การเพิ่มความเข้มข้นของไอออนซิลเวอร์ปริมาณน้อยมากโดยใช้ซิลิกาดัดแปรและตรวจวัดด้วยโฟลว์อินเจ็กชันเฟลมอะตอมมิกแอบซอร์บชันสเปกโทรเมตรี

นางสาวจิตวิไล เวฬุวนารักษ์

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PRECONCENTRATION OF TRACE SILVER ION USING MODIFIED SILICA AND DETERMINATION BY FLOW INJECTION FLAME ATOMIC ABSORPTION SPECTROMETRY

Miss Jitwilai Waluvanaruk

A Thesis Submitted in Partial Fulfillment of the Requirements
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Thesis Title	PRECONCENTRATION OF TRACE SILVER ION USING MODIFIED SILICA AND DETERMINATION BY FLOW INJECTION FLAME ATOMIC ABSORPTION SPECTROMETRY
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จิตวิไล เวฬุวนารักษ์: การเพิ่มความเข้มข้นของไอออนซิลเวอร์ปริมาณน้อยมากโดยใช้ซิลิกาดัดแปรและตรวจวัดด้วยโฟลว์อินเจ็กชันเฟลมอะตอมมิกแอบซอร์บชันสเปกโทรเมตรี (PRECONCENTRATION OF TRACE SILVER ION USING MODIFIED SILICA AND DETERMINATION BY FLOW INJECTION FLAME ATOMIC ABSORPTION SPECTROMETRY) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: อ.ดร. ภัสสร์พล งามอุโฆษ, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ. ดร. วัลภา เอื้องไมตรีภิรมย์, 84 หน้า.

การดัดแปรซิลิกาเจลที่มีอนุพันธ์ของเบนโซไทเอโซล คาลิกซ์[4]เอรีน (CU1CHO) สังเคราะห์ขึ้นโดยเชื่อมต่อด้วยชิฟเบส (**APS-CU1**) ทำการพิสูจน์เอกลักษณ์ของ **APS-CU1** ด้วยเทคนิคดีอาร์ยูวี, ฟูเรียร์ทรานสฟอร์มอินฟาเรดสเปกโทรสโกปี และเทอร์โมแกรวิเมตริกอะนาไลซิส **APS-CU1** ใช้เป็นเฟสของแข็งในการสกัดไอออนของซิลเวอร์ในระบบแบทช์และคอลัมน์ โดยสภาวะที่เหมาะสม คือ พีเอส 6-7 ใช้เวลาในการสกัด 10 นาที พบว่าความจุสูงสุดของเฟสของแข็งเท่ากับ 12.2 มิลลิกรัม(ไอออนซิลเวอร์)ต่อกรัม จากนั้นทำการศึกษาความสามารถในการเพิ่มความเข้มข้นของไอออนซิลเวอร์ร่วมกับเทคนิคเฟลมอะตอมมิกแอบซอร์ชันสเปกโทรเมตรี ไอออนของซิลเวอร์ถูกดูดซับโดยไหลผ่านสารละลายด้วยอัตราการไหลที่ 3.0 มิลลิลิตรต่อนาที และถูกชะด้วยไทโอซัลเฟตความเข้มข้น 0.1 โมลต่อลิตร ปริมาตร 250 ไมโครลิตรที่อัตราการไหล 1.5 มิลลิลิตรต่อนาที ทำการตรวจสอบประสิทธิภาพของระบบภายใต้สภาวะที่เหมาะสม โดยการเติมสารละลายมาตรฐานไอออนซิลเวอร์ที่ความเข้มข้น 20 และ 50 ไมโครกรัมต่อลิตร พบว่าขีดความสามารถต่ำสุดในการตรวจวัดเท่ากับ 8.8 ไมโครกรัมต่อลิตร ส่วนความสามารถในการให้ค่าคืนกลับเท่ากับ 100.2 และ 99.5 เปอร์เซ็นต์ ตามลำดับ และความแม่นในการวิเคราะห์ (%RSD) เท่ากับ 6.1 และ 3.3 เปอร์เซ็นต์ ตามลำดับ ที่ความเข้มข้น 20 และ 50 ไมโครกรัมต่อลิตร ตามลำดับ วิธีนี้สามารถประยุกต์ใช้ในการตรวจหาปริมาณไอออนซิลเวอร์ที่มีปริมาณน้อยในน้ำตัวอย่าง เช่น น้ำดื่มและน้ำประปา ได้ผลการวิเคราะห์เป็นที่น่าพอใจ

ภาควิชา ....เคมี.......... ลายมือชื่อนิสิต ....
สาขาวิชา ....เคมี.......... ลายมือชื่อ อ. ที่ปรึกษาวิทยานิพนธ์หลัก ....
ปีการศึกษา ......2553.......... ลายมือชื่อ อ. ที่ปรึกษาวิทยานิพนธ์ร่วม ....

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JITWILAI WALUVANARUK: PRECONCENTRATION OF TRACE SILVER ION USING MODIFIED SILICA AND DETERMINATION BY FLOW INJECTION FLAME ATOMIC ABSORPTION SPECTROMETRY. ADVISOR: PASSAPOL NGAMUKOT, Ph.D., CO-ADVISOR: ASST. PROF. WANLAPA AEUNGMAITREPIROM, Ph.D., 84 pp.

Silica gel chemically modified with benzothiazole calix[4]arene derivative (**CU1-CHO**) was synthesized via Schiff's base (**APS-CU1**). The **APS-CU1** was characterized by DR-UV and FT-IR spectroscopy and thermal gravimetric analysis. The **APS-CU1** was used as an effective sorbent for solid phase extraction (SPE) of Ag(I). The sorption properties of Ag(I) were studied in batch and column methods. The optimum pH was found to be 6-7 with the contact time of 10 min. A maximum capacity of the sorbent under the optimum conditions was 12.2 mg of Ag(I) per gram of sorbent. The on-line flow injection pre-concentration coupled with FAAS (FI-FAAS system) base on SPE for determination of Ag(I) was investigation. The solution of Ag(I) was passed directly through sorbent at flow rate of 3.0 mL min^{-1} and then eluted by 250 µL of 0.1 mol L^{-1} thiosulfate at the flow rate of 1.5 mL min^{-1}. The analytical characteristics performance of FI-FAAS system were studied under optimum conditions using spiked solution with standard Ag(I) at 20 and 50 µg L^{-} The detection limit of 8.8 µg L^{-1} was obtained. The accuracy of the proposed method was evaluated and percentage of recovery were 100.2% and 99.5%, respectively. The percent relative standard deviations (%RSD) at 20 and 50 µg L^{-1} were 6.1% and 3.3%, respectively. The developed method was successfully applied to the determination of trace Ag(I) in real water samples such as drinking water and tap water.

Department: ......Chemistry......	Student's Signature ....
Field of Study: ......Chemistry......	Advisor's Signature ....
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Thanks to my family for their love, financial support and encouragement.

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LIST OF ABBREVIATIONS

mg L^{-1} = Miligram per liter
μg L^{-1} = Milcrogram per liter
mol L^{-1} = Mole per liter
mmol L^{-1} = Millimole per liter
cm^{-1} = Wave number
°C = Degree Celsius
DI = Deionized water
g = Gram
mL = Milliliter
mmol = Millimole
h = Hour
min = Minute
o.d = Outer diameter
mm = Millimeter
nm = Nanometer
cm = Centimeter
ppm = Part per million
ppb = Part per billion
ppt = Part per trillion
LOD = Limit of detection
LOQ = Limit of quantitation
MDL = Method detection limit
MQL = Method quantitation limit

CHAPTER I

INTRODUCTION

1.1 Statement of the problem

Recently, heavy metals are widely used in many fields such as electronic, batteries and food industries. Silver can enter into the environment via industrial wastewaters which can also cause environmental and health problems. Therefore, an effective removal of silver ion from water or various industrial effluents is a very important procedure and has attracted considerable research and practical interest.

Moreover, the low-level detection of silver in environmental is necessary for the analysis. Flame atomic absorption spectroscopy (FAAS) is commonly used for determination of many elements in various samples which offers many advantages, such as simple, rapid, low cost of operation, high precision and accuracy. Nevertheless, the sensitivity of FAAS is not sufficiently high for the determination of metals at trace level. Therefore, the pre-concentration and separation steps were required prior to the determination of trace metals by increasing the analyte concentration in order to improve the sensitivity of method.

The solid phase extraction (SPE) is a method for pre-concentration of metals which has several advantages over other techniques, reducing the disposal cost, high pre-concentration factor, eliminate interferences, stable, reusability of solid phase and automated analysis [1]. However, the conventional batch or column procedure for pre-concentration based on SPE is usually effective but it is tedious, time-consuming, requires large sample and reagent volume and analyte losses. Thus, on-line flow injection pre-concentration coupled with FAAS is an interesting alternative method because it is eliminated the drawbacks and improve the sensitivity for the determination of trace metals [2]. Thus, on-line flow injection coupled with column based on SPE have been developed which offers many advantages, for example, high pre-concentration factor, simple, rapid, reduce the samples and reagents consumption, less risk of contamination and analyte losses and automation [3].

The sorbents used in SPE, silica gel especially modified with various organic compounds containing target functional groups such as nitrogen, sulfur and oxygen to improve the selectivity of the sorption have been studied. Because the silica gel has a large surface area and the surface consists of the silanol group and siloxane which are easy to modify. Furthermore, the solid phase as sorbent material has been used in on-line flow injection pre-concentration and determination of trace metals by FI system.

In this work, the benzothiazole calix[4]arene (**CU1**) was chosen as a chelating agent chemically modified on silica gel, because **CU1** containing S and N atom preformed to bind with metal ion. Thus, the sorbent was prepared and the sorption properties toward Ag(I) from aqueous solution was studied. Then, the on-line pre-concentration coupled to FAAS was designed for determination of Ag(I).

1.2 Research objective

This research aims to synthesize benzothiazolecalix[4]arene derivative chemically modified on silica gel as solid phase adsorbent. Furthermore, this sorbent was packed in column for on-line pre-concentration coupled to flame atomic absorption spectrometry (FAAS) for determination of trace Ag(I) in real samples.

1.3 Scope of the research

The scopes of this work were synthesis of benzothiazole calix[4]arene derivative chemically modified on silica gel and its characterization by ^{1}H-NMR spectrometry, mass spectrometry, elemental analysis, DR-UV-vis spectrometry, FT-IR spectrometry and thermal gravimetric analysis. Then, the prepared sorbent was used to sorbed Ag(I) in both batch and column methods before applying the sorbent as a solid phase material in on-line pre-concentration method. In batch method, parameters such as solution pH, extraction time, sorption capacity, type and concentration of eluent were studied. In column method, parameters such as the flow rate of metal and eluent solutions, reusability of sorbent and sample volume were studied. The parameters of sample and eluent flow rate, sample volume and interfering ions were studied in on-line pre-concentration method.

Furthermore, the proposed method was applied in real sample as drinking water and tap water with spike method.

1.4 Benefits of the research

We have successfully modified **CU1** onto silica gel which could be used as sorbent for extraction of Ag(I) in real sample and determination by flow injection coupled with on-line flame atomic absorption spectrometry.

CHAPTER II

THEORY AND LITERATURE REVIEW

The determination of heavy metals in the environment, various industries, foods and medicine is very important because heavy metals can be contaminated and hurmful to human or animal due to their toxic effects [4]. The determination of heavy metals can be carried out using in several techniques but in many cases the sensitivity of the analytical instrument is not sufficient for the analysis of a sample. Therefore, the development of methods for determination of heavy metals is required to improve the accuracy and precision.

2.1 Heavy metals in environment

Heavy metals are one group of pollutants in the environment. They may come from nature, human and especially industrial activities. The maximum acceptable concentration of some heavy metals in drinking water and industrial wastewater limited by the Pollution Control Department (PCD) are summarized in Table 2.1 [5].

Table 2.1 Maximum acceptable concentration of heavy metals in drinking water and industrial wastewater

Heavy metals	Maximum acceptable concentration (mg L^{-1})	
	Drinking water	Industrial wastewater
Arsenic	0.05	0.25
Cadmium	0.01	0.03
Copper	1.00	2.00
Chromium	0.05	0.75
Lead	0.05	0.20
Mercury	0.002	0.005
Silver	0.05	5.00

Silver ion is an importance heavy metal, because the silver ion can be marked antibacterial properties. Nowadays, silver compound is widely used to produce in the industries of alloys, medicine, dental amalgam, jewelry, electronic devices, photographic material, battery, food (such as disinfect water for drinking) and in catalysis [6]. However, silver has been recognized as a toxic element for biological system event at low concentration. The main toxicity of silver depends on inorganic ligand presented in solution, alkalinity and pH of solution. Figure 2.1 shows the stability of silver salts at different pH of aqueous solution.

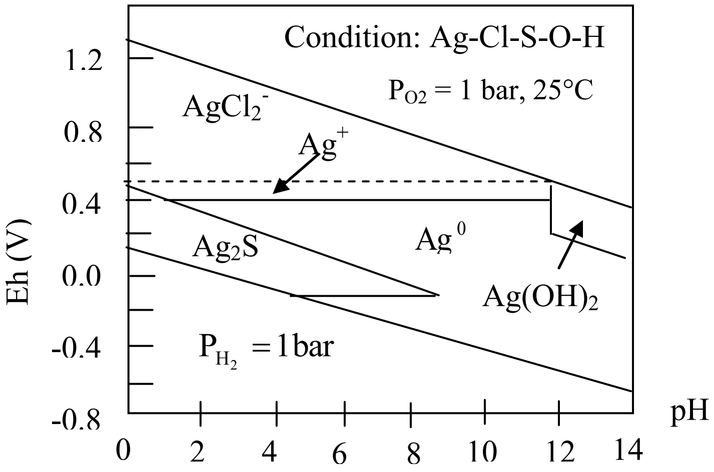


Figure 2.1 Eh-pH stability of silver [Redrawn from Bradl, H. B [7]]

Therefore, an effective removal of silver ion from water or various industrial effluents is very important and has attracted considerable research and practical interest.

2.2 Determination of heavy metal

The determination of heavy metals can be carried out by several techniques such as flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption spectrometry (GFAAS), inductively coupled plasma atomic emission spectrometry (ICP-AES) or inductively-coupled plasma atomic optical emission spectrometry (ICP-OES) and inductively-coupled plasma mass spectrometry (ICP-MS). However, the direct determination of trace amount of metals by atomic spectrometry techniques is not possible due to its low sensitivity that represent in term of detection limit shown in Table 2.2 [8]. The comparison of performance of atomic spectrometry techniques is summarized in Table 2.3 [9, 10].

Table 2.2 Typical detection limits (μg L^{-1}) for various elements by atomic spectrometry techniques

Element	FAAS	GFAAS	ICP-AES	ICP-MS
Ag	3	0.02	0.2	0.003
Cu	2	0.1	0.04	0.003
Cd	1	0.02	0.07	0.003
Cr	4	0.06	0.08	0.02
Ni	3	1	0.2	0.005
Pb	5	0.2	1	0.007
Zn	1	0.01	0.1	0.008

Table 2.3 Comparison of performance of atomic spectrometric techniques

Criterion	FAAS	GFAAS	ICP-AES	ICP-MS
Limit of detection	high ppb	sub ppb	sub ppb-ppt	sub ppt
Analytical capability	single element	single element	multielement	multielement
Sample throughput	~ 3-10 sec/sample	~ 2-3 min/sample	~ 1-35min/sample	~ 1-4 min/sample
Dynamic range	mid ppm range	low ppm range	high ppm range	mid ppm range
Elements applicable	>68	>50	>70	>80
Sample volume require	large	very small	medium	very small to medium
Ease of use	very easy	moderately easy	easy	moderately easy
Method development	easy	difficult	moderately easy	difficult
Operating cost	low	high	medium	very high

FAAS is common used for the determination of trace metal because this technique provides the detection limit in sub-ppm (mg L^{-1}) level, element specific, very fast, easy to set up and to use and low capital cost. However, this technique has a poor detection limit than other techniques. Thus, the pre-concentration step is required to increase the concentration of the metal before determination of analytes.

2.3 Solid phase extraction (SPE) [12]

Solid phase extraction is the most common technique used in sample-preparation for the separation of selected analytes from the solution and sorption on solid phase or pre-concentration prior to the measurement by instruments in order to improve the sensitivity of the analytical method. SPE offers several advantages such as reduce the toxic organic solvent for the extraction, reduce time of the analysis, automation and provide high recovery and pre-concentration factor.

SPE method always consists of four success steps (illustrated in Figure 2.2) as follows:

Conditioning: In the first step, the conditioning step is used to prepare the sorbent to be compatible with solution of sample and remove the impurities or air containing in the sorbent.

Loading: In the second step, the sample is throughout and react with a solid sorbent. The efficiency of the sorption of the analyte on sorbent depends on sample volume, pH of sample, sample flow rate and sample matrix.

Washing: In the third step, the solution is used to wash and remove the matrix on the solid sorbent for cleaning up before desorption into the next step.

Eluting: In the last step, the analyte retained on the sorbent is eluted by a suitable eluent to ensure efficient recovery of the analyte.

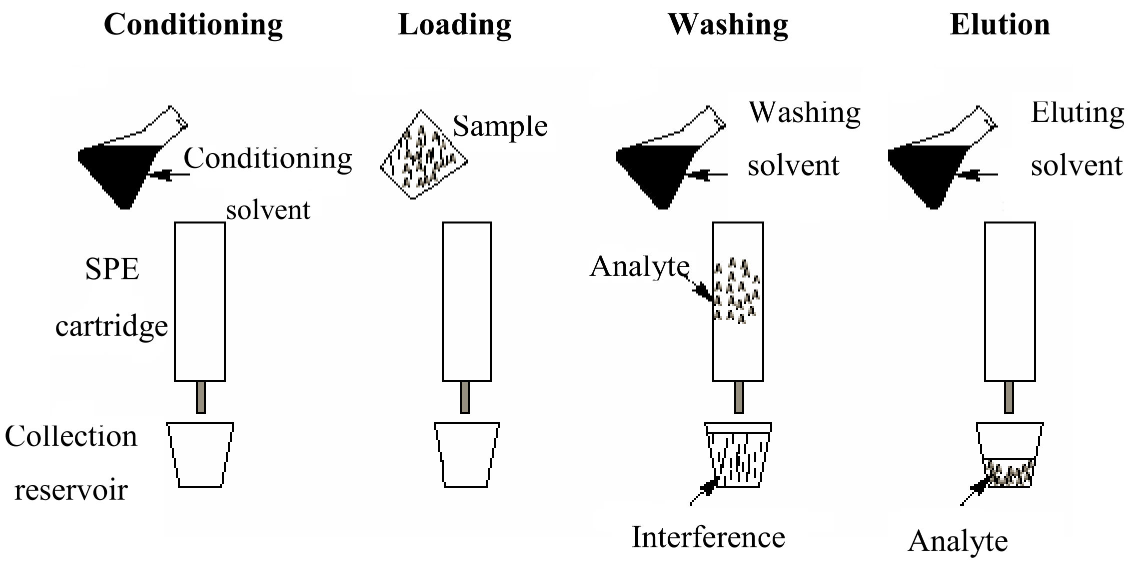


Figure 2.2 Four typical steps of SPE method [12]

2.3.1 Sorption process of elemental on the sorbent

The analyte retained on the sorbent depends on the mechanism retention of analyte and the nature of the sorbent. Three types of the interaction between analyte and sorbent are described below:

Adsorption: The adsorption mechanism usually occurs with non polar sorbent material such as silica C-18. The interaction between sorbent and analyte occurs through van der Waals forces or hydrophobic interaction depends on the polarity or hydrophobic behaviors of sorbent and analyte.

Chelation: The chelation interaction occurs between electron acceptor of metal and electron donor of ligand through a coordination covalent bond.

The selectivity of ligand towards metal ion depends on the hard soft acid base principle (HSAB) which affected the binding ability of metal ions onto chelating ligand. The binding ability depends on the nature of donor atom of ligand, charge and size of metal ions, pH of solution for metal extraction and the nature of solid support.

According to the acids and bases represent in tern of hard or soft by Pearson HSAB are shown in Table 2.4.

Table 2.4 Hard and soft acid and base species [13]

	Lewis acids	**Lewis bases**
Hard	H^+, Li^+, Na^+, K^+	F^-, Cl^-
	Be^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}	H_2O, OH^-, O^{2-}
	BF_3, BCl_3, $B(OR)_3$	ROH, RO^-, R_2O, CH_3COO^-
	Al^{3+}, $Al(CH_3)_3$, $AlCl_3$, AlH_3	NO_3^-, ClO_4^-
	Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{3+}	CO_3^{2-},SO_4^{2-}, PO_4^{3-}
		NH_3, RNH_2, N_2H_4
Borderline	$B(CH_3)_3$	Br^- NO_2^-, N_3^-
	Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+},	SO_3^{2-}
	Pb^{2+}, Rh^{3+}, Ir^{3+}, Ru^{3+}, Os^{2+}	$C_6H_2NH_2$, C_5H_5N, N_2
Soft		H^-
	BH_3, Tl^+, $Tl(CH_3)_3$	I^-
	Cu^+, Ag^+, Au^+, Cd^{2+}, Hg_2^{2+},	H_2S, HS^-, S^{2-}
	Hg^{2+}, CH_3Hg^+, Pd^{2+}, Pt^{2+}, Pt^{4+},	RSH, RS^-, R_2S
	Br_2, I_2	SCN^-, CN^-, RNC, CO
		$S_2O_3^{2-}$
		R_3P, $(RO)_3P$, $R_3AsC_2H_4$, C_6H_6

Ion exchange: The mechanism of ion exchange is based on the ion exchange between counter ion of the functional group on the sorbent and analyte ions. Two types of commercial ion-exchange sorbent are cation-exchanger and anion-exchanger. The strong ion exchanger, such as sulfonic acid groups (cation- exchange) or quaternary amines (anion-exchanger) can exchange ions at any pH. The weak ion exchange, such as carboxylic groups (cation- exchange) or primary, scecondary and tertiary amine (anion-exchanger) can exchange only at the pH values greater or less than the pK_a.

