 Mechanism of solid phase extraction process

The mechanism(s) of solid phase extraction process involves interactions between analytes molecules or ions and the SPE sorbent [28]. Understanding the mechanism permits an analyst to design or choose an appropriate SPE. The most common retention mechanisms in SPE are based on van der Waals forces, hydrogen bonding, dipole-dipole forces and cation-anion interactions. Each SPE sorbent involves one or more of these properties. Four modes of SPE are classified as follows.

- Reversed phase SPE: A reverse phase SPE involves a nonpolar SPE sorbent and a polar or quasi-polar liquid phase. The polarity of analytes dissolved in the liquid phase is middle between nonpolar and semi-polar. The attractive forces between the analytes and the SPE sorbent are based on nonpolar-nonpolar interactions (always called van der Waals forces or dispersion forces). A nonpolar solvent is usually used to elute the adsorbed analytes from the SPE sorbent. Carbon-based, polymer-based, and bonded silica-based SPE sorbents are often employed in reversed phase SPE.
- Normal phase SPE: A normal phase SPE is a contrast to a reverse phase SPE. It involves a polar SPE sorbent and a mid- to nonpolar liquid phase. The analytes dissolved in the liquid phase is semi-polar or polar compounds. The attractive forces between the analytes and the SPE sorbent are based on dipole-dipole forces and cation-anion interactions. A polar solvent, being more polar than the sample matrix is usually used to elute the adsorbed analytes from the SPE sorbent. Bonded silica-based sorbents with polar functional groups (also called chemically modified silica gel) is typically used in this SPE mode.

- Ion exchange SPE: An ion exchange SPE is mainly based on the electrostatic attractions between cationic analytes and anionic functionality of SPE sorbent (called cation exchange SPE) and anionic analytes and cationic functionality of SPE sorbent (called anion exchange SPE). Silica or polymer with aliphatic sulfonic acid groups is always used in cation exchange SPE, while silica or polymer with an aliphatic quaternary amine group bonded to the sorbent surface is often used in anion exchange SPE.

Figure 2.2 shows the method for selecting a mechanism to determine SPE procedure.

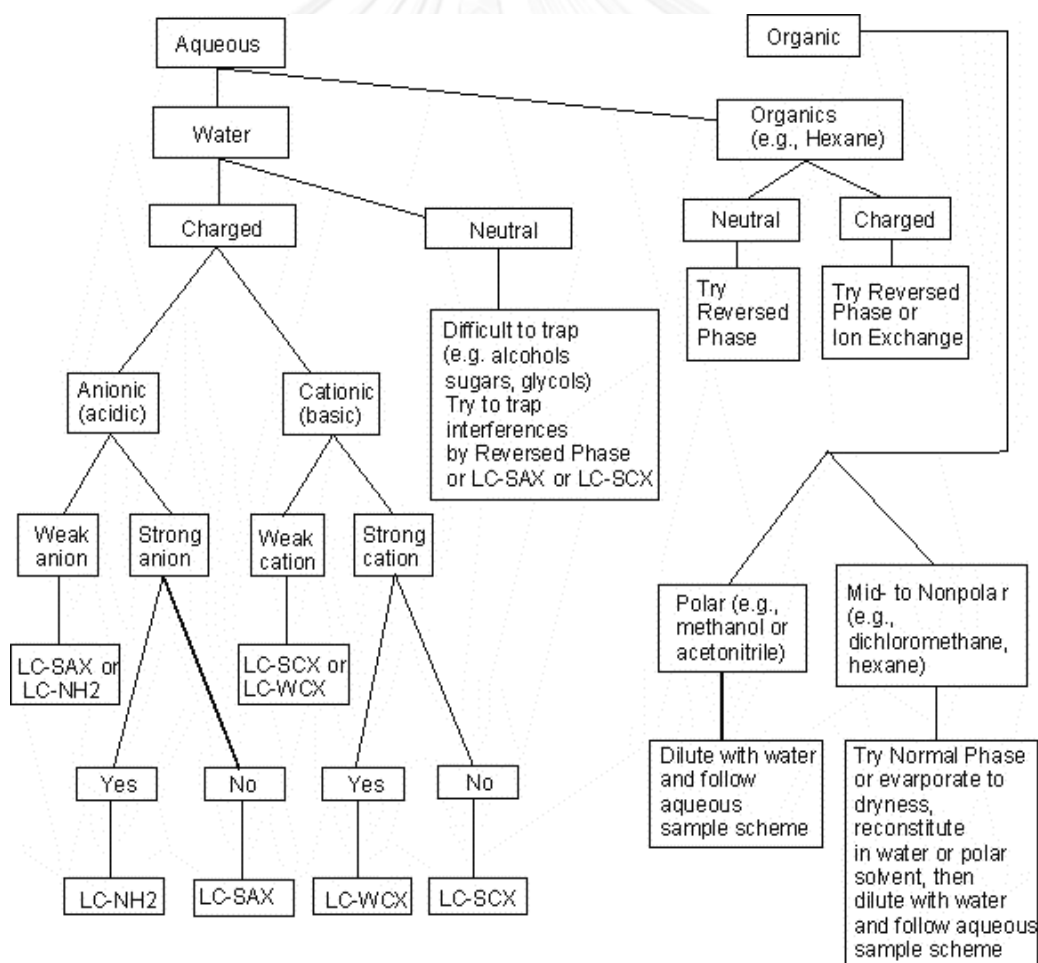


Figure 2.2 Choice of SPE procedure [28].

### 2.4.3 Selection of solid sorbent

A suitable SPE sorbent is carefully selected in order to obtain the best efficiency in analyte separation from a sample matrix. The main requirements for a solid sorbent in the analysis of elements are as follows:

- (i) The possibility to extract a large number of trace elements over a wide pH range (along with selectivity towards major ions),
- (ii) The fast and quantitative sorption and elution, in particular, sorbents that allow fast reaction rates are preferred to achieve faster extraction as well as higher loading capacities.
- (iii) A high capacity,
- (iv) Regenerability,
- (v) Accessibility

In the present, a large number of SPE sorbents are available, and the most frequently used groups of sorbents are chemically modified silica gel, polymeric sorbents (resin), and graphitized or porous carbon.

#### 2.4.3.1 Silica and bonded silica sorbent

Silica-based sorbents offer good mechanical strength and can be used in a wide pH range (typically in the range of 2 to 7.5). Silica surface is heterogeneous with silanol groups. Silica surface can be easily modified to tune its applicability towards different types of analytes. The functionality can be non-polar (e.g. C18), polar (e.g. –COOH or NH<sub>2</sub>), ionic or mixed-mode (e.g. C8/cation exchange). Silica-based sorbents can be used in reversed-phased (RP), normal-phase (NP) and ion-exchange (IE) SPE.

The most used silica-based SPE sorbent in trace element analysis are –SO<sub>3</sub><sup>–</sup> and –N<sup>+</sup>(CH<sub>3</sub>)<sub>3</sub> ionic functional groups. The extraction mechanism is based on ion exchange. The –SO<sub>3</sub><sup>–</sup> silica is a strong cation exchanger (SCX) for the extraction of cationic analytes from solution. The N<sup>+</sup>(CH<sub>3</sub>)<sub>3</sub> silica is a strong anion exchanger (SAX) for extraction of anionic analytes.

Sorbents for solid phase extraction and separation mechanisms for solid phase separations are listed in Table 2.5.

**Table 2.5** Sorbents for solid phase extraction and separation mechanisms for solid phase separations

Sorbent	Structure	Analyte type	Dissolving solvent	Elution solvent
Reversed phase				
Octadecyl (C18)	$-(CH_2)_{17}CH_3$	Nonpolar	methanol/water, acetonitrile/water	For nonpolar analytes: hexane, chloroform For polar analytes: methanol
Octyl (C8)	$-(CH_2)_7CH_3$			
Ethyl (C2)	$-CH_2CH_3$			
Cyclohexyl	$-CH_2CH_2-C_6H_{12}$			
Phenyl	$-CH_2-C_6H_6$			
Normal phase (bonded)				
Cyano (CN)	$-(CH_2)_3CN$	Slightly- moderately polar - strongly polar	hexane, chloroform	methanol
Amino (NH <sub>2</sub> )	$-(CH_2)_3NH_2$			
Diol (COHCOH)	$(CH_2)_3OCH_2CHOHCH_2OH$			
Normal phase (adsorption)				
Kieselguhr (Diatomaceous Earth)	-SiOH	Slightly- moderately polar - strongly polar	hexane, chloroform	methanol (dependent on type of analyte)
Silica gel	-SiOH			
Florisil	Mg <sub>2</sub> SiO <sub>3</sub>			
Alumina (neutral)	Al <sub>2</sub> O <sub>3</sub>			
Ion exchange (anion and cation Exchange)				
Amino (NH <sub>2</sub> )	$-(CH_2)_3NH_2^+$	Anion exchange - ionic acid	water or buffer (pH=pKa +2)	1) Buffer (pH=pK <sub>a</sub> +2) 2) pH where sorbent or analyte is neutral 3) Solvent with high ionic strength
1°, 2°- Amino (NH/NH <sub>2</sub> )	$-(CH_2)_3NH^+CH_2CH_2NH_2$			
Quaternary Amine (N <sup>+</sup> )	$-(CH_2)_3N^+(CH_3)_3$	Cation exchange - ionic base	water or buffer (pH=pKa-2)	1) Buffer (pH=pK <sub>a</sub> -2) 2) pH where sorbent or analyte is neutral 3) Solvent with high ionic strength
Carboxylic acid (COOH)	$-(CH_2)_2COO^-$			
Propyl sulfonic acid (SO <sub>2</sub> OH)	$-(CH_2)_3SO_2O^-$			
Aromatic sulfonic acid (ArSO <sub>2</sub> OH)	$-(CH_2)_3-C_6H_4-SO_2O^-$			

### 2.4.3.2 Polymeric sorbents

The most widely used polymeric sorbents are the styrene-divinylbenzene copolymers (PS-DVB). They offer the large surface area of surface areas of 700-1,200 m<sup>2</sup>/g which allow higher retention of analytes. PS-DVB bearing aromatic sites is suitable for nonpolar compounds which allow  $\pi$ - $\pi$  interactions with unsaturated analytes. Similar to silica, PS-DVB can be chemically modified to be suitable for trace element, known as chelating resin. However, these sorbents are not available in pre-packed cartridges because they required laborious purification steps before use.

### 2.4.3.3 Graphitized or porous carbon

Activated carbon is a sorbent with high specific microporous large surface areas containing polar groups. Graphitized carbon blacks (GCBs) are obtained from heating carbon blacks at very high temperature in an inert atmosphere. Their surface area is still low (about 100 m<sup>2</sup>/g). Carbon-based sorbent is non-specific and sometimes non-porous material. They can simultaneously extract neutral, cationic and anionic compounds via van der Waals forces. It is not suitable for a specific analyte especially in metal speciation analysis.

