การสังเคราะห์คีเลติงเรซินที่มีเบนโซไทเอโซลสำหรับการเพิ่มความเข้มข้นของไอออนโลหะที่เป็นพิษ

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SYNTHESIS OF BENZOTHIAZOLE-BASED CHELATING RESIN
FOR PRECONCENTRATION OF TOXIC METAL IONS

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สมสุภางค์ มีศรี : การสังเคราะห์คีเลติงเรซินที่มีเบนโซไทเอโซลสำหรับการเพิ่มความเข้มข้นของไอออนโลหะที่เป็นพิษ. (SYNTHESIS OF BENZOTHIAZOLE-BASED CHELATING RESIN FOR PRECONCENTRATION OF TOXIC METAL IONS) อ.ที่ปรึกษา: ผศ.ดร.อภิชาติ อิ่มยิ้ม, อ.ที่ปรึกษาร่วม: ผศ.ดร.ณรงค์ ประไพรักษ์สิทธิ์ 81 หน้า. ISBN 974-14-2880-4.

ทำการดัดแปรพื้นผิวพอลิสไตรีนไดไวนิลเบนซีน (PS-DVB) ให้มีหมู่เบนโซไทเอโซล โดยเริ่มจากการดัดแปรพื้นผิวพอลิสไตรีนไดไวนิลเบนซีนให้มีหมู่อะมิโน (amino-PS-DVB) จากนั้นนำมาทำปฏิกิริยากับสารประกอบ เอธิล 2-เบนโซไทเอโซลิลอะซิเตต (BA) ได้เรซิน ที่มีการเชื่อมต่อด้วยพันธะเอไมด์ (BA-PS-DVB) และนำ amino-PS-DVB มาทำปฏิกิริยาไดอะโซไทเซชันแล้วทำปฏิกิริยากับ BA ได้เรซินที่มีการเชื่อมต่อด้วยพันธะเอโซ (azo-BA-PS-DVB) จากนั้นตรวจสอบผลิตภัณฑ์ที่เตรียมได้ด้วยเทคนิคการวิเคราะห์ปริมาณของธาตุที่เป็นองค์ประกอบ (EA) และเทคนิคอินฟราเรดสเปกโทรสโคปี (IR) นำเรซินทั้ง 2 ชนิดที่เตรียมได้มาใช้สกัดไอออนของแคดเมียม ทองแดง และตะกั่วในน้ำ ก่อนนำมาทำการวิเคราะห์ปริมาณด้วยเทคนิคเฟลมอะตอมมิกแอบซอร์พชันสเปกโทรเมตรี (FAAS) โดยทำการศึกษาถึงภาวะที่เหมาะสมสำหรับการสกัดแบบแบทช์ ได้แก่ค่า pH ของสารละลาย ระยะเวลาที่ใช้ในการสกัด และค่าความจุการดูดซับ พบว่า pH ที่เหมาะสมสำหรับการสกัดด้วยเรซินทั้ง 2 ชนิดสำหรับไอออนของแคดเมียม ทองแดงและตะกั่ว เท่ากับ 8.0, 7.0 และ 6.0 ตามลำดับ เวลาที่ทำให้การสกัดเข้าสู่สมดุลอยู่ในช่วง 10 ถึง 20 นาที จากการศึกษาถึงพฤติกรรมการดูดซับพบว่าเป็นไปตามรูปแบบของแลงเมียร์ ในระบบคอลัมน์พบว่าอัตราการไหลผ่านคอลัมน์ของสารละลายโลหะแคดเมียม ทองแดง และตะกั่วบนเรซิน BA-PS-DVB และ เรซิน azo-BA-PS-DVB ที่เหมาะสมอยู่ ในช่วง 2.5 และ 4.0 มิลลิลิตรต่อนาที ตามลำดับ และสามารถชะโลหะที่ถูกดูดซับอยู่บนเรซินได้ด้วยกรดไนทริกเข้มข้น 0.5 ถึง 2.0 M พบว่าเรซิน azo-BA-PS-DVB มีความสามารถในการเพิ่มความเข้มข้นไอออนของแคดเมียมได้ถึง 50 เท่า และพบว่าเรซินทั้ง 2 ชนิดมีความสามารถในการเพิ่มความเข้มข้นไอออนของทองแดงได้เท่ากับ 20 และ 50 เท่าตามลำดับ วิธีการสกัดแบบคอลัมน์นี้ให้ผลที่น่าเชื่อถือ โดยให้ค่าการได้กลับคืนของไอออนของแคดเมียมและทองแดงเท่ากับ 71.2 และ 74.0% ตามลำดับ และ พบว่าเมื่อทำการทดลองซ้ำ 15 ครั้ง % RSD มีค่าต่ำกว่า 10 %

ภาควิชา..........................เคมี.......................... ลายมือชื่อนิสิต..........สมสุภางค์ มีศรี..........
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SOMSUPANG MEESRI: SYNTHESIS OF BENZOTHIAZOLE-BASED CHELATING RESIN FOR PRECONCENTRATION OF TOXIC METAL IONS. THESIS ADVISOR: ASSIST.PROF.APICHAT IMYIM, Ph.D., THESIS COADVISER: ASSIST.PROF NARONG PRAPHAIRAKSIT, Ph.D., 81 pp. ISBN 974-14-2880-4.

Polystyrene-divinylbenzene resin (PS-DVB) was functionalized with a benzothiazole group on the surface. PS-DVB with amino group was initially prepared by nitration and reduction reactions and subsequently treated with ethyl 2-benzothiazolyacetate (BA) to obtain the chelating resin with an amide linkage (BA-PS-DVB). On the other hand, the amino-PS-DVB was diazotized and coupled with BA to get the chelating resin with an azo linkage (azo-BA-PS-DVB). The resins were characterized by elemental analysis (EA) and infrared spectroscopy and then evaluated for its extraction of Cd(II), Cu(II) and Pb(II) ions in water before their determinations by flame atomic absorption spectrometry (FAAS). Extraction conditions were optimized for batch method such as pH of solution, extraction time, and adsorption isotherm. The optimum pH for the extraction of Cd(II), Cu(II) and Pb(II) are 8.0, 7.0 and 6.0, respectively while the equilibrium time of all three ions were reached within 10-20 minutes. Adsorption behavior of all metal ions followed Langmuir adsorption isotherm. In column method, the optimum flow rates of metal sorption onto BA-PS-DVB and azo-BA-PS-DVB column were 2.5 and 4.0 mL min^{-1}. Metal ions sorped onto columns were eluted by 0.5 to 2.0 M HNO_3. The preconcentration factors of Cd(II) and Cu(II) on azo-BA-PS-DVB and Cu(II) on BA-PS-DVB were 50, 50, and 20, respectively. The present column method gave good validation results: 71.2 and 74.0 %recovery for Cd(II) and Cu(II) and overall %RSD less than 10% (n=15).

Department………………… Chemistry……………. Student's signature….Somsupang Meesri…..
Field of study……………..... Chemistry……………. Advisor's signature………………….
Academic year …………………2005…………….…. Co-advisor's signature………………...

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

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LIST OF SYMBOLS AND ABBREVIATIONS

FAAS	Flame atomic absorption spectrometry
EA	Elemental analysis
FTIR	Fourier transforms infrared spectrometry
ATR-FTIR	Attenuated total reflectance fourier transforms infrared spectrometry
NMR	Nuclear magnetic resonance spectrometry
ICP-OES	Inductively coupled plasma optically emission spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
SPE	Solid-phase extraction
LLE	Liquid-liquid extraction
BA	Ethyl 2-benzothiazolyacetate
PS-DVB	Polystyrene-divinylbenzene
RSD	Relative standard deviation
LOD	Limit of detection
LOQ	Limit of quantitation
IDL	Instrument detection limit
MDL	Method detection limit

CHAPTER I

INTRODUCTION

1.1 Statement of the problem

The determination of metal ions at trace level is very important in context of environmental protection, food and agricultural chemistry as well as high purity materials. However, the direct determination of metal ions at trace levels is limited due to their low concentrations and matrix interferences. In trace analysis, therefore, preconcentration and separation of trace elements from the matrix is frequently necessary to improve the selectivity and detection limit of their determination. For this purpose, several methods have been proposed and used for preconcentration and separation of trace elements according to the nature of the samples, the concentration of the analytes and the measurement techniques. These methods include ion-exchange, co-precipitation, solvent extraction and chemical and bio-sorption.

Of all the preconcentration methods, solid-phase extraction (SPE) is one of the most effective multielement preconcentration methods because it can provide more flexible working conditions together with good stability, selectivity, high concentrating ability, high capacity for metal ions and simple operation [1]. In addition, SPE reduce solvent use, solvent exposure, disposal cost, and extraction time for sample preparation. Consequently, demands for trace element determination and concentration using these modified resins are ever increasing.

The most widely used polymeric sorbents are the polystyrene-divinylbenzene (PS-DVB) copolymers, which have a hydrophobic surface and overcome many of the limitations of bonded silicas, especially those related to the limited pH stability or the presence of the silanol groups. In general, PS-DVB resins have greater analyte retention, mainly for polar compounds, than bonded silicas, because their hydrophocic surface contains a relatively large number of active aromatic sites that allow π-π interactions. Nevertheless, sorbents based on PS-DVB have some drawbacks, such as their lack of selectivity and low breakthrough volumes for highly polar compounds, which leads to their incomplete extraction from predominantly aqueous samples. These drawbacks can be largely overcome by using modified resins obtained by attaching polar groups, such as acetyl, hydroxymethyl and benzoyl derivative, to the aromatic ring on the PS-DVB [2].

In this research, the modification of PS-DVB by functionalization with a benzothiazole group is excellent because of its strong affinity for transition metal ions and no leaching ligand was found during the operation.

1.2 Objectives

This research focused on developing a method for determination of trace levels of Cd(II), Cu(II) and Pb(II) in aqueous solution using a new chelating resin as adsorbent in solid-phase extraction (SPE) technique followed by the determination by flame atomic absorption spectrometry (FAAS)

1.3 Scope of the research

Initially, a new benzothiazole containing chelating ligand was synthesized and coupled to polymeric sorbent through an amide and azo linkage. Then the optimization for extraction and preconcentration characteristics of Cd(II), Cu(II) and Pb(II) ions in water using batch and column methods were carried out followed by the determination of metal ions by flame atomic absorption spectrometry (FAAS).

Various parameters influencing the extraction of this resin such as pH of solution, extraction time, concentration of eluent, amount of sorbent and sorption capacity as well as the effect of interference ions such as Na^+, Ca^{2+}, Mg^{2+}, and Cl^- were studied using batch method.

Consequently, the optimum condition from the batch method was employed for a preconcentration of metal ions in water samples using column method. The parameters e.g. concentration of eluent, flow rate and sample volume were investigated. Finally, the optimum method for preconcentration of metals in aqueous solution was validated and applied to the determination of metals in water samples.

1.4 The benefits of this research

In this work, we have combined the advantages of high porosity and ease of surface modification of PS-DVB together with benzothiazole derivative for the extraction and preconcentration of Cd(II), Cu(II) and Pb(II) in aqueous solution. The resin could be reused after regenerating with acid solution.

CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 Heavy metals in the environment

Metals are described as “heavy metals” when they, in their standard state, have a specific gravity (density) of more than 5 g cm^{-3} [1]. Some of them, such as copper, nickel, chromium and iron, are essential in very low concentrations for the survival of all forms of life. These are described as essential trace elements. Only when they are present in greater quantities, can these, like the heavy metal lead, cadmium and mercury which are already toxic in very low concentrations, cause metabolic anomalies. Here, the boundary between the essential and the toxic effect is somewhat problematic. In addition to its natural occurrence, heavy metals are normally brought into the environment by human activities, which has dramatically influenced and modified natural cycles. The major activities include mining and ore processing, coal and fuel combustion, industrial processing, agricultural, domestic, transportation and nuclear activities.

Heavy metals show a great tendency to from complexes, especially with nitrogen, sulphur and oxygen containing ligands of the biological milieu [3]. Chronic toxicity, caused by repeated exposure over long periods of time, is much more relevant. Besides the fact that mercury, cadmium and arsenic are highly toxic, some other heavy metals such as iron, copper, zinc, manganese, cobalt, nickel, tin and selenium are essential to many organisms. An undersupply of these, so called trace metals, leads to deficiency, while an oversupply results in toxic effects.

In accordance with the toxicity data and scientific studies, standard and guidelines have been proposed by various governments and institutions. List of maximum levels recommended for contaminants by the Joint FAO/WHO Codex Alimentarius Commission, third series are shown in Table 2.1.

Table 2.1 List of maximum levels of heavy metal contaminants at human tolerance [4]

Contaminant	Maximum level of human intake/day (mg of contaminant / kg of body weight)
Arsenic	0.05
Cadmium	0.0067-0.0083
Copper	0.5
Lead	0.05
Mercury (total)	0.005
Methyl mercury	0.0033

2.2 Background information of metals [1,3]

2.2.1 Cadmium

Cadmium is an extremely toxic and very hazardous heavy metal. Kidney damage and when present at high level, bone damage, are the main effects. Inhalation of its vapors can lead to lung damage.

Cadmium also is a frequent material in industrial waste discharges and has been introduced into the water systems largely through mining operations. It has also been employed in metal planting, where it is used as a sacrificial coating to prevent rust on steel. Some applications of the element are summarized in Table 2.2.

Table 2.2 Major uses of Cadmium

Industry	Applications
Batteries	Rechargeable NiCd batteries use about 70% of Cd production
Metal plating	For corrosion resistance, especially marine and aerospace applications
Plastic stabilizer	Used in poly(vinyl chloride) to protect against sunlight and heat degradation

2.2.2 Copper

Copper is an essential metal for many organisms, including humans. It is used in enzymes that modify redox reactions and in some oxygen-carrying systems. Its function in these is associated frequently with its ability to exist as both Cu(I) and Cu(II). The most common state is Cu(II). Like many essential metal, large amounts are toxic.

2.2.3 Lead

The toxicity of lead in the environment has caused extensive concern in recent years. Pb(II) forms comparatively covalent bonds with some appropriate donor groups in complexes, generally favoring sulfur and nitrogen over oxygen donors, and it may own some of its physiological action to replacement of other metals in some enzymes. Low levels have subtle effects on the nervous system, while higher levels can lead to many symptoms, such as severe effects on the nervous system, including loss of sight and hearing, as well as symptoms of gout, headache, insomnia, anemia, kidney damage, diarrhea, stomach pains, intestinal paralysis and eventually death.

Various technological sources of lead (Table 2.3) can serve as origins of ingested lead in humans and consequent lead poisoning. Like other heavy metals strongly bound by biological complexing agents, lead is cumulative poison and can act through long-term ingestion of relatively small quantities. Lead can be retained in the body for long periods, especially in bones, where it can replace some of the calcium. Table 2.3 shows some applications of lead.