2.3.2 Sorbent in SPE techniques

The good properties of sorbent in SPE are important for effective sorption of analyte. The solid sorbent requires high sorption capacity, wide working pH range, fast and quantitative sorption and elution, reusability and accessibility.

Sorbents can be divided into inorganic-based and organic-based as given below [12].

Inorganic-based

- silica gel: the advantages of this sorbent are not swell, stable under various conditions and chelating agent can be loaded on surface but have narrow pH stability range.
- other inorganic oxides (TiO_2, Al_2O_3,MgO): this sorbent may be influenced by the contain of the high concentration of phosphates and sulfate which decrease sorption of trace elements and major cations such as Na^+, K^+, Ca^{2+} and Mg^{2+} are weakly adsorbed on TiO_2.

Organic-based

This type can be categorized into polymeric and non-polymeric sorbents.

- Polymeric sorbent, such as polystyrene-divinylbenzene (PS-DVB: Amberlite XAD series) is able to use in entrie pH range but not suitable for extraction any metal due to hydrophobicity and slow kinetics.
- non-polymeric sorbent (such as carbon) have a large surface area and can sorption both organic compound and metals but this sorbent also has heterogeneous surface leading to low reproducibility and can act as catalysis of oxidation.

In order to develop sorbent for increasing the sorption efficiency and selectivity towards the analyte, the sorbent is usually immobilized with various chelate functional groups. Several procedures to prepare the modified sorbents are given below

i) direct physical adsorption (loading) of chelating agent onto the surface of sorbent but this procedure has some disadvantage such as leaking out of chelating agent,

ii) modification via covalent bond (grafting) between sorbent and chelating agent.

The modification of sorbent via covalent bond is widely used because it has strong interaction between the sorbent surface and functional groups. Thus, silica gel was chosen to improve the sorbent for solid phase extraction because it has the high surface area, fast sorption of metal ions and good mechanical stability.

In addition, The surface of silica gel containing silanol group (Si-OH) which it may present as the free form or the condensesd form of siloxane bridges (Si-O-Si) by siloxane bond [14]. The surface of silica gel is shown in Figure 2.3. The modification of silica surface may occur via the reaction between siloxane (Si-O-Si) or the silanol (Si-OH) group and the organic functional group. The reaction with the silanol group is generally accepted as the main modificationpathway. The organosilane such as aminopropyltriethoxysilane [15,16] and 3-mercaptopropyltriethoxysilane [17,18] are widely used to modify on silica gel.

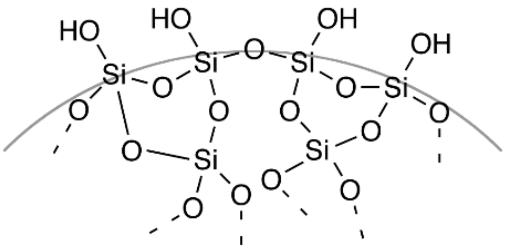


Figure 2.3 Surface of silica gel [19]

2.4 Flow injection (FI) and determination by atomic absorption spectrometry

Flow injection analysis is an automatic method analysis for trace element and it is widely used for pre-concentration or separation before determination with spectrometry techniques, such as FAAS, GFAAS, ICP-AES and ICP-MS. These methods are suffered from the contamination, loss of analyte, reduced residence times, reliability and high reproducibility [20]. Thus a flow injection technique is mostly used with widespread applications for quantitative chemical analysis.

In order to combine the determination step with the sample pre-concentration, the on-line flow injection system is often used to connect to the analytical instrument in order to reduce the analysis time and give the continuous determination.

Moreover, the typical flow injection analysis manifold consists of the pump, injection vale, column and a flow-though detector such as FAAS.

FI on-line pre-concentration coupled with FAAS for determination of metals have been developed and solid phase extraction has been coupled to system to pre-concentration.

2.5 Literature review

The determination of trace element in real sample and the pre-concentration step is required in order to improve the detection limit. The preliminary pre-concentration may be performed in batch or column to confirm the quantitative of the analyte. In order to combine the flow injection on-line pre-concentration with solid sorbent as solid phase extraction are mainly used because it offers such as low detection limit, low reagent consumption, high sample throughput, low matrix effect and high reproducibility.

Sorbent used as packing materials for pre-concentration is not always accommodated to flow on-line pre-concentration. Thus, the requirements for on-line materials are the mechanical properties which must be strong enough to the high flow rate and the rapid kinetic of sorption and complete desorption by an appropriate eluent [21]. Thus, silica gel was chosen as sorbent because it has a good chemical and physical properties for trace metal [22]. The modification of silica gel can be prepared by chemically sorption between surface of silica gel and the functional group of chelating ligand.

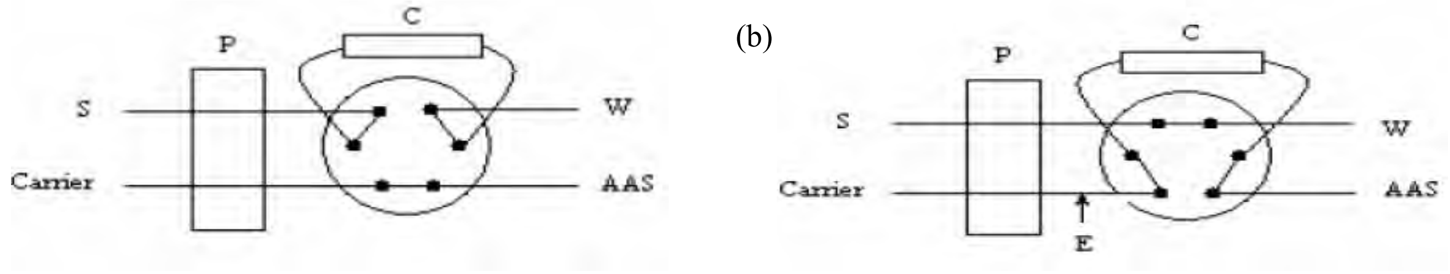
Previous researches studied about the modification of chelating ligand on sorbent used for the determination of silver ion (Ag(I)) batch or column were shown in Table 2.5.

The related publication in FI on-line pre-concentration coupled FAAS based on SPE reported in previous research can be explained below and a schematic diagram was presented in Figure 2.4

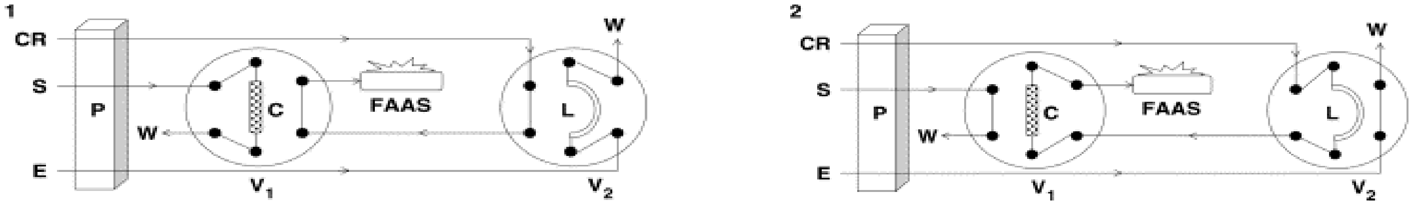
Table 2.5 Comparison of the ligands for determination of Ag(I)

Type of sorbent	Ligand	Applications	Method of determination	ref
Silica gel	3-aminopropyltriethoxysilane	Separation and preconcentration of vanadium, silver, manganese, and lead	GFAAS	[23]
Silica gel	2-mercaptobenzothiazole	Preconcentration and determination of silver ion	FAAS	[24]
Silica gel	2,4,6-trimorpholino-1,3,5-triazin	Separation, preconcentration and determination of silver ion	FAAS	[25]
Silica gel	dithizone	Determination of silver(I), Hg(II) and Pb(lI)	FAAS	[26]
Silica gel	2-[(2-mercapt ophenylimino) methy]phenol	Determination of trace amount of silver ion	FAAS	[27]
Silica gel	Azo-thioethr crowns containing 1,10-phenanthrone	Separation, preconcentration and determination of trace silver ion	FAAS	[28]
Silica	5-(4dimethylaminobenzylidene)-rhodanine	Separation, preconcentration and determination of silver ion	FAAS	[29]
Polyacrylamid	Benzil derivative of polyacrylohydrazide (BPAH)	Separation, preconcentration and measurement of silver(I) ion	FAAS	[30]

1) Shayessteh *et.al* [31]


(a) (b)

2) Valfredo *et.al* [32]



3) Coetzee *et.al* [33]

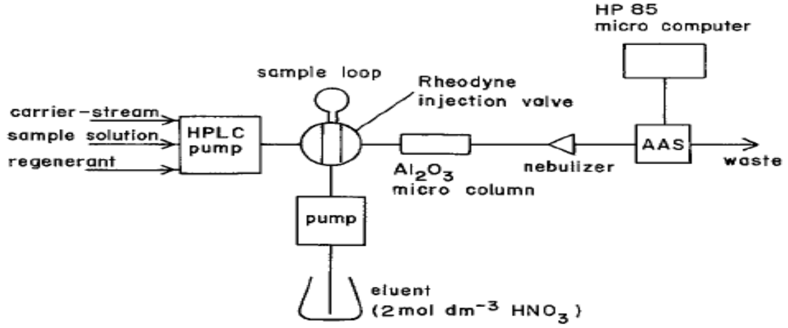


Figure 2.4 The system design for FI-FAAS

Shayessteh *et.al* [31] used an immobilized salen 2, 2′-[3 aza-1, 5-pentanedyle bis(nitrilomethylidyne)]-bis-phenol on alumina to determined of silver, zinc and copper in water and biological sample by flow injection on-line pre-concentration-FAAS. This method showed a good performance, it can be achieved the pre-concentration factor of 125, 210 and 166 for silver, zinc and copper respectively. The % RSD of silver, zinc and copper lower than 4.5 was obtained. The limit of detection of 0.3, 0.8 and 0.47 μg L^{-1} were obtained for silver, zinc and copper respectively.

Valfredo *et.al* [32] used Amberlite XAD-2 functionalized with Nitroso R salt to apply in an online system for pre-concentration of cobalt. This proposed method was successfully applied for determination of cobalt. The pre-concentration factor obtained was 223 with the detection limits of 0.39 μg L^{-1}.

Coetzee *et.al* [33] used a micro column of activated alumina in the basic form was used and coupled with flame atomic absorption for the pre-concentration and determination of silver. The results showed that the pre-concentration and detection limits were obtained 50 and 4.0 μg L^{-1} respectively.

In the previous research, benzothiazole calix[4]arene (**CU1**) was used as an ionophore for selective membrane electrodes that showed high selectivity to silver ion over interfering ions because calix[4]arene devivative containing S and N atoms were bound with metal ion [34]. Moreover, there has not been previously reported on the use of silica gel modified with benzothiazole calix[4]arene (**CU1**) for extraction of silver ions. Thus, the **CU1** was chosen for the modification on silica gel as sorbent material in flow injection on-line pre-concentration coupled FASS (FI-FAAS).

CHAPTER III

EXPERIMENTAL SECTIONS

3.1 Apparatus

The apparatuses used in this research are listed in Table 3.1. The operation parameters for silver ion determination by flame atomic absorption spectrometry are shown in Table 3.2.

Table 3.1 Apparatus lists

Apparatus	Model (company)
1. Nuclear magnetic resonance spectrometer	Mercury Plus 400 Varian
2. CHN analyzer	PE 2400 Series II (Perkin-Elmer)
3. Mass spectrometer in ESI mode	Micromass Platform II HP 1050
4. Fourier transforms infrared spectrometer	Impact 410 (Nicolet)
5. DR-UV-vis spectrophotometer	UV-2550, CPS-240A (SHIMADZA)
6. Flame atomic absorption spectrometer	AAnalyst 100 (Perkin-Elmer)
7. Thermal gravimetric analyzer	Pyrisl (Perkin-Elmer)
8. Centrifuge	Centaur 2 (Sanyo)
9. pH meter	Inlab®Basic BNC (Mettler TOLEDO)
10. Stirrer	Gem : MS 101
11. Peristaltic pump	REGLO Analog MS-4/8 model ISM 827 (ISMATEC®)

Table 3.2 Operating conditions for determination of silver ion by FAAS

Wavelength	328.30 nm
Slit width	0.70 nm
Lamp current	15.0 nm
C_2H_2 flow-rate	2.0 L min^{-1}
Air flow-rate	4.0 L min^{-1}

3.2 Chemicals

3.2.1 Chemicals

All chemicals in experiments were analytical reagent grade except ethyl acetate (Commercial grade), listed in Table 3.3.

Table 3.3 Chemical lists

Chemical	Supplier
Acetronitrile[a]	Lab Scan
Aminothiophenol	Merck
Bromoacetronitrile	Aldrich
Calcium nitrate	Merck
Dichloromethane	Merck
Dichloromethyl methyl ether	Merck
Ethyl acetate	Zen Point
Hydrochloric acid	Merck
Magnesium sulphate anhydrous	Panreac Quimica Sau
Magnesium nitrate	Merck
Methanol[a]	Lab Scan
Nitric acid 65%	Merck
Potassium nitrate	Merck
Single standard solution Ag(I) (1,000 mg L^{-1})	BDH

Table 3.3 Chemical lists (continued)

Chemical	Supplier
Sodium hydroxide	Merk
Sodium nitrite	Merck
Tin(IV)chloride	Aldrich
This(hydroxyl methyl)-amino methane	Carlo Erba
Thiosulfate	Merck
Toluene[a]	Lab Scan

[a] Toluene, acetronitrile and methanol were dried over calcium hydride under nitrogen atmosphere for moisture removal before used.

3.3 Characterization technique

The characterization of the synthesized material was confirmed by using several characterization techniques.

3.3.1 ^{1}H-NMR spectroscopy

NMR spectrum was recorded on a nuclear magnetic resonance spectrometer for identification of ligand structure. The deuterated solvent ($CDCl_3$) was used to dissolve the sample.

3.3.2 ESI mass spectrometry

The determination of molecular weight was recorded on a MALDI-TOF Micromass Platform.

3.3.3 Elemental analysis (EA)

The elemental analysis was used for measurement amounts of carbon, hydrogen and nitrogen to investigate the elemental composition of functionalized silica.

3.3.4 Fourier transform infrared (FT-IR) spectroscopy

Infrared spectra were recorded in the range of 400-4,000 cm^{-1} in transmittance mode with KBr pellet technique**.** FT-IR was used for characterization of functional groups or chemical bond on the modified material.

3.3.5 Diffuse reflectance UV-vis (DR-UV-Vis) spectroscopy

DR-UV was used for absorbance measurement of the immobilized ligand onto sorbent in the range of 200-800 nm.

3.3.6 Thermal gravimetric analysis (TGA)

TGA is a technique for measurement of a weight loss of material as a function of temperature by using a heating rate of 20 °C min^{-1} under nitrogen atmosphere. The weight loss at different temperatures indicates the composition between the modified material and the unmodified material.

3.4 Synthesis of benzothiazole calix[4]arene derivative chemically modified silica gel (APS-CU1)

3.4.1 Synthesis of benzothiazole calix[4]arene (CU1)

In the preparation of benzothiazole calix[4]arene, the procedure given by *Ngeontae, W. et al.* was applied [34]. The synthetic pathway of **CU1** is presented in Figure 3.1

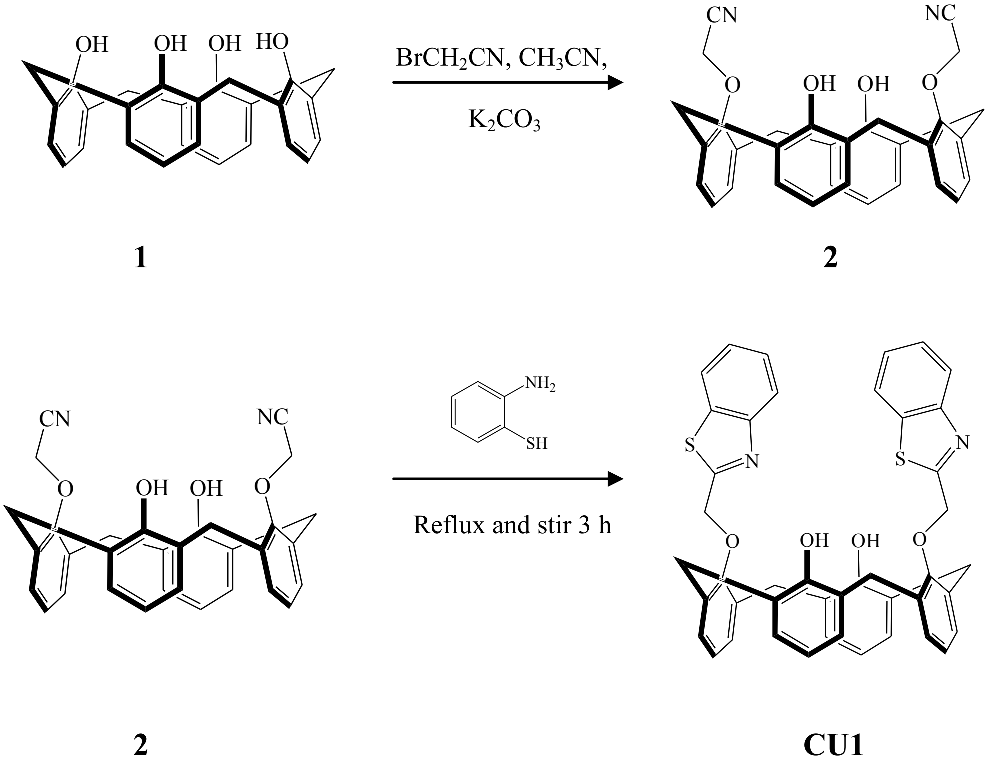


Figure 3.1 Synthetic pathways of **CU1**

In the first step, 25,26,27,28-tetrahydroxycalix[4]arene or **1** (3.05 g, 7.2 mmol) and potassium carbonate (3.34 g, 24.2 mmol) were dissolved in acetonitrile (60 mL) in a 100 mL two-necked round-bottomed flask and the mixture was stirred under nitrogen atmosphere at room temperature for 1 h. Then bromoacetonitrile (2.4 mL) was added and the mixture was heated at reflux at 90 °C under nitrogen atmosphere for 7 h. The mixture was cooled to room temperature and filtered. Then, the mixture was evaporated nearly dryness and treated with methanol to yield 25,27-bis(cyanomethoxy)-26,28-dihydroxycalix[4]arene **(2)** (2.407g, 66 %).

Next step, the mixture of compound **2** (1.02 g, 2.0 mmol) and aminothiophenol (0.5 mL, 4.7 mmol) was stirred and heated at reflux under nitrogen atmosphere for 3 h. The mixture was subsequently cooled to room temperature. Dichloromethane and methanol were added to precipitate 25,27-di(bebzothiazolyl)-26,28hydroxy-calix[4] arene (**CU1**) (1.081g, 74%).

Characterization data for 2

^{1}H-NMR ($CDCl_3$, 400 Mz, δ (ppm)): 7.15 (d, *J*=7.6 Hz, 4H, m-*HAr*OH), 6.89 (d, *J* =7.6 Hz, 4H, *m-HAr*OCH_2), 6.8-6.73 (t, *J*=7.2 Hz, 4H, *p-HAr*OH and *p-HArO*CH_2), 6.05 (s, 2H, OH), 4.85 (s, 4H, CH_2CN), 4.26 (d, *J* = 13.2 Hz, 4H, ArCH_2Ar), 3.62 (d, *J*=13.6 Hz, 4H, ArCH_2Ar)

Charecterization data for CU1

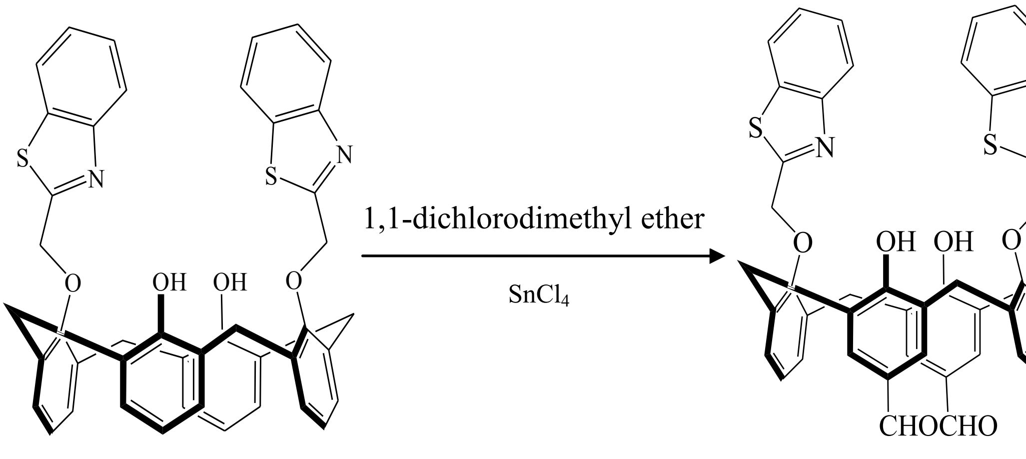
^{1}H-NMR ($CDCl_3$, 400 Mz, δ (ppm)): 8.09 (d, *J*=8.0 Hz, 2H, BT*ArH*), 7.51 (t, *J*=7.8 Hz, 2H, BT*ArH*), 7.45 (d, *J*=8.6 Hz, 2H, BT*ArH*), 7.33 (t, *J*=7.6 Hz, 2H, BT*ArH*), 7.25 (s, 2H, OH), 7.15 (d, *J*=7.6 Hz, 4H, *m-HAr*OH), 6.89 (d, *J* =7.6 Hz, 4H, *m-HAr*CH_2), 6.78 (t, *J*=7.2 Hz, 2H, *p-HAr*OH), 6.72 (t, *J*=7.4 Hz, 2H, *p-HAr*CH_2), 5.45 (s, 4H, ArOCH_2), 4.46 (d, *J* = 13.2 Hz, 4H, ArCH_2Ar), 3.62 (d, *J* = 13.6 Hz, 4H, ArCH_2Ar)

Elemetal Analysis for **CU1**: Anal calc: %C 73.51, %H 4.77 %N 3.90
Found: %C 72.0, %H 5.13 %N 3.85

3.4.2 Synthesis of benzothiazole calix[4]arene derivative (CU1-CHO) [35,36]

In a 100 mL two-necked round-bottomed flask, benzothiazole calix[4]arene or **CU1** (0.50 g, 0.70 mmol) and chloroform (20 mL) were done around -10ºC by using dry ice to control temperature under nitrogen atmosphere for 30 min.