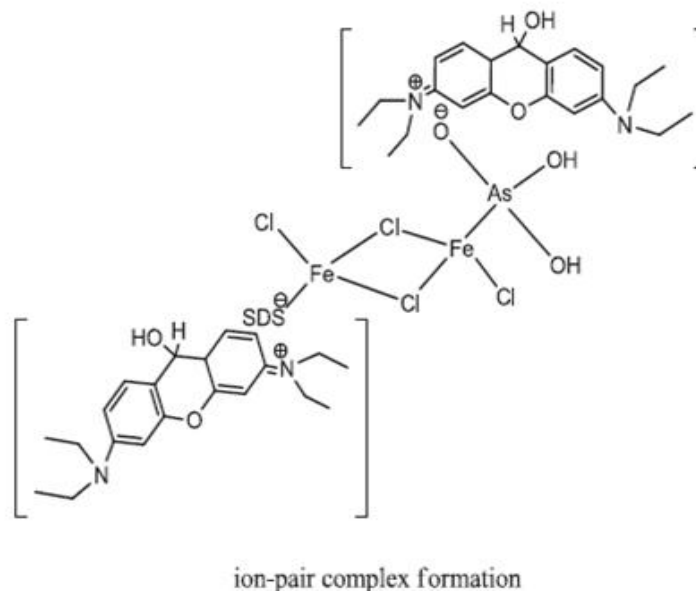
## 2.5 Literature Review

Speciation of arsenic has been more and more developed due to the difference in toxicity of each arsenic species. The most effective speciation method for arsenic is liquid chromatography coupled with mass spectrometry (LC-MS). However, complicated operation and high instrumental cost are main limitation of this technique. Therefore, simple or classical separation methods have been employed instead of LC-MS. In 2004, Zhang [22] have developed a sensitivity and accuracy method for speciation of arsenic in water by co-precipitation separation. Arsenic solution was mixed with ammonium pyrrolidine dithiocarbamate (APDC) solution and Ni(II) solution. The pH of the sample solution was adjusted to the pH range of 2–3. The sample solution was allowed to stand for 1 h at the room temperature for quantitative formation of the co-precipitate. The co-precipitate obtained containing trivalent species of arsenic was submitted to the AAS determination for As(III), because As(V) was hardly co-precipitated by the complex of Ni-(PDC)<sub>2</sub> in the same pH condition. In order to co-precipitate total arsenic in the sample solution, As(V) was reduced to As(III) before the procedure of co-precipitation. As(V) concentration in the sample solution can be calculated by the difference in concentration between As(III) and total arsenic. The detection limit was 0.02 ng/mL. The recoveries for spike of arsenic ranged from 97.1 to 103.4% for As(III) and from 96.6 to 99.4% for As(V). Moreover, Tuzen et al. [21] have studied the speciation of As(III) and As(V) by co-precipitation separation in the same way. Their procedure was based on the co-precipitation of As(V) on the precipitate of aluminum(III) hydroxide while As(III) was not co-precipitated.

Cloud point extraction (CPE) is one of separation method; it is used to selective separation arsenic species for speciation of arsenic. Ulusoy et al. [19] used cloud point extraction for separation and preconcentration of arsenic in water prior to hydride generation atomic absorption spectrometry (HGAAS). As(III) has formed an ion-pairing complex with Pyronine B (Figure 2.3) in the presence of sodium dodecyl sulfate (SDS) at pH 10.0 and the ion-pairing complex was extracted into the non-ionic surfactant, polyethylene glycol *tert*-octylphenyl ether (Triton X-114). After phase



separation, the surfactant-rich phase was diluted with 1 mol/L HCl and 0.5 mL of 3.0% (w/v) Antifoam A. Under the optimized conditions, a preconcentration factor of 60 and a detection limit of 0.008  $\mu\text{g/L}$  were achieved.



**Figure 2.3** The ternary complex of As(III)–Pyronine B–SDS [19]

Solid phase extraction is a separation method which has been widely used for separation of arsenic species. Some solid phase sorbents including non-polar C-18, polytetrafluoroethylene fiber, ion-exchange resin, controlled pore glass, cartridge, Diaion® HP-2MG resin, modified mesoporous  $\text{TiO}_2$ , activated carbon, porous graphitic carbon, and cetyltrimethylammonium bromide (CTAB)-modified alkyl silica have been employed for the separation and preconcentration of arsenic. Zhang et al [24] developed a method for the multi-element inorganic speciation of As(III, V), Se(IV, VI) and Sb(III, V) in natural water using solid phase extraction. Titanium dioxide ( $\text{TiO}_2$ ) was used to adsorb inorganic arsenic. For As(III) was co-precipitated with Pb-PDC, dissolved in dilute nitric acid, and then determined. The concentrations of As(V) can be calculated by the difference of the concentrations obtained by the determinations. Detection limits was found to 0.10  $\mu\text{g/L}$ . Chen et al. [11] used 3-(2-aminoethylamino) propyltrimethoxysilane (AAPTS) modified ordered mesoporous silica for the speciation of inorganic arsenic. The adsorption behaviors of As(III) and

As(V) on AAPTS modified ordered mesoporous silica were investigated. It was found that As(V) can be selectively adsorbed on the micro-column within the pH of 3–9, while As(III) could not be retained in the studied pH range and passed through the micro-column directly. Then, As(V) was eluted by 1 mol/L HCl and determined by ICP-OES. The limit of detection was 0.05  $\mu\text{g/L}$ . Erdoğan et al. [29] demonstrated the use of hybrid nano  $\text{ZrO}_2/\text{B}_2\text{O}_3$  sorbent for the speciation and determination of As(III), As(V) and total As in water samples. The hybrid sorbent was successfully applied for preconcentration and speciation of As(V) from various samples with a recovery of  $99\pm 5\%$ . The analytical limit of detection was found at 9.25 ng/L. Adsorption capacities of the hybrid sorbent were 98.04 mg/g for As(V). Xiong et al. [30] used cetyltrimethylammonium bromide (CTAB)-modified alkyl silica sorbent for speciation of inorganic arsenic. The limits of detection of As(V) was 0.15  $\mu\text{g/L}$ . The relative standard deviations for nine replicate determinations at 5.0  $\mu\text{g/L}$  level of As(V) was 4.0%. The calibration graphs of the method for As(V) and Se(VI) were linear in the range of 0.5–1000.0  $\mu\text{g/L}$  with a correlation coefficient of 0.9936.

Moreover, an online-solid phase extraction was developed for the speciation of arsenic to overcome the loss of analyte, improve the precision and increase the sample throughput. For example, Sigrist et al. [31] presented the analysis of As(III) and total arsenic in water samples by solid phase extraction using trimethylaminopropyl-based resin coupled with a flow system and HG-AAS detection. The limit of detection of As(III) and total arsenic were 0.5 and 0.6  $\mu\text{g/L}$ , respectively. Issa et al. [32] developed a method for separation and determination of inorganic arsenic and organic arsenic in drinking, natural and wastewater. Three resins including a strong base anion exchange (SBAE) and two hybrid (HY) resins: HY-Fe and HY-AgCl were used. The adsorption of each arsenic species depended on the pH of the solution. Quantitative separation of inorganic arsenic (As(III), As(V)) and organic arsenic was successful by using SBAE column. At pH less than 8, As(III) was not adsorbed onto SBAE, allowing the determination of As(III). HY-Fe could adsorb all species except for DMA. While HY-AgCl could retain As(III) and AS(V). Thus both MMA and DMA could be determined in the eluent. All separated arsenic species were determined by ICP-OES. Overall recoveries were in the range of 88-102%.

## CHAPTER III

### EXPERIMENTAL

#### 3.1 Apparatus

##### 3.1.1 Inductively coupled plasma optical emission spectrometer (ICP-OES)

An arsenic concentration was analyzed by an ICP-spectrometer model iCAP 6500 DUO (Thermo). The operation conditions for determination of arsenic by ICP-OES are shown in Table 3.1

**Table 3.1** ICP-OES conditions for determination of arsenic

Parameter	ICP-OES condition
Wave length	189.042 nm
RF power	1150 W
Auxiliary gas flow	0.5 L/min
Nebulizer gas flow	0.6 L/min
Coolant gas flow	12 L/min
Analysis pump rate	50 rpm

##### 3.1.2 pH meter

All solutions in this work were adjusted their pH value by a pH meter model SevenCompact pH/Ion s220 (Mettler-Toledo).

### 3.2 Chemical

All chemicals used in this research are analytical grade listed in Table 3.2

**Table 3.2** Chemicals lists

Chemicals	Supplier
Silver nitrate	Merck
The stock solution of 1000 ppm As(V)	Merck
The stock solution of 1000 ppm As(III)	Fluka
Dimethylarsenic acid/Cacodylic acid	Aldrich
Nitric acid	Merck
Hydrochloric acid	Merck
Sodium hydroxide	Sigma-Aldrich
Methanol	Merck

### 3.3 Solution preparation

#### Arsenic solution

Arsenic stock solution was used as follows: 1000 mg/L As(III): arsenic(III) atomic absorption standard solution (1 mg/mL As in 2% HNO<sub>3</sub>), 1000 mg/L As(V): arsenic(V) atomic absorption standard solution (1 mg/mL As in 0.5 mol/L HNO<sub>3</sub>) and 1000 mg/L DMA: DMA stock solution was prepared by dissolving 0.092 g of cacodylic acid (CH<sub>3</sub>)<sub>2</sub>AsO(OH) with 50 mL of 1% HNO<sub>3</sub>.

#### Silver nitrate solution

Silver nitrate solution (0.1 mol/L) was prepared by dissolving approximate amount of AgNO<sub>3</sub> with Milli-Q water. The obtained solution was used to prepare silver chloride-silica-based sorbents and added into arsenic solutions for separation of arsenic species by the silver chloride-silica-based column.

### Hydrochloric acid solution

Hydrochloric acid solution (1.0 mol/L) was prepared by diluting the concentrated hydrochloric acid with deionized water. The obtained solution was used in the elution of arsenic species from the silver chloride-silica-based column.

### Nitric acid solution

Nitric acid solution (0.1, 0.5, 1.0 mol/L and 1 %v/v) was prepared by diluting the concentrated nitric acid with deionized water. The obtained solution was used in the elution of arsenic species from a strong anion exchange column and solution pH adjustment.

### Sodium hydroxide solution

Sodium hydroxide solutions (0.1, 0.5 and 1.0 mol/L) were prepared by dissolving approximate amount of sodium hydroxide with deionized water and used for solution pH adjustment.

## 3.4 Strong anion exchange and silver chloride-silica-based columns

Strong anion exchange columns were purchased from Vertical Chromatography Co., Ltd. (VertiPak<sup>TM</sup> SAX Tubes). The columns are in the form of *n*-propyl(trimethyl) ammonium chloride silica (SAX column): 2 cm-diameter, 9 cm-length, 10 mL of volume, and 200 mg of packed adsorbent amount. The specifications of the column are shown in Table 3.3.

**Table 3.3** Specifications of the strong anion exchange columns

Specifications	
Phase	SAX (Trimethylaminopropyl)
Base	Irregular-shape silica
Particle size ( $\mu\text{m}$ )	50
Pore size ( $\text{\AA}$ )	60
Surface area ( $\text{m}^2/\text{g}$ )	500
Carbon load (%)	7
Bonding	Trifunctional
Capacity (meq/g)	0.6
End cap	No
pH stability	2-9

The preparation methods of silver chloride-silica-based columns were applied from the US Patent 4724082 [33]. Silver chloride-silica-based column was prepared by passing 10 mL of 0.1 mol/L  $\text{AgNO}_3$  through a strong anion exchange column. Then, the column was cleaned with deionized water for washing off residual  $\text{AgNO}_3$ . The color of the sorbent changed from white to light gray. The columns were kept away from light.

### 3.5 Optimization of adsorption arsenic

#### Effect of pH

The effect of pH was firstly studied for the adsorption behavior of arsenic species (As(III), As(V) and DMA) on SAX column and silver chloride-silica-based column. Ten milliliter of arsenic standard solution (1.0 mg/L) of each As(III), As(V) and DMA were adjusted pH value at 5-9 by using HNO<sub>3</sub> and NaOH solution. The arsenic solution was passed through the column in a loading step. In an elution step, Ten milliliter of eluent solution was passed through the column. The loading and eluting flow rates used in extraction were fixed at 2 mL/min. The obtained solutions from the loading and elution steps were analyzed for arsenic concentration by ICP-OES. An adsorption percentage of each arsenic species on the column was calculated for investigating adsorption behavior.