Table 2.3 Major uses of Lead

As metal	
Batteries	Most widely used in lead-acid storage batteries
Water pipes	No longer used; some may still exist in old construction
As inorganic lead	
Paints	White pigment and base, also colored pigments; now removed from indoor paints
Stabilizer in plastic	Heat stabilizer in poly(vinyl chloride)
As organic lead	
Gasoline additives	Generally phased out except in undeveloped nations

2.3 Determination of metals

Selection of determinative technique is often dictated by equipment and concentration level of metals in samples. Ease of operation, number of analyses, and familiarity of personnel with techniques are also influential factors. Spectrometric techniques commonly used include flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption spectrometry (GFAAS) or electro thermal vaporization atomic absorption spectrometry (ETAAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS) and X-ray fluorescence spectrometry (XRF).

FAAS is often used to determine many elements in samples because of the ease and affordability. However, detection limits of FAAS are relatively high for some elements. GFAAS is the method of choice when sensitivity is the major concern. Nevertheless, this technique is very sensitive to variations in the composition of matrix. Therefore, removal of the matrix components and preconcentration of the analyte are often necessary.

ICP-AES has also been used for routine elemental analysis. Sequential or simultaneous mode ICP-AES allows rapid analysis, dramatically improving throughput. Detection limit in ICP-AES are generally slightly better than FAAS.

ICP-MS has been used for the determination of metals. Its most effective and very fast response time, exceptional detection limit: 0.1-10 ppt, high resolution and high sensitivity have made this technique ideal but the cost of the required instrumentation may be prohibitive to many laboratories.

In Table 2.4, the comparison of detection limits of some metals by using various techniques is summarized.

Table 2.4 Detection limits (ng/mL)[a] for some elements by atomic spectroscopy techniques [5]

Element	**Flame AA**	**Electrothermal AA**[b]	**Flame Emission**	**ICP Emission**	**ICP-MS**
Cd	1	0.02	2000	0.07	0.003
Cu	2	0.1	10	0.04	0.003
Pb	5	0.2	200	1	0.007

[a] The detection limits are calculated at 3σ. The detection limits listed are representative of the techniques and do not represent the performance of particular instrument. All ICP-MS performance; lower levels of detection are available in magnetic sector ICP-MS systems.

[b] Based on 10 μL sample

2.4 Preconcentration of trace elements

Various techniques have been applied to the determination of trace heavy metals in biological samples. Direct instrumental analysis of these samples is difficult because of many complexes and large matrices, which always affect the capability of the instrument to obtain the correct results in normal operation [6]. In addition to the interference of matrices in the analysis, some metals have concentration in the mg L^{-1} or μg L^{-1} range, which are near or below the limit of detection of the instrument. The preconcentration step can solve the problems and lead to easy determination. There are various methods of preconcentration, including co-precipitation, solvent extraction and solid-phase extraction.

2.5 Sorption

To understand any of solid-phase extraction techniques, it is first necessary to understand the physical-chemical processes of sorption [7]. Schwarzencach *et al*. [8] make the distinction between ***absorption*** meaning into a three-dimension matrix, like water uptake in a sponge, and ***adsorption*** as meaning onto a two-dimensional surface. Absorption, also referred to as a ***partitioning*,** occurs when analytes pass into the bulk of the extracting phase and are retained. Adsorption is the attraction of an analyte to a solid that results in accumulation of the analyte's concentration at porous surfaces of the solid. Absorption results from weaker interactive forces than adsorption. Because adsorption and/or absorption processes are sometimes difficult to distinguish experimentally and often occur simultaneously, the general term ***sorption*** will be used here when referring to these processes. The term ***sorbent*** will refer to the solid extracting phase, including certain solid-supported liquid phases. To predict and optimize extraction, it is important for the analyst to be aware of the nature of the sorbent used.

Although different processes may dominate in different situations, it can be assumed that multiple steps occur during sorption of an organic compound from liquids "into" or "onto" a solid phase. Any of the steps may become a rate-limiting process in controlling sorption of an analyte. The analyte may interact with a solid-phase sorbent in at least four ways:

1. Through absorption, the analyte may interact with the sorbent by penetrating its three-dimensional structure; similar to water being absorbed by a sponge. Three-dimensional penetration into the sorbent is a particularly dominating process for solid-supported liquid phases. In the absorption process, analytes do not compete for sites; therefore, absorbents can have a high capacity for the analyte.

2. The analyte may interact two-dimensionally with the sorbent surface through adsorption due to the intermolecular forces such as van der Waals or dipole-dipole interactions. Surface interactions may result in displacement of water or other solvent molecules by the analyte. In the adsorption process, analytes may compete for sites; therefore, adsorbents have limited capacity. Three steps occur during the adsorption process on porous sorbents: ***film diffusion*** (when the analyte passes through a surface film to the solid-phase surface), ***pore diffusion*** (when the analyte passes through pores of the solid-phase), and ***adsorptive reaction*** (when the analyte ***binds, associates*** or ***interacts*** with the sorbent surface).

3. If the compound is ionogenic (or ionizable) in aqueous solution, there may be an electrostatic attraction between the analyte and charge sites on the sorbent surface. Sorbents specifically designed to exploit these types of ionic interactions are referred to as ***ion-exchange*** (either anion or cation-exchange) ***sorbents***.

4. Finally, it is possible that the analyte and the sorbent may be chemically reactive toward each other such that the analyte becomes covalently bonded to the solid-phase sorbent. This type of sorption is generally detrimental to analytical recovery and may lead to slow or reduced recovery, also termed ***biphasic desorption***. All of these interactions have the potential of operating simultaneously during sorption.

2.6 Solid-phase extraction

2.6.1 What is solid-phase extraction? [9-10]

Solid-phase extraction (SPE) is a method of sample preparation that concentrates and purifies analytes from solution by sorption onto a disposable solid-phase cartridge, followed by the elution of the analyte with an appropriate solvent for instrumental analysis. The mechanisms of retention include reversed phase, normal phase, and ion-exchange. Traditionally, sample preparation consisted of sample dissolution, purification, and extraction that were carried out with liquid-liquid extraction (LLE). The disadvantages with liquid-liquid extraction include the use of large volume of organic solvent, cumbersome glassware, and cost. Furthermore, liquid-liquid extraction often creates emulsions with aqueous samples that are difficult to extract, and therefore, is not easily automated. These difficulties can be overcome with solid-phase extraction.

Initially, SPE was based on the use of polymeric sorbent, such as XAD resins (polymeric adsorbents), which were packed in small disposable columns for use on a drug analysis. The early environmental applications consisted of both XAD resins and bonded-phase sorbent, such as C-18 [7]. These pre-columns were used for sample trace enrichment prior to liquid chromatography and were often done on-line, which means at the same times as liquid chromatography. However, these first, steel, on-line pre-columns were quickly were replaced with an off-line column made of plastic in order to be both inexpensive and disposable. Eventually, the term solid-phase extraction was coined for these low-pressure extraction columns. Thus, solid-phase extraction is an analogous term to liquid-liquid extraction, and in fact, solid-phase extraction might also be called liquid-solid extraction. However, it is the term solid-phase extraction or the acronym SPE that has become the common name for this procedure.

2.6.2 Basic principles [11]

An SPE method always consists of three to four successive steps, as illustrated in Figure 2.1. First, the solid sorbent should be conditioned using an appropriate solvent, followed by the same solvent as the sample solvent. This step is crucial, as it enables the wetting of the packing material and the solvation of functional groups. In addition, it removes possible impurities initially contained in the sorbent or the packing. Also, this step removes the air present in the column and fills the void volume with solvent. The nature of the conditioning solvent depends on the nature of the solid sorbent.

The second step is the percolation of the sample through the solid sorbent. Depending on the system used, volumes can range from 1 mL to 1 L. The sample may be applied to the column by gravity, pumping, aspirated by vacuum or by an automated system. The sample flow rate through the sorbent should be low enough to enable efficient retention of the analytes, and high enough to avoid excessive duration. During this step, the analytes are concentrated on the sorbent. Even though matrix components may also be retained by the solid sorbent, some of them pass through, thus enabling some purification (matrix separation) of the sample.

The third step, which is optional, is the washing of the solid sorbent with an appropriate solvent, having low elution strength, to eliminate matrix components that have been retained by the solid sorbent, without displacing the analytes. A drying step may also be advisable, especially for aqueous matrices, to remove traces of water from the solid sorbent. This will eliminate the presence of water in the final extract, which, in some cases, may hinder the subsequent concentration of the extract and/or the analysis.

The final step consists of the elution of the analytes of interest by an appropriate solvent, without removing retained matrix components. The solvent volume should be adjusted so that quantitative recovery of the analytes is achieved with subsequent low dilution. In addition, the flow rate should be correctly adjusted to ensure efficient elution.

It is often recommended that the solvent volume be fractionated into two aliquots, and before the elution to let the solvent soak the solid sorbent.

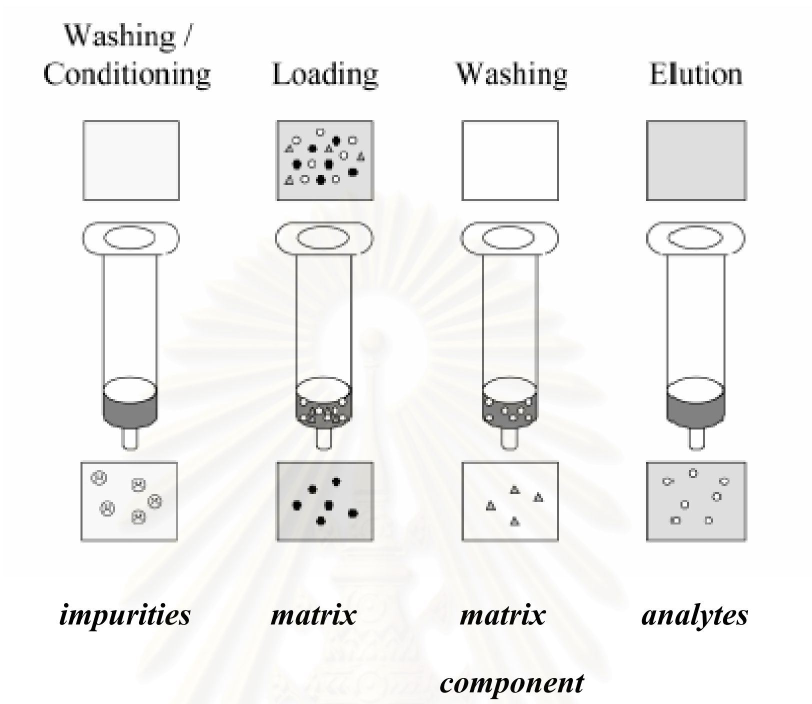

Figure 2.1 SPE operation steps

V. Camel / Spectrochimica Acta Part B 58 (2003) 1177-1233.

2.6.3 Retention of trace elements on the sorbent

Adsorption of trace elements on the solid sorbent is required for preconcentration as illustrated in Figure 2.2. The mechanism of retention depends on the nature of the sorbent, and many include simple adsorption, chelation or ion-exchange. Also, for trace elements, ion-pair solid-phase extraction may be used.

Adsorption: Trace elements are usually adsorbed on a solid-phase through van der Waals forces or hydrophobic interaction. Hydrophobic interaction occurs when the solid sorbent is highly non-polar (reversed phase). The most common sorbent of this type is octadecyl-bonded silica (C_{18}-silica). More recently, reversed polymeric phases have emerged, especially the styrene-divinylbenzene copolymer that provides additional π-π interaction when π-electrons are present in the analyte. However, because most trace element species are ionic, they will not be retained by such sorbents.

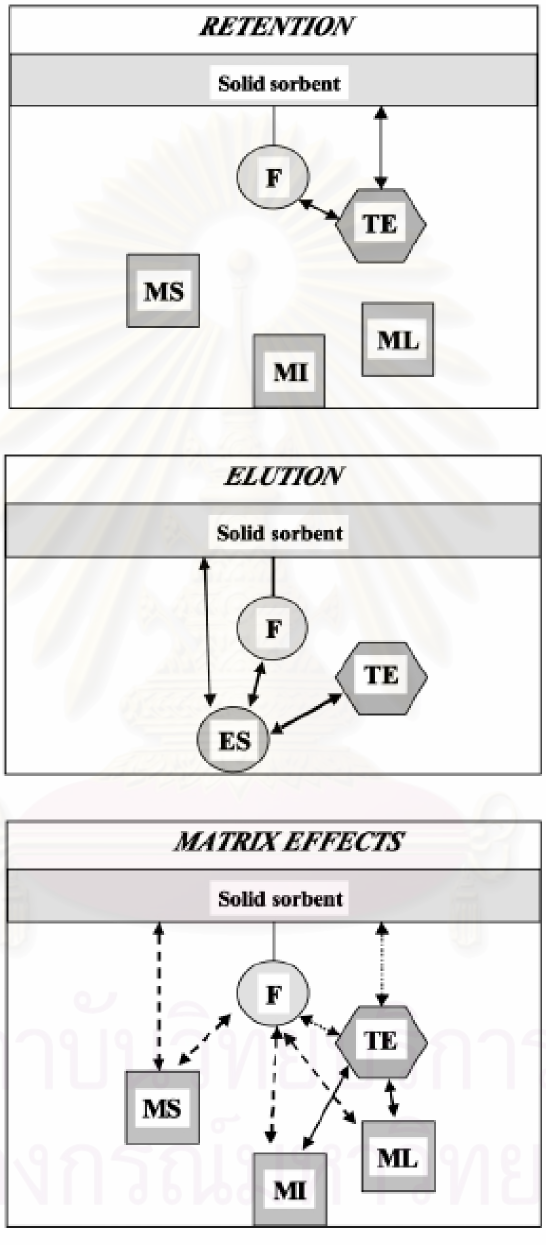

Figure 2.2 Interactions occurring at the surface of the solid sorbent. F= functional group; TE= trace element; MS= matrix solvent; MI= matrix ions; ES= elution solvent.

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Chelation: Several functional group atoms are capable of chelating trace elements. The atoms most frequently used are nitrogen (e.g. N present in amines, azo groups, amides, nitriles), oxygen (e.g. O present in carboxylic, hydroxyl, phenolic, ether, carbonyl, phosphoryl groups) and sulfur (e.g. S present in thiols, thiocarbamates, thioethers). The nature of functional group will give an idea of the selectivity of the ligand towards trace elements. In practice, inorganic cations may be divided into 3 groups:

group I-'hard' cations; these ions preferentially react via electrostatic interactions (due to gain in entropy caused by changes in orientation of hydration water molecules); this group includes alkaline and alkaline-earth metals (Ca^{2+}, Mg^{2+}, Na^{+}) that form rather weak outer-sphere complexes with only hard oxygen ligands.

group II-'borderline' cations; these ions have an intermediate character; this group contains Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Mn^{2+}. They possess affinity for both hard and soft ligands.

group III-'soft' cations; these ions tend to form covalent bonds, such as Ag^{+}, Pd^{2+}, Cd^{2+}, Pt^{2+} and Hg^{2+}. Hence, Cd^{2+} and Hg^{2+} possess strong affinity for intermediate (N) and soft (S) ligands.