1,1-Dichlorodimethyl ether (1.50 mL, 15.40 mmol) and 0.1 M tin(IV)chloride (7.70 mL, 7.70 mmol) were added and the mixture was stirred at room temperature for 1 h. Dichloromethane (200 mL) was added and the mixture was acidified with 3 M HCl (100 mL). The organic phase was separated and dried over magnesium sulfate and filtered. After evaporating the organic solvent, the crude product was purified on a column chromatography (SiO_2) using 10% ethyl acetate in dichloromethane as eluent to give a white solid (0.45 g, 82 %). The synthetic pathway of **CU1-CHO** is shown in Figure 3.2.



CU1 **CU1-CHO**

Figure 3.2 Synthetic pathway of **CU1-CHO**

Characterization data for CU1-CHO

^{1}H-NMR ($CDCl_3$, 400 Mz, δ (ppm)): 9.80 (s, 2H, Ar*CHO*), 8.23 (s, 2H, OH), 8.09 (d, *J*=8.0 Hz, 2H, BT*ArH*), 7.71 (s, 4H, *m-HAr*OH) 7.51 (t, *J*=7.8 Hz, 2H, BT*ArH*), 7.45 (d, *J*=7.6 Hz, 2H, BT*ArH*), 7.33 (t, *J* =7.6 Hz, 2H, BT*ArH*), 6.99 (d, *J* =7.6 Hz, 4H, *m-HAr*CH_2), 6.88 (t, *J*=7.0 Hz, 2H, *p-HAr*CH_2), 5.05 (s, 4H, ArOCH_2), 4.46 (d, *J*=13.2 Hz, 4H, ArCH_2Ar), 3.62 (d, *J*=13.6 Hz, 4H, ArCH_2Ar)

Elemetal Analysis for **CU1-CHO** + $CH_3COOCH_2CH_3$+CH_2Cl_2:

Anal calc: %C 64.62, %H 4.68, %N 2.96

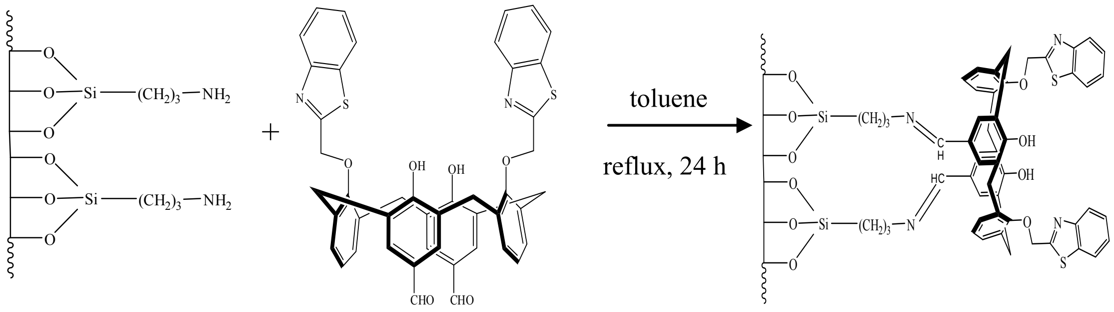
Found: %C 65.78, %H 4.32, %N 3.19

MALDI-TOF for [$C_{46}H_{34}N_2O_6S_2$] Anal calc: *m/z* = 774.19, Found: *m/z* = 775.3

FT-IR (ν in cm^{-1})**:** 1,700 (C=O stretching), 2,700-2,900 (C-H stretching**)**

3.4.3 Preparation of benzothiazole calix[4]arene derivative chemically modified silica gel (APS-CU1)

In a 100 mL two-necked round-bottomed flask, aminopropyl silica gel or **APS** (1.0 g), synthesized as described in literature [37], was refluxed and stirred under nitrogen atmosphere with **CU1-CHO** (100 mg) in toluene (50 mL) for 24 h. The mixture was cooled to room temperature. The suspended solid was filtered, washed with dichloromethane and dried in vacuum under ambient temperature. The product was kept in a desiccator. The synthetic pathway of **APS-CU1** is shown in Figure 3.3.



APS **CU1-CHO** **APS-CU1**

Figure 3.3 Synthetic pathway of **APS-CU1**

Characterization data for APS-CU1

FT-IR (ν in cm^{-1})**:** 1,100 (Si-O stretching), 800-500 (Si-O-Si stretching), 1,650 (C=N stretching)

DR-UV-vis spectrum (λ in nm): 343

3.5 Sorption and desorption of metal ion onto APS-CU1

The metal sorption properties of **APS-CU1** were studied in batch and column methods before applying in flow injection on-line pre-concentration. In batch method, various parameters such as solution pH, extraction time and sorption capacity were studied. In column method, the flow rate of sample and eluent, type of eluent, sample volume and the reusability of **APS-CU1** were studied.

3.5.1 Preparation of reagents

All solutions were prepared by using DI water.

a) Silver standard solutions

Silver standard solutions were prepared by dilution of 1,000 mg L^{-1} stock standard solution to the desired concentrations with buffer solution. The pH value was adjusted using sodium hydroxide and nitric acid solutions.

b) Nitric acid solution

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

c) Sodium hydroxide solutions

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

e) Thiosulfate solutions

Thiosulfate solutions were prepared by dissolving an appropriate amount of thiosulfate in DI water.

f) Tris-buffer solutions

Tris-buffer solutions were prepared by dissolving an appropriate amount of Tris (hydroxyl methyl)-amino methane in DI water.

g) Interfering ions solution

The solutions of interfering ions at 10, 100, 1,000 mg L^{-1} were prepared by dissolving an appropriate amount of $NaNO_3$, KNO_3, $Ca(NO_3)_2{\cdot}4H_2O$ and $Mg(NO_3)_2{\cdot}6H_2O$ in DI water.

3.5.2 Batch Method

All experiments were performed in triplicate.

3.5.2.1 Effect of pH

The effect of pH on extraction efficiency was studied by varying the pH range between 2.0 to 9.0. The pH of the solution was controlled by 0.005 mol L^{-1} This-buffer and adjusted pH with sodium hydroxide solutions (1% and 5% w/v) or nitric acid solution (1% and 5% v/v) to the desired value. The experiments were performed by using 20 mg of **APS-CU1** in a 5.0 mL of Ag(I) solution (10 mg L^{-1}). The mixture was mechanically stirred for 1 h. The sorbent was separated by centrifugation. The Ag(I) concentration of the supernatant was determined by FAAS.

3.5.2.2 Effect of extraction time

The mixture was prepared by mixing 20 mg of **APS-CU1** with a 5.0 mL of Ag(I) solution (10 mg L^{-1}) at pH 7. The influence of extraction time on extraction efficiency was studied by a varying a stirring time in the range of 1-60 minutes. The sorbent was then separated by centrifugation. Thereafter, the residual Ag(I) concentration was determined by FAAS.

3.5.2.3 Sorption capacity

In this experiment, the sorption capacity of Ag(I) on **APS-CU1** was studied by extraction the various concentrations of solution containing 5-140 mgL^{-1} of Ag(I) under the optimum pH with the suitable amount of **APS-CU1**. The mixture was mechanically stirred for 10 minutes at room temperature. The sorbent was then separated by centrifugation. Thereafter, the residual Ag(I) concentration was determined by FAAS.

3.5.2.4 Effect of eluent

The effect of the eluent on percent desorption of Ag(I) was studied. After the extraction step, under the optimum conditions, the sorbent was rinsed with 5.0 mL of DI water then separated by centrifugation. After that, 5.0 mL of various types and different concentrations of eluent was added. The mixture was mechanically stirred for 1 h. at room temperature. The sorbent was separated by centrifugation. Then, the concentration of Ag(I) in eluent was determined by FAAS.

3.5.2.5 Effect of elution time

For elution time, the suitable eluent was selected from the results of the experiment 3.5.2.4. The effect of elution time was investigated by varying the elution times in the range of 5-90 minutes by using the same procedure in experiment 3.5.2.4.

3.5.3 Column Method

A column was prepared by using a laboratory-made mini-column 4.7 mm in o.d. and 2.5 cm in length. All experiments were performed in triplicate.

3.5.3.1 Effect of sample flow rate

In this experiment, a mini-column was packed with 20 mg of **APS-CU1.** Then, 5.0 mL of 10 mg L^{-1} Ag(I) at pH 7 was passed through the column controlled by a peristaltic pump. The flow rate was varied between 1.0 to 5.0 mL min^{-1}. The Ag(I) in residual solution was then determined by FAAS.

3.5.3.2 Effect of eluent flow rate

In this study, the suitable eluent was selected from the results from the experiment 3.5.2.4. After the extraction step as mentioned in section 3.5.3.1, 5.0 mL of DI water was passed through the column. Then, 5.0 mL of eluent solution at different flow rates in a range of 1.0-5.0 mL min^{-1} was passed through the column. The concentration of Ag(I) in the eluent was determined by FAAS.

3.5.3.3 Reusability of APS-CU1

The reusability of **APS-CU1** was studied by using this sorbent for 8 cycles of sorption and desorption. Ag(I) was extracted and eluted under the optimum conditions from the results of experiment 3.5.3.1 and 3.5.3.2.

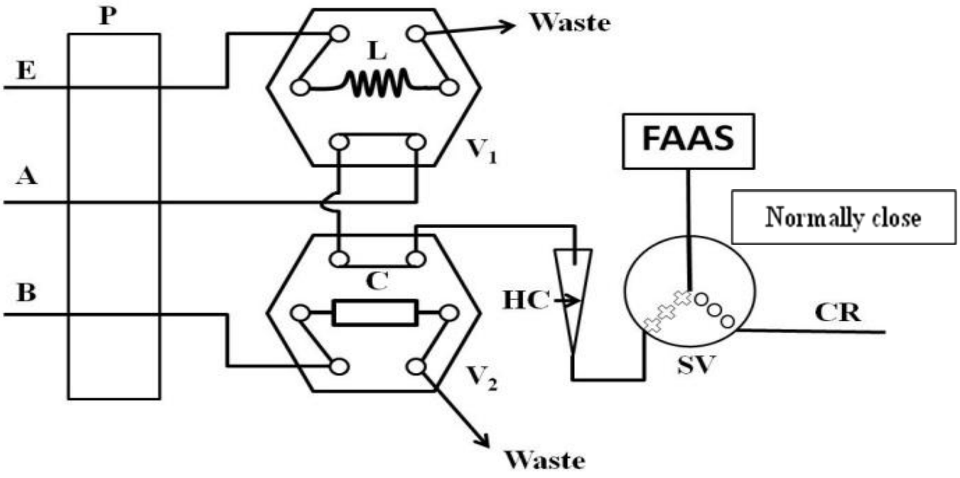
3.5.3.4 Effect of sample volume

The sample solutions of different volumes (5-250 mL) were spiked with 100 µL of 5 mg L^{-1} Ag(I) solution and passed through a mini-column packed with 20 mg of **APS-CU1** under the optimum flow rate. The concentration of Ag(I) was determined by FAAS.

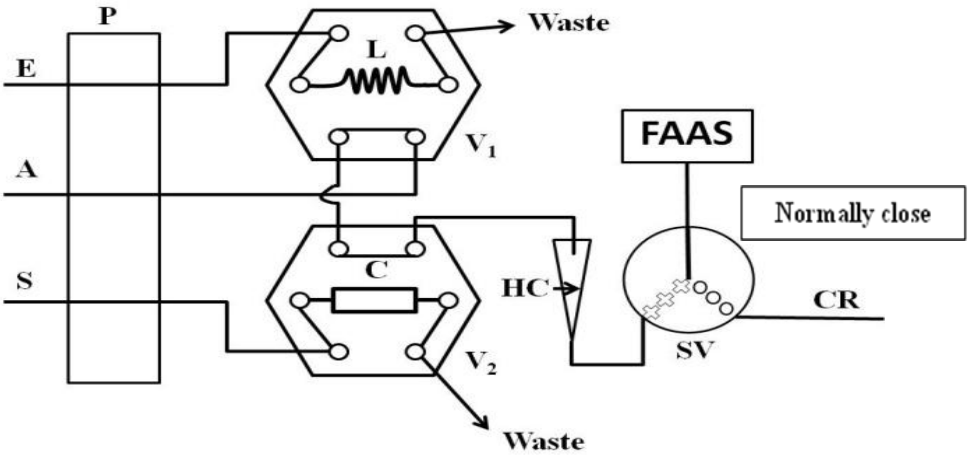
3.6 On-line preconcentration study

The on-line pre-concentration of the Ag(I) solution was studied with flow injection (FI) coupled to flame atomic absorption spectrometer system (FI-FAAS). This system was used for pre-concentration and determination of metal ion. The FI manifold in the on-line pre-concentration method was schematically shown in Figure 3.1. The FI-FAAS system consisted of one peristaltic pump, two six-port valves (V1, V2) and one solenoid valve (SV) which are connected to each other, eluent loop (L), a mini column (C) and holding chamber (HC). (Redrawn from ref [38]).

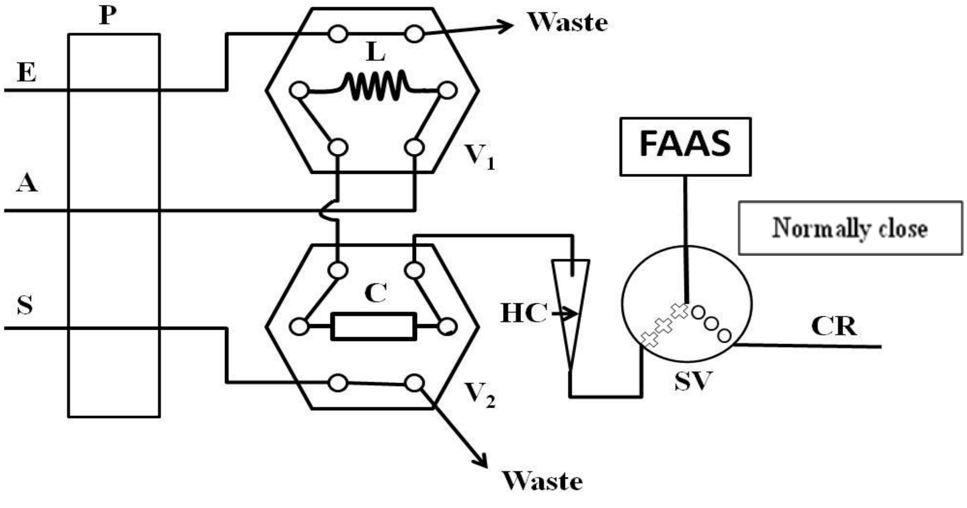
(A) Conditioning step



(B) Sorpting step



(C) Eluting step



(D) Measuring step

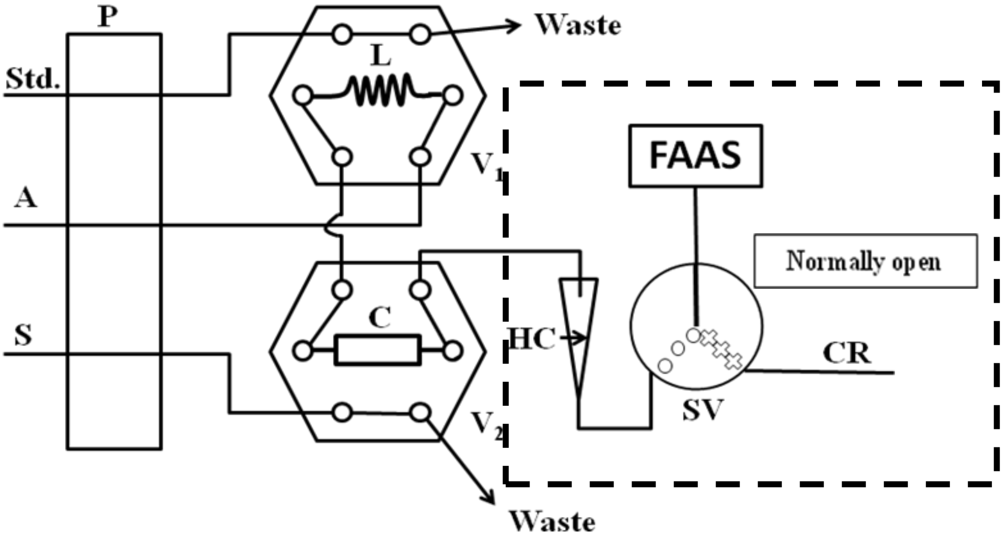


Figure 3.4 Schematic diagram for the on-line pre-concentration and determination of Ag(I). B = buffer, S = sample, A = air, CR = carrier (DI water), E = eluent (0.1 mol L^{-1} thiosulfate), P = peristaltic pump, C = mini-column packed with 20 mg of **APS-CU1**, V_1 and V_2 = six port valves, HC = holding chamber, SV = solenoid valve, L = eluent loop 250 µL, W = waste, FAAS = flame atomic absorption spectrometer.

The procedures in FI-FAAS system for the pre-concentration and determination of Ag(I) consisted of four steps:

(A) **Conditioning step**: The buffer was passed through the mini-column on V2. In this step, the surface of **APS-CU1** was activated for Ag(I) binding.

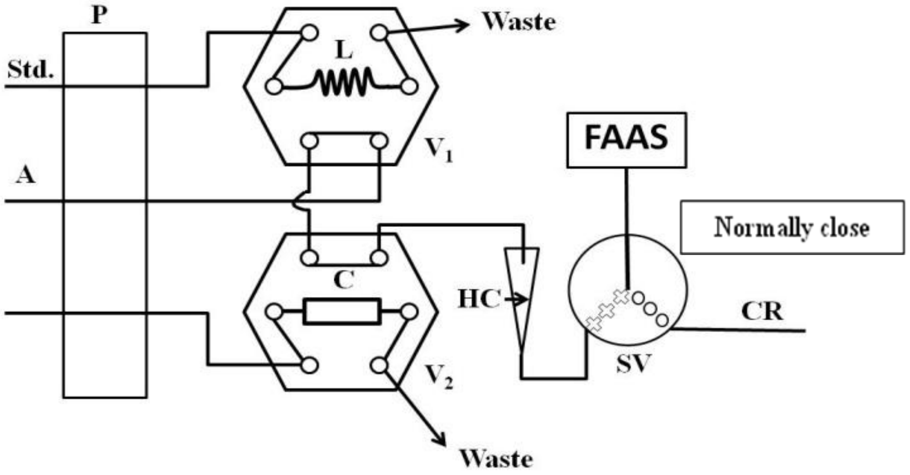
(B) **Sorpting step**: A solution of Ag(I) at optimum pH was pumped through the mini-column. Ag(I) was extracted on the mini-column and then the effluent was flowed toward waste. Carrier was directly taken via SV to the nebulizer of the flame atomic absorption spectrometer while the eluent was filled in the eluent loop (L).

(C) **Eluting step**: When V2 and V1 were simultaneously turned on, valve V1 was connected to V2 and the carrier transported the eluent through the mini-column. The retained Ag(I) in the mini-column was eluted and driven into the holding chamber.

(D) **Measuring step**: When SV was energized, the retained Ag(I) from holding chamber was transferred to the nebulizer of FAAS and the eluate was taken directly to FAAS for determination.

The procedure for performing calibration curves without pre-concentration (direct determination) was shown in Figure 3.2 and consisted of two steps:

(1) Loading step



(2) **Determining step**

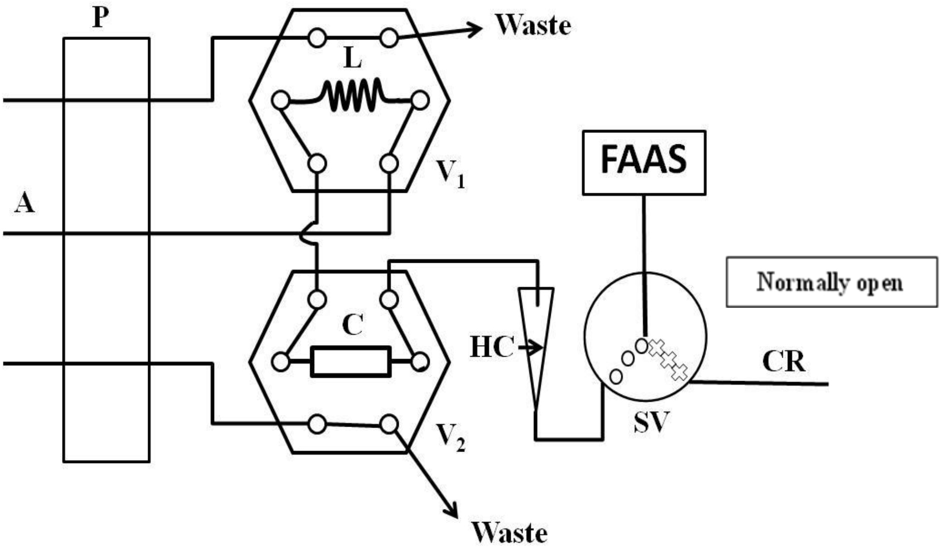


Figure 3.5 Schematic diagram of direct determination by the on-line system. Std. = standard, Ag(I) solution, CR = carrier (DI- water), P = peristaltic pump, V_1 and V_2 = six port valves, HC = holding chamber, SV = solenoid valve, L = eluent loop 250 µL, W = waste, FAAS = flame atomic absorption spectrometer.