#### Effect of flow rate

In this experiment, the effect of flow rate was investigated at 1-2 mL/min. First, the SAX column was cleaned and the flow rate was calibrated with deionized water. Then, Ten milliliter of As(V) solution (0.3 mg/L) at the optimum pH was passed through the column and eluted using 10 mL of 1 mol/L HCl. The arsenic concentration in the solutions obtained from the loading and eluting steps was determined.

#### Effect of eluent concentration

The effect of eluent concentration was investigated by testing with the SAX column. In the eluting step, the column was eluted with different eluent concentrations (0.5 and 1.0 mol/l HCl). Ten milliliter of As(V) solution (0.3 mg/L) at the optimum condition was passed through the column. Then, As(V) was eluted by passing HCl solution through the column. The loading and eluting flow rates used in extraction were fixed at 2 mL/min. The arsenic concentration in the obtained solution was determined by ICP-OES.

### 3.6 Speciation of arsenic

#### 3.6.1 Strong anion exchange column

Ternary mixtures arsenic containing 0.3 mg/L of As(III), 0.3 mg/L of As(V) and 0.3 mg/L of DMA was prepared by diluting their 1000 mg/L stock solution. The solution pH was adjusted 7. Ten milliliter of deionized water was pumped through the column (using a manifold system SUPELCO Figure 3.1) for cleaning the column. Then, Ten milliliter of arsenic solution was pumped through the column. After that, arsenic species adsorbed on the column was eluted by passing 10 mL of 1 mol/L HCl through the column. The loading and eluting flow rates used in extraction were fixed at 2 mL/min. The arsenic concentration in obtained solutions was determined by ICP-OES.



**Figure 3.1** Photograph of vacuum manifold system.



### 3.6.2 Silver chloride-silica-based column

Ternary mixtures arsenic containing 0.3 mg/L of As(III), 0.3 mg/L of As(V) and 0.3 mg/L of DMA were prepared by diluting the 1000 mg/L stock solutions. The solution pH was adjusted at pH 9 by using HNO<sub>3</sub> solution and NaOH solution. The solution was added with 100  $\mu$ L of 0.1 mol/L AgNO<sub>3</sub> and stirred for 2 min. The silver chloride-silica-based column was set with the vacuum manifold system. The column was washed with deionized water. Then, 10 mL of the arsenic solution was pumped through the column. After that, arsenic species adsorbed on the column was eluted by passing 10 mL of 1 %v/v HNO<sub>3</sub> through the column. The loading and eluting flow rates used in extraction were fixed at 2 mL/min. The arsenic concentration in obtained solutions was determined by ICP-OES.

#### 3.6.2.1 Effect of the addition of AgNO<sub>3</sub>

The addition of AgNO<sub>3</sub> in arsenic solution was studied for investigating adsorption of arsenic species on the silver chloride-silica-based column. 100  $\mu$ L of 0.1 mol/L AgNO<sub>3</sub> was added into 10 mL of As(III) solution. The pH of solution was adjusted in the pH range of 5-9. The solution was passed through the silver chloride-silica-based column and eluted with 10 mL of 1 %v/v HNO<sub>3</sub>. The arsenic concentration in obtained solutions was determined by ICP-OES. Finally, the adsorption percentage of arsenic on the column was calculated.

### 3.7 Method validation

The validation of the proposed method was studied under optimum condition. The accuracy, precision, instrument detection limit (IDL) and method detection limit (MDL) of the method were investigated and reported.

#### 3.7.1 Accuracy and precision

Ternary mixtures of arsenic containing 0.3 mg/L of As(III), 0.3 mg/L of As(V) and 0.3 mg/L of DMA was extracted by the SAX columns and the silver chloride-silica-based columns in 3 replicated. The obtained solutions were analyzed for arsenic concentration by ICP-OES. Concentrations of As(III), As(V) and DMA were calculated. The accuracy and precision of the method were calculated and showed in term of percent recovery (%recovery) and percent relative standard deviation (%RSD).

#### 3.7.2 Instrument detection limit (IDL) and method detection limit (MDL)

Blank solution (DI water) was analyzed. The signal of arsenic at wavelength 189.042 nm was recorded by ICP-OES in 10 replicates. The instrument detection limit was calculated from the mean of signal blank and 3 times of standard deviation.

The method detection limit was determined in 7 replicates. Each blank solution was spiked with low arsenic species (2 and 5 times the instrument detection limit). Then, the solution was extracted by the SAX column and the arsenic concentration was determined by ICP-OES. The method detection limit was calculated from the standard deviation and Student's test value.

### 3.8 Influence of coexisting ion interference

The proposed method was subject to the study of influence of coexisting ion interference. The 100-fold diluted industrial wastewater samples were spiked with  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$  in the range of 10-200 mg/L. The separation of arsenic species was studied in comparison to a non-spiked industrial wastewater sample. The sample solution was subject to arsenic species separation under the chosen conditions by SAX and silver chloride-silica-based columns. The arsenic concentration in the obtained solutions was determined by ICP-OES. Finally, the adsorption percentage of arsenic on both columns was calculated.

### 3.9 Speciation of arsenic species in industrial waste water

The proposed method was applied for the speciation of arsenic species in two industrial wastewaters received from PTT public Company Limited. The accuracy and precision of the method were determined by using spiked and represented in terms of percent recovery (%recovery) and percent relative standard deviation (%RSD). The industrial wastewater was diluted 100 times with deionized water. The obtained solution was employed as non-spiked sample. Then, the As(III), As(V) and DMA standard solutions were added in non-spiked samples at the concentration of 0.3 mg/L. The obtained solutions were employed as arsenic-spike samples. Both non-spiked and arsenic-spike samples were subjected to arsenic species separation under the chosen conditions of the proposed method for speciation of arsenic and the obtained information was calculated for %recovery and %RSD.

## CHAPTER IV

### RESULTS AND DISCUSSION

#### 4.1 Preparation of silver chloride-silica-based sorbent

Silver chloride-silica-based sorbent (Si-AgCl) is one of the sorbent that was used to extract arsenic species in this work. The silver chloride-silica-based sorbent was prepared by passing silver nitrate solution through strong anion exchange silica based sorbent (Si-Cl) to change the functional group of the sorbent from *n*-propyl(trimethyl) ammonium chloride silica, Si-Cl to Si-AgCl, as shown in Figure 4.1. Then the sorbent was washed with deionized water several times for removing residual AgNO<sub>3</sub> which might interfere the extraction of arsenic species. The columns were kept away from light.

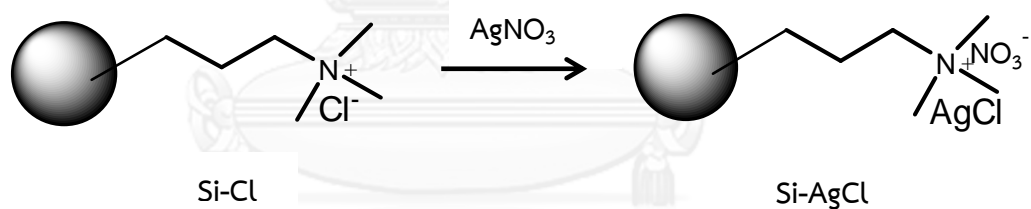
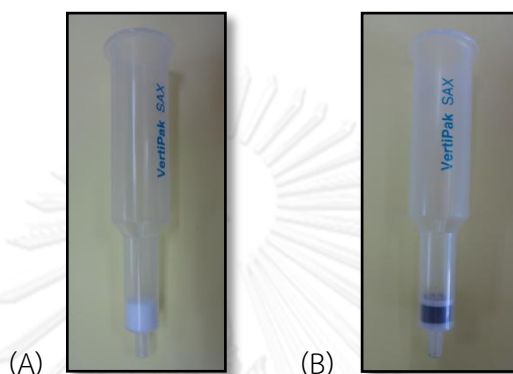


Figure 4.1 Preparation of silver chloride-silica-based sorbent

The appearance and color of the columns are shown in Figure 4.2, (A) a SAX column before passing silver nitrate solution was white sorbent, while (B) a silver chloride-silica-based column was dark purple. The color change may be attributed to the auto-reduction of  $\text{Ag}^+$  to  $\text{Ag}(0)$  catalyzed by light during the preparation step.



**Figure 4.2** Pictures of columns (A) Si-Cl, (B) Si-AgCl.

#### 4.2 Optimization of arsenic adsorption

Various parameters affecting the speciation of arsenic species were evaluated such as the effects of pH, flow rate, and eluent concentrations. The adsorption and elution efficiencies were the main factors to assess the performance of the prepared columns. Each experiment was done in 3 replicates.

The adsorption and elution efficiencies of arsenic species on the columns were presented in term of percent adsorption (or %Adsorption) and percent elution (or %Elution). The percent adsorption and percent elution of As(III), As(V) and DMA were determined by Equations 4.1 and 4.2.

$$\% \text{Adsorption} = \frac{C_i - C_f}{C_i} \times 100 \quad (4.1)$$

$$\% \text{Elution} = \frac{C_{eluted}}{C_i - C_f} \times 100 \quad (4.2)$$

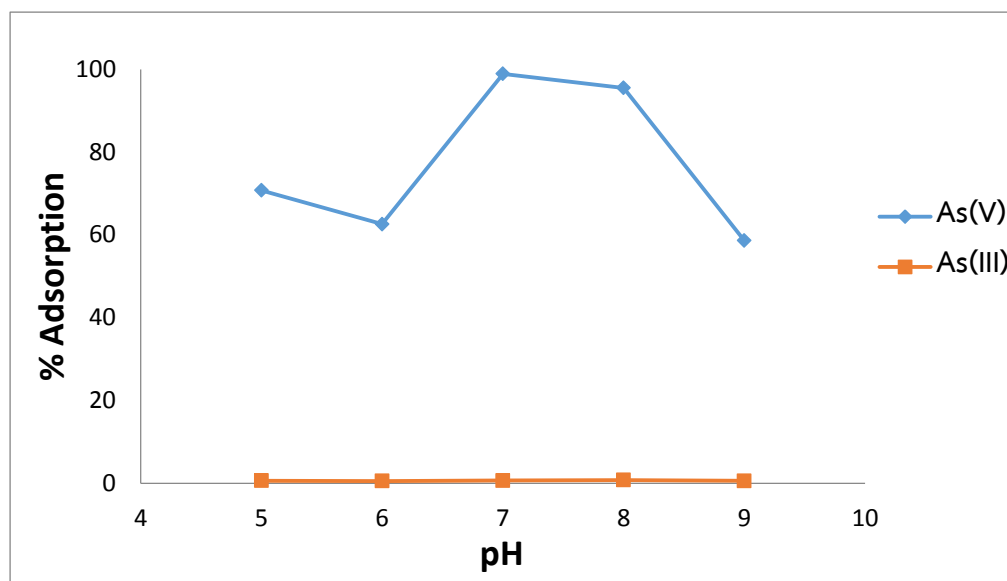
Where  $C_i$  = the initial concentration of arsenic in the solution before extraction (mg/L)  
 $C_f$  = the final concentration of arsenic in the solution after extraction (mg/L)  
 $C_{eluted}$  = the concentration of arsenic in eluted solution (mg/L)

#### 4.2.1 Effect of pH

The pH of solution plays as a key role in the adsorption and separation of different species on the sorbent materials by solid phase extraction because three arsenic species (As(III), As(V) and DMA) can show different ionic character at various pH values. The effect of pH was studied on the SAX column and the silver chloride-silica-based column.