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

Chelating agents may be directly added to the sample for chelating trace elements, the chelates being further retained on an appropriate sorbent. An alternative is to introduce the functional chelating group into the sorbent. For that purpose, three different means are available: (1) the synthesis of new sorbents containing such groups (*new sorbents*); (2) the chemical bonding of such groups on existing sorbents (*functionalized sorbents*); (3) the physical binding of the groups on the sorbent by impregnating the solid matrix with a solution containing the chelating ligand (*impregnated, coated or loaded sorbents*). The latter remains the most simple to be used in practice. Its main drawback is the possible flush of the chelating agent out of the solid sorbent during sample percolation or elution that reduces the life time of the impregnated sorbent.

Binding of metal ions to the chelate functionality is dependent on several factors: (1) nature, charge and size of the metal ion; (2) nature of the donor atoms present in the ligand; (3) buffering conditions which favor certain metal extraction and binding to active donor or groups; and (4) nature of the solid support (e.g. degree of cross-linkage for a polymer)

Ion-pairing: When a non-polar sorbent is to be used, an ion-pair reagent can be added to the sorbent. Such reagents contain a non-polar portion (such as a long aliphatic hydrocarbonate chain) and a polar portion (such as an acid or base). The non-polar portion interacts with the reversed-phased non-polar sorbent, while the polar portion forms an ion-pair with the ionic species present in the matrix (that could be either free metallic species in solution or complexes).

Ion-exchange: Ion-exchange sorbents usually contain cationic or anionic functional groups that can exchange the associated counter-ion. Strong and weak sites refer to the fact that strong sites are always present as ion-exchange sites at any pH, while weak sites are only ion-exchange sites at pH values greater or less than the pK_a. Strong sites are sulfonic acid groups (cation-exchange) and quaternary amines (anion-exchange), while weak sites consist of carboxylic acid groups (cation-exchange) or primary, secondary and tertiary amines (anion-exchange). These groups can be chemically bound to silica gel or polymers (usually a styrene-divinylbenzene copolymer), the latter allowing a wider pH range.

An ion-exchanger may be characterized by its capacity, resulting from the effective number of functional active groups per unit mass of the material. The theoretical value depends upon the nature of the material and the form of the resin. However, in the column operation mode, the operational capacity is usually lower than the theoretical one, as it depends on several experimental factors, such as flow-rate, temperature, particle size and concentration of the feed solution. As a matter of fact, retention on ion-exchangers depends on the distribution ratio of the ion on the resin, the stability constants of the complexes in solution, the exchange kinetics and the presence of other competing ions. Even though ion-exchangers recover hydrate ions, charged complexes and ions complexed by labile ligands, they are of limited use in practice for preconcentration of trace elements due to their lack of selectivity and their retention of major ions.

2.6.4 Elution of trace elements from sorbent

The same kind of interactions usually occurs during the elution step. This time, the type of solvent must be correctly chosen to ensure stronger affinity of the trace element for the solvent, to ensure disruption of its interaction with the sorbent (as illustrated in Figure 2.2). Thus, if retention on the sorbent is due to chelation, the solvent could contain a chelating reagent that rapidly forms a stronger complex with the trace metal. Elution may also be achieved using an acid that will disrupt the chelate and displace the free trace element. Similarly, if retention is due to ion-exchange, its pH dependence enables the use of eluents with different pH to be used, such as acids.

Of prime importance is to selectively elute only the target species. So, if they are more strongly retained on the sorbent than the interferent compounds, a washing step with a solvent of moderate elution strength is highly advisable before elution of the target species with the appropriate solvent.

2.6.5 Step-by-step method development guide

Development of an SPE method can be considered as two-step procedure. First, the most appropriate sorbent for the application should be chosen. Optimization of the most influential parameters should then be undertaken. Obviously, optimization should initially be performed using spiked synthetic solutions, but it must be followed by the use of certified reference materials or spiked real samples, as matrix components (such as ligands or other ions) may change the trace element retention on the sorbent, thereby decreasing recoveries of the target species.

2.6.5.1 Selection of solid sorbent

The nature and properties of the sorbent are of prime importance for effective retention of metallic species. Careful choice of the sorbent is thus crucial to development of SPE methodology. In practice, the main requirements for a solid sorbent are; (1) the possibility to extract a large number of trace elements over a wide pH range (along with selectivity towards major ions); (2) the fast and quantitative sorption and elution; (3) a high capacity; (4) regenerability; and (5) accessibility. In particular, sorbents that allow fast reaction rates are preferred to achieve faster extraction as well as higher loading capacities. Hence, sorbents based on hydrophilic macroporous polymers and cellulose or on fibrous materials provide excellent kinetic properties. [12]

The broad variety of sorbents available explains one of the most powerful aspects of SPE, which is selectivity. Sorbents can be mainly categorized as inorganic based ones (silica gel SiO_2, alumina Al_2O_3, magnesia MgO and other oxide species) and organic based ones (natural polymers, as well as synthetic polymers).

2.6.5.1.1) Inorganic based sorbents

Inorganic based sorbents are mainly made of silica gel even though other *inorganic oxides* may be used.

a) Silica gel. Silica gel based sorbents present the advantages of mechanical, thermal and chemical stability under various conditions. They frequently offer a high selectivity towards a given metal ion. However, all silica based sorbents suffer from different chemical limitations, namely the presence of residual surface silanol groups (even after an end-capping treatment) and a narrow pH stability range.

b) Other inorganic oxides. Adsorption on inorganic oxides may also be influenced by the presence of salts in the matrix. In particular, high concentrations of phosphates and sulfates may decrease trace element retention on titania (TiO_2). On the opposite, major cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) are weakly adsorbed on titania.

2.6.5.1.2) Organic based sorbents

Organic based sorbents may be divided into polymeric and non-polymeric sorbents. Polymeric sorbents have been, by far, the most used of trace element preconcentration having the advantage over boned silica in that they can be used over the entire pH range.

a) *Polystyrene-divinylbenzene based sorbents.* Macroporous hydrophobic resins of the Amberlite XAD series are good supports for developing chelating matrices. Amberlite XAD-1, XAD-2, XAD-4, and XAD-16, are polystyrene-divinylbenzene (PS-DVB) resins with a highly hydrophobic character and no ion-exchange capacity. In addition to the hydrophobic interaction that also occurs with C_{18}-silica, such sorbents allow π-π interactions with aromatic analytes.

Due to the hydrophobic character of PS-DVB, retention of trace elements on such sorbents requires the addition of a ligand to the sample.

b) Divinylbenzene-vinylpyrrolidone copolymers. Sorbents made of divinylbenzene-vinylpyrrolidone (DVB-VP) copolymers has been successfully applied to the determination of polar organic compounds in water samples. It is more convenient to used, compared to classical sorbents, as it can dry out during the extraction procedure without reducing its ability to retain analytes. In addition, it is stable over the entire pH range. However, until now, no application related to the preconcentration of trace elements has been reported.

c) Carbon sorbents. Due to their large surface areas (300-1000 m^2/g), these sorbents are well-recognized for their very strong sorption both for trace organic compounds and trace elements. Metal chelates may also be retained on this sorbent after addition of a proper chelating agent to the sample such as amino acids, dithizone. The ligand should be chosen to avoid a strong interaction with the activated carbon otherwise complete dissociation of the metal chelate.

The main drawback when using activated carbon is their heterogeneous surface with active functional groups that often leads to low reproducibility. In addition, these sorbents are very reactive and can act as catalysts for oxidation and other chemical reactions.

2.6.5.2 Influential parameters

The main experiment variables that affect analyte recovery by SPE have been extensively. There are three main steps for SPE application, conditioning, loading, and elution. In each step, many parameters influence the efficiency of the SPE.

2.6.5.2.1) Conditioning parameters

a) Washing step: A washing step is highly recommended, especially when ultra traces of elements are determined.

b) Conditioning solvent: The nature of the conditioning solvent must be appropriate to the nature of the solid sorbent to ensure a good wettability of the functional groups. The sorbent should further be conditioned by a solvent whose nature is similar to that of the sample. Thus, for aqueous samples, the solvent will be water with a pH and ionic strength similar to that of the sample.

2.6.5.2.2) Loading parameters

a) Sample volume to be percolated: An important parameter to control in SPE is the breakthrough volume, which is the maximum sample volume that should be percolated through a given mass of sorbent after which analytes start to elute from the sorbent resulting in non-quantitative recoveries.

b) Sample flow rate: The sample flow rate should be optimized to ensure the quantitative retention along with minimization of the time required for sample processing.

c) Sample pH: The sample pH is of prime importance for efficient retention of the trace elements on the sorbents. Its influence strongly depends on the nature of the sorbent used. When the retention of trace elements is based on chelation (either in the sample or on the solid sorbent), the sample pH is also a very important factor as most chelating ligands are conjugated bases of weak acid groups and accordingly, they have a very strong affinity for hydrogen ions.

d) Sample matrix: The presence of existing ligands in the sample matrix may affect trace element retention when stable complexes are formed in the sample with these ligands, as trace elements are less available for further retention. Thus, if metals are present in the sample as strong complexes, they may not dissociate, there is no retention of the free metal on the sorbent. For example, the reduction in the retention of Cu(II) on Amberlite CG50 occurs in the presence of ligand such as glycine. The presence of ions other than the target ones in the sample may also cause problems during the SPE step. In particular, due to their usually high levels; they may hinder the preconcentration step by overloading the sorbent or cause interferences during spectrophotometric analysis. Therefore, their influence should be studied before validating a SPE method. Sometimes the addition of a proper masking agent (such as EDTA or thiourea for example) may prevent the effect of interferences due to ions in the sample. Finally, the ionic strength of the sample is another parameter to control for an efficient SPE, as it may influence the retention of trace elements.

2.6.5.2.3) Elution parameters

a) Nature of the solvent: The eluting solvent should be carefully chosen to ensure efficient recovery of the retained target species and quantitative recovery as far as possible and compatible with the analysis technique.

b) Solvent flow rate: As a rule, the higher the flow rate, the larger the solvent volume required for complete elution.

c) Solvent volume: The volume of the used solvent should be as low as possible in order to obtain a high concentration of analytes but it should ensure the complete elution of the analytes.

The elution step should enable sufficient time and elution volume to permit the metallic species to diffuse out of the solid sorbent pores.

2.7 Applications of SPE to the determination of trace element

Classical liquid-liquid extractions (LLE) of trace elements are usually time-consuming and labor-intensive. In addition, they require strict control of extraction conditions, such as temperature, pH and ionic strength. For all these reasons, several procedures tend to be replaced by SPE methods. This technique is attractive as it reduces consumption of and exposure to solvents, their disposal costs and extraction time. It also allows the achievement of high recoveries, along with possible elevated enrichment factors. However, as different results between synthetic and real samples may be observed, recoveries should be estimated in both cases as far as possible. In addition, SPE can be interfaced on-line with analytical techniques, such as liquid chromatography (LC) or atomic absorption spectrometry (AAS). Its application for preconcentration of trace metals from different samples is also very convenient due to sorption of target species on the solid surface in a more stable chemical from than in solution. Finally, SPE affords a broader range of applications than LLE due to the large choice of solid sorbents [9-11].

2.7.1 Preconcentration

LLE requires the use of large volumes of high purity solvent, thereby affording limited preconcentration factors. The use of SPE enables the simultaneous preconcentration of trace elements and removal of interferences, and reduces the usage of organic solvents that are often toxic and may cause contamination. Upon elution of the retained compounds by a volume smaller than the sample volume, concentration of the extract can be easily achieved. Hence, concentration factors of up to 1000 may be attained.

2.7.2 Preservation and storage of the species

SPE allows on-site pre-treatment, followed by simple storage and transportation of the pre-treated samples with stability of the retained metallic species for several days. This point is crucial for the determination of trace elements, as the transport of the sample to the laboratory and its storage until analysis may induce problems, especially changes in the speciation. In addition, the space occupied by the solid sorbents is minimal and avoids storage of bulky containers and the manpower required to handle them.

2.7.3 High selectivity

SPE offers the opportunity of selectively extracting and preconcentrating only trace elements of interest, thereby avoiding the presence of major ions. This is crucial in some cases, such as with spectrophotometric detection, since the determination of heavy metals in surface waters may necessitate the removal of non-toxic metals, such as Fe or Zn, when they occur at high concentrations. It may also be possible to selectively retain some particular species of a metal, thereby enabling speciation.

2.7.4 Automation and possible on-line coupling to analysis techniques

SPE can be easily automated, and several commercially available systems have been recently reviewed [13]. Home-made systems have also been reported [14]. In addition, SPE can be coupled on-line to analysis techniques. On-line procedures avoid sample manipulation between preconcentration and analysis steps, so that analyte losses and risk of contamination are minimized, allowing higher reproducibility [15]. In addition, all the sample volume is further analyzed, which enables smaller sample volume to be used. However, in the case of complex samples, off-line SPE should be preferred due to its greater flexibility, and the opportunity to analyze the same extract using various techniques.

a) On-line coupling to liquid chromatography. On-line systems mainly use a micro-column. The sorbent is chosen not only for its efficiency in trapping analytes, but also for its compatibility with the stationary phase packed into the chromatographic column. Indeed, it is highly recommended to use the same packing in the pre-column and the chromatographic column to prevent losses in efficiency upon analysis. For the case of two different sorbents being used, the retention of the analytes in the pre-column should be lower than that in the analytical column to ensure band refocusing at the head of the chromatographic column. On-line systems with several detectors have been reported, such as UV detector [16] or inductively coupled plasma mass spectrometer (ICP-MS) [17], with detection limits in the 0.05–50 μg L^{-1} range. Detection limits with this technique as low as 0.5 ng L^{-1} could even be achieved by detection at the maximum absorption wavelength using a photodiode array UV detector [18]. The coupling of SPE to LC via flow injection has also been reported using cold vapor atomic absorption spectrometry (CV-AAS) as the detection, enabling enrichment factors approximately 850 [19].

b) On-line coupling to atomic absorption spectrometry. Olsen *et al*. [20] and Fang *et al*. [21-22] were the first describe an on-line flow injection (FI) sorbent extraction preconcentration system for flame atomic absorption spectrometry (FAAS) using micro-columns packed with a cation-exchanger. Later, they also proposed a system for on-line flow injection sorbent extraction preconcentration with electrothermal vaporization atomic absorption spectrometry (ET-AAS) using lead as a model trace element [23]. The sorbent should provided for rapid sorption and desorption of analytes to be used in FI systems [24]. In addition, it should be provided for a high selectivity.

c) On-line coupling to ICP-AES or ICP-MS. The first report of FI on-line preconcentration coupled to inductively coupled plasma atomic emission spectrometry (ICP-AES) appeared nearly twenty years ago [25]. Since then, several studies have used this coupling with different sorbents such as ZrO_2 or functionalized silica gel, for example.

d) On-line coupling to spectrophotometry. Spectrophotometry offers the advantage of requiring inexpensive and very common instrumentation. Solid-phase spectrophotometry (SPS) has also been reported with FI systems due to its simplicity and low detection limits. The solid sorbent is packed in either commercially available or customized flow cells. With such systems the retained analytes are periodically removed from the flow cell using an acid or a complexing solution [26, 27, and 28].