(1) **Loading step**: A standard Ag(I) solution having concentration in the range of 0.2-1.2 mg L^{-1} was continuously pumped through the eluent loop, while the carrier was taken directly from SV to the nebulizer of the flame atomic absorption spectrometer.

(2) **Determining step**: When V2 and V1 simultaneously turned on, valve V1 was connected to V2 and the standard Ag(I) solution was transferred through the carrier in the line into the holding chamber. When SV was energized, the standard Ag(I) solution from holding chamber was flushed to the nebulizer of FAAS.

In this experiment, the on-line pre-concentration method was studied by using spiked samples with standard Ag(I) and performed according to Figure 3.4. The calibration curve without pre-concentration was performed according to Figure

3.5. The effect of various parameters such as sample flow rate, eluent flow rate and sample volume were also investigated. All experiments were performed in triplicate.

3.6.1 Comparision of the calibration curve between direct method and pre-concentration method

The signal of Ag(I) standard solution having concentration in the range of 0.25-1.25 mg L^{-1} was recorded after pre-concentration by using 5.0 mL of sample and 250 μL of eluent. Moreover the signal of Ag(I) solution (0.25-1.25 mg L^{-1}) was measured by FAAS direct determination.

3.6.2 Effect of sample flow rate

Effect of sample flow rate on Ag(I) on-line pre-concentration-FAAS was an important parameter to be optimized in order to obtain quantitative retention of metal ion on sorbent. 5.0 mL of 25 μg L^{-1} of Ag(I) solution at pH 6.5 was passed through an on-line pre-concentration system (FI-FAAS) using a flow rate in the range 1.0-6.0 mL min^{-1}. The retained Ag(I) was eluted by 250 μL of 0.1 mol L^{-1} thiosulfate at a flow rate of 1.5 mL min^{-1}. The concentration of Ag(I) in the eluate was determined by FAAS.

3.6.3 Effect of eluent flow rate

5.0 mL of 25 μg L^{-1} of Ag(I) solution at pH 6.5 was passed through an on-line pre-concentration system (FI-FAAS) at an optimum flow rate (3.0 mL min^{-1}). The retained Ag(I) was eluted by 250 μL of 0.1 mol L^{-1} thiosulfate at different flow rate in the range of 1.0 - 4.0 mL min^{-1}. The concentration of Ag(I) in the eluate was determined by FAAS.

3.6.4 Effect of sample volume

The 5.0 and 10.0 mL of sample solutions containing 25 μg L^{-1} of Ag(I) at pH 6.5 was passed through an on-line pre-concentration system (FI-FAAS) at an optimum flow rate (3.0 mL min^{-1}). The retained Ag(I) was eluted by 250 μL of 0.1 mol L^{-1} thiosulfate at flow rate of 1.5 mL min^{-1}. The concentration of Ag(I) in the eluate was determined by FAAS.

3.6.5 Effect of interfering ion

The comparison of Ag(I) solution (25 μg L^{-1}) in the present of nitrate salts of Na^+, K^+, Ca^{2+}, Mg^{2+} and Ag(I) solution in the absent of nitrate salts. Therefore, the nitrate salts of Na^+, K^+, Ca^{2+}, Mg^{2+} were added to Ag(I) solution (25 μg L^{-1}) with concentrations of 10, 100 and 1,000 mg L^{-1} at pH 6.5. The solution was passed through an on-line pre-concentration system (FI-FAAS) at an optimum flow rate (3.0 mL min^{-1}). The retained Ag(I) was eluted by 250 μL of 0.1 mol L^{-1} thiosulfate at a flow rate of 1.5 mL min^{-1}. The concentration of Ag(I) in the eluate was determined by FAAS.

3.7 Method validation

In this part, the method validation was performed by using spiked samples with standard Ag(I) solution under two concentration levels: 20 and 50 μg L^{-1}. The analysis was repeated in 10 times at the optimum conditions as shown in Table 3.4. The calibration curve was prepared by direct injection of 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 mg L^{-1} standard Ag(I) solutions. Then, the sample solution (5.0 mL) spiked with 20 and 50 μg L^{-1} of Ag(I) solution at pH 6.5 was passed through an on-line pre-concentration system (FI-FAAS) at 3.0 mL min^{-1}. The retained Ag(I) was eluted by 250 μL of 0.1 mol L^{-1} thiosulfate solution at flow rate of 1.5 mL min^{-1}. The concentration of Ag(I) in the eluate was determined by FAAS. The accuracy and precision were represented in term of the percentage of recovery and the relative standard deviation (%RSD), respectively. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated from the standard deviation of 10 measurements of blank solution (0.1 mol L^{-1} thiosulfate) using FI-FAAS without pre-concentration. In addition, method detection limit (MDL) and method quantitation litmit (MQL) of method were calculated by relating with the pre-concentration factor (P.F.).

Table 3.4 Optimum conditions for Ag(I) pre-concentration and determination by FI-FAAS.

Concentration of Ag(I) at pH 6.5	20 and 50 µg L^{-1}
Sample volume	5.0 mL
Sample flow rate	3.0 mL min^{-1}
Eluent volume	250 µL of 0.1 mol L^{-1} thiosulfate
Eluent flow rate	1.5 mL min^{-1}

3.8 Real sample analysis

To demonstrate the applicability of the FI-FAAS method in the determination of Ag(I) in real samples, the real sample was spiked with the standard solution of Ag (I) at two concentration levels (20 and 50 µg L^{-1}). The calibration curve was prepared at 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 mg L^{-1} of the standard Ag(I) solution.

The water samples used in this experiment were drinking water and tap water (5.0 mL), then spiked at 0.1 and 0.25 µg of Ag(I) and adjusted the pH to 6.5. The sample solution was passed through an on-line pre-concentration column under the optimum conditions as mentioned in Table 3.4. The concentration of Ag(I) in the eluate was determined by FAAS. Furthermore, the concentration of Ag(I) in real sample solutions determined by FI-FAAS method were compared to the result from inductively coupled plasma atomic optical emission spectrometry (ICP-OES) without the pre-concentration. The operating conditions of ICP-OES measurement determined Ag(I) as showed in Table 3.5 and the calibration curve was prepared at 10, 20, 30, 40, 50 and 60 µg L^{-1} of the standard Ag(I) solution. The experiment was performed in 10 replicates. The accuracy and precision were represented as %recovery and %RSD, respectively.

Table 3.5 The source setting conditions for determination of Ag(I) by ICP-OES

Wavelength	328.30 nm
Radiofrequency	1150.0 nm
Auxilliary gas flow	0.5 L min^{-1}
Nebulizer gas flow	0.7 L min^{-1}
Coolant gas flow	12.0 L min^{-1}

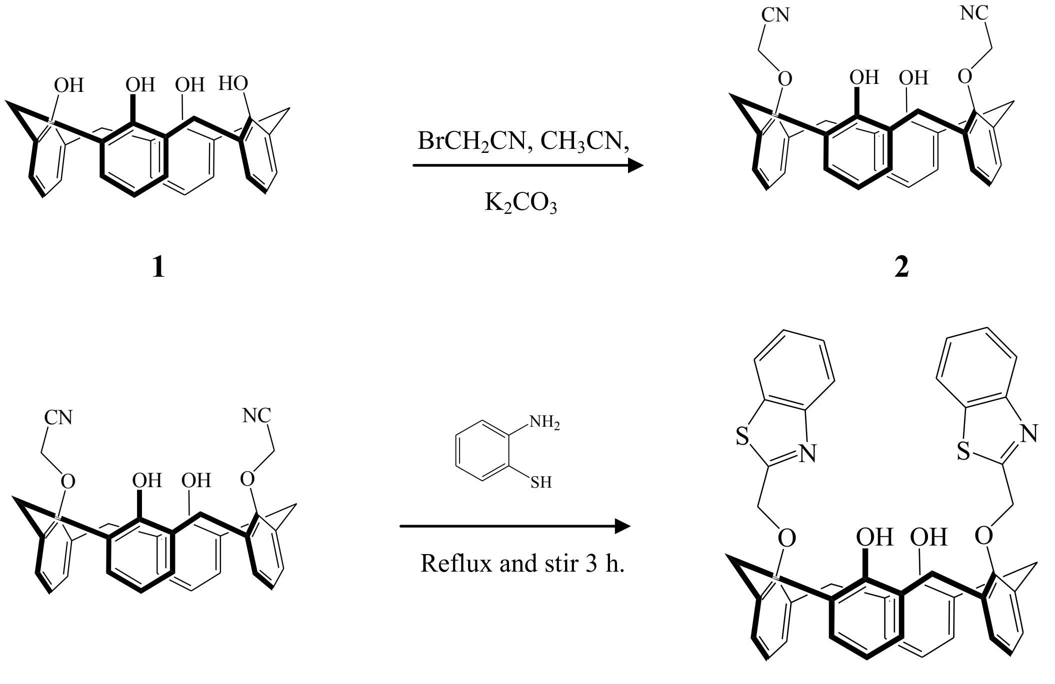
CHAPTER IV

RESULTS AND DISCUSSION

4.1 Synthesis and characterization of modified silica with CU1

In this research, **CU1** was chosen as a chelating ligand for silver ion because this molecule contains sulfur and nitrogen atoms acting as soft binding sites. Moreover, this molecule also has calix[4]arene building block that suitable for metal-ligand complex**.**

4.1.1 Synthesis and characterization of benzothiazole calix[4]arene (CU1)



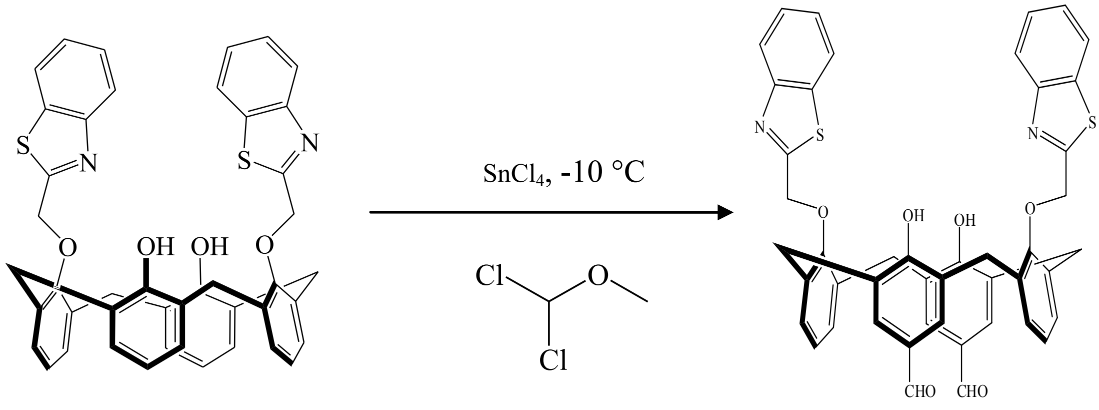
1 **2**

2 **CU1**

In the first step of the synthesis of **CU1**, the cyano group was linked to calix[4]arene via necleophilic substitution in the presence of potassium carbonate in acetronitrile to generate a cyano compound **2**. The compound **2** was characterized by ^{1}H-NMR spectroscopy. ^{1}H-NMR spectrum showed the characteristic signal of CH_2CN at 4.85 ppm and the characteristic signal of aromatic of calix[4]arene presented at 7.15-6.05 ppm. These results indicated that the cyano group was coupled with calix[4]arene.

The compound **2** was reacted with aminothiophenol via condensation reaction. The **CU1** was purified by dissolving in dichloromethane. Methanol was then added to precipitate **CU1**. The **CU1** was characterized by ^{1}H-NMR spectroscopy and elemental analysis. ^{1}H-NMR spectrum showed the characteristic signal of aminothiophenol (BT) at 8.09-7.33 ppm and the characteristic signal of aromatic of calix[4]arene presented at 7.25-5.45 ppm. The elemental analysis showed the composition of 72.0%, 5.13% and 3.85% for C, H and O, respectively.

4.1.2 Synthesis and characterization of CU1-CHO



CU1 **CU1-CHO**

CU1-CHO was synthesized via electrophilic aromatic substitution between **CU1** and 1,1-dichlorodimethyl ether in the presence of tin(IV)chloride as a catalyst. The reaction was performed in one step and the product was obtained as a white solid. The **CU1- CHO** was characterized by ^{1}H-NMR spectroscopy, elemental analysis, FT-IR and mass spectrometry. ^{1}H-NMR spectrum showed the characteristic signal of Ar*CHO* at 9.80 ppm. The elemental analysis showed that **CU1-CHO** contained C 65.78%, H 4.32% and N 3.19% and mass spectrum showed the molecular weight corresponded with the calculated result.

FT-IR spectra of **CU1** and **CU1-CHO** were illustrated in Figure 4.1. The difference between **CU1** and **CU1-CHO** were observed. The characteristic peak of **CU1-CHO** was found at around 1,700 cm^{-1} due to C=O stretching vibration of aldehyde group and a peak in the range of 2,700-2,900 cm^{-1} corresponding to the C-H stretching vibration [39].

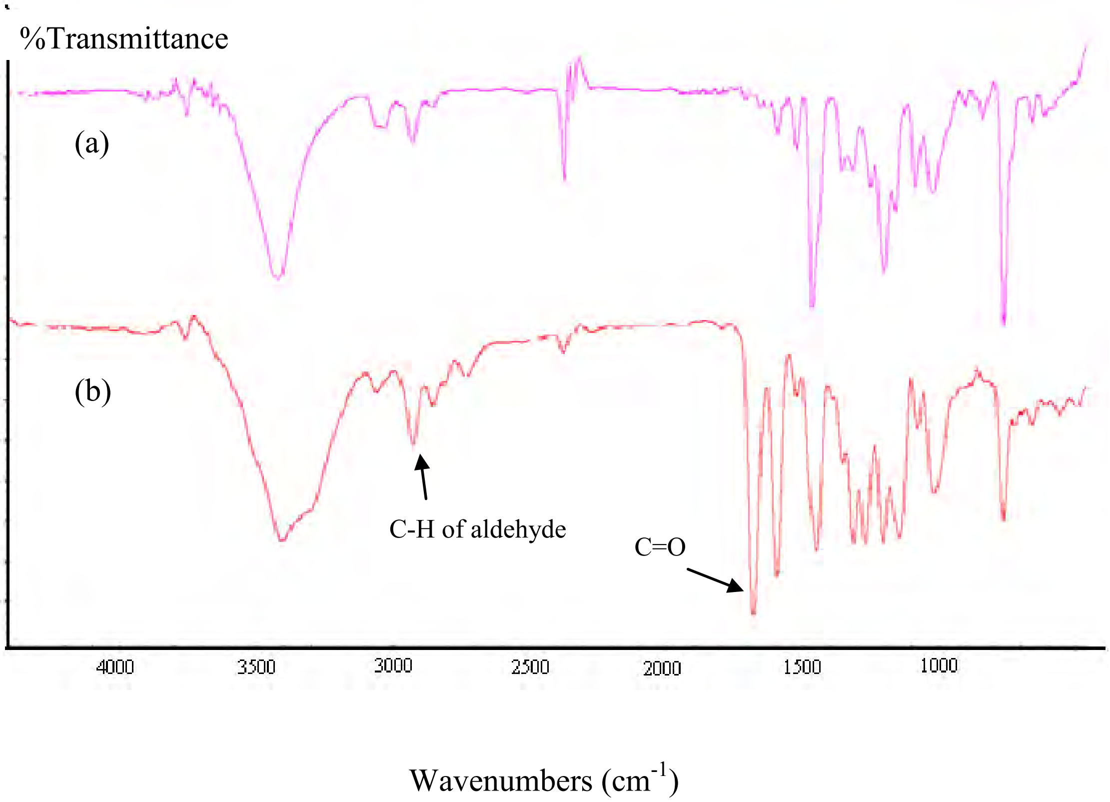


Figure 4.1 FT-IR spectra of (a) **CU1** and (b) **CU1-CHO**

4.1.3 Synthesis and characterization of APS-CU1

The **APS-CU1** synthesis was carried out via Schiff's base formation between amino groups (act as nucleophile) of **APS** and the carbonyl carbon atom (act as electrophile) of **CU1-CHO** by condensation reaction. The mixture was heated under reflux at 100 °C to ensure a complete reaction.

The characterization of **APS-CU1** was confirmed by FT-IR spectroscopy, DR-UV-vis spectroscopy and TGA.

FT-IR spectra of **APS** and **APS-CU1** are presented in Figure 4.2. The FT-IR spectra showed some minor differences between **APS** and **APS-CU1**. The characteristic peak of **APS** corresponding to Si-O stretching vibration was observed at 1,100 cm^{-1}. The Si-O-Si stretching vibration around 800-500 cm^{-1} and N-H bending vibration at 1,640 cm^{-1} can also be observed [39]. In this cases, a peak of **APS-CU1** at 1,650 cm^{-1} was attributed to C=N stretching vibration indicating that **CU1-CHO** was bonded with **APS** through Schiff's base bond.

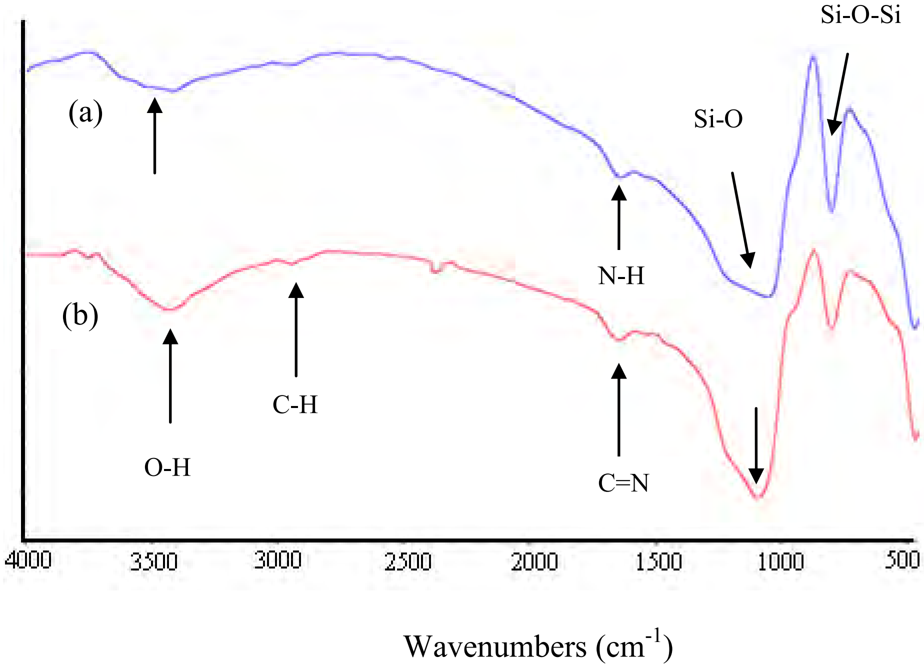


Figure 4.2 FT-IR spectra of (a) **APS** and (b) **APS-CU1**

DR-UV spectrum of **APS-CU1** (illustrated in Figure 4.3) showed an absorption band at 343 nm which was different from that of **APS** and this peak matches the position of benzolethiazole [40], indicating that benzothiazole calix[4]arene derivative was successfully immobilized onto **APS**.