##### 4.2.1.1 Strong anion exchange column

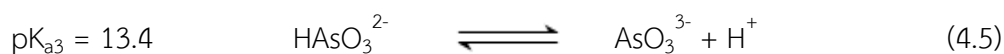
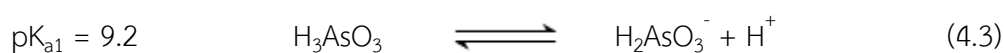
The effect of pH on the SAX column was investigated. The arsenic standard solutions of As(III) and As(V) after being adjusted their pH value in the range of 5-9 were passed through a SAX column at a fixed solution flow rate of 2 mL/min controlled by a vacuum pump connected with a manifold. The effect of pH was expressed by percent adsorption of As(III) and As(V) as shown in Figure 4.3.



**Figure 4.3** Effect of pH on the adsorption percentage of As(III) and As(V) on strong anion exchange column, concentration of As(III and V): 1.0 mg/L.

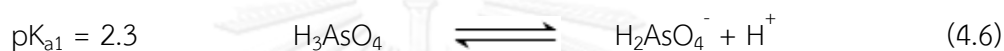
The result showed that As(III) was not adsorbed and passed through the SAX column at all studied pH. On the contrary, As(V) was quantitatively adsorbed and retained on the Si-Cl column at the pH values of 7 and 8 while the adsorption of As(V) at pH 9 was decreased probably due to the instability of silica in basic conditions and the effect of  $\text{OH}^-$  competition in anion exchange mechanism.

The different adsorption behavior of As(III) and As(V) on the SAX column can be explained by their  $\text{pK}_a$  and different ionic characters. The distribution of As(III) and As(V) species, as shown in Figure 4.4, describes their ionic character of As(III) and As(V) at any pH value of solution. As(III) has  $\text{pK}_a$  of 9.2, 12.1 and 13.4, as shown in Equations 4.3-4.5 [34].



As(III) exists as uncharged species in the form of  $\text{H}_3\text{AsO}_3$  at pH of solution less than 9 while the amino group on the SAX column existed as positive charge in the form of  $-\text{N}^+(\text{CH}_3)_3$ . Thus, As(III) was not adsorbed and passed through the column because there was no electrostatic effect with the sorbent.

On the contrary, As(V), having  $\text{pK}_a$  of 2.3, 7.0 and 11.5 (Equations 4.6-4.8) [34], was presented mainly in negative charged species. At the studied pH range of 5-9, As(V), indicating in the form of  $\text{H}_2\text{AsO}_4^-$  and  $\text{HAsO}_4^{2-}$ , can be attracted by  $-\text{N}^+(\text{CH}_3)_3$  via electrostatic interaction and can be adsorbed on the column.



The pH value of 7.0 showed the highest percent adsorption of As(V). Therefore, pH 7.0 was selected as optimum pH for separation arsenic species by SAX column.



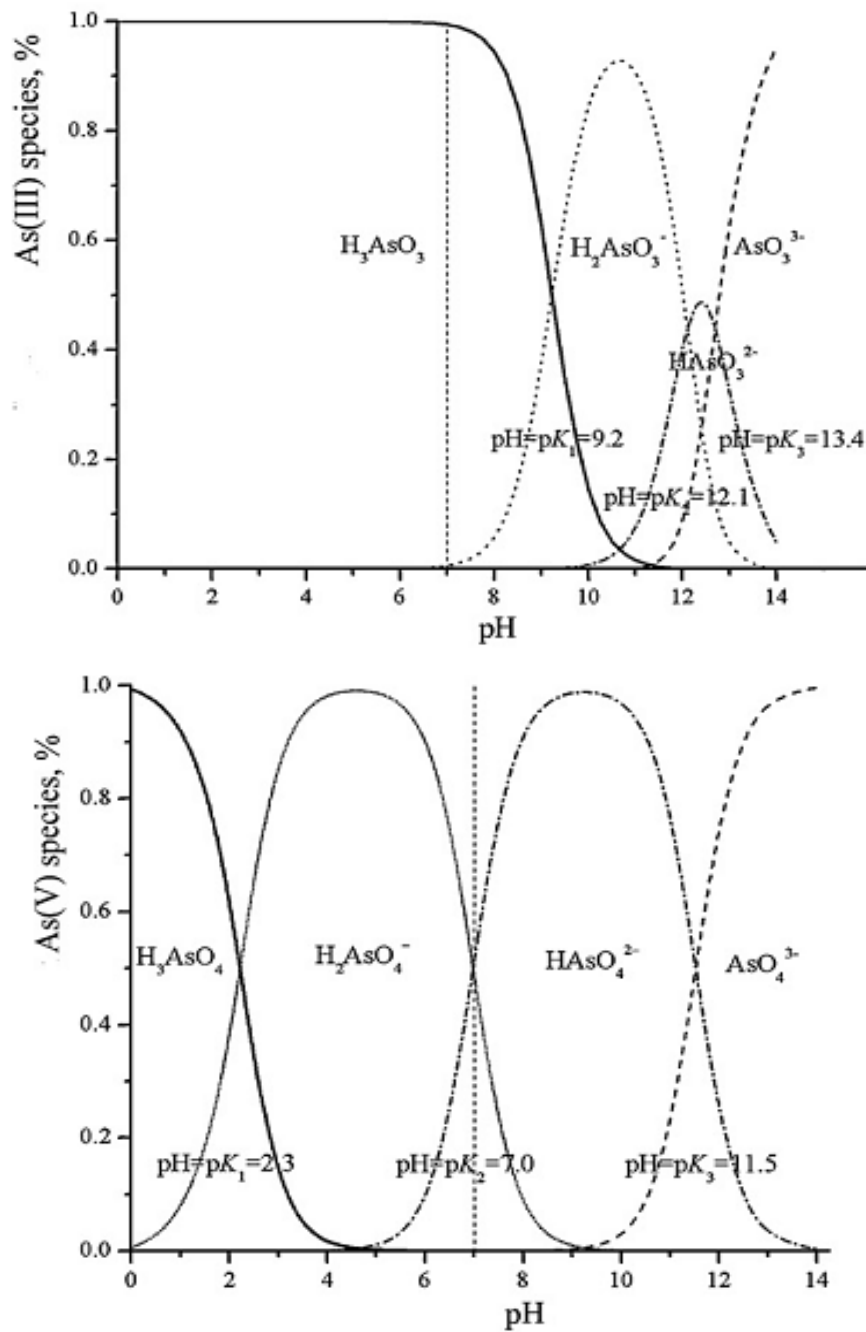
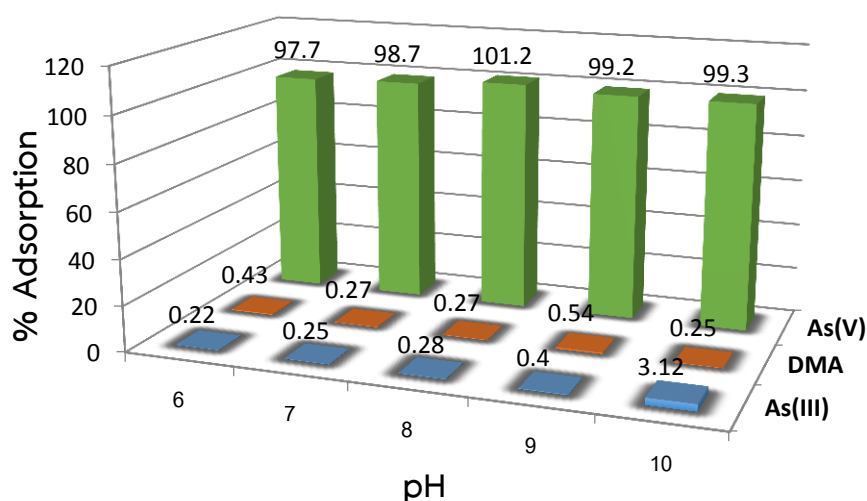


Figure 4.4 The distribution of As(III) and As(V) at various pH in the solution [32].

#### 4.2.1.2 Silver chloride-silica-based column

The effect of solution pH in the range of 6-10 (to ensure the dissociation of  $\text{H}_3\text{AsO}_3$ ) on the modified column, silver chloride-silica-based column (Si-AgCl), was studied to assess the adsorption behavior of arsenic species by passing standard solutions of interested arsenic species through the silver chloride-silica-based column and the elution was done by using 1% (v/v) nitric acid. The percent adsorption of As(III), As(V) and DMA on the column were shown in Figure 4.5.



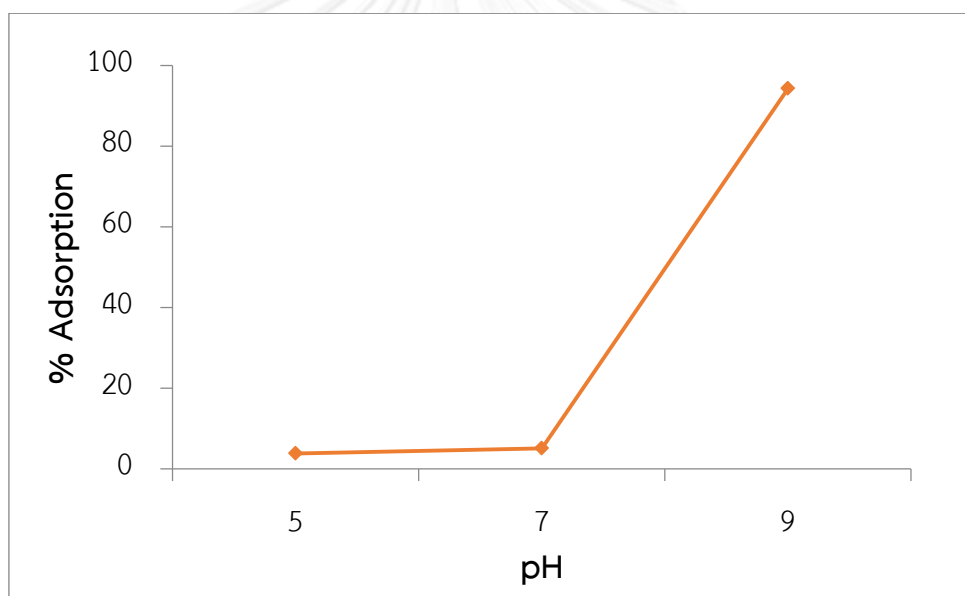
**Figure 4.5** Effect of pH on the adsorption percentage of As(III), As(V) and DMA On the silver chloride-silica-based column, concentration of As(III/V/DMA): 1.0 mg/L.

The silver chloride-silica-based column was desired in this work for the separation of inorganic arsenic (As(III) and As(V)) and organic arsenic (DMA). However, the result did not meet the expectation. It was found that As(III) and DMA were not adsorbed on the silver chloride-silica-based column. The percent adsorption of As(III) and DMA at the studied pH were as low as 0.22-3.12 and 0.25-0.54, respectively. On the contrary, As(V) is one of arsenic species that can be bonded and adsorbed on the column. The interaction of As(V) and silver chloride-silica-based column was depicted in Equation 4.9.