2.8 Sorption isotherms

Two important physiochemical aspects for the evaluation of the adsorption process as a unit operation are the equilibrium of the adsorption and the kinetics. Equilibrium studies give the capacity of the adsorbent. The equilibrium relationships between adsorbent and adsorbate are described by adsorption isotherms, usually the ratio between the quantity adsorbed and that remaining in solution at a fix temperature at equilibrium. There are two types of adsorption isotherms: Langmuir adsorption isotherms and Freundlich adsorption isotherms [29].

A number of different equations can be used to predict theoretical adsorption capabilities for different adsorbents. For this research, the Langmuir isotherm equations have been used to predict adsorption capabilities of metals on solid sorbent [30].

2.8.1 Langmuir isotherm

Langmuir [31] proposed the first isotherm model which assumed monolayer coverage of the adsorbent surface. The most commonly used expression of the Langmuir equation for describing adsorption data for solid-liquid systems is

$$\frac{x}{m}=\frac{K_L M C_e}{1+K_L M C_e} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots .... (2.1)$$

where x = amount or concentration of the solute adsorbed in *mg*

m = mass of the adsorbent in *kg*

C_e = equilibrium concentration of the solute in *mg/L*

K_L, M = constants

The linearized expression of this equation is

$$\frac{1}{x/m}=\frac{1}{K_L M C_e}+\frac{1}{M} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots ... (2.2)$$

This equation is called the “Double-Reciprocal Langmuir” equation and is more suitable for situations in which the distribution of equilibrium concentrations tends to be skewed towards the lower end of the range of the equilibrium concentrations. An EPA report [32] concluded that the Langmuir constant or affinity parameter (K_L) is related to the bonding energy between the adsorbed ion and the adsorbent, but that specific functional relationship is uncertain. Kinniburgh [33] note that K_L is the best estimate from the slope of the adsorption isotherm at very low concentrations. The constant M is accepted as the adsorption maximum of the adsorbent with respect to the specific solute and is interpreted as the maximum amount or concentration that the adsorbent can retain.

2.9 Literature review

Polymeric resin has been widely used as a solid support for metal extraction in SPE technique, especially polystyrene-divinylbenzene (PS-DVB), due to its high porosity and ease of surface modification [34]. The modification methods of PS-DVB was recently reported [35]; can be divided into two main methods; impregnation and functionalization with chelating agents or ligands containing electron donor atoms, such as nitrogen, oxygen and sulfur.

For the impregnation method of PS-DVB, ammonium pyrrolidine dithiocarbamate (APDC) was used for preconcentration of Bi(III), Cd(II), Co(II), Cu(II), Fe(II), Ni(II) and Pb(II) in drinking water and sea water [36]. 8-hydroxyquinoline (8-HQ) was tested by Isshiki *et al.* [37] for preconcentration of Cr(III) from sea water. The use of 1,5-diphenylcarbazone was reported for preconcentration of Cr(III) from tap water [38]. Dithizone was used for preconcentration of Hg(II) and methyl mercury in water [39]. 1-(2-pyridylazo)-2 naphthol (PAN) was used for Cu(II) preconcentration from sea water [40]. However, in practice, the resins prepared by impregnation of the ligands are difficult to reuse, due to partial leaching of the ligands during the elution process thus resulting in poor repeatability.

To overcome this problem, the resin may be chemically functionalized. Several chemical modifications of PS-DVB have recently been reviewed, but only a few are commercially available. The ligands were generally coupled to a methylene or an azo spacer on the resin. The use of Alizarin Red-S [41] and Pyrocatechol Violet [42] was reported for preconcentration of Zn(II), Cd(II), Ni(II) and Pb(II) in water and salicylic acid [43] was used for preconcentration of Zn(II) and Pb(II) in water. Chromotropic acid, pyrocatechol, thiosalicylic acid, xylenol orange was functionalized on PS-DVB for preconcentration of Pb(II) in water through an azo spacer [44]. The use bis-(*N,N'*-salicylidene)1,3-propanediaminewas report for preconcentration of Cu(II), Ni(II), Co(II), Zn(II), Fe(II), Mn(II), Pb(II), Cd(II) and Cr(III) in water [45]. *o*-vanilline thiosemicarbazone was used for preconcentration of elements in rare earth such as, La(III), Ce(III), Th(IV), U(VI), Cu(II) Zn(II) and Pb(II) in water [46-47]. *o*-aminophenol [48] and used 2-(methylthio)aniline [49] were functionalized on PS-DVB for preconcentration of Cu(II), Cd(II), Co(II), Hg(II), Ni(II), Zn(II) and Pb(II) through an azo spacer and their has amine group on resin. The results showed that the resin has low selectivity.

However, from the literature, it can be observed that chelating agents are macromolecule, difficult to synthesize and expensive. The objective of this research is to synthesize a new chelating agent that is a small molecule containing donor atoms such as O, N and S and functionalize on a polymeric sorbent through an amide linkage (-CO-NH-R) and azo linkage (-N=N-). The chelating agent is a benzothiazole derivative, as illustrated in Figure 2.3. This ligand was chosen due to the ease of preparation and its selectivity for Cu(II), as previously reported by Abbotto *et al.* [50].

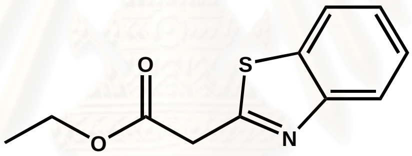


Figure 2.3 Structure of ethyl 2-benzothiazolylacetate

CHAPTER III

EXPERIMENTAL SECTIONS

3.1 Apparatus

3.1.1 UV-vis spectrophotometer

UV-vis spectrophotometer model HP 8453 (Hewllet Packard) was used for the characterization of ethyl 2-benzothiazolyacetate (BA).

3.1.2 Flame atomic absorption spectrometer

Flame atomic absorption spectrometer (FAAS) model AAnalyst 100 (Perkin-Elmer) was used for the determination of metal concentration. The instrumental parameters are listed in Table 3.1.

Table 3.1 FAAS conditions for determination of metals concentration in solutions

Operating conditions	**Cd**	**Cu**	**Pb**
Wavelength (nm)	228.8	324.8	283.3
Slit width (nm)	0.70	0.70	0.70
Lamp type	HCL[a]	HCL[a]	HCL[a]
Lamp current (mA)	4	15	10
C_2H_2 flow-rate (mL min^{-1})	3	3	3
Air flow-rate (mL min^{-1})	10	10	10

[a] Hallow Cathode Lamp

3.1.3 Fourier transforms infrared spectrometer

Fourier transforms infrared spectrometer (FTIR) model Nicolet Impact 410 was used for the characterization of all polymers.

3.1.4 Attenuated total reflectance fourier transforms infrared spectrometer

Attenuated total reflectance fourier transforms infrared spectrometer (ATR-FTIR) model Nicolet Magna-IR 750 was used for the characterization of all polymers.

Table 3.2 ATR-FTIR conditions for the characterization of all polymers

Operating conditions	
Internal Reflection Element (IRE)	Ge Hemi-sphere
Incidence angle	45 °
No. of scan	128
Detector	DTGS KBr
Beam spliter	KBr

3.1.5 Nuclear magnetic resonance spectrometer

Nuclear magnetic resonance spectrometer (NMR) model Varian Mercury + 400 was used for the characterization of ethyl 2-benzothiazolyacetate (BA).

3.1.6 CHNS/O analyzer

CHNS/O analyzer PE 2400 Series II (Perkin-Elmer) was used for elemental analysis of all polymers.

3.1.7 pH meter

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

3.1.8 Peristaltic pump

A peristaltic pump REGLO Analog MS-4/8 model ISM 827 (ISMATEC®) was used for the control of the flow of solutions passing through the column.

3.2 Chemicals

All chemicals were ACS grade listed in Table 3.3.

Table 3.3 Chemicals lists

Chemicals	Supplier
2-Aminothiophenol	MERCK
Calcium chloride	Cenco
Calcium nitrate	BDH
Dichloromethane	MERCK
Dow Styrene-DVB, 18-100 mesh	SUPELCO
Ethanol	MERCK
Ethyl cyanoacetate	MERCK
Hydrochloric acid 37%	MERCK
Nitric acid 65%	MERCK
Potassium hydroxide	MERCK
Potassium nitrate	BDH
Single standard solution for Cd, Cu and Pb (1000 mg L^{-1})	BDH
Sodium hydroxide	MERCK
Sodium nitrate	CARLO ERBR
Sodium sulphate	CARLO ERBR
Stannous chloride Dihydrate	Fluka
Sulfuric acid 98%	LAB-SCAN
Toluene	Fisher Scientific

3.3 Methodology

3.3.1 Preparation of chemicals and reagents

All solutions were prepared by using deionized water.

a) Working standard solutions

Working standard solutions of Cd(II), Cu(II) and Pb(II) were prepared by stepwise dilution of 1000 mg L^{-1} stock standard solution to the required concentrations.

b) Interference ions solutions

Solutions A; containing of Cd(II) and Cu(II) 1.0 mg L^{-1} and other ions; $NaSO_4$, KNO_3 and $CaCl_2$ 1 g L^{-1} (pH 6.0-7.0).

Solutions B; containing of Pb(II) 5.0 mg L^{-1} and other ions; $Ca(NO_3)_2$, $NaNO_3$ and KNO_3 1 g L^{-1} (pH 5.0-6.0).

c) Potassium hydroxide solutions

The KOH solutions (1%, 5%, 10% and 20%, w/v) were prepared by dissolving the appropriate amount of KOH in deionized water.

d) Hydrochloric acid solutions

Hydrochloric acid (1 M and 4 M), were prepared daily by direct dilution from the concentrated solution.

e) Nitric acid solutions

Nitric acid solutions were prepared daily by direct dilution from the concentrated solution.

f) Sodium hydroxide solution

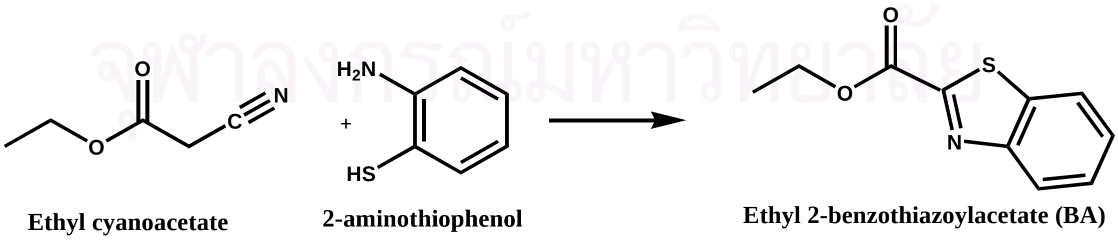
Sodium hydroxide solutions (2 M and 10% w/v) were prepared daily by dissolving the appropriate amount of NaOH in deionized water.

g) Sodium nitrite

Sodium nitrite 1 M, was prepared daily by dissolving the appropriate amount of $NaNO_2$ in deionized water.

3.3.2 Preparation of chelating agent

A mixture of ethyl cyanoacetate (6.00 g, 53.0 mmol) and 2-aminothiophenol (6.63 g, 53.0 mmol) was stirred for 2 hours at 120 °C under nitrogen atmosphere. The reaction was presented by scheme 3.1. The product was characterized by ^{1}H-NMR.



Scheme 3.1 Synthesis of chelating agent

3.3.3 Preparation of nitro-PS-DVB

PS-DVB beads, (10 g, AR grade), was stirred with 4 M HCl (15 mL) for 12 hours, filtered, rinsed with deionized water until free from acid, rinsed by ethanol, and dried in air. The resin was then treated with 20 mL of concentrated HNO_3 and 50 mL of concentrated H_2SO_4 and the mixture was stirred at 60 °C for 1 hour. Thereafter, the reaction mixture was poured into an ice-water mixture, labeled as ***nitro-PS-DVB***.

3.3.4 Preparation of amino-PS-DVB

The nitrated resin (from 3.3.3) was filtered, rinsed with deionized water until free from acid and further refluxed with a reducing mixture containing 40 g of $SnCl_2$, 45 mL of concentrated HCl, and 50 mL of ethanol at 90 °C for 12 hours. The final product was filtered, rinsed with deionized water and 2 M NaOH and air-dried, labeled as ***amino-PS-DVB***.

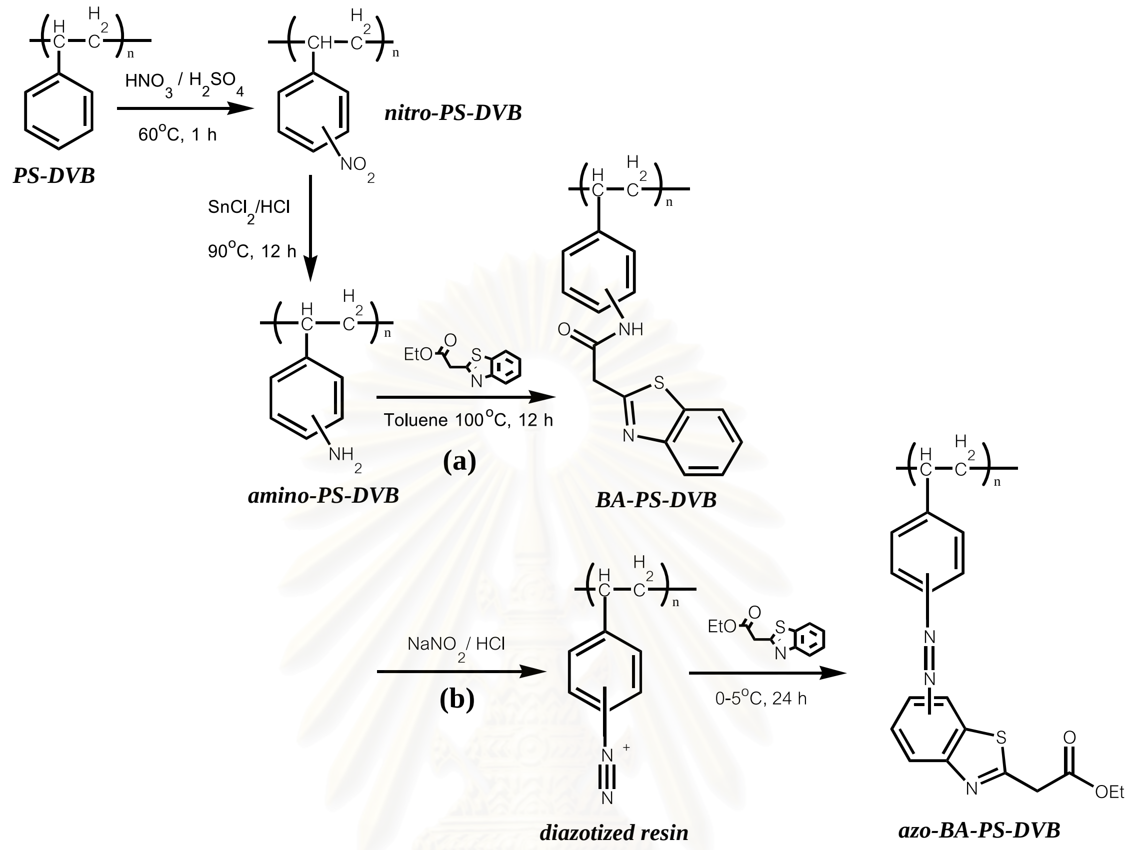
3.3.5 Preparation of chelating resins

(a) amino-PS-DVB was refluxed with BA (0.5 g) in toluene (30 mL) at 100 °C for 12 hours. The resin was filtered, rinsed with ethanol and air-dried, so-called ***BA-PS DVB***.