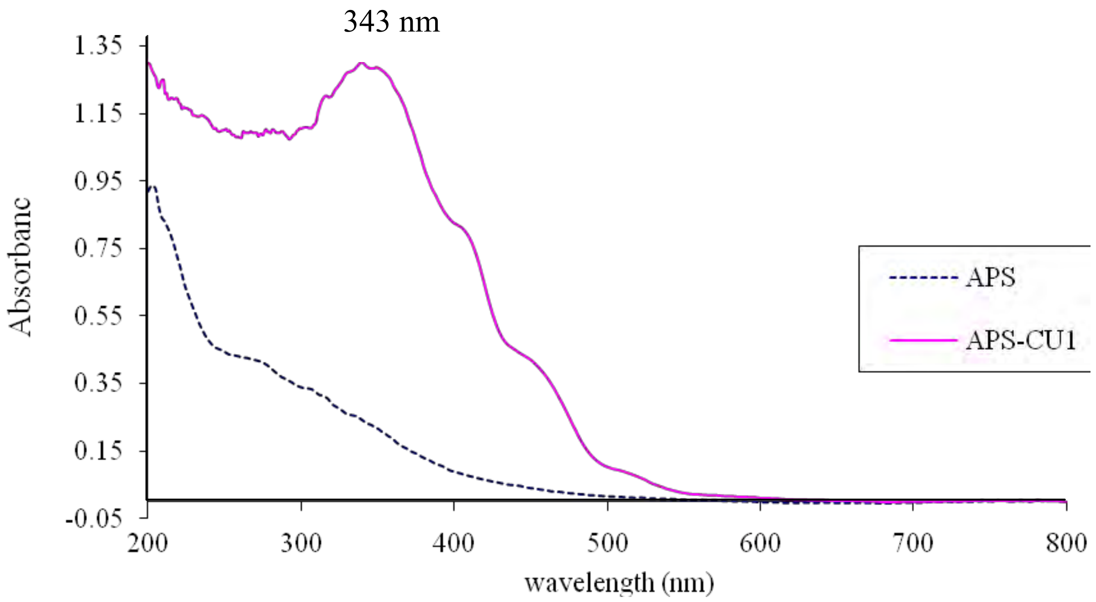
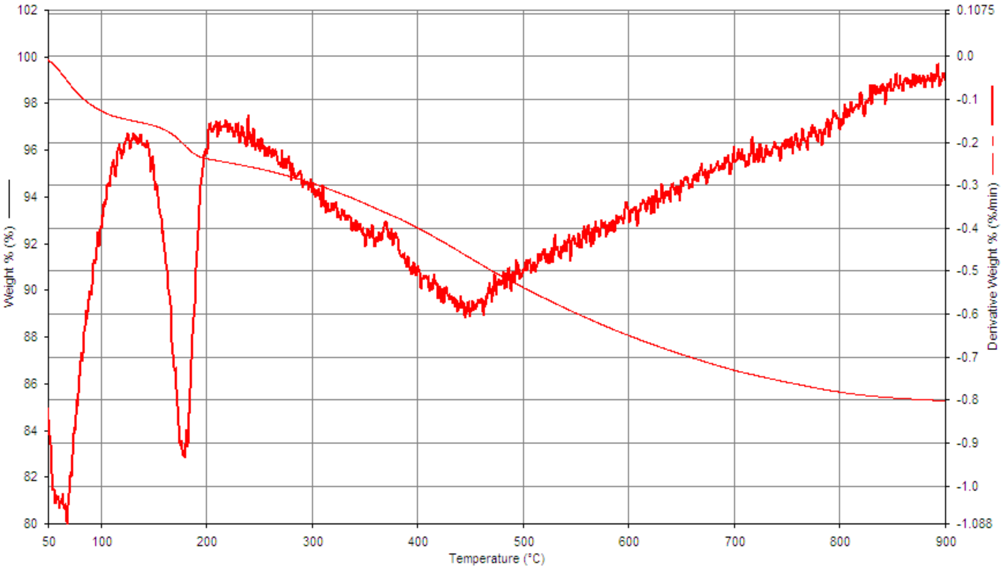


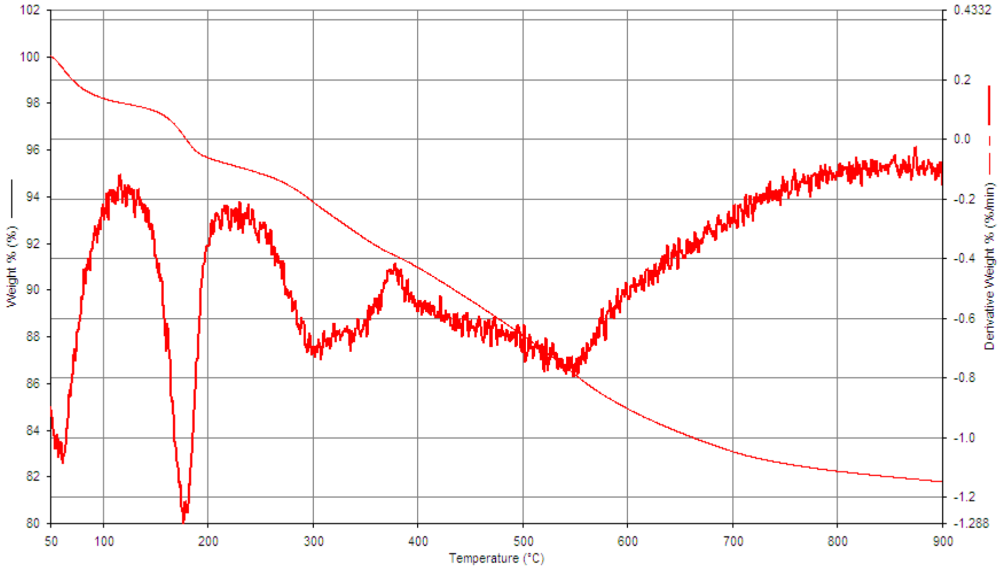
Figure 4.3 DR-UV-vis spectra of **APS** and **APS-CU1**

In addition, TGA was carried out to confirm the successful synthesis of **APS** and **APS-CU1**. The results of TGA are illustrated in Figure 4.4. The TAG curves showed the different decomposition temperatures between the modified and unmodified sorbent. The loss of water between 50ºC and 200ºC was corresponded to the physical adsorption of water. For **APS** (see Figure 4.4 (a)), 10.25% weight loss in the temperature range of 200-800ºC corresponding to the loss of aminopropyl modified on silica gel was observed.

The TGA curve of **APS-CU1** (see Figure 4.4 (b)) showed the weight loss in two steps. In the first step, the weight loss of 2.69 % at the temperature lower than 400 ºC corresponded to the loss of ligand modified on **APS**. The second step, the increase in the weight loss of 3.29% between 400ºC and 800ºC corresponded to the loss of aminopropyl moiety. This indicated that the chelating ligand was presented on the **APS**.



(a)



(b)

Figure 4.4 TGA curves of: (a) aminopropyl silica gel (**APS**) and (b) **APS-CU1**

4.2 Speciation analysis of silver

Nowadays the silver nanoparticles (AgNPs or Ag(0)) are wildly used in cosmetic and medical products because of their antibacterial property. The increasing demand of silver nanoparticles usage requires an environmental evaluation. A direct determination of silver nanoparticles is generally measured by UV-Vis spectrometry. The maximum absorption band of silver nanoparticles has been found at 375-400 nm [41]. However, UV-Vis spectrometry is not sensitive enough for a determination of silver nanoparticles at trace level. Moreover, silver nanoparticles (Ag(0)) can be oxidized to silver ion (Ag(I)) by oxygen. The information of the speciation analysis of silver is therefore required. Unfortunately, the available analytical technique for speciation of silver at trace concentration is rarely reported.

In this work, **APS-CU1** was used as sorbent in the solid phase extraction for either the separation of Ag(I) from a solution containing both Ag(I) and Ag(0) or the preconcentration before determination with FAAS. Only Ag(I) ought to be extracted onto **APS-CU1** whereas Ag(0) did not. The speciation analysis of silver was performed by indirect method. Ag(0) was oxidized to Ag(I) by using oxidizing agent as hydrogen peroxide followed by solid phase extraction. The total concentration of Ag(I) was obtained. The concentration of Ag(I) without oxidation subtracted from the total concentration of Ag(I) was the concentration of Ag(0).

4.2.1 Preliminary study on Ag(I) and Ag(0) extraction

Firstly, pH of solution was studied to prove the hypothesis mentioned above. The effect of pH was studied at pH ranging from 5.0 to 11.0. The pH solution was adjusted with nitric acid or ammonia solutions. The experiments were performed by using 20 mg of **APS-CU1** in 5.0 mL of Ag(I) or Ag(0) solution (10 mg L^{-1}). The mixture was mechanically stirred for 10 min. The sorbent was separated by centrifugation. The silver concentration of the supernatant was determined by FAAS.

The extraction efficiency is represented in term of %sorption which can be calculated form the change of the metal concentration in solution by equation (4-1):

$$\text{sorption (\%)} = \frac{C_i - C_{eq}}{C_i} \times 100 \qquad (4\text{-}1)$$

where C_i = the initial concentration of metal ion in the solution before extraction

C_{eq} = the equilibrium concentration of metal ion remaining in the solution after extraction

The sorption results are shown in Figure 4.5. The extraction efficiencies of Ag(I) and Ag(0) were insignificant different at pH 5-7 with >90% sorption. They decreased rapidly between pH 9 to pH 11. However, % sorption of Ag(I) was superior in comparison with that of Ag(0) at pH 10-11.

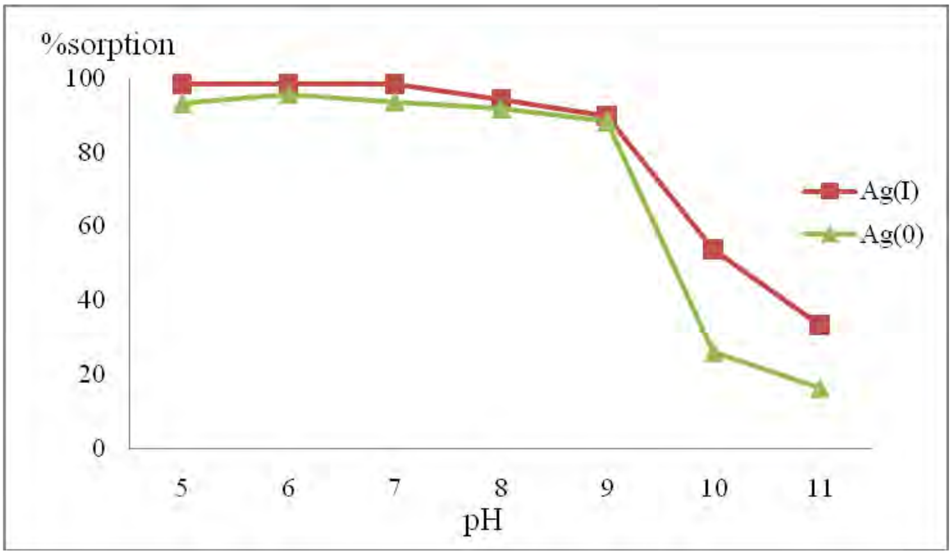


Figure 4.5 Effect of pH on Ag(I) and Ag(0) extraction

Moreover, the UV-vis spectrum of the solution of Ag(0) after extraction was also recorded. The absorption band of Ag(0) at around 400 nm was not observed at pH 5-9 while this characteristic absorption band was still occurred at pH 10-11. But the absorbance decreased in comparison with that of the initial Ag(0) solution. The possible hypothesis for theses phenomena was Ag(0) was changed to Ag(I) under desired condition and then Ag(I) was extracted by **APS-CU1**. How Ag(0) changed to Ag(I) was probably due to **APS-CU1** which was able to reduce Ag(0). Thus, the reducing property of **APS-CU1** was investigated by the reaction between **APS-CU1** and potassium permanganate ($KMnO_4$) solution. A 0.1 g of **APS-CU1** was titrated in acid solution with 3.0 mL of 0.001 mol L^{-1} $KMnO_4$ to end point. **APS** was also titrated in the same manner as **APS-CU1** using 4.5 mL of 0.001 mol L^{-1} $KMnO_4$ to end point while the end point of siliga gel (0.1 g) was used only one drop of 0.001 mol L^{-1} $KMnO_4$. These results indicated that both **APS** and **APS-CU1** had the reducing property.

As **APS-CU1** was unable to speciate Ag(0) and Ag(I) at any pH and the reducing property of sorbent, the further experiments were focused on the study of Ag(I) sorption with **APS-CU1**.

4.3 Sorption and desorption study

The **APS-CU1** was used as sorbent in the sorption and desorption for Ag(I) in batch and column methods before applying in on-line pre-concentration. The various parameters such as solution pH, extraction time, sorption capacity, flow rate of sample and eluent solutions, type of eluent, sample volume and the reusability of **APS-CU1** were studied.

The metal ion extraction efficiency and the elution efficiency were represented in term of %sorption and %desorption. The latter can be calculated form the change of the metal concentration in solution by equation (4-2).

$$\text{desorption (\%)} = \frac{C_e}{C_s} \times 100 \qquad (4\text{-}2)$$

where C_s = the concentration of metal ion sorbed on sorbent

C_e = the concentration of metal ion eluted from sorbent

4.3.1 Batch Method

4.3.1.1 Effect of solution pH

The influence of the solution pH on the extraction depended on the binding site of ligand and metal ion. In acid solution, the binding sites of the ligand containing N and S atom in benzothiazole group can be protonated so the ligand doesn't form complex with metal ion. In basic solution, the binding site is ready to form complex with metal ion. However, the metal ion is present in different species in solution e.g. metal-hydroxide form. Therefore, the effect of pH on the extraction should be first studied.

The initial pH of Ag(I) solution was varied in the range of 2.0-9.0. The results of extraction of Ag(I) on **APS–CU1** are shown in Figure 4.6

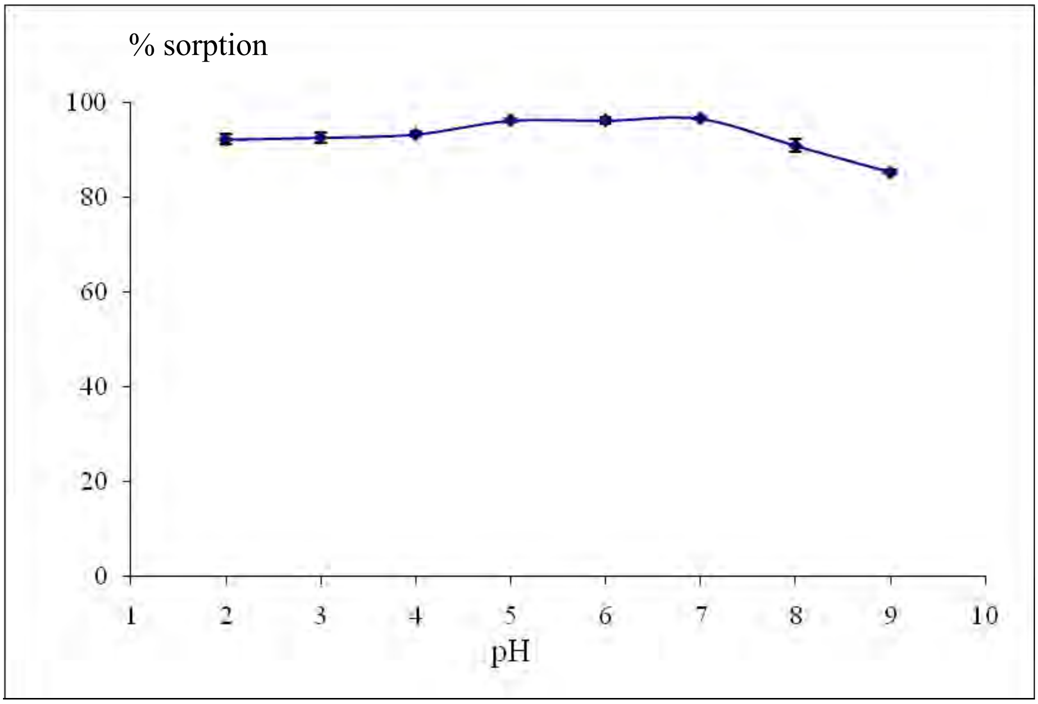


Figure 4.6 Effect of pH on Ag(I) extraction

The results showed that silver ions were sorbed quantitatively by **APS-CU1** between pH 2 to 7. %Sorption decreased gradually at pH > 8 because of the occurrence of the precipitate as Ag(I) hydroxide form. However, the colour changed of the sorbent in comparison with the sorbent before use under acidic medium (pH 2-5) was observed probably due to the decomposition of imine bond. Moreover, the modified silica gel was not stable in alkali solution [42]. Thus, the solution pH of 7 was chosen as the optimum pH for further studies.

4.3.1.2 Effect of extraction time

In this experiment, the effect of the extraction time was investigated to obtain the optimum extraction time. The experiment was performed by increasing extraction time from 1-60 min. The results are shown in Figure 4.7.

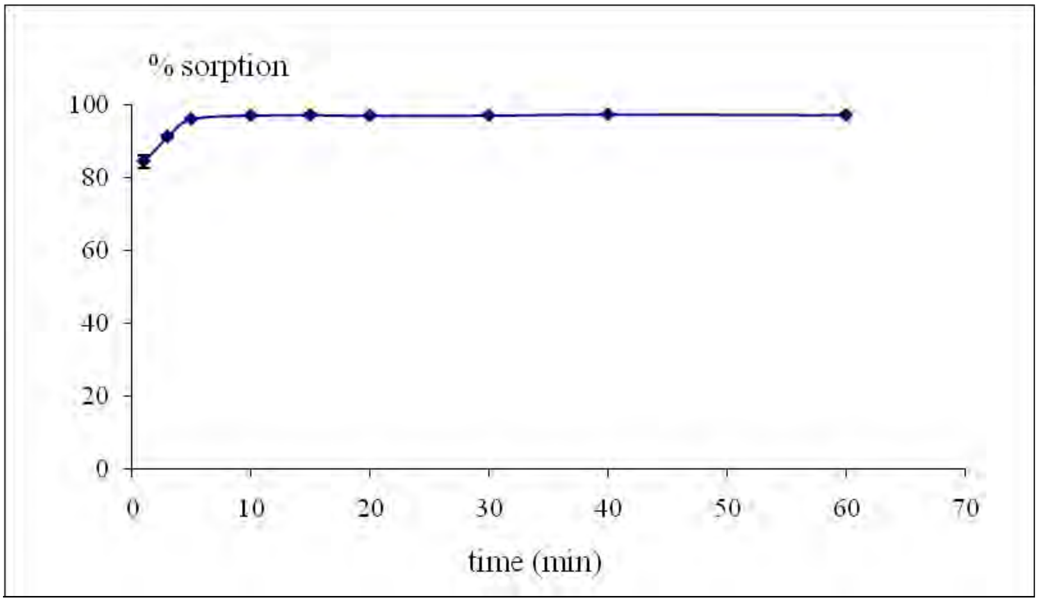


Figure 4.7 Effect of extraction time on extraction of Ag(I)

The extraction efficiency was increased when the extraction time increased due to the longer contact time between binding site and metal ion. Thus, the extraction time of 10 min was chosen because it showed fast kinetic of extraction and it was suitable for the column system.

4.3.1.3 Sorption capacity

The experiment was performed by extraction the various initial concentrations of Ag(I) with a constant weight of **APS-CU1** at room temperature.

The sorption capacity can be calculated by equation (4-3) as follows:

$$\text{Sorption capacity} = \frac{N_b - N_a}{m_{sorbent}} \qquad (4\text{-}3)$$

Where N_b = the amount of metal ion in the solution before extraction (mg)

N_a = the amount of metal ion remaining in the solution after extraction (mg)

$m_{sorbent}$ = mass of **APS-CU1** (g)

The result illustrated in Figure 4.8 showed the maximum sorption capacity of Ag(I) on the **APS-CU1** with in the initial concentration of 60 mg L^{-1}. The estimated maximum sorption capacity was found to be 12.2 mg g^{-1}.

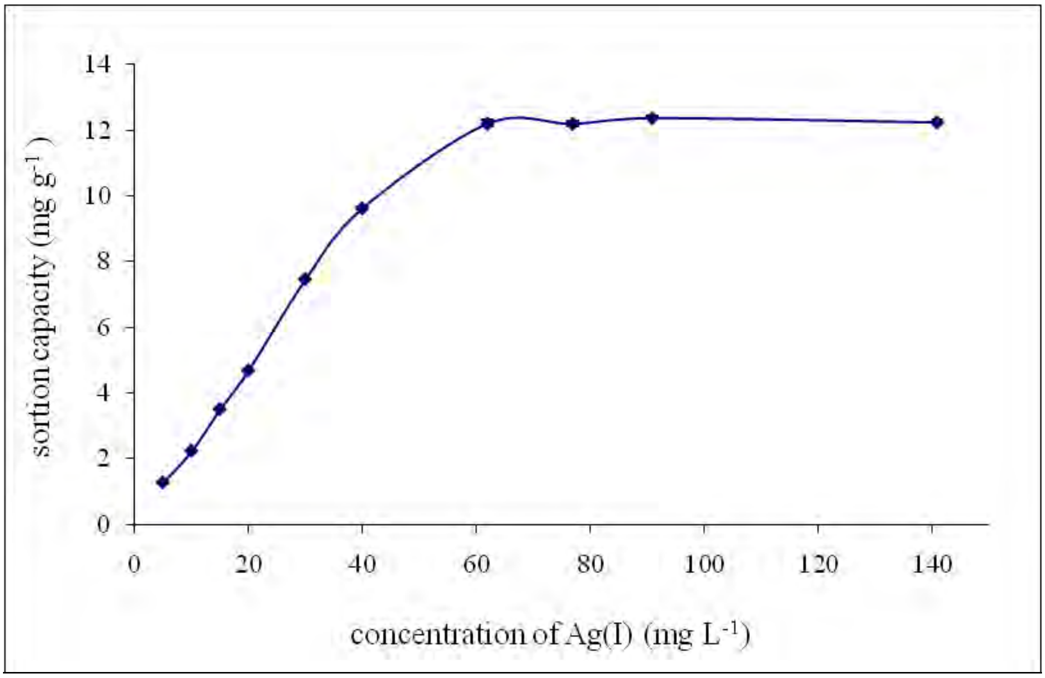


Figure 4.8 Sorption capacity of Ag(I) on **APS-CU1**

From the sorption capacity in the Figure 4.8 can be described the sorption behavior of metal ions on sorbent at equilibrium by several adsorption isotherms [43]. Langmuir and freundlich isotherm are two models used in the study of sorption behavior. Langmuir isotherm is based on the assumption that analytes are chemically sorbed on the sorbent surface at a fixed number of well defined sites. Each site can be held on one analyte molecule with the monolayer adsorption [44]. The Langmuir isotherm is represented by equation (4-4) as follows:

$$\frac{C_e}{q} = \frac{1}{b_{qm}} + \frac{C_e}{q_m} \qquad (4\text{-}4)$$

where C_e = equilibrium concentration of the analyte metal ion in aqueous solution (mg L^{-1} or mol L^{-1})

q = sorption capacity of sorbent (mg g^{-1})

q_m = maximum sorption capacity of sorbent (mg g^{-1})

b = Langmuir constant related to energy of sorption

While, Freundlich isotherms is based on the assumption that the uptake of metal occur on the heterogeneous surface by monolayer or multilayer sorption [44, 45]. The Freundlich isotherm is represented by equation (4-5) as follows:

$$\log_q = \log K_f + \frac{1}{n} \log C_e \qquad (4\text{-}5)$$

where K_f = Freundlich constant related to sorption capacity (mg g^{-1})

n = the numerical value of Freundlich constant

The Langmuir and freundlich isotherm plots are shown in Figure 4.9 and 4.10, respectively.