#### 4.2.1.3 Effect of $\text{AgNO}_3$ addition

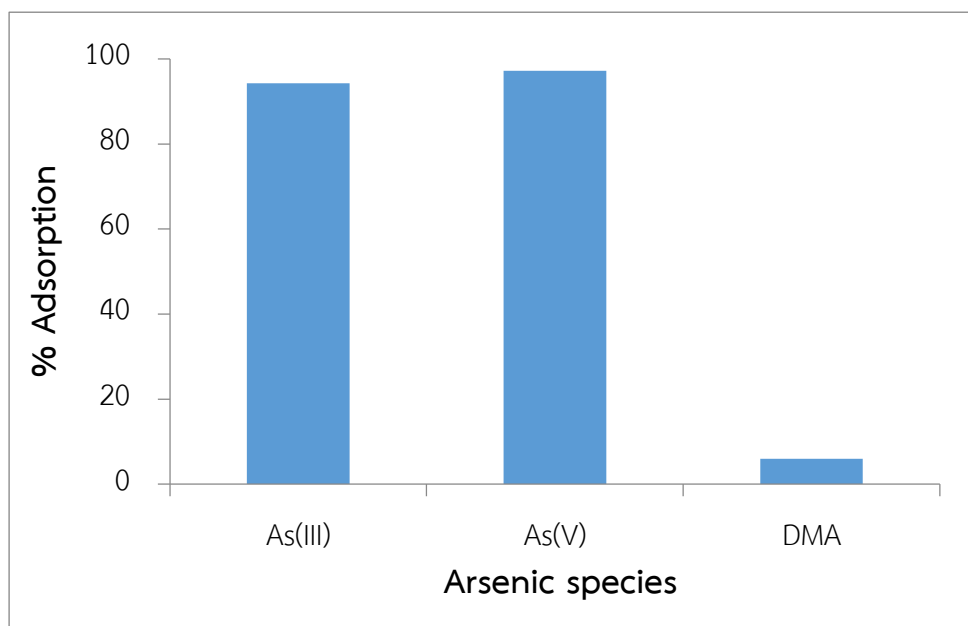
From the preliminary investigation, the adsorption behavior of As(III), As(V) and DMA on the silver chloride-silica-based column did not meet the expectation. Therefore, the study of the effect of  $\text{AgNO}_3$  addition was performed under the hypothesis that As(III) could be adsorbed on the column and the separation of inorganic arsenic and organic arsenic would be successful. A small amount (200  $\mu\text{L}$ ) of 0.1 mol/L  $\text{AgNO}_3$  was added into the standard solution of As(III). The solution pH value was adjusted in the range of 5-9 and the extraction by the Si-AgCl column was then performed. The result was shown in Figure 4.6.



**Figure 4.6** Effect of the addition of  $\text{AgNO}_3$  on adsorption of As(III) on the silver chloride-silica-based column, concentration of As(III) = 1.0 mg/L.

It was found that at pH 5 and 7, As(III) was not retained and passed through the silver chloride-silica-based column while As(III) was adsorbed on the column at pH 9 and the percent adsorption of As(III) was as high as 94.29% because As(III) was possibly precipitated with  $\text{AgNO}_3$  in silver arsenite form and struck on the column before the elution with nitric acid.

As the addition of  $\text{AgNO}_3$  was successful to separate As(III) from the solution and adsorbed onto the silver chloride-silica-based column, thus the addition of  $\text{AgNO}_3$  was tested for the case of As(V) and DMA at solution pH of 9. The results are shown in Figure 4.7.

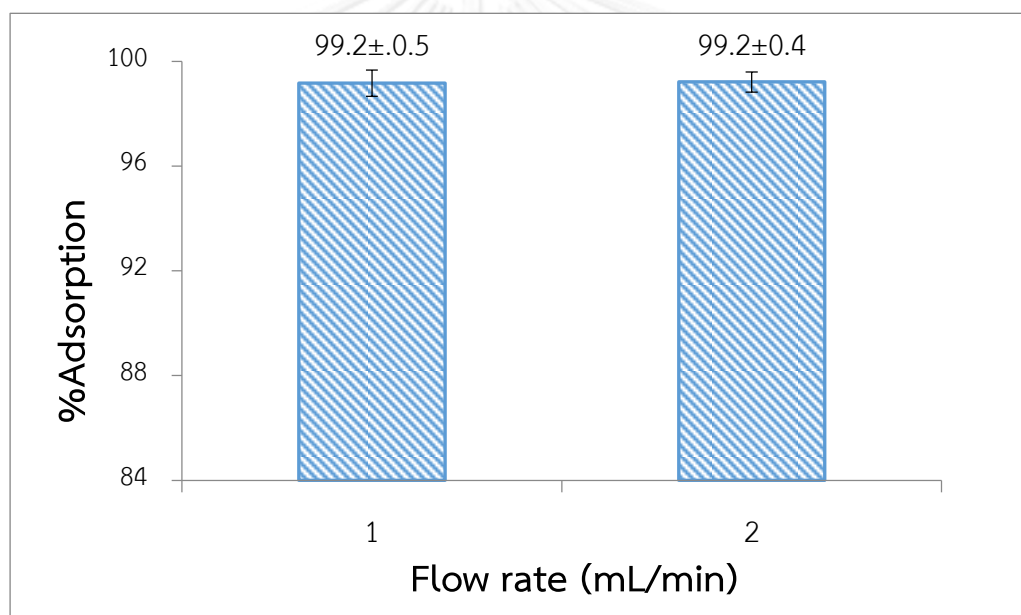


**Figure 4.7** Adsorption percentage of arsenic species on the silver chloride-silica-based column, concentration of As(III/V/DMA): 1.0 mg/L.

The results showed that As(III) and As(V) could very well adsorbed on the silver chloride-silica-based column while DMA was not retained and passed through the column. This suggested that the addition of  $\text{AgNO}_3$  into arsenic solutions facilitated the separation of inorganic arsenic and organic arsenic by the silver chloride-silica-based column. As(III) and As(V) might form nuclei in form of silver arsenite and silver arsenate, respectively while DMA might not interact with  $\text{Ag}^+$  and directly passed through the column. Therefore, the separation of arsenic species by the silver chloride-silica-based column in this research was based on the system in which  $\text{AgNO}_3$  was added at pH 9.

#### 4.2.2 Effect of flow rate

A flow rate of solution is an important parameter in an extraction by SPE which affect to the adsorption of arsenic on the column. If the flow rate is too fast it may cause a low adsorption. The effect of flow rate at 1 and 2 mL/min was investigated for the extraction of arsenic on the SAX column (Figure 4.8). It was found that the percent adsorption at the flow rates of 1 and 2 mL/min in the extraction of the SAX column and the silver chloride-silica-based column was not different with more than 99 % adsorption. For the reason of time saving in experiments, the flow rate at 2 mL/min was chosen for the separation of arsenic.

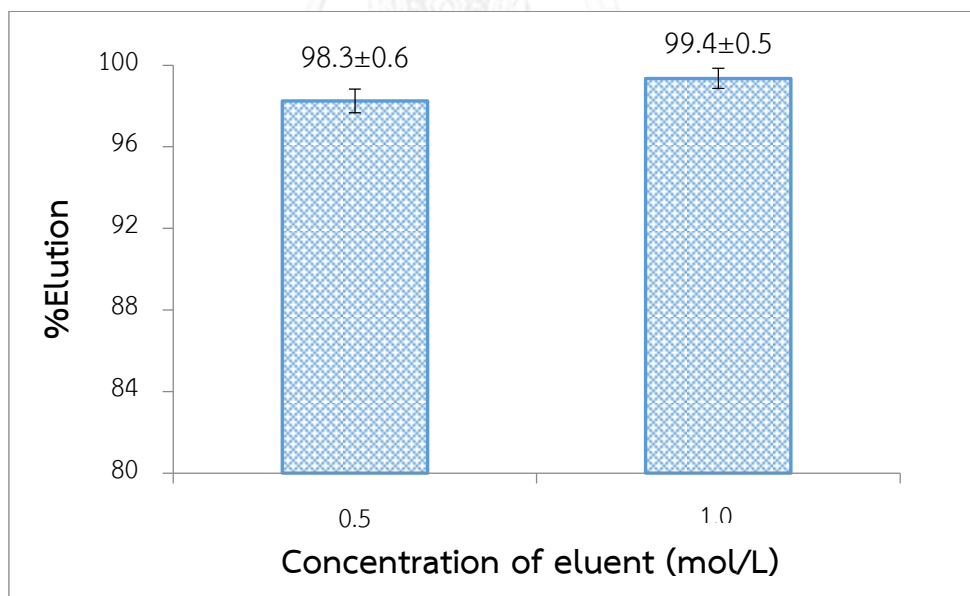


**Figure 4.8** Effect of flow rate on the adsorption percentage on strong anion exchange column, concentration of As(V) = 0.3 mg/L.

#### 4.2.3 Effect of eluent concentration

The eluent concentration can affect an elution of analyte adsorbed on the column. At low eluent concentrations, the elution of arsenic species previously adsorbed on the column was probably poor and giving a low recovery.

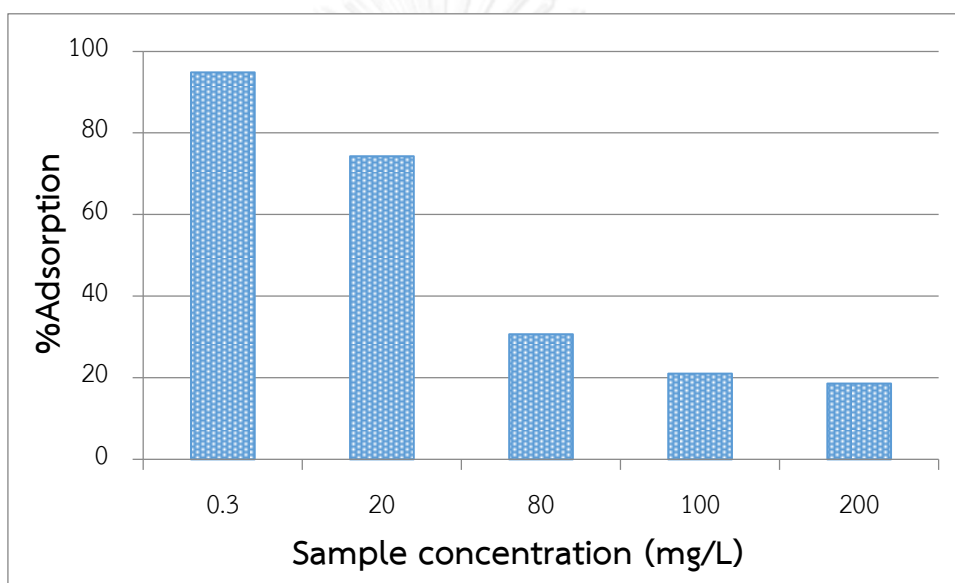
In this experiment, the effect of eluent concentrations was studied only on the SAX column because using low concentration of nitric acid (1% v/v nitric acid) allowed the elution of arsenic species from the silver chloride-silica-based column with good elution efficiency. The eluent concentration of hydrochloric acid at 0.5 and 1.0 mol/L was investigated in order to provide an enough chloride ions for replacing arsenic species (As(V)) that was previously adsorbed on the column. The percent elution calculated from Equation 4.2 was shown in Figure 4.9. The elution percentages of arsenic species using 0.5 and 1.0 mol/L HCl were similar with more than 98 % elution. The hydrochloric acid concentration of 1.0 mol/L was then selected for the elution of arsenic species for the SAX column.



**Figure 4.9** Effect of eluent concentration on percent elution using the strong anion exchange column, concentration of As(V) = 0.3 mg/L.

#### 4.2.4 Effect of sample concentration

In order to assess a suitable sample concentration for the extraction, the sample concentration was varied from 0.3-500 mg/L. Under an optimal condition, 10 mL of sample solution was passed through the SAX column containing 200 mg of sorbent. The effect of sample concentration on percent adsorption was shown in Figure 4.10.



**Figure 4.10** Effect of sample concentration on percent adsorption on the strong anion exchange, concentration of As(V) in the range of 0.3-200 mg/L.