(b) amino-PS-DVB was suspended in an ice-water mixture (250 mL) and treated with 1 M HCl and 1 M $NaNO_2$ until the reaction mixture showed a permanent dark blue color with starch-iodide paper. The diazotized resin was filtered, washed with ice-cold water and reacted with BA (0.5 g in 250 mL of 10% NaOH solution) and stirred at 0-5 °C for 24 hours. The resin was filtered, washed with deionized water and air-dried, so-called ***azo-BA-PS-DVB***.

The UV-vis absorbance of the BA solution before and after the reaction was measured in order to investigate and elucidate whether the reaction had taken place.

The preparation procedure was summarized in Scheme 3.2



Scheme 3.2 Synthesis of chelating resins

3.4 Characterization

3.4.1 Characterization of chelating agent

The chelating agent was characterized by ^{1}H-NMR and UV-vis spectrophotometer.

3.4.2 Characterization of resins

All resins obtained in each stage were characterized by EA and FTIR with KBr pellet method.

3.5 Extraction study

The study of extraction was divided into two schemes, batch and column methods.

3.5.1 Batch method

The effect of various parameters influencing the extraction efficiency was investigated. The parameters taken in account were pH, extraction time and interference ions. In addition, the sorption capacity and desorption of the metals were studied.

3.5.1.1 pH effect on extraction

A Cd(II) solution (5.0 mL) containing 1.0 mg L^{-1} was placed in a test tube after being adjusted its pH ranging from 2.0-8.0. BA-PS-DVB (0.10 g) or azo-BA-PS-DVB (0.05 g) was added into the test tube and the mixture was stirred for 30 minutes. The resins were separated. The remained amount of Cd(II) in the solution was determined by FAAS.

The study of Cu(II) and Pb(II) was performed in the same procedure but the initial concentration of Pb(II) was 5.0 mg L^{-1} and its pH was adjusted ranging from 2.0-6.0.

3.5.1.2 Extraction time

In order to determine the rate of loading of Cd(II), Cu(II) and Pb(II) on the resins, batch experiments were carried out under the following conditions;

0.10 g of BA-PS-DVB and 0.05 g of azo-BA-PS-DVB was separately contacted and stirred with 5.0 mL of 1.0 mg L^{-1} Cd(II) solution after adjusting its pH to the optimum values at different contact time of 5, 10, 20, 30, 40, 50 and 60 minutes. The remained amount of Cd(II) in the solution was determined by FAAS.

The study of Cu(II) and Pb(II) was performed in the same procedure but the initial concentration of Pb(II) was 5.0 mg L^{-1}.

3.5.1.3 Effect of eluent

The elution of retained metal from the resins was investigated using 5 mL of eluting agent. A starting solution contained 1.0 mg L^{-1} of Cd(II) (pH 6-7, 5.0 mL) was contacted with 0.10 g of BA-PS-DVB or 0.05 g of azo-BA-PS-DVB for 30 minutes. Nitric acid was used as an eluent; its concentration was varied from 0.1 to 2.0 M. The amount of Cd(II) in the stripped solution was determined by FAAS.

The desorption of Cu(II) and Pb(II) was performed in the same manner but the initial concentration of Pb(II) was 5.0 mg L^{-1}.

3.5.1.4 Sorption capacity

The study of the adsorption isotherm was investigated by varying concentration of solution containing 1.0-5.0 mg $L^{-1}$of Cd(II) with 0.10 g of BA-PS-DVB or 0.05 g of azo-BA-PS-DVB for 20 minutes under optimum conditions for each metal at 25.0 $\pm$ 1.0 $^{\circ}$C. The resin was separated. The remained amount of Cd(II) in the solution was determined by FAAS.

The extraction of Cu(II) and Pb(II) was performed in the same manner but the initial concentration of Pb(II) was 5.0-8.0 mg L^{-1}.

3.5.1.5 Effect of interference ions

Solutions A; 0.20 g of BA-PS-DVB or 0.10 g of azo-BA-PS-DVB was stirred with 10.0 mL of this solution (under optimum conditions for each metal). The remained amount of Cd(II) and Cu(II) in the solution was determined by FAAS.

Solutions B; 0.10 g of BA-PS-DVB or 0.05 g of azo-BA-PS-DVB was stirred with 5.0 mL of this solution (under optimum conditions). The remained amount of Pb(II) in the solution was determined by FAAS.

3.5.2 Column method

3.5.2.1 Column preparation

A mini-column with 2.79 mm i.d. was packed with 0.10 g of BA-PS-DVB or 0.05 g of azo-BA-PS-DVB.

3.5.2.2 Effect of flow rate

The flow rate of the sample solution affects the extraction efficiency for each metal. A starting solution contained 0.5 mg $L^{-1}$of Cd(II) (pH 6-7, 5.0 mL) was passed through the prepared column, at different flow rate from 0.5 to 8.0 mL min^{-1} (controlled with the peristaltic pump).

The study of Cu(II) and Pb(II) was performed in the same manner but the initial concentration of Cu(II) and Pb(II) was 0.5 and 3.0 mg L^{-1}, respectively.

3.5.2.3 Effect of eluent

The elution of retained metal from the resins was investigated using 5 mL of eluting agent. A starting solution contained 0.5 mg $L^{-1}$of Cd(II) (pH 6-7, 5.0 mL) was passed through the column (under optimum flow rate). Nitric acid was used as eluents, its concentration was varied from 0.1 to 2.0 M. The amount of Cd(II) in the stripped solution was determined by FAAS.

The desorption of Cu(II) and Pb(II) was performed in the same manner but the initial concentration of Cu(II) and Pb(II) was 0.5 and 3.0 mg L^{-1}, respectively.

3.5.2.4 Effect of sample volume

To deal with real samples, the maximum applicable sample volume must be considered. For this purpose; 25, 50, 100, 250 and 500 mL of sample solutions were investigated. For Cd(II) solution, 250 µL of 10 mg $L^{-1}$of Cd(II) was spiked into each solutions with different volume and the metal solution was passed through the column packed with 0.10 g of BA-PS-DVB or 0.05 g of azo-BA-PS-DVB (under the optimum conditions), after that the metal was eluted from the column by an appropriate eluent. The amount of Cd(II) in the eluent was determined by FAAS.

The study of Cu(II) and Pb(II) was performed in the same way but for the Pb(II) solution, 250 µL of 50 mg L^{-1} of Pb(II) was spiked into each solutions with different volume.

3.6 Method validation

Under the optimum conditions, the method validation was performed by repeating the same experiment 15 times. The accuracy and precision were calculated as the recovery and relative standard deviation (RSD), respectively. Recoveries were investigated by using the sample solutions containing spiked metals.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Characterization

4.1.1 Characterization of chelating agent

The results were illustrated in the followings.

4.1.1.1 ^{1}H-NMR

The ^{1}H-NMR spectrum of ethyl 2-benzothiazolyacetate (BA) was obtained with chemical shifts as followed: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.02 (d, 1H, J = 8), 7.88 (d, 1H, J = 8), 7.47 (t, 1H, J = 8), 7.39 (t, 1H, J = 8), 4.25 (q, 2H, J = 7.2), 4.16 (s, 2H), 1.30 (t, 3H, J = 7.2). The result indicated that ethyl 2-benzothiazolyacetate was successfully synthesized.

The ^{1}H-NMR spectrum of ethyl 2-benzothiazolyacetate was depicted in Figure 4.1.

4.1.1.2 UV-vis spectrophotometry

The UV-visible spectrum of ethyl 2-benzothiazolyacetate (BA) was shown in Figure 4.2. The absorption band at 230 and 260 nm was found. This wavelength would be further used to determine the amount of BA.

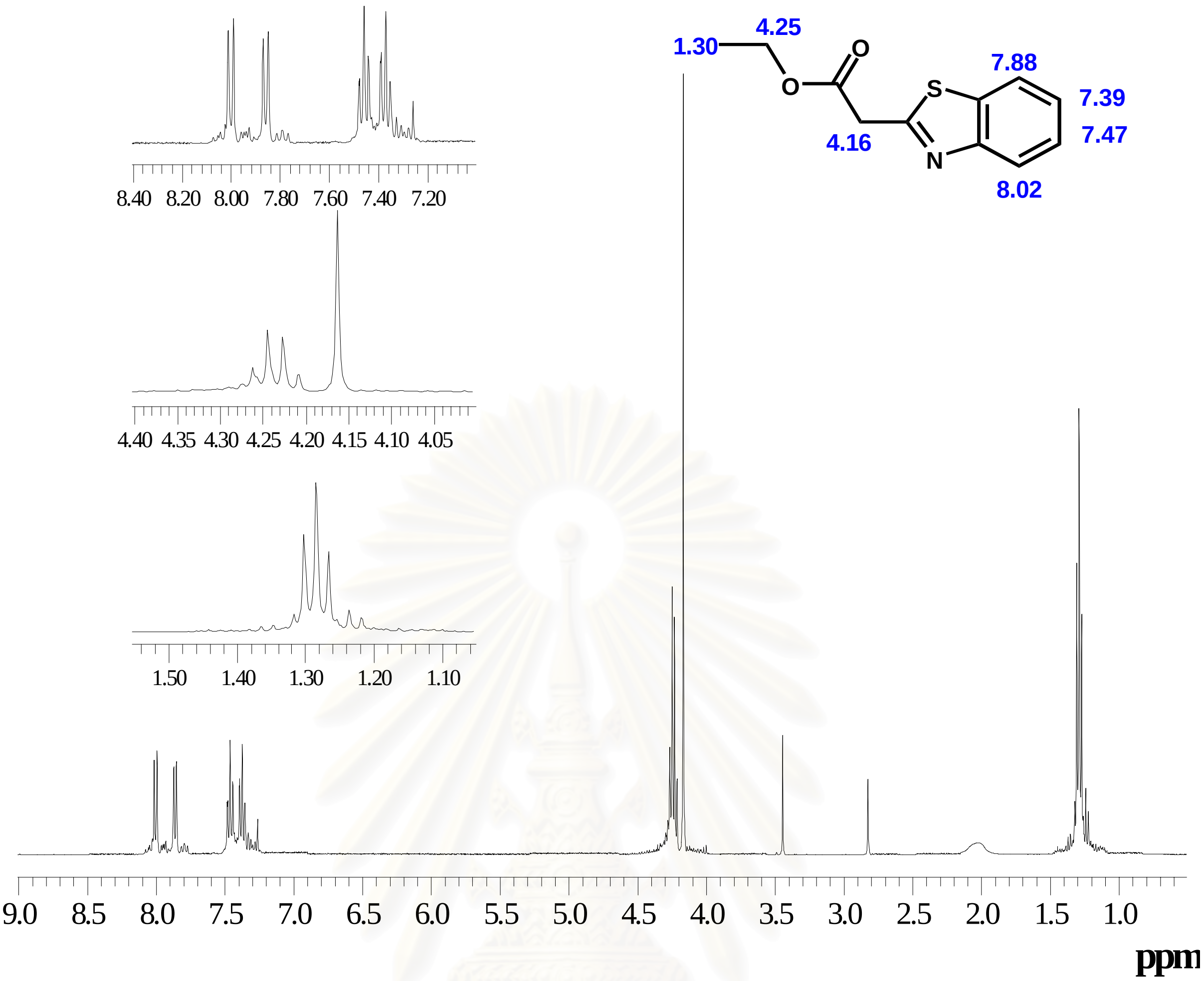


Figure 4.1 ^{1}H-NMR spectrum of ethyl 2-benzothiazolyacetate

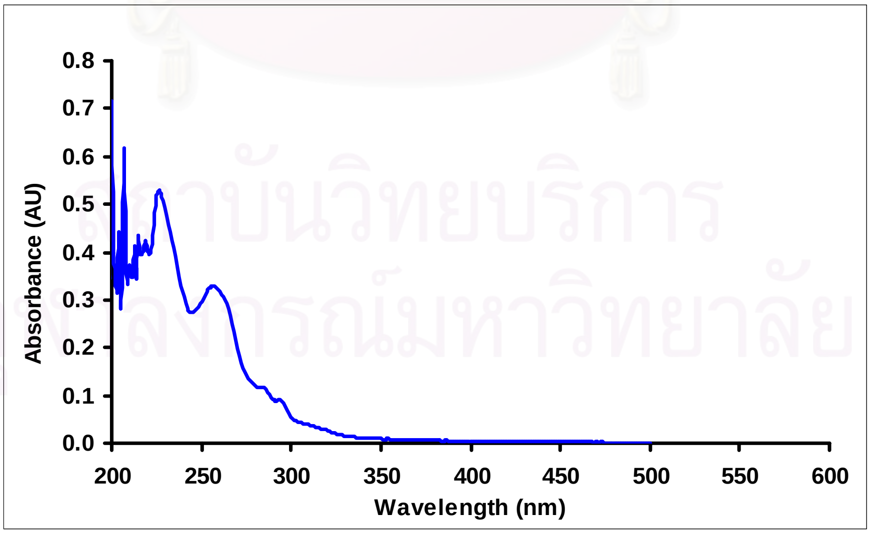


Figure 4.2 UV-visible spectrum of ethyl 2-benzothiazolyacetate

4.1.2 Investigation of the coupling reaction

During both amidation reaction and coupling-diazotization reaction between BA and amino-PS-DVB, the UV-vis absorbances of the BA solutions before and after the reactions were measured. The objective of this experiment was to investigate whether the reaction had taken place by comparing the absorbance at 230 and 260 nm. According to Beer's law, the concentration or the amount of ligand is proportional to the absorbance, if the absorbance of BA solution decrease, it indicates that the BA reacts and binds with the solid phase. Considering the results shown in Figure 4.3, the absorbance of BA solution after the reaction slightly decreased for amidation reaction and significantly decreased for coupling-diazotization reaction. This observation revealed that both reactions had taken place and azo-BA-PS-DVB contained more chelating agent than BA-PS-DVB.

4.1.3 Characterization of resins

All resins obtained in each stage were characterized by EA and IR.