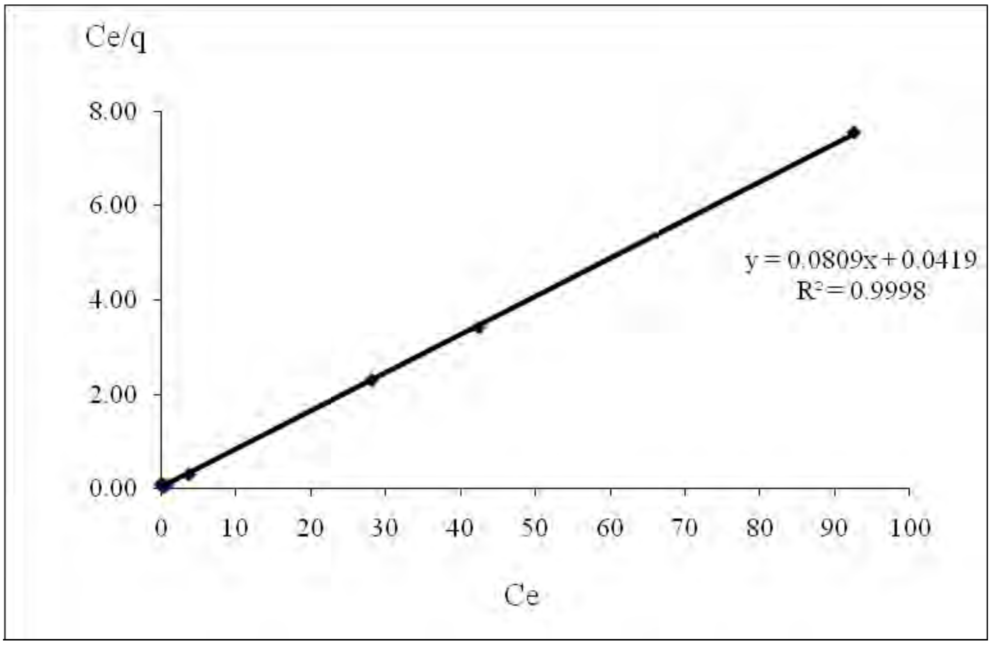


Figure 4.9 Langmuir isotherm plot of sorption of Ag(I) onto **APS-CU1**

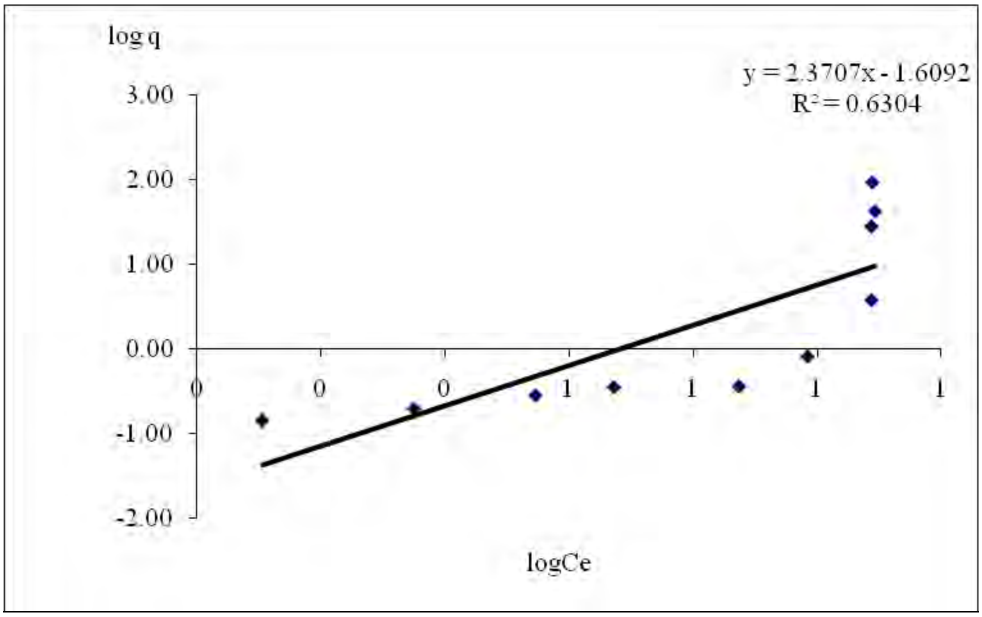


Figure 4.10 Freundlich isotherm plot of sorption of Ag(I) onto **APS-CU1**

The Langmuir and Freundlich isotherm parameter were also calculate and listed in Table 4.1 and 4.2, respectively.

Table 4.1 Parameters of Langmuir isotherm for sorption of Ag(I)

Equation	R^2	$q_{m,cal}$ (mg g^{-1})	$q_{m,exp}$ (mg g^{-1})	$b\times10^4$(mol L^{-1})
y = 0.0809x+0.0419	0.9998	12.36	12.28	0.5178

Table 4.2 Parameters of Freundlich isotherm for sorption of Ag(I)

Equation	R^2	K_f (mg g^{-1})	n
y = 2.3707x-1.6092	0.6304	2.65	0.4128

From the results shown in Table 4.1 and 4.2, the correlation coefficients (R^2) of Ag(I) obtained by fitting the experimental data to the Langmuir isotherm model ($R^2 > 0.99$). In the case of Freundlich isotherm shows the $R^2 < 0.99$ that indicated that the Freundlich isotherm model was not valid for these ions. Therefore, the sorption behavior of Ag(I) on **APS-CU1** follows to Langmuir isotherm which it was assumed that the Ag(I) are chemically sorbed on the sorbent.

4.3.1.4 Effect of eluent

In this experiment, the desorption of Ag(I) was studied by using different types and concentrations of eluent. HNO_3 was chosen to elute Ag(I) because it was widely used and compatible with AAS technique. Moreover, thiosulfate and thiourea were general complexing agent for Ag(I) with high formation constants ($k_f = 1.7.0\times10^{13}$) [46]. The results of the elution efficiency are presented in Table 4.3.

Table 4.3 Eluent efficiency on Ag(I) desorption

Eluent type	Concentration (mol L^{-1})	%Desorption[a]
Thiourea	0.1	85.6 ± 0.4
	0.3	88.6 ± 0.7
	0.5	90.5 ± 0.8
	0.7	95.0 ± 0.7
Thiosulfate	0.07	90.4 ± 0.1
	0.1	96.9 ± 0.2
HNO_3	0.1	15.3 ± 0.6

[a] mean value ± SD, n=3

The results showed that thiourea and thiosulfate provided higher elution efficiencies than HNO_3. Because the Schiff-base bond (-C=N-) of **APS-CU1** was probably broken to aminopropylsilica and **CU1** in acid solution.

In order to choose the most effective desorption of Ag(I) from the sorbent, the various concentrations of thiourea and thiosulfate were examined. When compare the concentrations of thiourea and thiosulfate, the results showed that both 0.7 mol L^{-1} thiourea and 0.1 mol L^{-1} thiosulfate gave the elution of Ag(I) from sorbent more than 95%. Then, thiosulfate was chosen, because the utilized concentration was less than thiourea and the toxic waste can be reduced. According to Hard-Soft Acid-Bases (HSAB) theory, the sulfur atom of thiosulfate ($S_2O_3^{2-}$) act as a soft base, which tend to form complex with soft acid like Ag(I). Therefore, thiosulfate was chosen as a eluent throughout the whole experiment.

4.3.1.5 Effect of elution time

The elution time was investigated by using a 0.1 mol L^{-1} thiosulfate solution as an eluent to optimize the suitable time resulting in the maximum desorption. The results illustrated in Figure 4.11, showed that the elution time was showed rapidly with more than 90% desorption within 25 min. Thus, the desorption of Ag(I) was able to be applied to the column method.

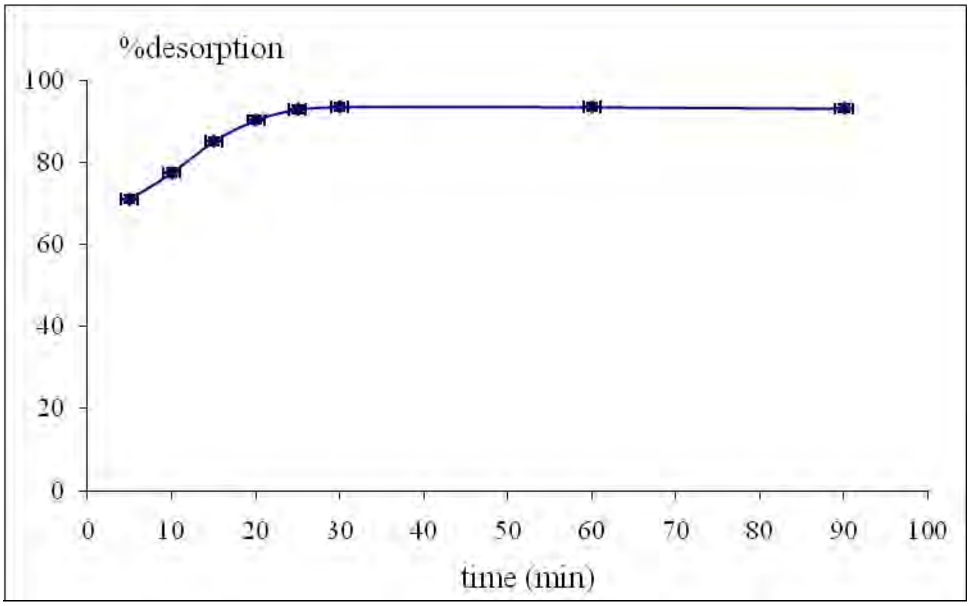


Figure 4.11 Effect of elution time

4.3.2 Column Method

In the column method, a column was prepared by using a laboratory-made mini-column 4.7 mm o.d. and 2.5 cm length packed with 20 mg of **APS-CU1**. Various parameters such as flow rates of sample and eluent, sample volume and reusability of **APS-CU1** were studied before applying in on-line pre-concentration.

4.3.2.1 Effect of sample flow rate

The sample flow rate is an important parameter to obtain the maximum extraction efficiency because the sorption of metal ion depends on the contact time between metal ion and sorbent. The sorption of metal ion on sorbent occurs incompletely at a high flow rate because the solution is passed quickly through the column. When using a low flow rate, a long sorption time resulting in high sorption efficiency but prolonging analysis time is obtained.

The effect of sample flow rate was investigated in range of 1.0-5.0 mL min $^{-1}$. The results are shown in Figure 4.12.

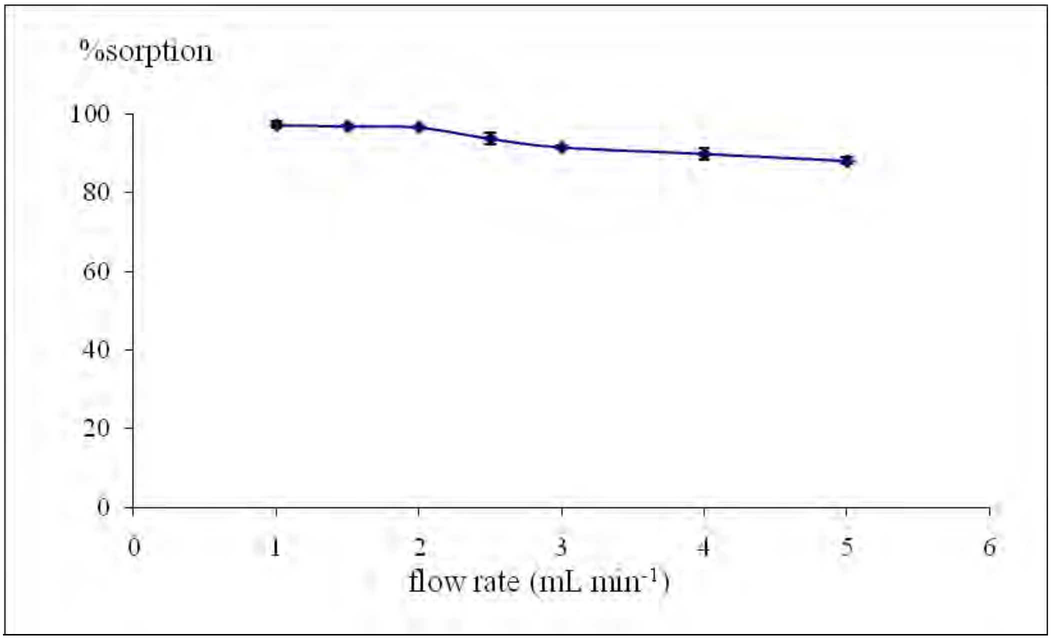


Figure 4.12 Effect of sample flow rate

The extraction efficiency was found to be 2.0 mL min $^{-1}$ because when the flow rate increased, %desorption decreased due to the short contact time between sorbent and Ag(I) solution.

Therefore, the sample flow rate of 2.0 mL min $^{-1}$ was chosen to study in the further experiment.

4.3.2.2 Effect of eluent flow rate

The eluent flow rate was applied to maintain the maximum elution efficiency. A 5.0 mL of 0.1 mol L^{-1} thiosulfate solutions were used as eluent at different flow rates in a range of 1.0-5.0 mL min^{-1}.

From Figure 4.13, the flow rate of 1.0 mL min^{-1} was found as a suitable flow rate to elute Ag(I) quantitatively. At higher eluent flow rate, the desorption efficiency decreased because the solution of eluent passed quickly thought column. Therefore, the elution flow rate at 1.0 mL min^{-1} was chosen.

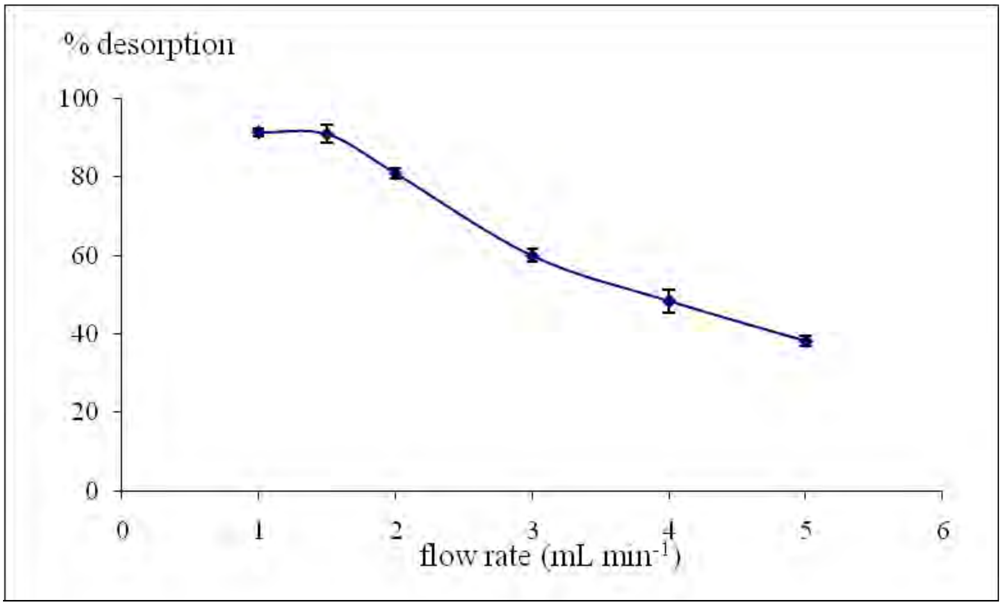


Figure 4.13 Effect of eluent flow rate

4.3.2.3 Reusability of APS-CU1

Reusability is an important property of a sorbent. Several cycles of sorption and desorption were performed under the optimum conditions (as mentioned in the Table 4.4.) to investigated the reusability of **APS-CU1**.

Table 4.4 Optimum conditions for Ag(I) sorption and desorption on column method

Sorption	Concentration of Ag(I)	10 mg L^{-1}
	Solution pH	6-7
	Sample flow rate	2.0 mL min^{-1}
	Sample volume	5.0 mL
Desorption	Eluent type	Thiosulfate
	Eluent concentration	0.1 mol L^{-1}
	Eluent flow rate	1.0 mL min^{-1}
	Eluent volume	5.0 mL

The reusability of **APS-CU1** is considered in term of %recovery which can be calculated by equation (4-6) as follows:

$$\%\text{ recovery} = \frac{N_e}{N_i} \times 100 \qquad (4\text{-}6)$$

where N_e = the amount of metal ion eluted form sorbent (mg)

N_i = the initial amount of metal ion (mg)

The reusability study of **APS-CU1** on column method was considered in term of %recovery as a function of cycle number. The results illustrated in Figure 4.14 showed that %recovery of Ag(I) in the determination of each cycle did not change significantly within 6 cycles of sorption and desorption. The %recovery decreased to lower than 80 % after 6 cycles of sorption and desorption due to the reduction of number of the available active sites.

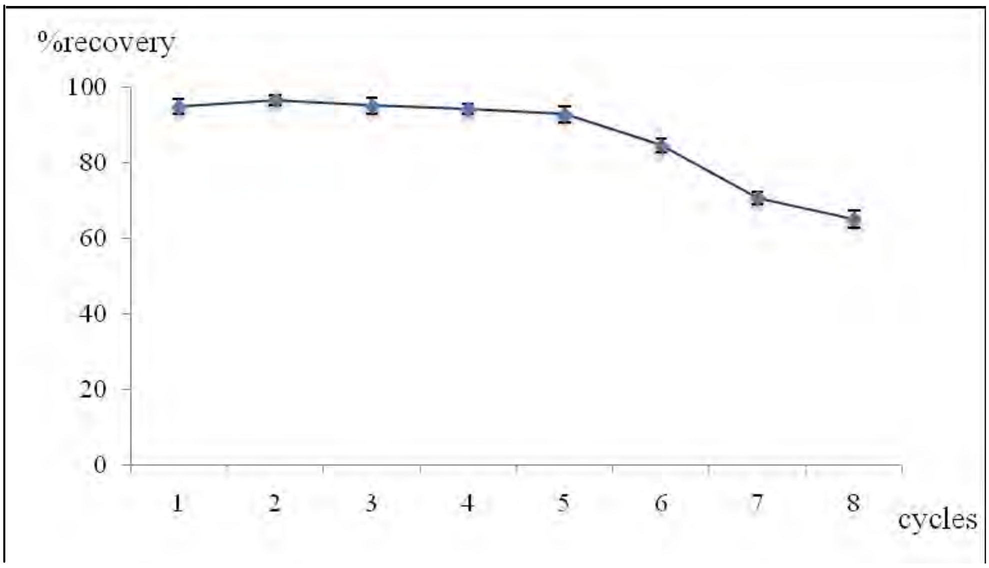


Figure 4.14 Reusability of **APS-CU1** on column method

4.3.2.4 Effect of sample volume

The sample volume is an important parameter affecting on pre-concentration factor. The sample solutions of different volumes (5-250 mL) were spiked with 100 µL of 5 mg L^{-1} Ag(I) solution and passed through a mini-column under the optimum conditions. The results are presented in Figure 4.15

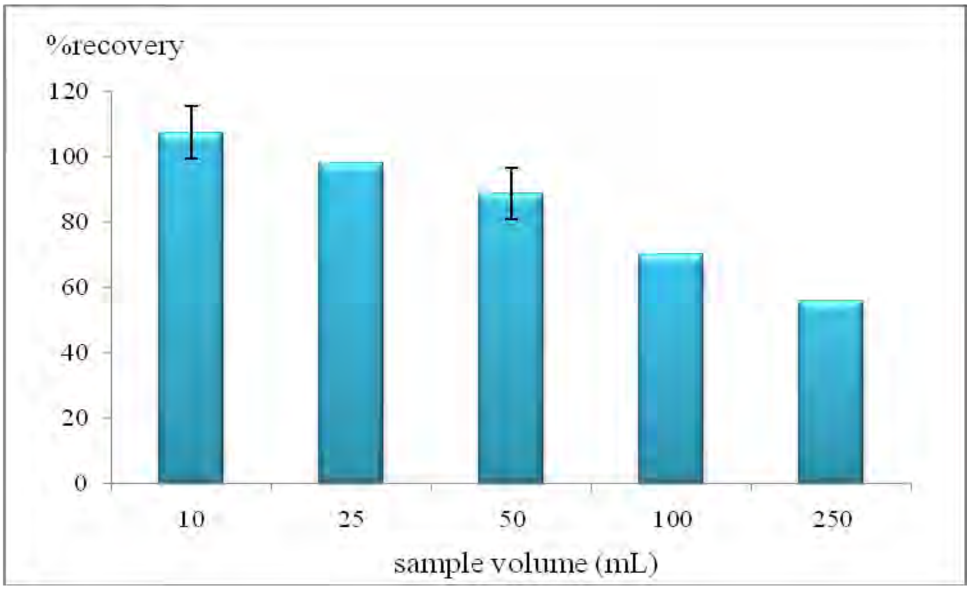


Figure 4.15 Effect of sample volume on %recovery

The maximum sample volume percolated through the column and provided the acceptable recoveries were 10.0, 25.0 and 50.0 mL when comparison with the acceptable values as shown in Table 4.5. The sample volume 100 and 250 mL showed the unacceptable recoveries because of the extracted Ag(I) was desorbed back to the sample solution under competition process between sample amounts and active sites of **APS-CU1**.

Table 4.5 Acceptable values of analyte recovery and precision of the determination of analyte at different concentrations [47].

Unit	Mean recovery, %	RSD,%
100%	98-102	1.3
10%	98-102	2.8
1%	97-103	2.7
0.1%	95-105	3.7
100 ppm	90-107	5.3
10 ppm	80-110	7.3
1 ppm	80-110	11
100 ppb	80-110	15
10 ppb	65-115	21
1 ppb	40-120	30

4.3.3 On-line pre-concentration

In order to obtain the optimum parameters, the various parameters such as the influence of the sample flow rate, eluent flow rate, sample volume and effect of interfering ion by the on-line pre-concentration system were studied.

However, this system was also modified by adding the holding chamber (HC) because of the incompatibility between flow rate pass through the column and flow rate of FAAS nebulizer. The holding chamber in the FI-FAAS system served as temporary sample storage before injecting into the FAAS. In this manner, the sample flow rate at column outlet would be automatically reduced and compatible with the flow rate at FAAS nebulizer inlet.