It was found that the percent adsorption of a sample concentration at 0.3 mg/L was 94.8% suggesting that arsenic species can be quantitatively adsorbed on the column. The adsorption percentages were continuously decreased to 74.3, 30.7, 21.0 and 18.6 when the sample concentration rose up to 20, 80, 100 and 200 mg/L, respectively. Therefore, the sample concentration in the extraction process was recommended for 0.3 mg/L which gave 90 % adsorption under the optimal condition.

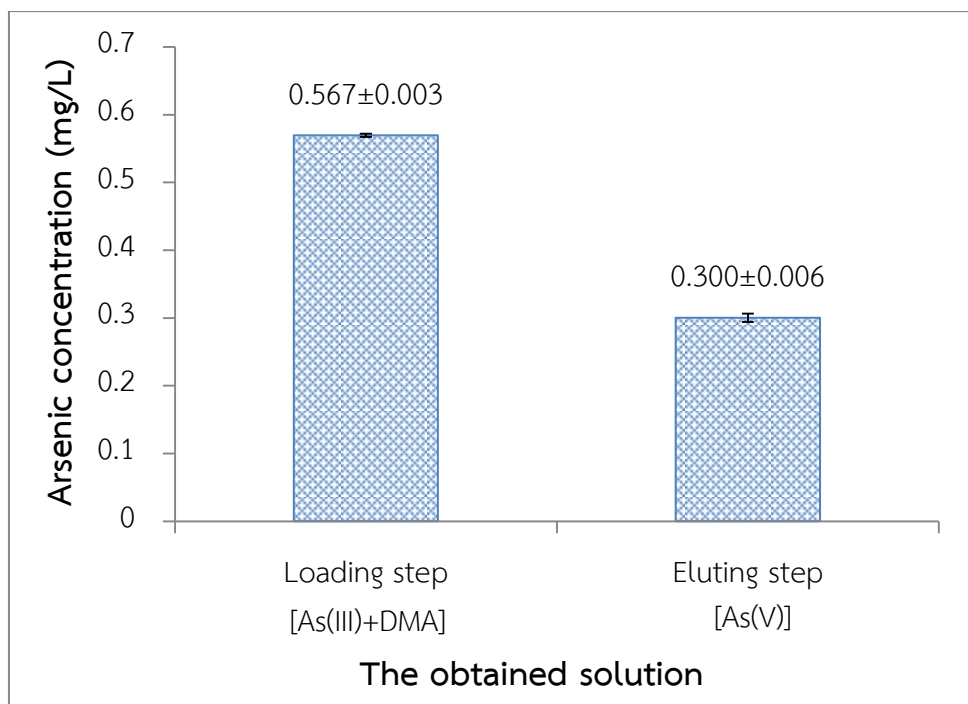
### 4.3 Speciation of arsenic

Based on the preliminary study of the effect of pH, the adsorption behavior of As(III), As(V) and DMA was investigated using single arsenic species standard solution. Therefore, in this experiment, the ternary mixture of three arsenic species of As(III), As(V) and DMA was tested for the adsorption on the SAX column and the silver chloride-silica-based column.

#### 4.3.1 Strong anion exchange column

The ternary mixture of arsenic containing 0.3 mg/L of As(III), 0.3 mg/L of As(V) and 0.3 mg/L of DMA was passed through the SAX column under optimal conditions. The separation was divided into two steps including loading and eluting steps. In the loading step, the solution was passed through the column and in the eluting step, the adsorbed arsenic on the column was eluted with 1.0 mol/L hydrochloric acid. The arsenic concentration in the obtained solutions from the loading and eluting steps was analyzed by ICP-OES and showed in Figure 4.11.



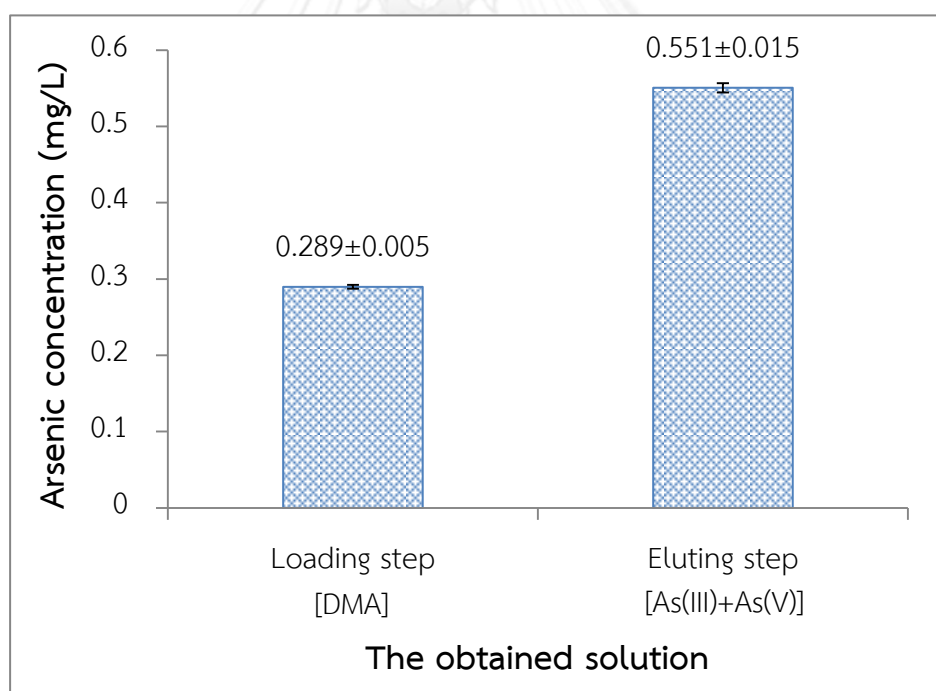


**Figure 4.11** Arsenic concentration in the obtained solutions from the SAX column: concentrations of As(III/V/DMA) = 0.3, 0.3 and 0.3 mg/L, flow rate = 2.0 mL/min.

The SAX column was a selective column for the separation of As(V) from the ternary mixture of arsenic species. The results reveals that arsenic concentration of loading step was 0.567 mg/L which was the sum of As(III) and DMA (0.3 mg/L each) that could not be adsorbed on the SI-Cl column and directly passed through the column. The recovery of As(III) and DMA was 94.5%. Meanwhile the arsenic concentration in the solution obtained from the eluting step corresponded to the concentration of As(V) that was consequently eluted from the column using hydrochloric acid. The As(V) concentration was 0.30 mg/L corresponding to 100% recovery. These results reveal that the SAX column was capable to separate As(V) from As(III)/DMA and the concentration of As(V) was directly determined from the solution of eluting step.

### 4.3.2 Silver chloride-silica-based column

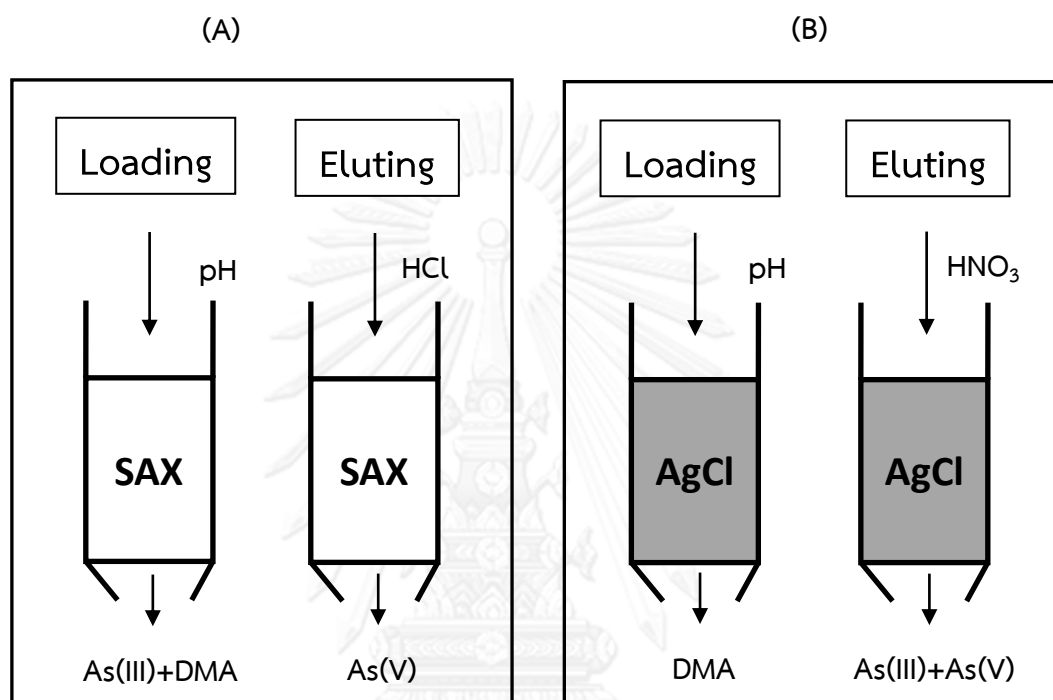
The ternary mixtures of arsenic containing 0.3 mg/L of As(III), 0.3 mg/L of As(V) and 0.3 mg/L of DMA after adding a small volume of  $\text{AgNO}_3$  solution was extracted using the silver chloride-silica-based column. The arsenic concentration of obtained solutions from the extraction was shown in Figure 4.12. The silver chloride-silica-based column allows the separation of inorganic arsenic (As(III) and As(V)) and organic arsenic (DMA). From the result, the arsenic concentration of loading step was 0.289 mg/L (corresponding to 96.3% recovery) which was the concentration of DMA, that cannot be adsorbed on the column, while As(III) and As(V) were retained on the column and consequently eluted by nitric acid solution. The concentration of eluting step (As(III) and As(V)) was 0.551 mg/L (corresponding to 91.8% recovery). In conclusion, this procedure allows the determination of DMA in mixture solutions.



**Figure 4.12** Arsenic concentration of the obtained solution from the silver chloride-silica-based column: concentrations of As(III/V/DMA) = 0.3, 0.3 and 0.3 mg/L, flow rate = 2.0 mL/min.

### 4.3.3 Calculating the concentration of each arsenic species

The separation of As(III), As(V) and DMA with the SAX column and the silver chloride-silica-based column was summarized in Figure 4.13.



**Figure 4.13** Scheme of the separation of arsenic species using (A) strong anion exchange column and (B) silver chloride-silica-based column.

The concentration of each arsenic species was determined directly or calculated from the subtraction from the total arsenic concentration. The concentration of As(V) was directly measured in the eluent solution of the SAX column (Figure 4.13(A)) while the concentration of DMA was directly measured in the effluent solution of the silver chloride-silica-based column and the concentration of As(III) could be calculated from Equation 4.10.

$$C_{As(III)} = C_{As(total)} - C_{As(V)} - C_{DMA} \quad (4.10)$$

Where  $C_{As(III)}$  = the calculated concentration of As(III)

$C_{As(\text{total})}$  = the total arsenic concentration determined directly by ICP-OES in the starting solution

$C_{As(V)}$  = the concentration of As(V) in the eluate of SAX column

$C_{DMA}$  = the concentration of DMA in the effluent of Si-AgCl column

#### 4.4 Method validation

Speciation of arsenic species: As(III) As(V) and DMA, in water by solid phase extraction with the SAX columns and silver chloride-silica-based columns was employed, the method under the optimum condition was validated in order to check its accuracy and precision. Moreover, the instrument detection limit (IDL) and the method detection limit (MDL) were also evaluated.