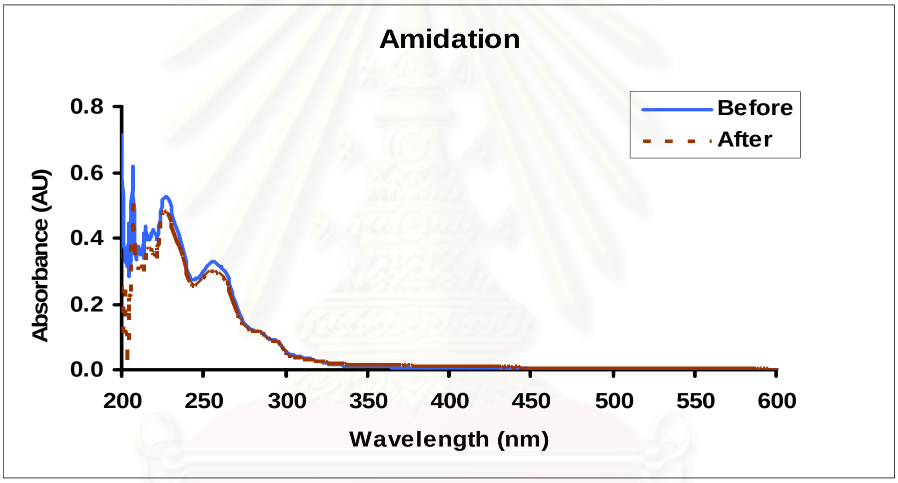
4.1.3.1 Elemental analysis

The EA results of nitro-PS-DVB resin showed that the nitration reaction on the surface of PS-DVB was undertaken. Due to an azo group (-N=N-) in azo-BA-PS-DVB, the ratio of nitrogen to carbon (0.0916) was higher than BA-PS-DVB (0.0767).

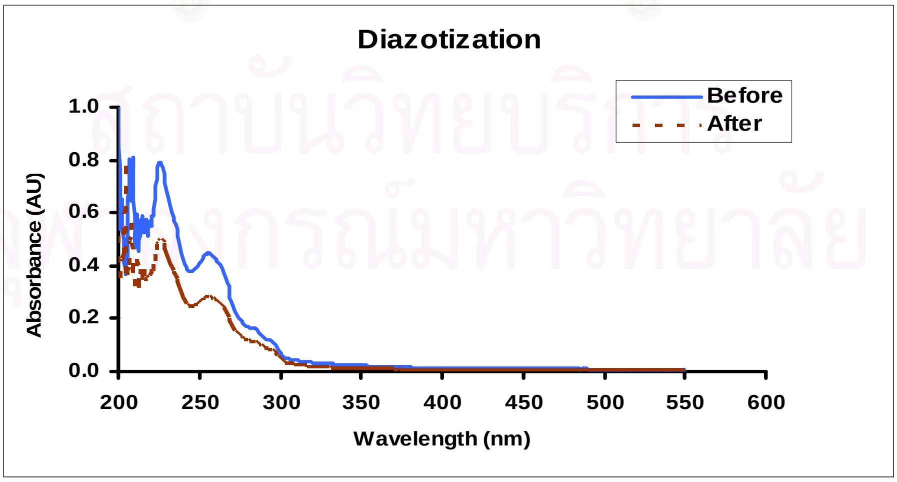
The EA results were illustrated in Table 4.1.

Table 4.1 Elemental analysis results of resins

Sample	C (%)	H (%)	N (%)	N/C
PS-DVB	92.5	7.48	-	-
nitro-PS-DVB	70.3	6.62	6.20	-
amino-PS-DVB	65.3	5.98	6.41	-
BA-PS-DVB	74.4	6.44	5.71	0.0767
azo-BA-PS-DVB	70.5	6.35	6.46	0.0916



(a)



(b)

Figure 4.3 UV-visible spectra of amidation (a) and diazotization reaction (b)

4.1.3.2 Infrared spectroscopy

The resin was characterized by FTIR with KBr pellet method. The IR spectra were depicted in Figure 4.4.

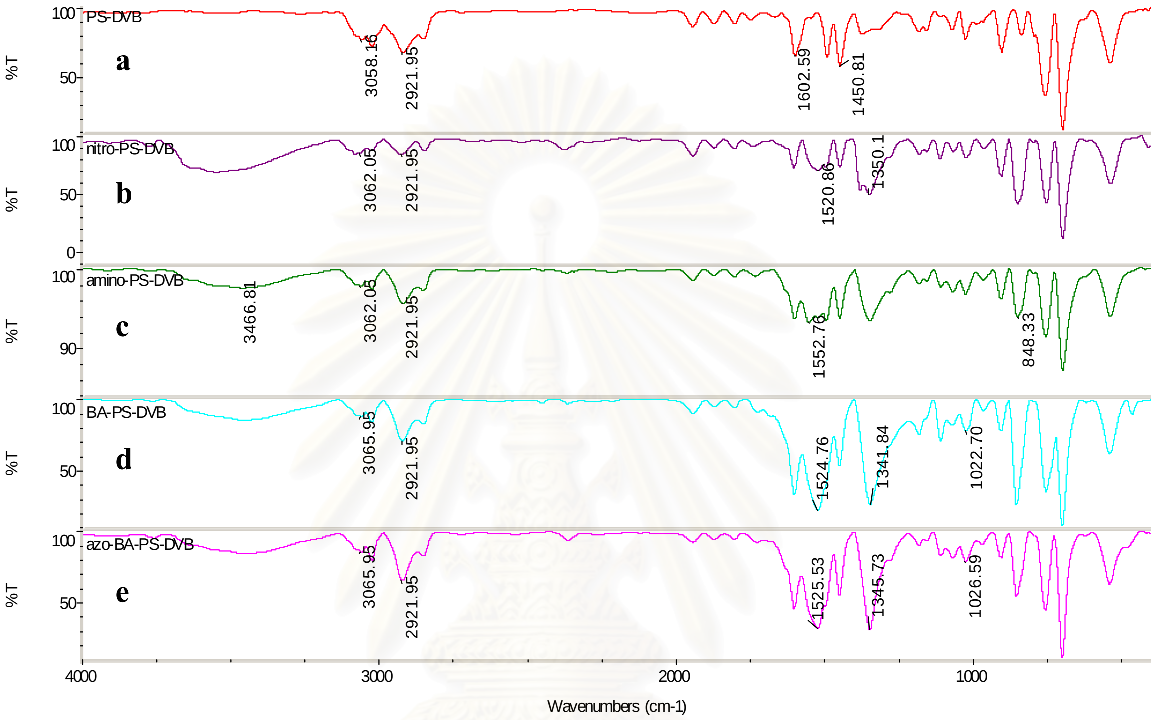


Figure 4.4 IR spectra of benzothiazole derivative resin based on PS-DVB (a) PS-DVB, (b) nitro-PS-DVB, (c) amino-PS-DVB, (d) BA-PS-DVB and (e) azo-BA-PS-DVB

The IR bands of nitro (1350, 1520 cm^{-1}), amine (3200-3500 cm^{-1}), mono-substituted amide (1530, 1680, 3250-3500 cm^{-1}), and azo (1540 cm^{-1}) groups were observed for nitro-PS-DVB, amino-PS-DVB, BA-PS-DVB and azo-BA-PS-DVB, respectively. The benzothiazole derivative resin based on polystyrene-divinylbenzene bead was successfully synthesized.

4.1.3.3 Attenuated total reflectance fourier transforms infrared spectroscopy

The ATR-FTIR was used to characterize the resin in order to confirm the functional groups on the surface of the resins. The result was shown in Figure 4.5.

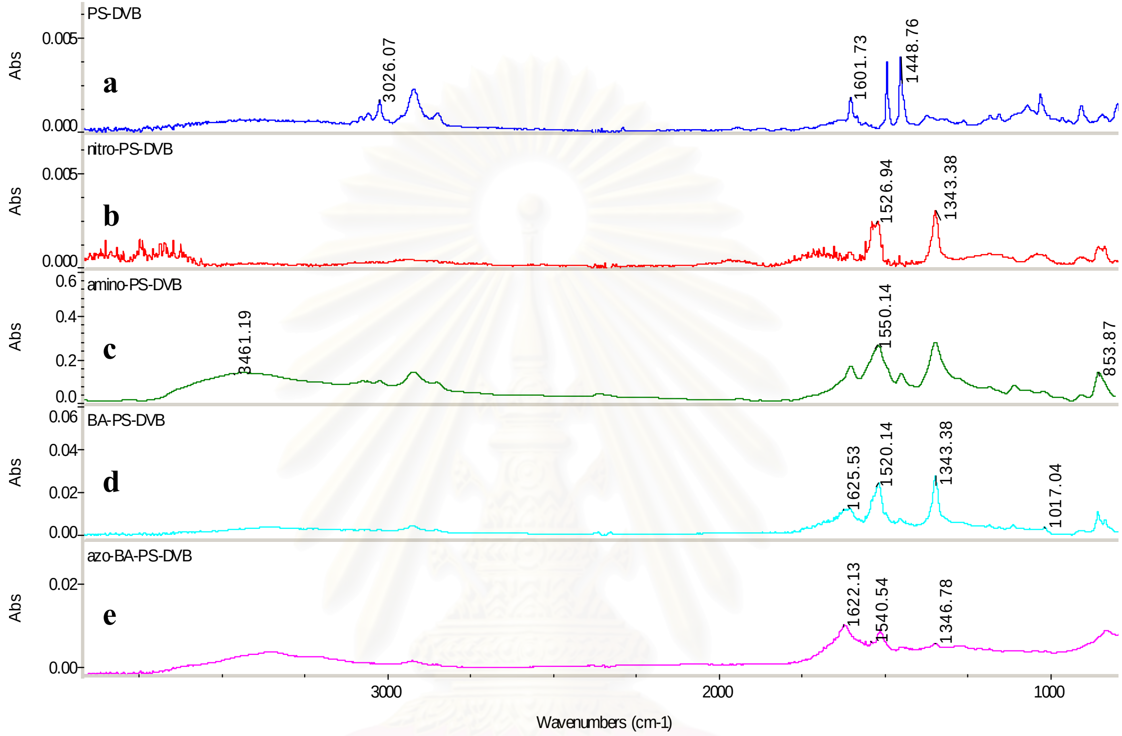


Figure 4.5 ATR-FTIR spectra of benzothiazole derivative resin based on PS-DVB (a) PS-DVB, (b) nitro-PS-DVB, (c) amino-PS-DVB, (d) BA-PS-DVB and (e) azo-BA-PS-DVB

From figure 4.5, the bands of nitro (1320, 1520 cm^{-1}), amine (3200-3500 cm^{-1}), mono-substituted amide (1520, 1680, 3250-3500 cm^{-1}), and azo (1540 cm^{-1}) groups were observed. In conclusion, the benzothiazole derivative resin was successfully synthesized.

4.2 Extraction study

4.2.1 Batch method

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

4.2.1.1 Effect of the sample pH on the metal sorption

The metal sorption (mg/g resin) of the metal retained on the sorbents was calculated from the difference between the starting amounts of each metal (mg) and the amount of metal (mg) left in the filtrate according to equation 4.1.

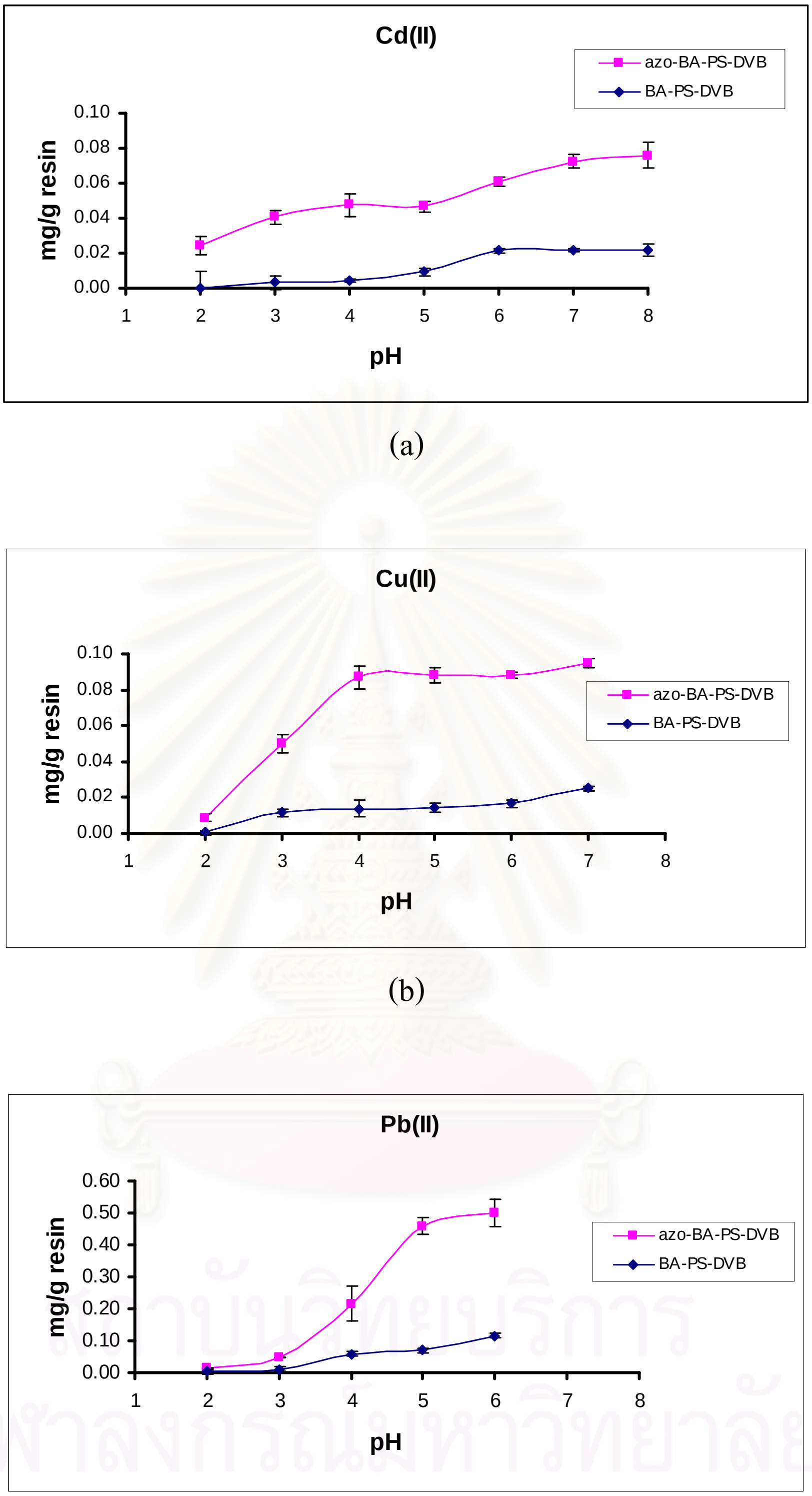
$$\text{Metal sorption} = \frac{N_s - N_f}{m} \qquad \text{(4.1)}$$

where N_s = the starting amounts of each metal (mg)

N_f = the amount of metal left in the filtrate (mg)

m = mass of sorbent (g)

The results of each metal sorption were illustrated in Figure 4.6. Regarding the results, when pH values of the solutions increased, the sorption percentages of the three metal ions increased. In acidic medium, nitrogen atoms acting as an electron donor might be protonated, resulting in positive charge of nitrogen that could rather not bind with metal ions. As a result, the sorption percentages were low. When pH values raised, the nitrogen atoms were probably deprotonated and they became electron donor for the metals. The complexation reaction was ameliorated, resulting in higher percent extraction.