4.3.3.1 Comparison of the calibration curve between direct method and pre-concentration method

In this experiment, the pre-concentration performance was studied in order to compare the calibration curve between direct method and pre-concentration method. The calibration curves were obtained by using two different concentration ranges:

1. Direct method: the concentration range of 0.25-1.25 mg L^{-1} of Ag(I) was performed according to Figure 3.2.
2. Pre-concentration method: the concentration range of 0.0125-0.0625 mg L^{-1} of Ag (I) was performed according to Figure 3.1.

Table 4.6 shows linear calibration equation of direct and pre-concentration method, the signals were not significantly different. The pre-concentration method in which the initial concentration was 20 times lower than that of the direct method gave the slope closed to the direct method signal. This indicated that the on-line pre-concentration method exhibited a good performance.

Table 4.6 Comparison of the calibration curves between direct method and pre-concentration method.

Calibration curve	Linear equation
Direct method	$y = 0.051x - 0.0019$ $R^2 = 0.9943$
Pre-concentration method	$y = 0.0497x - 0.0016$ $R^2 = 0.9947$

4.3.3.2 Effect of sample flow rate

In order to maintain the extraction efficiency depends on the contact time between metal ion and sorbent, the effect of sample flow rate on the determination of Ag(I) was studied. The investigation of flow rate in real system was intended to prove whether the pressure or increasing length from additional part in system affect on flow rate. The flow rate was studied in ranged from 1.0- 6.0 mL min^{-1} and perform according to Figure 3.1. The results are shown in Figure 4.16.

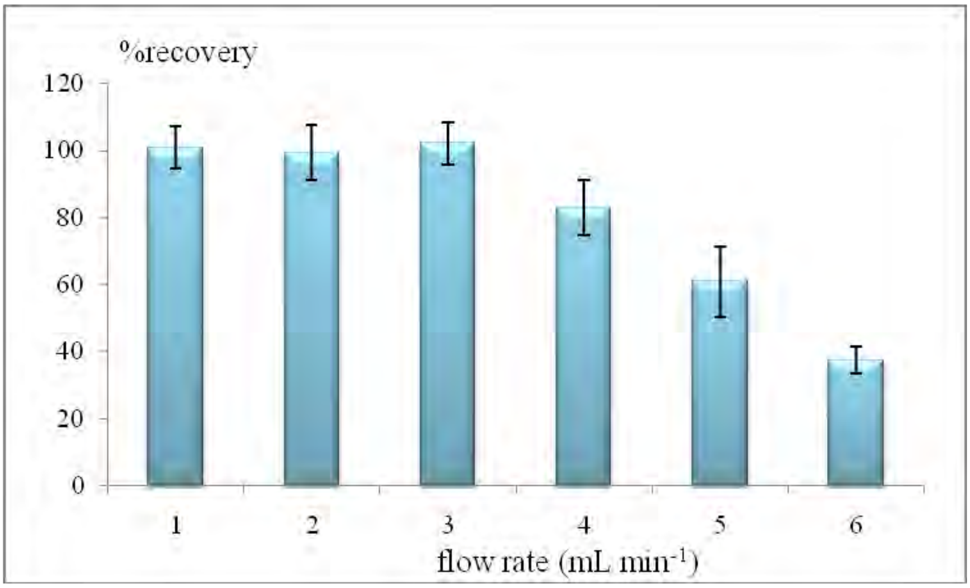


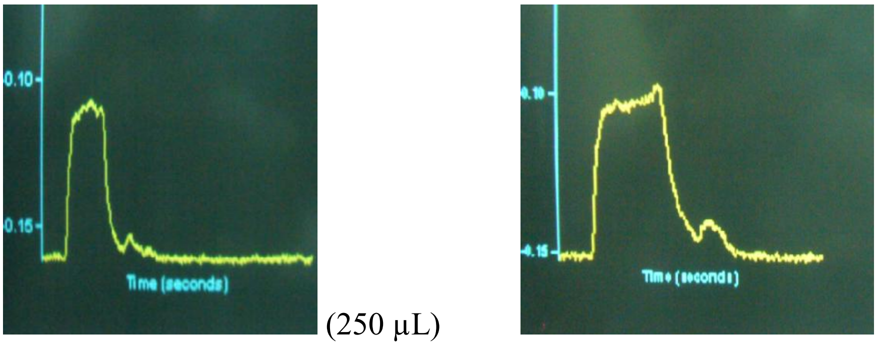
Figure 4.16 Effect of sample flow rate by FI-FAAS [conditions: **APS-CU1** = 20 mg, sample = 5.0 mL of 25 µg L^{-1} of Ag(I) pH = 6.5, eluent flow rate = 1.5 mL min^{-1}, eluent volume = 250 µL, [thiosulfate] = 0.1 mol L^{-1}]

The results were represented that, the recovery was obtained at a flow rate of 1.0-3.0 mL min^{-1}. Therefore, the sample flow rate of 3.0 mL min^{-1} was chosen to obtain the shorten analysis time.

4.3.3.3 Effect of eluent

The effect of eluent volume in on-line pre-concentration-FAAS system was studied to increase the elution efficiency from the column. The optimum volume should provide a sharp analytical signal (well-defined peak shape). It should also provide a complete elution with a highest pre-concentration efficiency. Moreover, the eluent flow rate must be compatible with the flow rate of FAAS nebulizer.

The effect of eluent volume was studied by directly passing 5.0 mL of 1.0 mg L^{-1} of Ag(I) standard solution to the nebulizer at different volumes of 250 μL and 500 μL and perform according to Figure 3.1. The results are shown in the Figure 4.17.



(250 μL) (500 μL)

Figure 4.17 Effect of eluent volume on the analytical signal

In this experiment, 250 μL of the eluent volume was chosen because it provide better peak shape than 500 μL. Because this system operated in peak height mode, the smaller eluent volume provide the higher sample throughput and less eluent consumption

The influence of the eluent flow rate on the determination of Ag(I) was studied at the flow rate in a range of 1.0- 4.0 mL min^{-1}. The results of Ag(I) on-line preconcentration are shown in Figure 4.18. The flow rate of 1.5 mL min^{-1} was found as a suitable low flow rate level of eluent to quantitatively elute Ag(I).

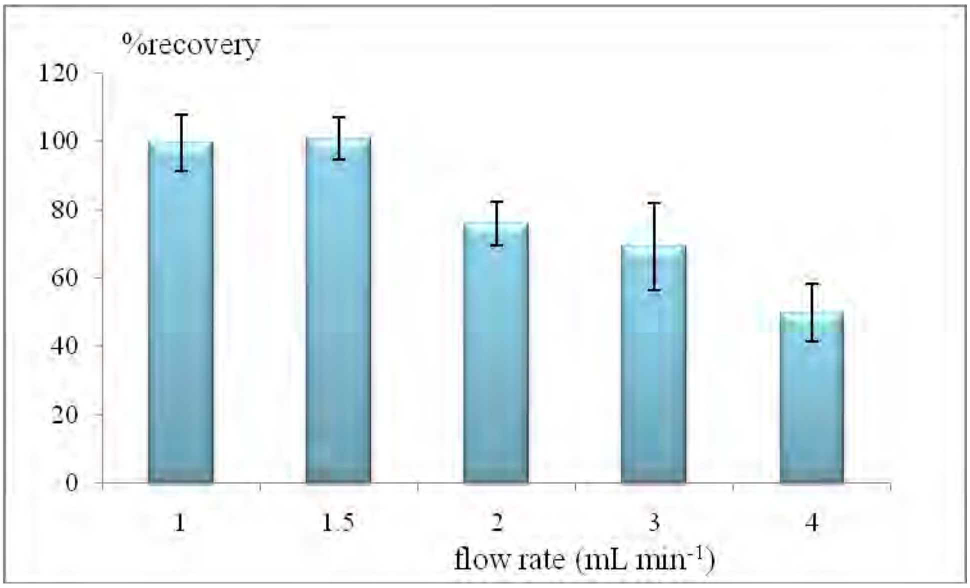


Figure 4.18 Effect of eluent flow rate by FI-FAAS [conditions: **APS-CU1** = 20 mg, sample = 5.0 mL of 25 µg L^{-1} of Ag(I) pH 6.5, sample flow rate = 3.0 mL min^{-1}, eluent volume = 250 µL, [thiosulfate] = 0.1 mol L^{-1}]

4.3.3.4 Effect of sample volume

In order to obtain the highest preconcentration factor, 5.0 and 10.0 mL of Ag(I) solution containing 25 µg L^{-1} was studied under the optimum conditions as follows:

APS-CU1 20 mg, sample = 5.0 mL of 25 µg L^{-1} of Ag (I) (pH 6.5), sample flow rate = 3.0 mL min^{-1}, eluent flow rate = 1.5 mL min^{-1}, eluent volume = 250 µL, [thiosulfate] = 0.1 mol L^{-1}.

The results are shown in Table 4.7.

Table 4.7 Effect of sample volume

sample volume (mL)	Pre-concentration factor	%recovery[a]
5.0	20	98.6 ± 5.5
10.0	40	84.3 ± 2.7

[a] mean±SD, n = 3

The results showed that 5.0 and 10.0 mL of sample yielded the acceptable recovery and high pre-concentration factor which can be calculated from the ratio between the initial volume of sample and the final volume which were 20 and 40 for 5.0 and 10.0 mL, respectively.

4.3.3.5 Effect of interfering ions

Common interfering ions such as Na^+, K^+, Ca^{2+}, Mg^{2+} could be found in the natural water. These ions may affect the sorption efficiency of Ag(I) by sorbent due to the competitive sorption with the analyte metal ions. Therefore, the effect of interfering ions was studied to evaluate the selectivity of **APS-CU1** for the determination of Ag(I) by using the FI-FAAS method.

The nitrate salts of Na^+, K^+, Ca^{2+}, Mg^{2+} were added to Ag(I) solution with concentrations of 10, 100 and 1,000 mg L^{-1}. The FI-FAAS method was performed under the optimum conditions as follows:

APS-CU1 20 mg, sample = 5.0 mL of 25 μg L^{-1} of Ag(I) pH 6.5, sample flow rate = 3.0 mL min^{-1}, eluent flow rate = 1.5 mL min^{-1}, eluent volume = 250 μL, [thiosulfate] = 0.1 mol L^{-1}.]

The results are shown in the Table 4.8. The interfering ions in high concentration did not affect the extraction efficiency of Ag(I) on **APS-CU1** in comparison with the Ag(I) solution in the absent of interfering ions.

Table. 4.8 Effect of interfering ions on the determination of Ag(I) by FI-FAAS

Interfering ion	Concentration.(mg L^{-1})	%recovery[a]
Without interfering ion	-	100.81 ±6.3
Na^+	10	101.6 ±4.6
	100	100.1 ±9.6
	1,000	93.9 ±7.0
K^+	10	103.2 ±13.3
	100	104.7 ±2.7
	1,000	95.5 ±7.1
Mg^{2+}	10	100.9 ±5.4
	100	97.9 ±4.6
	1,000	102.5 ±9.3
Ca^{2+}	10	100.5 ±10.0
	100	100.0 ±2.5
	1,000	98.5 ±2.5

[a] mean±SD, n = 3

4.4 Method validation [48]

After the optimum conditions obtained from the on-line pre-concentration Ag(I) using FI-FAAS, the method was validated by demonstration the performance of the method related to accuracy and precision which were reported in term of % recovery and %RSD, respectively. The accuracy was usually determined by spiked sample with the known concentration of the standard solution and the percentage of recovery values is calculated by equation (4.7) as follows:

$$\%\ \text{recovery} = \frac{N_{bs} - N_b}{N_s} \times 100 \qquad (4\text{-}7)$$

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

N_s = the amount of metal ion in spiked (mg)

N_b = the amount of metal determine in matrix blank or unspiked sample (mg)

The precision of this method was determined in term of relative standard deviation (%RSD) under optimum conditions, which is calculated by equation (4.8) as follows:

$$\%\ RSD = \frac{SD}{\overline{\overline{X}}} \times 100 \qquad (4.8)$$

where SD = the standard deviation

X = the mean value

The method detection limit was determined by using blank solution. The limit of detection (LOD) is the lowest concentration level of an analyte in the sample that can be determined from three times standard deviation of the signal from blank solution, thus LOD value is calculated by equation (4.9) as follows:

$$LOD_{(mgL^{-1})} = y_{(mgL^{-1})} + 3SD_{(mgL^{-1})} \qquad (4.9)$$

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

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

The limit of quantitation (LOQ) is the lowest concentration of an analyte that can be indicated quantitative result under conditions of the method and LOQ is calculated by equation (4.10) as follows:

$$LOQ_{(mgL^{-1})} = y_{(mgL^{-1})} + 10SD_{(mgL^{-1})} \qquad (4.10)$$

The method detection limit (MDL) and method quantitation limit (MQL) of the method can be determined by equations 4.11 and 4.12, respectively as follows:

$$MDL = \frac{LOD}{P.F.} \times 100 \qquad (4.11)$$

$$MQL = \frac{LOQ}{P.F.} \times 100 \qquad (4.12)$$

Where $P.F.$= Pre-concentration Factor = $\frac{\textit{sample volume (mL)}}{\textit{eluent volume(mL)}}$

Accuracy, precision, LOD, LOQ, MDL and MQL of spiked sample are summarized in Table 4.9.

Table 4.9 Accuracy, precision, LOD, LOQ, MDL and MQL of the proposed method of FI-FAAS for Ag(I) determination.

Ag(I) concentration (μg L^{-1})	Recovery (%)[a]	RSD (%)[a]	LOD (μg L^{-1})	LOQ (μg L^{-1})	MDL (μg L^{-1})	MQL (μg L^{-1})
20.0	100.2	6.1	8.8	16.0	0.44	0.8
50.0	99.5	3.3				

[a]mean value, n=10

The method validation was performed by using spiked sample with standard Ag(I) at 20.0 and 50.0 μg L^{-1}, respectively which covered the calibration range to test an accuracy and precision. It was found that the accuracy and precision of this proposed method for Ag(I) determination were acceptable at both concentration levels in comparison with the acceptable values shown in Table 4.3. LOD, LOQ, MDL and MQL values were found to be 8.8 μg L^{-1} and 16.0 μg L^{-1}, 0.44 μg L^{-1} and 0.8 μg L^{-1} respectively.

4.5 Real sample analysis

The FI-FAAS system was applied to determine Ag(I) in real samples using spiked method. The real samples for this work were drinking water and tap water. The results are represented as the percentage of recovery and %RSD shown in Table 4.10.

Table 4.10 Determination of Ag(I) in real sample by FI-FAAS in comparision with ICP-OES

Sample	Added (μg L^{-1})	Proposed method FI-FAAS		Reference method ICP-OES		*t*-stat (*t*-criti=2.20)
		Found[a] (μg L^{-1})	%Recovery	Found[a] (μg L^{-1})	%Recovery	
Drinking water	-	n.d.	n.d.	n.d.	n.d.	-
	20.0	19.7 ± 0.6	98.5	20.7 ± 0.3	103.5	1.86
	50.0	47.9 ± 1.5	95.8	50.3 ± 0.9	100.6	2.15
Tap water	-	n.d.	n.d.	n.d.	n.d.	-
	20.0	19.3 ± 0.6	96.5	20.9 ± 0.6	104.5	1.71
	50.0	48.1 ± 2.4	96.2	51.1 ± 1.4	102.2	2.07

n.d. = non detectable

[a]mean ± SD, n = 10

The results showed that the percentages of recovery of this proposed method were acceptable at both concentration levels in comparison with the acceptable values shown in Table 4.10. Moreover, %recovery of FI-FAAS was not significantly different in comparison with the reference method (ICP-OES) ($t_{critical}$ =2.20 > t_{exp} = 1.71-2.15, 95% of confidence level). Therefore, the proposed FI-FAAS method was able to be applied for determination of Ag(I) in real samples.

CHAPTER V

CONCLUSION

A chelating agent, benzothiazole calix[4]arene (**CU1-CHO**) was synthesized in three steps:

1) Nucleophilic substitution of calix[4]arene with bromoacetronitrile to generate compound 2,
2) Condensation reaction of compound 2 with aminothiophenol to generate **CU1**,
3) Electrophilic aromatic substitution between **CU1** and 1,1-Dichlorodimethyl ether to gernerate **CU1-CHO**.

The sorbent **(APS-CU1)** was successfully prepared in one step synthesis via Schiff's base formation between amino groups of **APS** and the carbonyl carbon atom of **CU1-CHO** by condensation reaction. The prepared sorbent was characterized by FT-IR, DR-UV-Vis and TGA.

The sorption and desorption of Ag(I) on **APS-CU1** were studied in batch and column methods:

In batch method: the suitable pH range for sorption of Ag(I) was pH 6.5-7.0. The extraction time to obtain the equilibrium of sorption was 10 min and the maximum sorption capacity was found to be 12.2 mg g^{-1} and the sorbent was quantitatively desorbed by using 0.1 mol L^{-1} thiosulfate.

In column method: the sample flow rate of 2.0 mL min^{-1} and eluent flow rate of 1.0 mL min^{-1} was found as suitable flow rate to quantitatively sorption and desorption Ag(I). The reusability of the sorbent did not significantly change within 6 cycles of sorption and desorption.

The data achieved from batch and column showed the good sorption and desorption on **APS-CU1**. Furthermore, the **APS-CU1** sorbent was used as a sorbent column in on-line flow injection pre-concentration coupled with FAAS (FI-FAAS system). The optimum conditions for determination of Ag(I) onto

APS-CU1 were investigated. The analytical performance by a proposed system were summarized in Table 5.1

Table 5.1 The analytical performance for determination Ag(I) by FI-FAAS

Features	
Sample volume	5.0 and 10.0 mL
Sample flow rate	3.0 mL min^{-1}
Eluent type	Thiosulfate 0.1 M
Eluent volume	250 µL
Eluent flow rate	1.5 mL min^{-1}
Linear ragne	10-48 µg L^{-1}
Dectection limit (LOD)	8.8 µg L^{-1}
Limit of quantitation (LOQ)	16 µg L^{-1}
Method detection limit (MDL)	0.44 µg L^{-1}
Method quantitation limit (MQL)	0.8 µg L^{-1}
Precision (%RSD, n=10)	6.1 (Ag(I) 20 µg L^{-1}
	3.3 (Ag(I) 50 µg L^{-1}
Accuracy (%recovery, n=10)	100.2 (Ag(I) 20 µg L^{-1}
	99.5 (Ag(I) 50 µg L^{-1}
Preconcentration factor	20 (sample 5.0 mL)
	40 (sample 10.0 mL)

In addition, the presence of Na^+, K^+, Ca^{2+}, Mg^{2+} as interfering ion at high concentration did not affect the extraction efficiency of Ag(I) sorption. The results indicated that **APS-CU1** has a selectivity to Ag(I).

The results also showed that the proposed FI-FAAS system could be used with good precision and accuracy. Finally, this system was successfully applied for the determination of Ag(I) in spiked drinking water and tap water.

Suggestion for future work

In order to enhance the effectiveness or obtain more convenient system, the on-line flow injection pre-concentration coupled with FAAS system used in this work should be developed to a fully automated system. Some user friendly software for data acquisition, data analysis, and instruments control should be adapted to the system for ease of use.

REFERENCES

[1] Jame, S. F. Analtical solid-phase extraction. USA : John Wiley and Sons, 1999.

[2] Flow injection analysis [Online]. Available from: http://www.scitopics.com/ Flow Injection Analysis.html [2010, March 5].

[3] Praveen, R. S., Naidu, G. R. K., and Rao, T. P. Dithiocarbamate functionlized or surface sorbed Merrifield resin beads as column materials for on line flow injection-flame atomic absorption spectrometry determination of lead. *Anal. Chim. Acta.* 2007, (600), 205.

[4] Bradl, H. B. Heavy metal in the environment. *Interface science and technology*, Vol. 6, 2005.

[5] Water Quality Standard [Online]. Pollution control Department, Ministry of Natural Resources and Environment. Available from http://www.pcd.go.th/info_serv/en_reg_std_water01.html. [2010, December 21].

[6] Herrerra, I., et al. Binding of silver(I) ions by Alfalfa biomass (Medicago Sativa): batch pH, time, temperature and ionic strength studies. *Journal of Hazardous*., 2003, (4), 1-14 .

[7] Bradl, H. B. Heavy metal in the environment. Vol.6. Germany:Elsevier, 2004.

[8] Skoog, D. A., West, D. M., Fundamentals of analytical chemistry. 8th ed, California, 2004.

[9] Yamplai, A., Chemically modified silica gel with 1,8-diaminoanthraquinone for preconcentration of heavy metal. Master’s Thesis, Department of Chemistry Faculty of Science Chulalongkorn University, 2007.

[10] Thermo Elemental [Online]. Avialable from: http://www.thermo.com/eThermo/CMA/PDFs/Articles/articlesFile_18407.pdf [2011, January 11].

[11] Camel, V. Solid phase extraction of trace elements. *Spectrochim Acta Part B: Atomic Spectroscory*. 2003, 58(7), 1177-1233.

[12] Solid phase extraction [Online]. Avialable from: http://www.google.co.th/imglanding?q=basic+step+of+solid+phase+extraction. [2011, January 5].

[13] Gary, L., and Miessler, D. A. T. Inorganic Chemistry. 3rd ed. New Jersey: Pearson Prentice Hall, PEARSON Education, 2004.

[14] Allen, L. H., Matijevic, E., and Meites, L. Exchange of Na^+ for the silanolic protons of silica. *J. Inorg. Chem*. 1971, (33), 1293-1299.

[15] Mahmoud, M. E. Comparison of metal sorption properties of three silica gel phase-physically adsorbed and chemically immobilized 1-aminothraquinone. *Anal. Lett*. 2002, (35), 1251-1267.

[16] Akl, M. A. A., Kenay, I. M. M., and Lasheen, R. R. Organically modified silica gel and flame atomic absorption spectrometry: employment for separation and preconcentration of nine trace heavy metals for their determination in netural aqueous system. *Microchem. J*. 2004, (78), 143-156.