##### 4.4.1 Accuracy and precision

The ternary mixture of arsenic standard at a concentration of 0.3 mg/L As(III), 0.3 mg/L As(V) and 0.3 mg/L DMA was a model solution of the method validation. The separation of these three arsenic species was performed by using SAX columns and silver chloride-silica-based columns. The concentrations of total As, As(V) and DMA were measured and the concentration of As(III) was calculated according to the Equation 4.10. The accuracy and precision of the proposed method were expressed in terms of percent recovery and percent relative standard deviation, respectively. The results were presented in Table 4.1. The percent recovery was calculated from Equation 4.11.

$$\% \text{Recovery} = \frac{C_{\text{found}}}{C_i} \times 100 \quad (4.11)$$

Where,  $C_{\text{found}}$  = the concentration of each arsenic species receiving from the proposed method

$C_i$  = the initial concentration of each arsenic species in sample solutions

**Table 4.1** Accuracy and precision of the proposed method (n=3)

Arsenic species	%Recovery	%RSD
As(III)	93.3±1.5	1.56
As(V)	100.1±2.0	2.03
DMA	96.6±1.7	1.74

The result showed that the percent recovery and percent relative standard deviation of As(III), As(V) and DMA were in the range of 93.3-101.1 and 1.56-2.03, respectively. It indicates an acceptable accuracy and precision of the proposed method according to the acceptance criteria for accuracy and precision of an analytical method [35]. Moreover, the MDLs were lower than the maximum allowed concentration in wastewater which is 0.25 mg/L, regulated by the Pollution Control Department of Thailand.

#### 4.4.2 Instrument detection limit (IDL) and method detection limit (MDL)

The instrument detection limit was calculated using Equation 4.12 [32] from the mean and standard deviation of signals of ten replicate measurements of blank solution and compared with a standard calibration curve.

$$S_{IDL} = X_{ave} + 3SD \quad (4.12)$$

Where

- $S_{IDL}$  = the signal at IDL concentration
- $X_{ave}$  = the mean of signal of the blank solution
- SD = the standard deviation of signal of the blank solution

For the method detection limit estimation, each arsenic species was spiked into a blank solution at a concentration of 10 µg/L. This concentration corresponded to approximately 5-fold level of IDL. The method detection limit was calculated by multiplying the standard deviation of the seven replicate measurements and the Student's t-value at 99% confidence level (3.14 for six degrees of freedom) expressed by Equation 4.13 [32].

$$\text{MDL} = t_{(n-1)} \times \sigma \quad (4.13)$$

Where  $t$  = the Student's t-value  
 $\sigma$  = the standard deviation of the concentration of the blank spiked level of 5-fold IDL

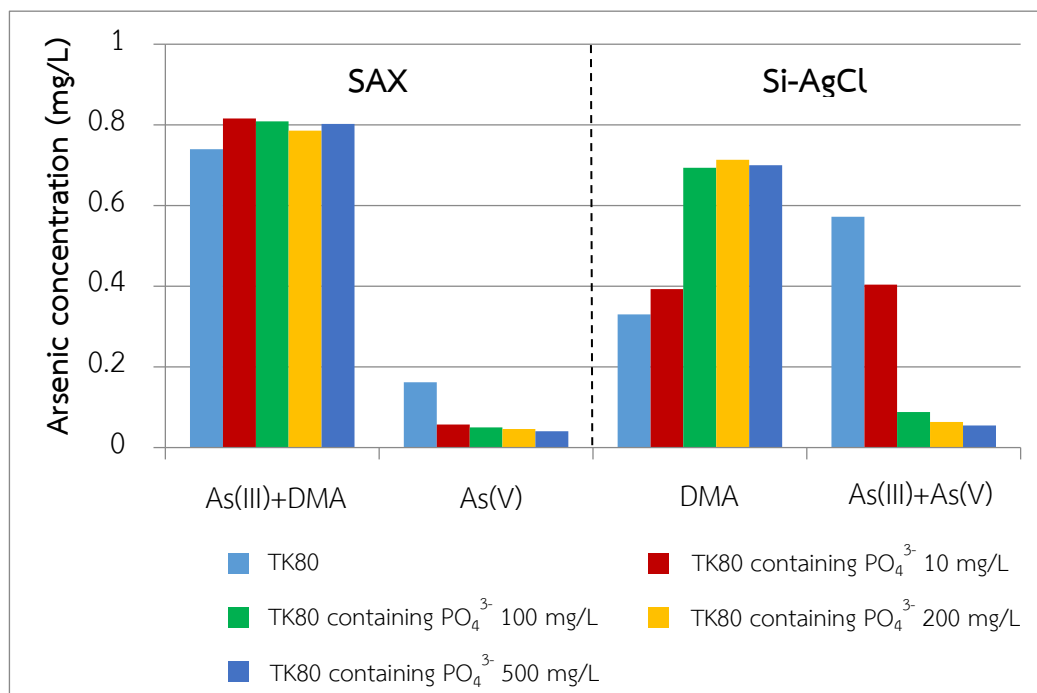
The instrument detection limit and the method detection limit were calculated and shown in Table 4.2.

**Table 4.2** Concentration at instrument detection limit and method detection limit of the proposed method

	Concentration ( $\mu\text{g/L}$ )
IDL	1.98
MDL of As(III)	2.30
MDL of As(V)	1.48
MDL of DMA	2.51

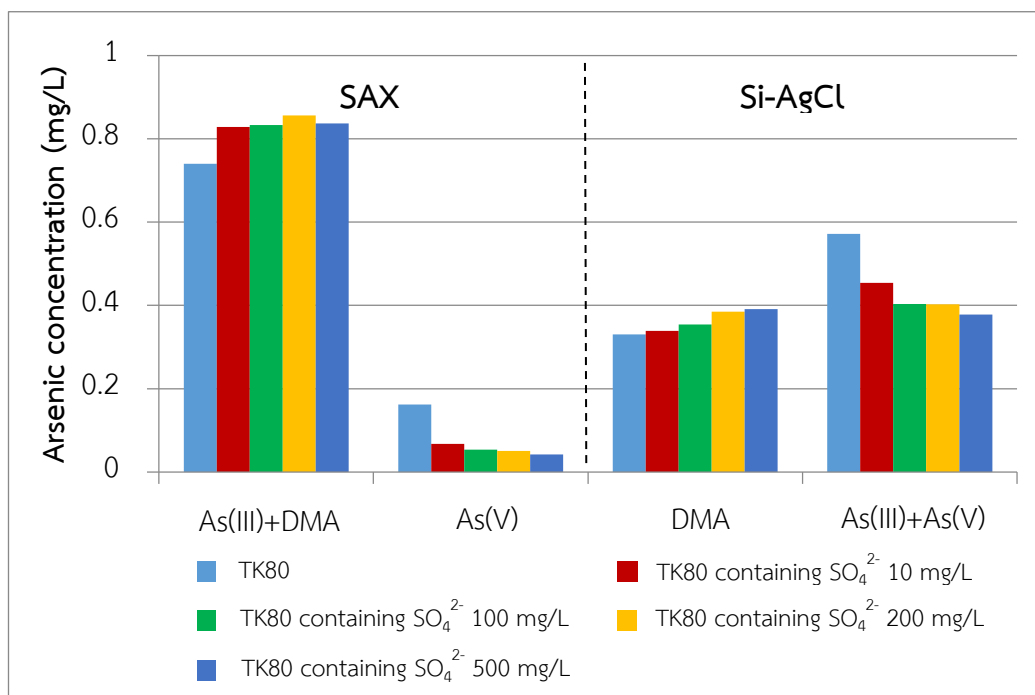
#### 4.5 Influence of coexisting ion interference

The influence of coexisting ions on the percent recovery of As(III), As(V) and DMA was investigated under optimal conditions. Sulfate and phosphate are considered as interferences in the arsenic adsorption process on the SAX column and the silver chloride-silica-based column due to their anionic charge and oxyanionic structure being close to those of arsenic species. The sulfate and phosphate concentrations at 10, 100, 200 and 500 mg/L were spiked in a real industrial wastewater sample (TK80). Since the amount of arsenic in the real sample is very high (>100 mg/L), it was then diluted 100 fold before preceding the proposed method. The arsenic concentration from the separation with SAX columns and silver chloride-silica-based columns were shown in Figure 4.14 and 4.15.



**Figure 4.14** Interfering effect of  $\text{PO}_4^{3-}$  in the separation of arsenic species: (left side) using strong anion exchange column, (right side) using silver chloride-silica-based column.

From Figure 4.14, the effect of  $\text{PO}_4^{3-}$  in the separation of arsenic species using the two columns was illustrated by a comparison of arsenic concentration between in the sample (TK80) and in the spiked sample containing  $\text{PO}_4^{3-}$ . On the SAX column, the samples having  $\text{PO}_4^{3-}$  showed a decrease of arsenic concentration in the eluting step (As(V)) while the arsenic concentration in loading step increased due to the interference of  $\text{PO}_4^{3-}$  to As(V) adsorbed on the column. In case of silver chloride-silica-based column,  $\text{PO}_4^{3-}$  affected the adsorption of arsenic species at samples containing  $\text{PO}_4^{3-}$  of 100, 200 and 500 mg/L.



**Figure 4.15** Interfering effect of  $\text{SO}_4^{2-}$  in the separation of arsenic species: (left side) using strong anion exchange column, (right side) using silver chloride-silica-based column.

From Figure 4.15, the separation performance of arsenic species in samples containing  $\text{SO}_4^{2-}$  by a SAX column was diminished. The adsorption of As(V) on the column decreased due to the presence of  $\text{SO}_4^{2-}$ . On the silver chloride-silica-based column, the concentration of arsenic in loading step of TK80 and TK80 containing  $\text{SO}_4^{2-}$  was not significantly different. It was indicated that  $\text{SO}_4^{2-}$  did not interfere the separation of DMA using the silver chloride-silica-based column.

The interfering effect of  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$  resulted possibly from the competition with arsenic anions.



#### 4.6 Speciation analysis of arsenic species in industrial wastewater

The proposed method was employed to speciate As(III), As(V) and DMA in an industrial wastewater sample. The method was also validated using a spike method. The wastewater sample was diluted 100 folds because it contains too high arsenic concentration and probably affected the separation efficiency. The non-spiked diluted and spiked diluted samples were passed through the SAX columns and the silver chloride-silica-based columns. The arsenic concentration of three species, percent recovery and percent relative standard deviation were calculated and shown in Table 4.3.

**Table 4.3** Speciation of As(III), As(V) and DMA in industrial wastewater sample and method validation results in term of accuracy and precision (n=3)

	Arsenic species		
	Arsenic concentration		
	As(III)	As(V)	DMA
100-fold diluted TK80 ( $\mu\text{g/L}$ )	725 $\pm$ 6	128 $\pm$ 2	44 $\pm$ 5
As-added sample ( $\mu\text{g/L}$ )	300	300	300
Found As ( $\mu\text{g/L}$ )	1013 $\pm$ 4	441 $\pm$ 9	350 $\pm$ 13
%Recovery	97.2 $\pm$ 0.7	104.3 $\pm$ 2.2	102.0 $\pm$ 2.6
%RSD	0.72	2.13	2.56

It was shown that the percent recovery and percent relative standard deviation of As(III), As(V) and DMA were in the range of 97.2-104.3 and 0.72-2.56, respectively. It indicated the acceptable accuracy and precision of the method employed in separation of arsenic species in real samples. The amounts of As(III), As(V) and DMA in TK80 sample were 725 $\pm$ 6, 128 $\pm$ 2, and 44 $\pm$ 5  $\mu\text{g/L}$ , respectively.