(a)

(b)

(c)

Figure 4.6 Effect of pH on metal sorption (n=3); (a) Cd(II), (b) Cu(II) and (c) Pb(II)

In case of BA-PS-DVB, the initial pH of the solution affected the sorption behavior of all metal ions. For Cd(II), the highest sorption capacity was observed with pH value 6.0-8.0. For Cu(II) and Pb(II), the optimum pH were 7.0 and 6.0, respectively. The highest sorption capacity of Cd(II), Cu(II) and Pb(II) 0.022, 0.025 and 0.116 mg g $^{-1}$were observed at their optimum pH values. It seems that the sorption should increase while the pH values increase; nonetheless, the experiments were not performed because the precipitation of metal hydroxide occurred in higher pH solutions.

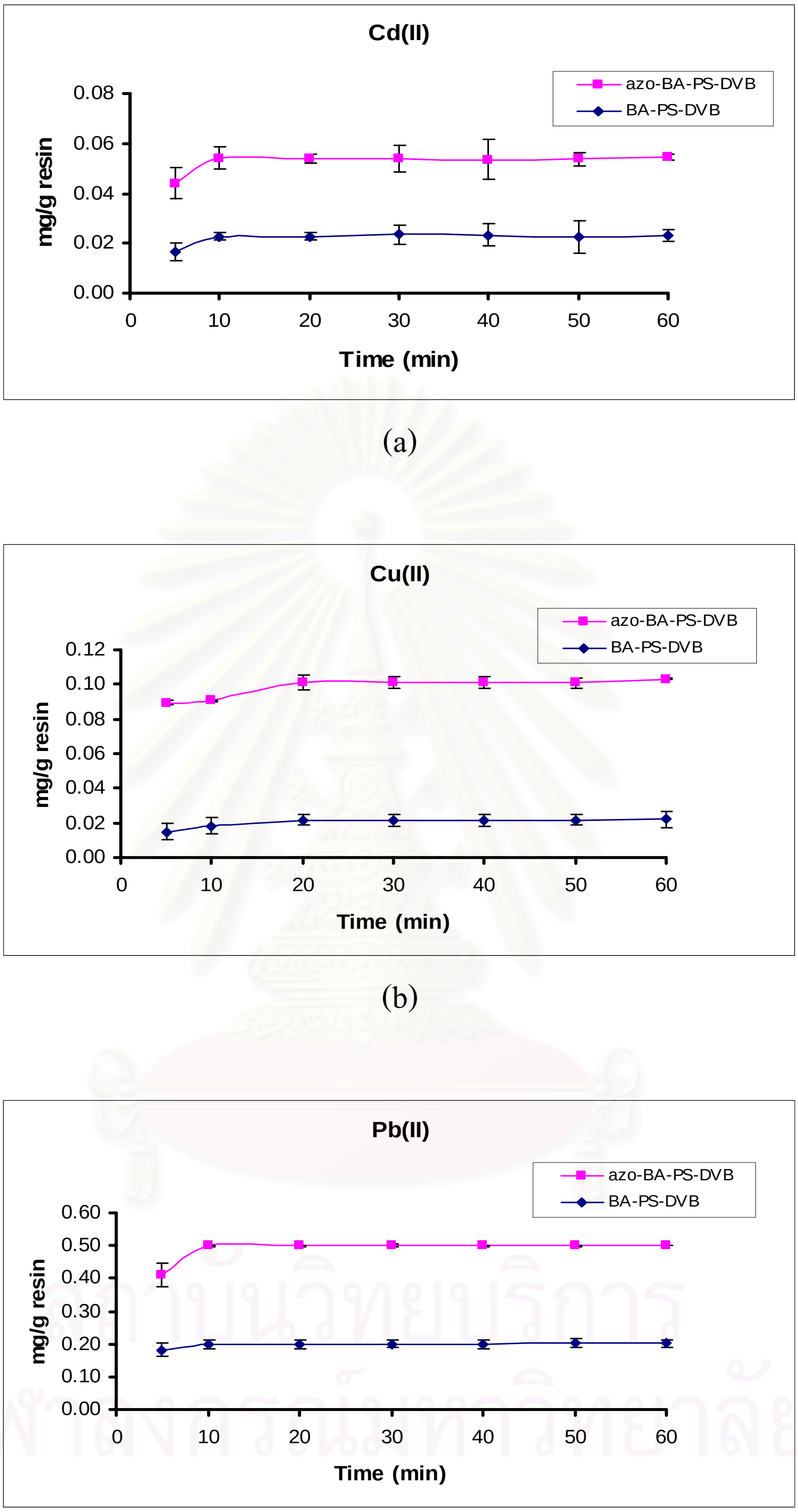
In case of azo-BA-PS-DVB, Cd(II), the highest sorption capacity was observed with pH value 7.0-8.0. For Cu(II) and Pb(II), the optimum pH were 7.0 and 6.0, respectively. The highest sorption capacity of Cd(II), Cu(II) and Pb(II) 0.076, 0.095 and 0.500 mg g $^{-1}$ were observed at their optimum pH values.

By comparing the two resins, azo-BA-PS-DVB is more efficient than BA-PS-DVB.

4.2.1.2 Effect of the contact time on the metal sorption

The rate of loading of metal ion onto the resin was determined by agitating the standard solution with the resin at optimum pH with different contact time. The results of each metal sorption were illustrated in Figure 4.7. The equilibration time for BA-PS-DVB and azo-BA-PS-DVB was achieved at around 10 minutes for Cd(II) and Pb(II) and 20 minutes for Cu(II).

The kinetic of the resin-metal interaction is sufficiently rapid for most of the metal ions at the optimum pH and it reflects a good accessibility of the chelating sites of the modified resin for metal ions.



(a)

(b)

(c)

Figure 4.7 Effect of contact time on metal sorption (n=3); (a) Cd(II), (b) Cu(II) and (c) Pb(II)

4.2.1.3 Desorption of metals

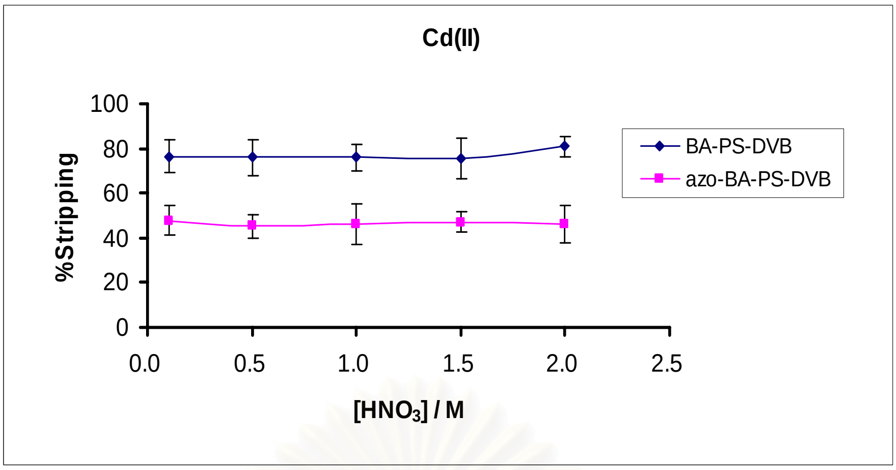
Desorption of the metals from the resin was performed with nitric acid of different concentrations at 30 minutes of desorption time. The percent stripping in this section was calculated from equation 4.2. The results are summarized in Figure 4.8.

$$\%\text{Stripping} = \frac{N_e}{N_a} \times 100 \qquad \text{.. (4.2)}$$

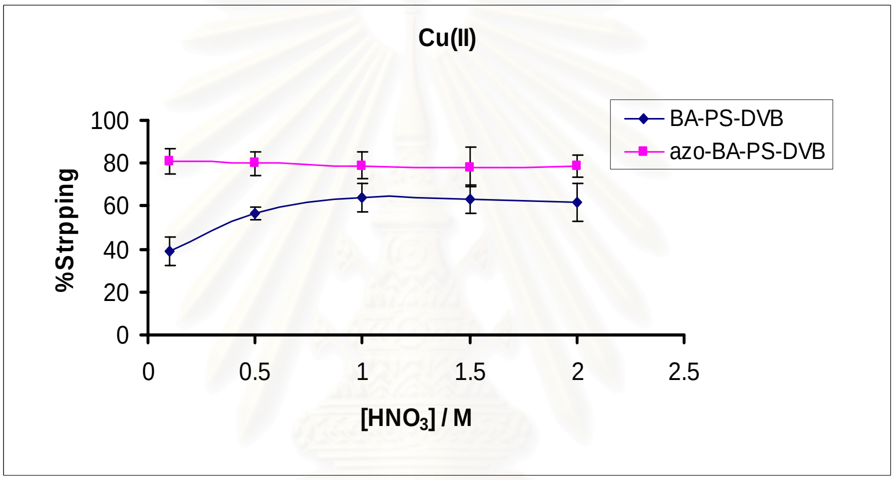
where N_a = amount of metal ions (mg) adsorbed on resin

N_e = amount of metal ions (mg) eluted from resin

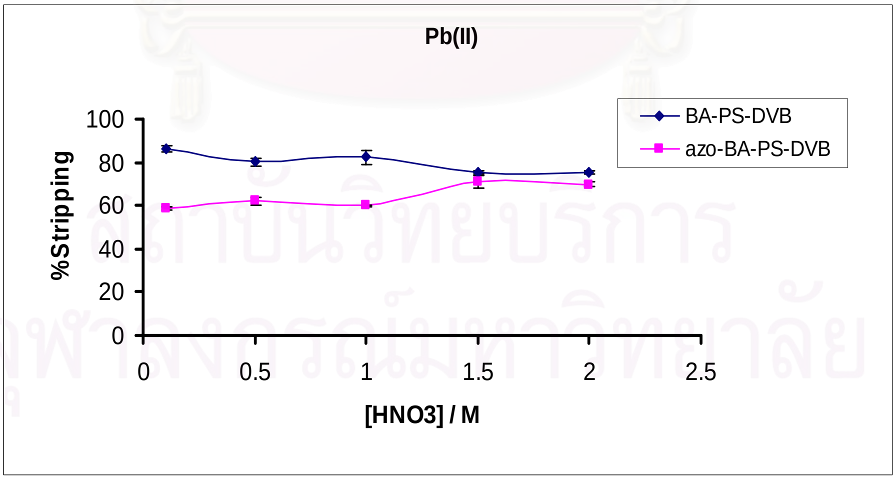
For BA-PS-DVB, it was observed that 1.0 M nitric acid was able to quantitatively elute Cd(II), Cu(II) and Pb(II) from the resin with the percent stripping of 76, 64 and 82, respectively. Thus, for azo-BA-PS-DVB, it was observed that 0.5 M nitric acid was appropriate to elute Cd(II) and Cu(II) and Pb(II) from the resin with the percent stripping of 45, 80 and 60, respectively. Due to the relatively low percent stripping, other eluents should be tried to desorp Cu(II) from BA-PS-DVB and Cd(II) and Pb(II) from azo-BA-PS-DVB.



(a)



(b)



(c)

Figure 4.8 Effect of nitric acid concentrations on metal desorption (n=3);

(a) Cd(II), (b) Cu(II) and (c) Pb(II)

4.2.1.4 Sorption capacity

The adsorption of Cd(II), Cu(II), and Pb(II) in mg $g^{-1}(N_f)$ onto resin as a function of the resin was investigated. The Langmuir treatment is based on the assumption that the maximum adsorption corresponds to saturated monolayer of adsorbed species on the resin surface and there is no migration of the metal ions in the plane of the surface. The data reveal that the adsorption process conforms to the Langmuir model [30], according to equation 4.3.

$$\frac{C}{N_f} = \frac{C}{N_s} + \frac{1}{bN_s} \quad \text{..} \quad (4.3)$$

where C = the concentration of solution in equilibrium (mol dm^{-3})

N_f = the concentration of cations adsorbed on surface (mol g^{-1})

N_s = the maximum amount of solute adsorbed per gram of surface (mol g^{-1})

b = binding association constant (L mol^{-1})

N_s and b is Langmuir constants related to adsorption capacity and energy of adsorption, respectively.

All these adsorption studies were based on the linearized form of the adsorption isotherm derived from the C/N_f as a function of C plot. The results showed that the adsorption isotherm of the resins for Cd(II), Cu(II), and Pb(II) obeyed Langmuir adsorption model, shown in Figure 4.9.

The maximum sorption capacity (N_s), binding association constant (b) and correlation coefficient (r) determined from the Langmuir plot are given in Table 4.2.

Table 4.2 Langmuir parameters and correlation coefficient calculated from experimental data

Metal- sorbent	BA-PS-DVB [a]			azo-BA-PS-DVB [a]		
	Cd(II)	Cu(II)	Pb(II)	Cd(II)	Cu(II)	Pb(II)
N_s (mmol g^{-1})	0.398	1.01	0.982	1.03	5.68	1.55
N_s (mg g^{-1})	44.7	64.2	204	116	361	321
b (L $mmol^{-1}$)	0.334	12.9	1.68	0.180	0.0360	1.03
r	0.9889	0.9911	0.9938	0.9782	0.9838	0.9741

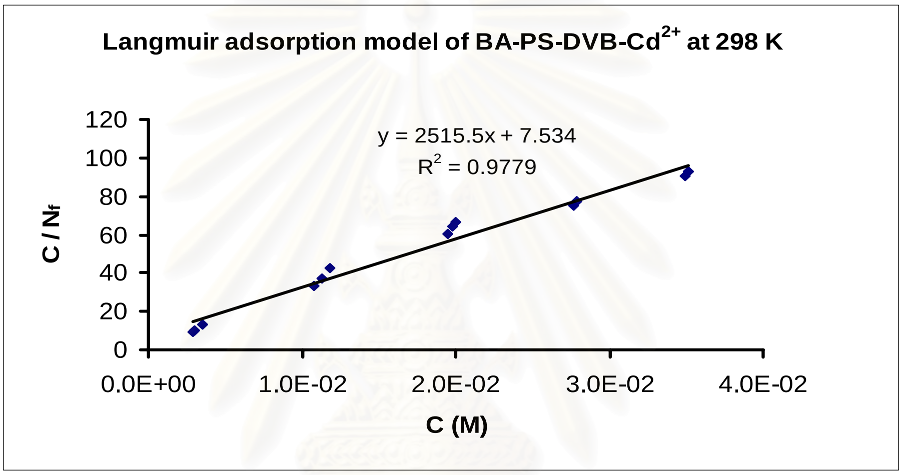
[a] Mean value, (n=3)

The maximum sorption capacity (N_s) on BA-PS-DVB determined from the Langmuir plot were 44.7, 64.2 and 204 mg g^{-1} for Cd(II), Cu(II), and Pb(II), respectively. In the same way, the adsorption capacities on azo-BA-PS-DVB were 116, 361 and 321 mg g^{-1}. These results confirmed the observation form the previous section that azo-BA-PS-DVB was more efficient than BA-PS-DVB.

Smaller binding association constant (b) corresponds to weaker binding affinity. Therefore, it was concluded that the azo-BA-PS-DVB appeared weaker binding affinity to metal ions than BA-PS-DVB. These results confirmed the observation form the previous section that the elution of metals from BA-PS-DVB was achieved by using higher concentration of nitric acid than from azo-BA-PS-DVB.