[17] Arakaki, L. N. H., et al. New thiol adsorbent grafted on silica gel: synthesis, characterization and employment for heavy metal adsorption. *J. Environ.Monit*. 2003, (5), 366-370.

[18] Liang, X., et al. Preparation and characterization of thiol-fucntionalized silica gel and application for sorption of Pb^{2+} and Cd^{2+}. *Colloid Surf. A-Physicochem.Eng.Asp*. 2009, (349), 61-68.

[19] Silica gel structure [Online]. Available from: http://upload.wikimedia.org/wikipedia/commons/b/b/Shematic_silica_gel_ surface.png [2011, January 5].

[20] Dunce, A. F., Cheregi, M., Calatayud, J. M., Mateo, J. V. G., and Enein, H. Y. A. *Crit. Rev. Anal. Chem.* 2003, (33), 57.

[21] Fang, Z. L. Flow injection separation and preconcentration. New York: VCH, 1993.

[22] Jal, P. K., Patel, S., and Mishra, B.K. Chemical modification of silica surface by immobilization of functional group for extraction concentration of mental ions. *Talanta*. 2003, (62), 1005-1029.

[23] Ekinci, C., and Koklu, U. Determination of vanadium, manganese, silver and lead by graphite furnace atomic absorption spectrometry after preconcentration on silica gel modified with 3–aminopropyltriethoxysilane. *Spectrochim Acta Part B*. 2000, (55), 1491-1495.

[24] Afsaneh, S., Nasser, I., and Narges, S. Directly silica boned analytical reagents: synthesis of 2-mercaptobenzothiazole-silica gel and its application as anew sorbent for preconcentration and determination of silver ion using solid phase extraction method. *Sep. Purif. Technol*. 2004, (40), 303-308.

[25] Tayyebeh, M., Abbas, A., Mohammad, A. Z., and Mohammad, S. Separation, preconcentration and determination of silver ion from water sample using silica gel modified with 2,4,6-trimorpholino-1,3,5-triazin. *J. Hazard Mater B*128. 2006, 67-72.

[26] Zaporozhets, O., Petruniock, N., and Sukhan, V. Determination of silver(I), Hg(II) and Pb(lI) by using silica gel loaded with dithizone and zinc dithizone. *Talanta*. 1999, (50), 865-873.

[27] Shabani, H. A. M., Dadfarnia, S., Jafari, A. A., and Shahbasi, Z. Flame atomic absorption spectrometric determination of trace amount of silver ion in aqueous sample after solid phase extraction using octadecyl silica membrane disks modified by 2-[(2-mercaptophenyliminomrthyl]phenol. *Can. J. Anal. Sci. Spect*. 2006, 54(4), 194-199.

[28] Mojtaba, S., et al. Separation, preconcentration and determination of trace silver ion in aqueous sample after solid phase extraction using octadecyl silica membrane disks modified with some recently synthesized mixed Aza-thioethr crowns containing 1,10-phenanthrone sub-unit and atomic absorption spectrometry, *Sep. Purif. Technol.* 2002, (28), 141-147.

[29] Gholamhassan, A., Javad, Z., Mohammad, R. S., and Sara, E. Solid phase selective and extractive preconcentration of silver ion from aqueous sample on modified silica gel with 5-(4dimethylaminobenzylidene)- rhodanine. *Anal. Sci*. 2009, (25), 711716.

[30] Absalan, G.; Akhond, M.; Ghanizadeh, A.Z.; Abedi, Z.A. and Tamami, B. Benzil derivative of polyacrylohydrazide as a new sorbent for separation, preconcentration and measurement of silver(I) ion. *Sep. Purif. Technol.* 2007 (56), 231–236

[31] Shayessteh, D., Ali, M. H. S., Abbas, A. J., Zahra, S., and Fatema, T. Silver, zinc and copper determination in water and biological sample employing FI-FAAS and a micro column of a immobilized Salen 2,2´-[3 Aza- 1, 5-pentanedyle bis(nitrilomethylidyne)]-bis-phenol on surfactant coated alumina. *Can. J. Anal. Sci. Spect*. 2006, 51(6), 302-311.

[32] Valfredo, A. L., et al. Amberlite XAD-2 functionalized with Nitroso R salt: synthesis and application in an online system for preconcentration of cobalt. *Anal. Chim. Acta*. 2003, (494), 87-95.

[33] Coetzee, P. P., Taljiaard, I., and de Beer, H. On-line preconcentraion of silver on activated alumina and determination in borehole water by flow injection atomic absorption spectrophotometry. *J.Anal. Chem.* 1990, (336), 202-204.

[34] Ngeontae, W., Janrungroatsakul, W., Morakot, N., Aeungmaitrepirom, W., and Tuntulani, T., New Silver Selective Electrode Fabricated From Benzothiazole Calix[4]arene: Speciation Analysis of Silver Nanoparticles. *Sensor and Actuat B* 134. 2008, 377–385.

[35] Sdira, S. B., et al. First synthesis of upper rim mono and dinitrone calix[4]arene derivative. *Supramol. Chem*. 2007, 19 (6), 393-398.

[36] Ngeontae, W., Aeungmaitrepirom, W., and Tuntulani, T. Synthesis of dithia diaza calix[4] arene derivative and its extraction properties towards Zn^{2+}, Ni^{2+}, Cu^{2+} and Cr^{3+}. *J. Sci. Chula. Univ.* 2006, 31 (2), 101-104.

[37] Ngeontae, W., Aeungmaitrepirom, W., and Tuntulani, T. Chemically modified silica gel with aminothioamidoanthraquinone for solid phase extraction and preconcentration of Pb(II), Cu(II), Ni(II), Co(II) and Cd(II) *Talanta.* 2007, (71), 1075–1082.

[38] Mameechai, N., On-line preconcentration of lead for the determination with flame atomic absorption spectrometry, Master’s Thesis, Department of chemistry Faculty of Science Chulalongkorn University, 2009.

[39] Pavia, D. L., Lampman, G. M., and Kriz, G. S. Introduction of spectroscopy. 3rd. United States: Brooks/Cole Thomson Learning, 2001.

[40] Gajdos, P., Magdolen, P., Zahradnik, P., and Foltinova, P. New Conjugated Benzothiazole-N-oxides: Synthesis and Biological Activity. *Molecules*. 2009, (14), 5382-5388.

[41] Synthesis and Study of Silver Nanoparticles [Online]. Available from: http://www.scribd.com/doc/37679769/Synthesis-and-Study-of-Silver-Nano-Particles [2011, Januarry 2].

[42] Landau, M. V., et al. Wetting stability of Si-mcm-41 mesoporous material in nutral, acid and basic aqueous solution. *Micropor. Mesopor. Mat*. 1999, (33), 149-163.

[43] Kinniburgh, D. G. General propose adsorption isotherm. *Environ. Sci. Technol*. 1986, (20), 895-904.

[44] Igwe, J. C., and Abia, A. A. Adsorption isotherm studies of Cd(II), Pb(II) and Zn(II) ions bioremediation from aqueous solution using unmodified and EDTA-modified maize cob. *Ecletica Quimica*. 2007, (32), 33-42.

[45] Zhai, Y., Wei, X., Zeng, G., Zhang, D., and Chu, K. Studies of sorbent derived from sewage sludge for the removal of Cd2+, Ni2+ in aqueous solution. *Sep. Purif. Technol*. 2004, (38), 191-196.

[46] Kotz, J. C., Treichel, P., and Townsent J.R. Chemistry and chemical reactivity. Vol. 2, Canada: Cengage Learning.

[47] Huber, L. Validation and quantification in analytical laboratories. Illinois: Interhharm Press. 1999.

[48] Miller, J. N. and Miller, J.C. Statistic and chemometrics for analytical chemistry. 4th ed. England: Pearson Education Limited, 2000.

APPENDIX

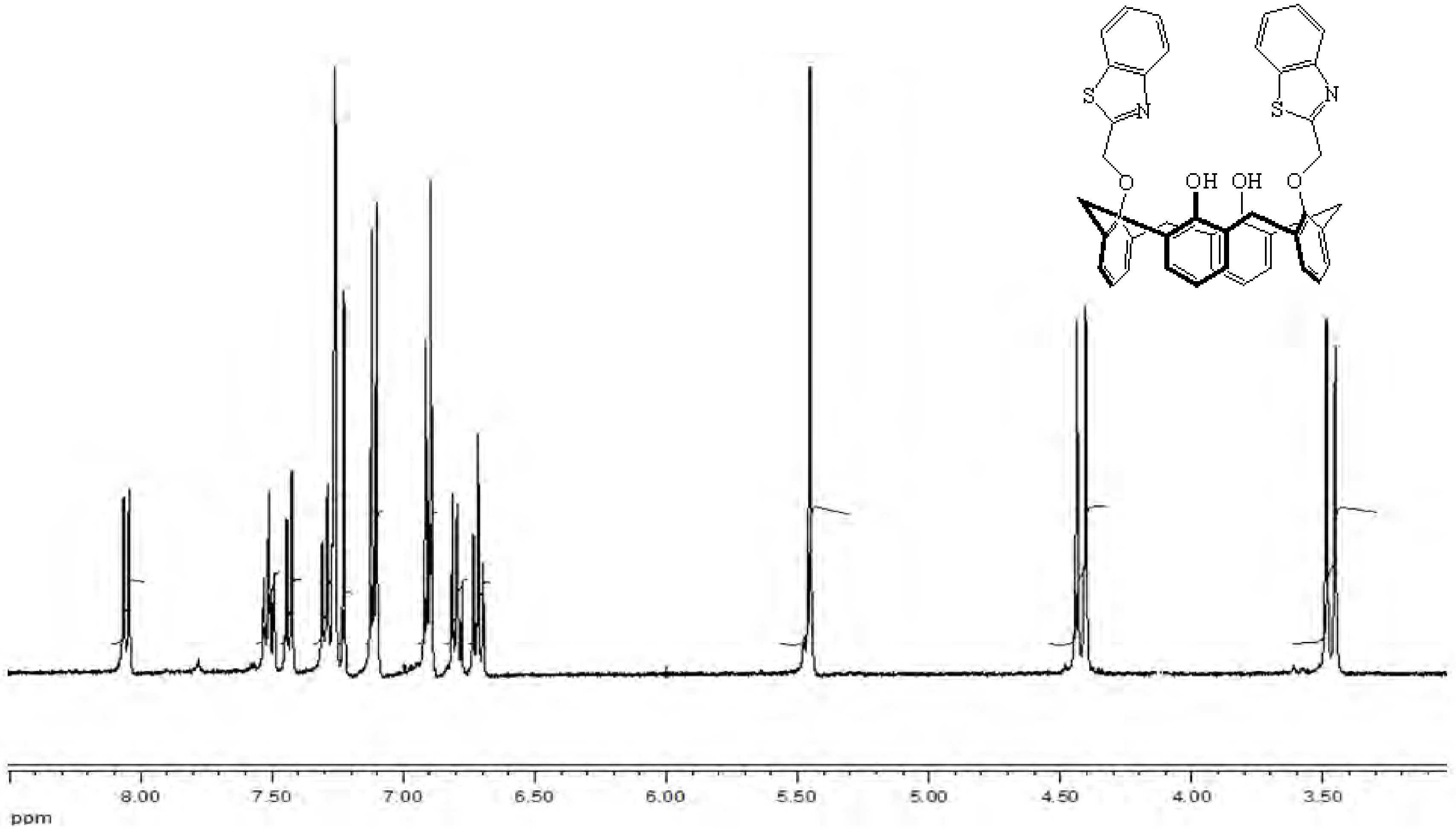


Figure A.1 ^{1}H-NMR spectrum (400 MHz,$CDCl_3$) of **CU1**

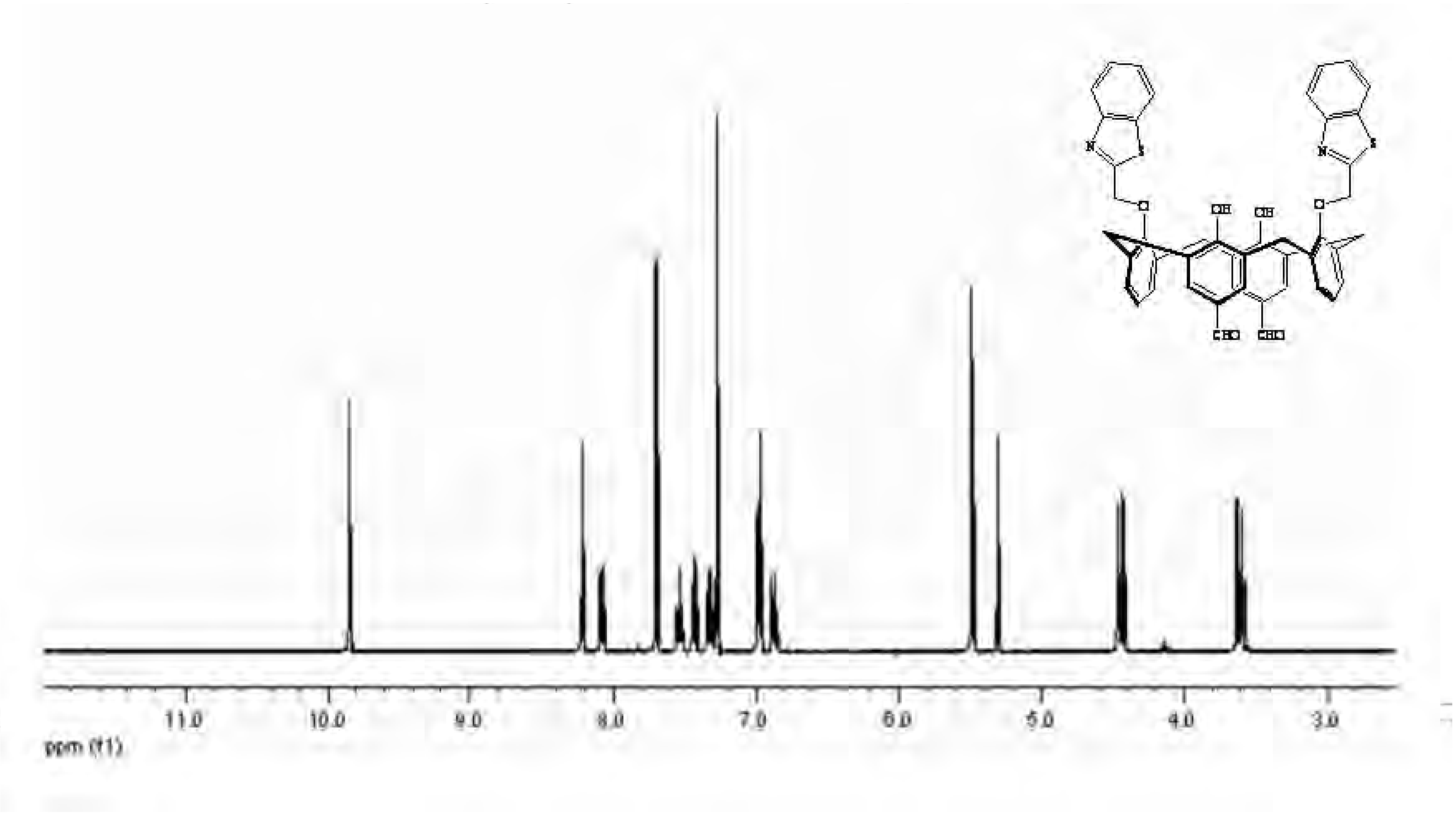


Figure A.2 ^{1}H-NMR spectrum (400 MHz,$CDCl_3$) of **CU1-CHO**

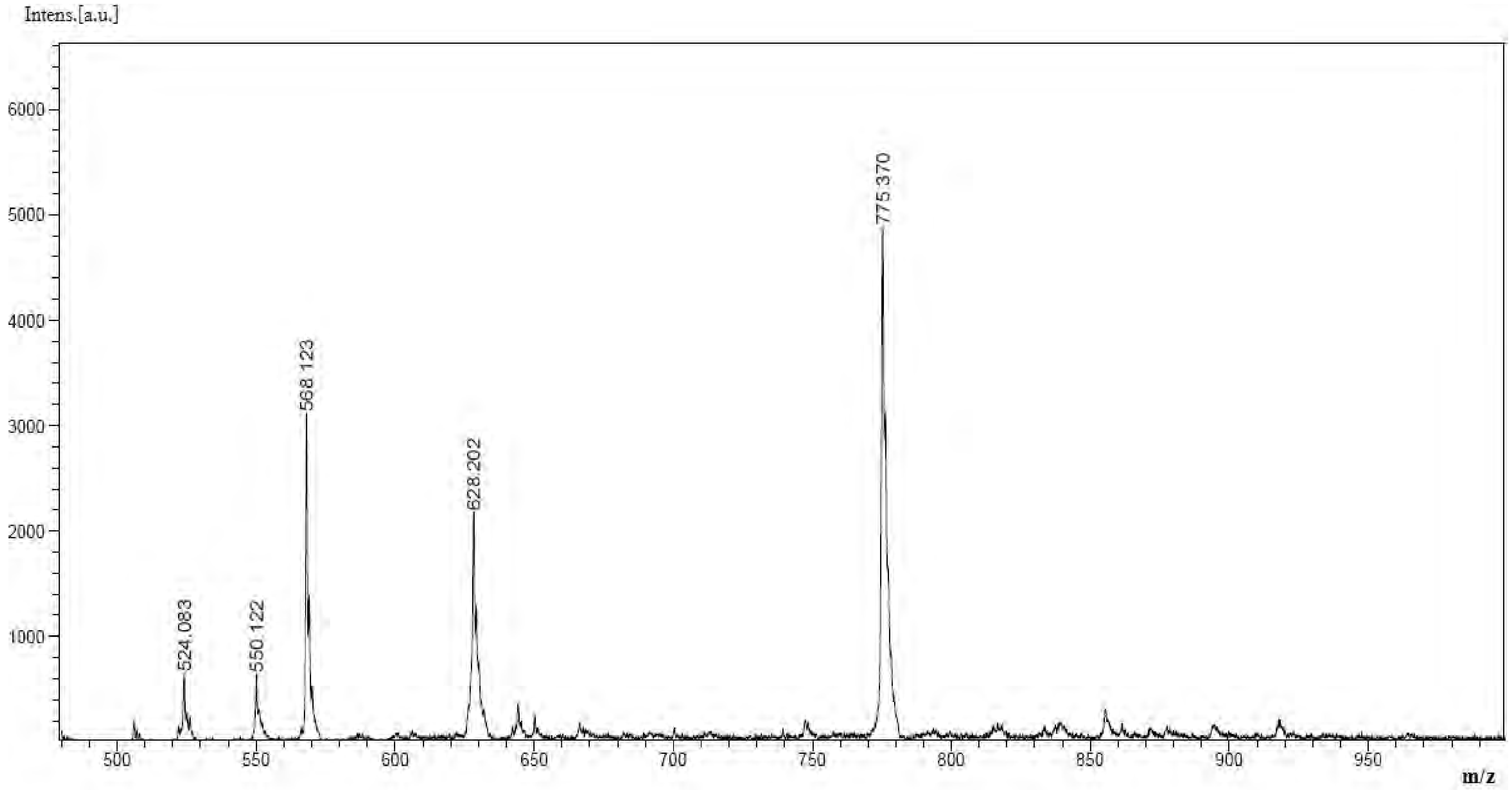


Figure A.3 Mass spectrum of **CU1-CHO**

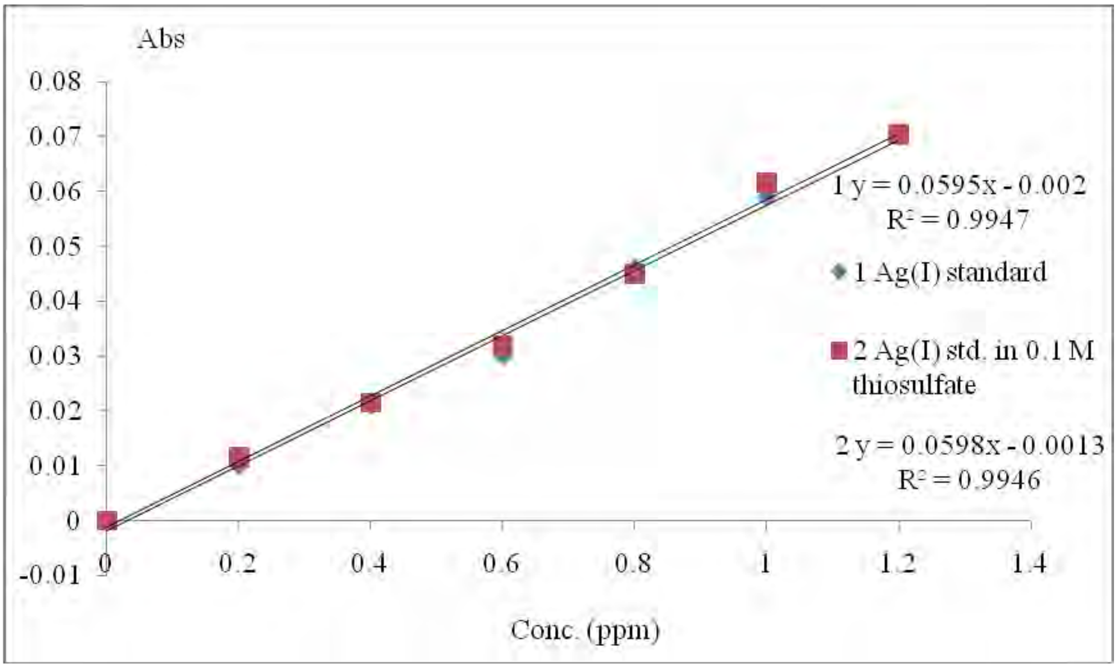


Figure A.4 Calibration curve of Ag(I) standard solution and Ag(I) standard in 0.1 M thiosulfate solution

VITA

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