## CHAPTER V

### CONCLUSION

#### 5.1 Conclusion

A new solid phase extraction method based on a selective adsorption, which is rapid, simple and economical, was developed for the speciation of arsenic by using a SAX column (*n*-propyl(trimethyl) ammonium chloride silica, Si-Cl) and a silver chloride-based column (Si-AgCl). The Si-AgCl column was prepared by passing silver nitrate solution through the SAX column. Effect of various parameters in extraction including pH value, flow rate, eluent concentration and sample concentration was investigated for finding optimal conditions in the separation of arsenic species. The detail of extraction with the strong anion exchange column and the silver chloride-based column was shown in Table 5.1.

**Table 5.1** The detail of extraction with strong anion exchange column and silver chloride-based column

Detail	Strong anion exchange	Silver chloride column
Structure	Si-(CH <sub>2</sub> ) <sub>3</sub> -N <sup>+</sup> -(CH <sub>3</sub> ) <sub>3</sub>	Si-AgCl
Amount of adsorbent	200 mg	200 mg
Sample volume	10 mL	10 mL
Sample pH	7	9
Flow rate (loading and eluting)	2 mL/min	2 mL/min
Eluent	HCl	HNO <sub>3</sub>
Eluent concentration	1 mol/L	1%(v/v)
Eluent volume	10 mL	10 mL

In the study of arsenic separation by SAX column, at pH solution 7, As(III) and DMA could not retain and passed through the column while only As(V) species can be absorbed on the column. In the case of silver chloride column which is used for the separation of organic arsenic and inorganic arsenic species, at pH 9, DMA was passed through the column while As(III) and As(V) can be retained and absorbed on the column. The concentration of As(V) can be measured directly in the eluent solution of the SAX column. The concentration of DMA is determined in the effluent solution from the silver chloride-silica-based column and the concentration of As(III) is calculated from the difference of total arsenic, As(III) and As(V).

Under the optimal conditions, the speciation of arsenic by the proposed method shows that the percent recovery, percent relative standard deviation and MDL of As(III), As(V) and DMA are in the range of 93.3-101.1, 1.56-2.03 and 1.48-2.51  $\mu\text{g/L}$ , respectively.

Finally, the proposed method was successfully applied for the speciation of arsenic in real industrial wastewater samples. Good accuracy and precision of the method in separation of arsenic species was obtained. The percent recovery and percent relative standard deviation of As(III), As(V) and DMA were in the range of 97.2-104.3 and 0.72-2.56, respectively. However,  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$  interfere the separation of arsenic by SAX column and silver chloride-silica-based column.

## 5.2 Suggestions for future work

- An elimination of  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$  such as using the precipitation with  $\text{Ca}^{2+}$  or  $\text{Ba}^{2+}$  should be further studied prior to analysis.
- The proposed method could be applied for other kind of samples such as ground water samples and/or food samples.

## REFERENCES

- [1] ATSDR. Toxicological profile for arsenic. Atlanta, GA: Agency for Toxic Substances and Disease Registry, U.S. Department of Health & Human Services, Public Health Services, 2007.
- [2] Smedley, P.L. and Kinniburgh, D.G. A review of the source, behaviour and distribution of arsenic in natural waters. Applied Geochemistry 17(5) (2002): 517-568.
- [3] Mandal, B.K. and Suzuki, K.T. Arsenic round the world: a review. Talanta 58(1) (2002): 201-235.
- [4] WHO. Guidelines for Drinking-water Quality. 4 ed. Geneva: World Health Organization Press, 2011.
- [5] Shiomi, K. Arsenic in the Environment, Part 2, Human Health and Ecosystem Effects. Wiley, 1994.
- [6] Saha, J.C., Dikshit, A.K., Bandyopadhyay, M., and Saha, K.C. . A review of arsenic poisoning and its effects on human health. Critical Reviews in Environmental Science and Technology 29(3) (1999): 281-313.
- [7] Karadjova, I.B., Lampugnani, L., Dedina, J., D'Ulivo, A., Onor, M., and Tsalev, D.L. Organic solvents as interferents in arsenic determination by hydride generation atomic absorption spectrometry with flame atomization. Spectrochimica Acta Part B: Atomic Spectroscopy 61(5) (2006): 525-531.
- [8] Shraim, A., Chiswell, B., and Olszowy, H. Speciation of arsenic by hydride generation-atomic absorption spectrometry (HG-AAS) in hydrochloric acid reaction medium. Talanta 50(5) (1999): 1109-1127.
- [9] Li, F., Wang, D.-D., Yan, X.-P., Su, R.-G., and Lin, J.-M. Speciation analysis of inorganic arsenic by microchip capillary electrophoresis coupled with hydride generation atomic fluorescence spectrometry. Journal of Chromatography A 1081(2) (2005): 232-237.
- [10] Becker, E., Rampazzo, R.T., Dessuy, M.B., Vale, M.G.R., Da Silva, M.M., Welz, B., and Katskov, D.A. Direct determination of arsenic in petroleum derivatives by graphite furnace atomic absorption spectrometry: A comparison between

- filter and platform atomizers. Spectrochimica Acta Part B: Atomic Spectroscopy 66(5) (2011): 345-351.
- [11] Chen, D., Huang, C., He, M., and Hu, B. Separation and preconcentration of inorganic arsenic species in natural water samples with 3-(2-aminoethylamino) propyltrimethoxysilane modified ordered mesoporous silica micro-column and their determination by inductively coupled plasma optical emission spectrometry. Journal of Hazardous Materials 164(2-3) (2009): 1146-1151.
- [12] Sahayam, A.C., Chaurasia, S.C., and Venkateswarlu, G. Dry ashing of organic rich matrices with palladium for the determination of arsenic using inductively coupled plasma-mass spectrometry. Analytica Chimica Acta 661(1) (2010): 17-19.
- [13] Kundu, S., Ghosh, S.K., Mandal, M., Pal, T., and Pal, A. Spectrophotometric determination of arsenic via arsine generation and in-situ colour bleaching of methylene blue (MB) in micellar medium. Talanta 58(5) (2002): 935-942.
- [14] Song, Y. and Swain, G.M. Total inorganic arsenic detection in real water samples using anodic stripping voltammetry and a gold-coated diamond thin-film electrode. Analytica Chimica Acta 593(1) (2007): 7-12.
- [15] Wang, P., Zhao, G., Tian, J., and Su, X. High-performance liquid chromatography-inductively coupled plasma mass spectrometry based method for the determination of organic arsenic feed additives and speciation of anionic arsenics in animal feed. Journal of Agricultural and Food Chemistry 58(9) (2010): 5263-5270.
- [16] Narukawa, T., Suzuki, T., Inagaki, K., and Hioki, A. Extraction techniques for arsenic species in rice flour and their speciation by HPLC-ICP-MS. Talanta 130 (2014): 213-220.
- [17] Amran, M., Lagarde, F., and Leroy, M. Determination of arsenic species in marine organisms by HPLC-ICP-OES and HPLC-HG-QFAAS. Mikrochim Acta 127(3-4) (1997): 195-202.
- [18] Gonzalez, A., Cervera, M.L., Armenta, S., and de la Guardia, M. A review of non-chromatographic methods for speciation analysis. Analytica Chimica Acta 636(2) (2009): 129-157.

- [19] Ulusoy, H.I., Akcay, M., Ulusoy, S., and Gurkan, R. Determination of ultra trace arsenic species in water samples by hydride generation atomic absorption spectrometry after cloud point extraction. *Analytica Chimica Acta* 703(2) (2011): 137-144.
- [20] Shemirani, F., Baghdadi, M., and Ramezani, M. Preconcentration and determination of ultra trace amounts of arsenic(III) and arsenic(V) in tap water and total arsenic in biological samples by cloud point extraction and electrothermal atomic absorption spectrometry. *Talanta* 65(4) (2005): 882-887.
- [21] Tuzen, M., Citak, D., Mendil, D., and Soylak, M. Arsenic speciation in natural water samples by coprecipitation-hydride generation atomic absorption spectrometry combination. *Talanta* 78(1) (2009): 52-56.
- [22] Zhang, Q. Differential determination of trace amounts of arsenic(III) and arsenic(V) in seawater by solid sampling atomic absorption spectrometry after preconcentration by coprecipitation with a nickel-pyrrolidine dithiocarbamate complex. *Analytica Chimica Acta* 508(1) (2004): 99-105.
- [23] Yu, C., Cai, Q., Guo, Z.-X., Yang, Z., and Khoo, S.B. Inductively coupled plasma mass spectrometry study of the retention behavior of arsenic species on various solid phase extraction cartridges and its application in arsenic speciation. *Spectrochimica Acta Part B: Atomic Spectroscopy* 58(7) (2003): 1335-1349.
- [24] Zhang, L., Morita, Y., Sakuragawa, A., and Isozaki, A. Inorganic speciation of As(III, V), Se(IV, VI) and Sb(III, V) in natural water with GF-AAS using solid phase extraction technology. *Talanta* 72(2) (2007): 723-729.
- [25] Simpson, N.J.K. and Wells, M.J.M. *Solid-phase extraction principle, technique, and application*. Taylor & Francis Group LLC, 2000.
- [26] Camel, V. Solid phase extraction of trace elements. *Spectrochimica Acta Part B: Atomic Spectroscopy* 58(7) (2003): 1177-1233.
- [27] Lucci, P., Pacetti, D., Frega, N.G., and Núñez, O. *Current Trends in Sample Treatment Techniques for Environmental and Food Analysis*. Chromatography - The Most Versatile Method of Chemical Analysis. 2012.

- [28] Zwir-Ferenc, A. and Biziuk, M. Solid phase extraction technique – trends, opportunities and applications. Polish Journal of Environmental Studies 15 (2006): 677-690.
- [29] Erdoğan, H., Yalçinkaya, Ö., and Türker, A.R. Determination of inorganic arsenic species by hydride generation atomic absorption spectrometry in water samples after preconcentration/separation on nano  $ZrO_2/B_2O_3$  by solid phase extraction. Desalination 280(1-3) (2011): 391-396.
- [30] Xiong, C., He, M., and Hu, B. On-line separation and preconcentration of inorganic arsenic and selenium species in natural water samples with CTAB-modified alkyl silica microcolumn and determination by inductively coupled plasma-optical emission spectrometry. Talanta 76(4) (2008): 772-779.
- [31] Sigrist, M., Albertengo, A., Beldomenico, H., and Tudino, M. Determination of As(III) and total inorganic As in water samples using an on-line solid phase extraction and flow injection hydride generation atomic absorption spectrometry. Journal of Hazardous Materials 188(1-3) (2011): 311-8.
- [32] Ben Issa, N., Rajakovic-Ognjanovic, V.N., Marinkovic, A.D., and Rajakovic, L.V. Separation and determination of arsenic species in water by selective exchange and hybrid resins. Analytica Chimica Acta 706(1) (2011): 191-198.
- [33] Boom, W.S. Ion Exchange Process Having Increase Flow Rates. US Patent 4724082: Dow Chemical Co., 1988.
- [34] Sharma, V.K. and Sohn, M. Aquatic arsenic: Toxicity, speciation, transformations, and remediation. Environment International 35(4) (2009): 743-759.
- [35] Huber, L. Validation and Qualification in Analytical Laboratories. Illinois: Interpharm Press, 1999.



APPENDIX

จุฬาลงกรณ์มหาวิทยาลัย  
**CHULALONGKORN UNIVERSITY**



## VITA

Mr. Nawapong Phattamajintatamrong was born on December 8, 1988 in Ratchaburi, Thailand. He received his Bachelor's Degree of Science from Department of Chemistry, Faculty of Science, Silpakorn University in 2009. After that, he continued to study further as graduate student at the Department of Chemistry, Faculty of Science, Chulalongkorn University and has become one of the member of Environmental Analysis Research Unit. The present address is 6/40 Rongnammun Road, Banpong, Banpong, Ratchaburi, Thailand, 70110. Contact number is 089-2725932.