By comparing the b values of the same metal from two resins, it was observed that the b value of BA-PS-DVB was higher than that of azo-BA-PS-DVB because of the difference in ligand structure. BA-PS-DVB has one multidentate binding site containing O, N, and S atoms, while azo-BA-PS-DVB has two binding sites, one is an azo group and another one is benzothiazole group. The binding mechanism of BA-PS-DVB might be only chelation between metal ion and the multidentate binding site, while the metal ion could adsorbed on azo-BA-PS-DVB by two mechanisms, an ion-exchange with azo group and chelation with benzothiazole group. The association constant (b) calculated from Langmuir plot of azo-BA-PS-DVB was an average value from these two binding mechanisms. Thus, the b value of azo-BA-PS-DVB was lower than the one of BA-PS-DVB.

Regarding the maximum sorption capacity (N_s), the higher number of binding sites on azo-BA-PS-DVB, the higher maximum sorption capacity.



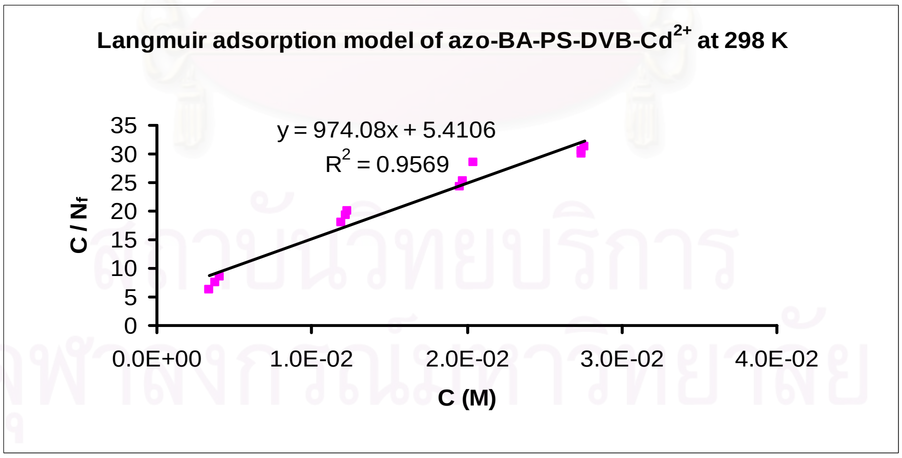
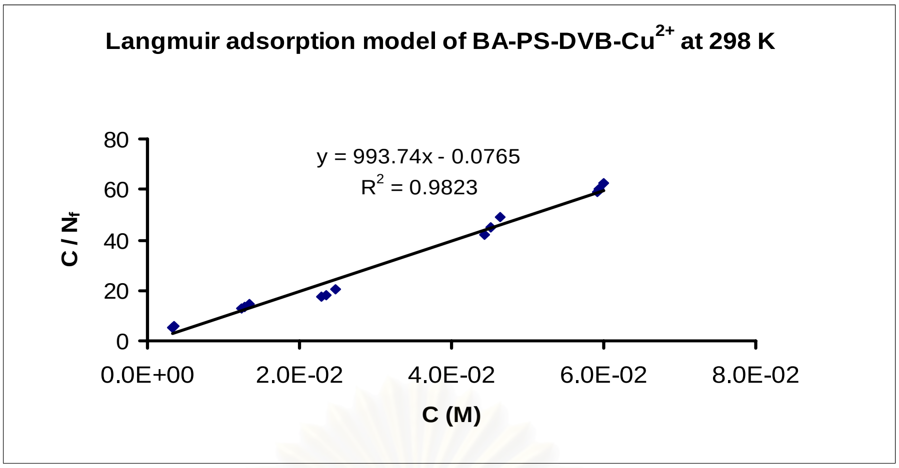


Figure 4.9 (a) Adsorption isotherm of Cd(II)



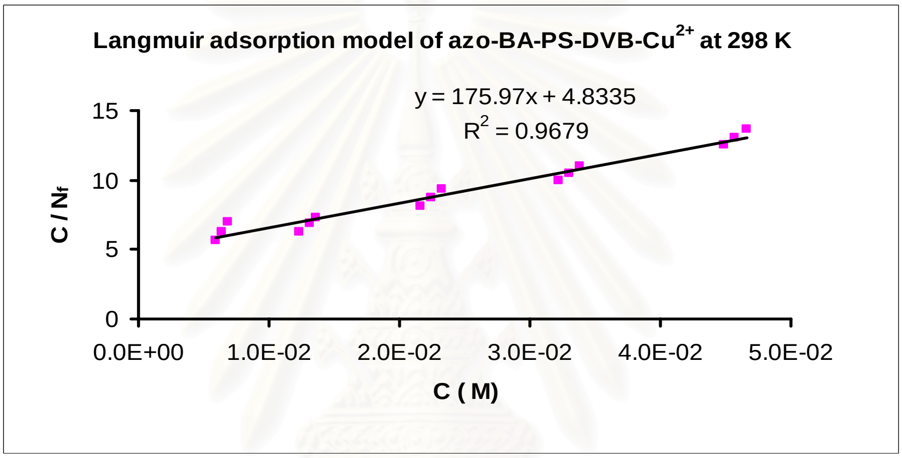
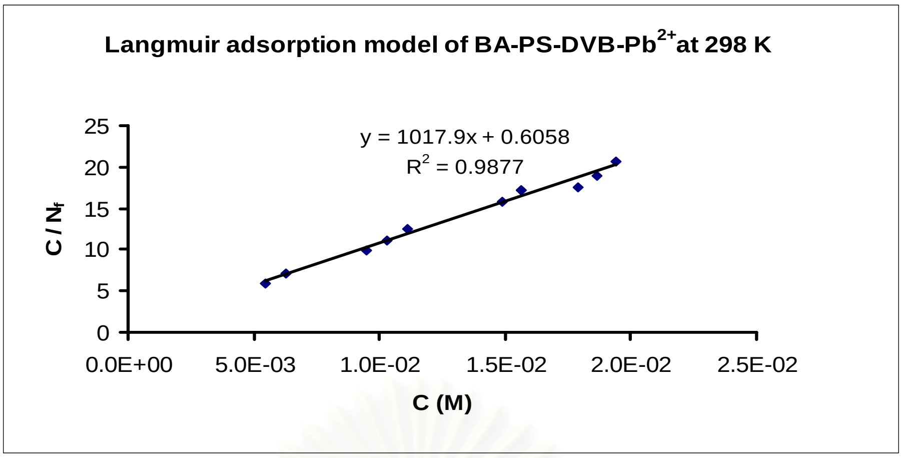


Figure 4.9 (b) Adsorption isotherm of Cu(II)



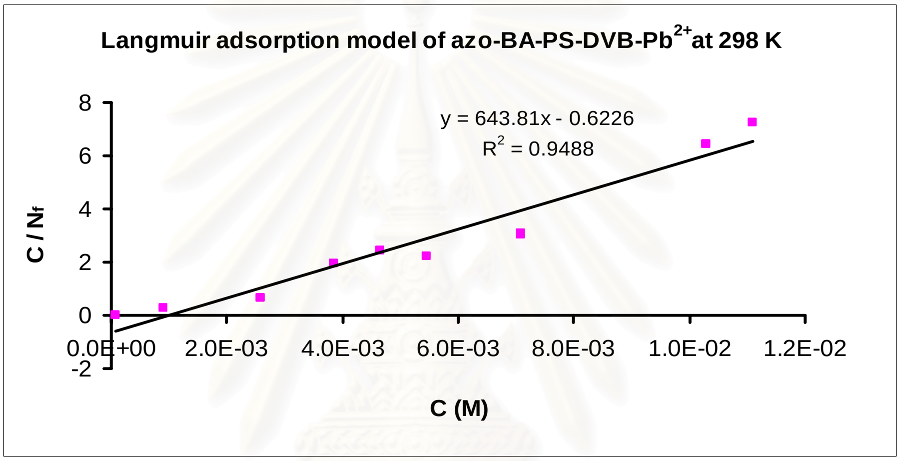


Figure 4.9 (c) Adsorption isotherm of Pb(II)

Figure 4.9 Adsorption isotherm of (a) Cd(II), (b) Cu(II) and (c) Pb(II) (n=3)

4.2.1.5 Selectivity of resins

The nature of functional group of chelating agent will give the selectivity of the ligand towards trace elements. According to the Hard Soft [Lewis] Acid Base Principle [HSAB]; Hard [Lewis] acids prefer to bind to hard [Lewis] bases and Soft [Lewis] acids prefer to bind to soft [Lewis] bases [52].

The result in mmol g^{-1} was shown in Table 4.3. These values were estimated from Langmuir plot.

Table 4.3 Selectivity of each metal ion calculated from experimental

Metals-sorbent	(mmol g^{-1})	
	BA-PS-DVB	**azo-BA-PS-DVB**
Cd(II)	0.398	1.03
Cu(II)	1.01	5.68
Pb(II)	0.982	1.55

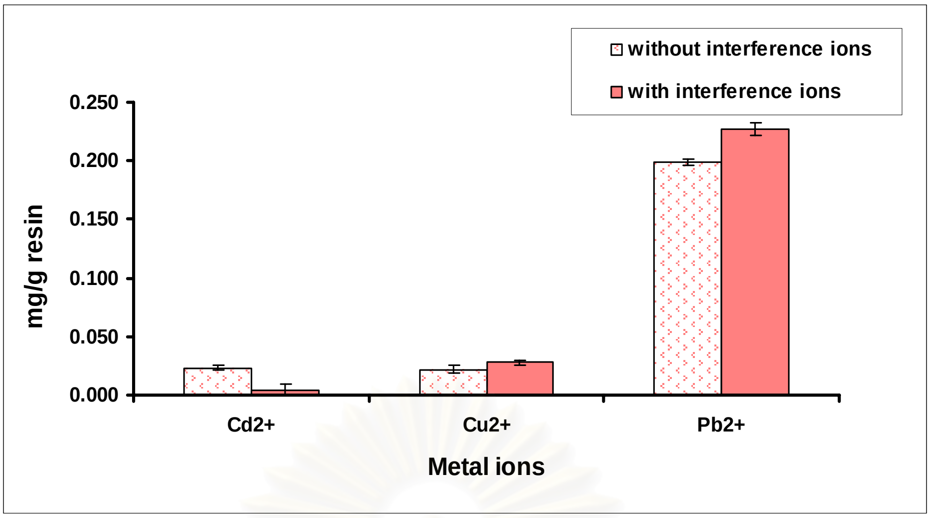
The selectivity order of metal ions on both BA-PS-DVB and azo-BA-PS-DVB were observed that Cu(II)> Pb(II)>Cd(II).

Cu(II) and Pb(II) are borderline Lewis acids. Borderline cations possess affinity for both hard and soft ligand. Then, ethyl 2-benzothiazolyacetate contains donor atoms such as nitrogen and sulfur which are soft ligands and oxygen which is hard ligand. Conversely, Cd(II) is a soft Lewis acid, that tends to form covalent bonds and possesses affinity for borderline (N) and soft (S) ligands. Therefore, the chelating agent prefers to bind to Cu(II) and Pb(II) than Cd(II).

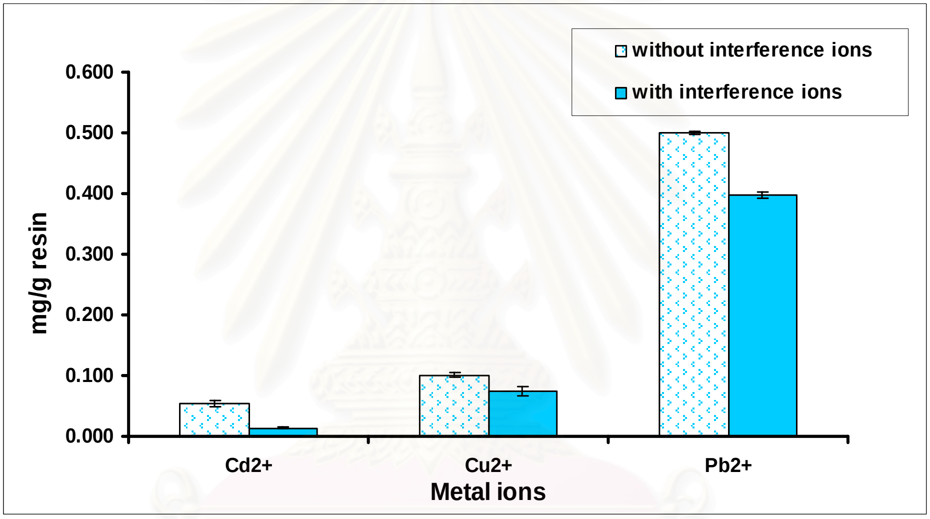
4.2.1.6 Effect of interference ions on the metal sorption

The effect of $NaSO_4$, KNO_3, $CaCl_2$, $Ca(NO_3)_2$ and $NaNO_3$ at concentration 1 g L^{-1} on the sorption of all metal ions (Cd(II) 1.0 mgl^{-1}, Cu(II) 1.0 mgl^{-1} and Pb(II) 5.0 mg L^{-1}) was studied.

The effect of interference ions on the metal sorption were illustrated in Figure 4.10.



(a) BA-PS-DVB



(b) azo-BA-PS-DVB

Figure 4.10 Effect of interference ions on the metal sorption (n=3)

The results showed that the interference ions affected Cd(II) sorption on BA-PS-DVB and azo-BA-PS-DVB. The interference ions did not significantly affect the sorption of Cu(II) on BA-PS-DVB, but they slightly decreased its sorption on azo-BA-PS-DVB. In case of Pb(II), the interference ions enhanced the sorption on BA-PS-DVB, but they quenched its sorption on azo-BA-PS-DVB. These results revealed that the active sites of both resins were different and the ionic strength of the solution had an influence on the sorption of all metals.

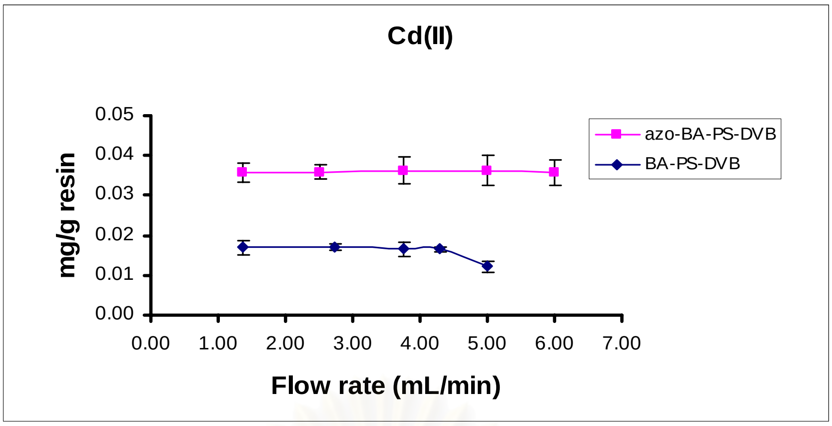
4.2.2 Column method

4.2.2.1 Effect of flow rates

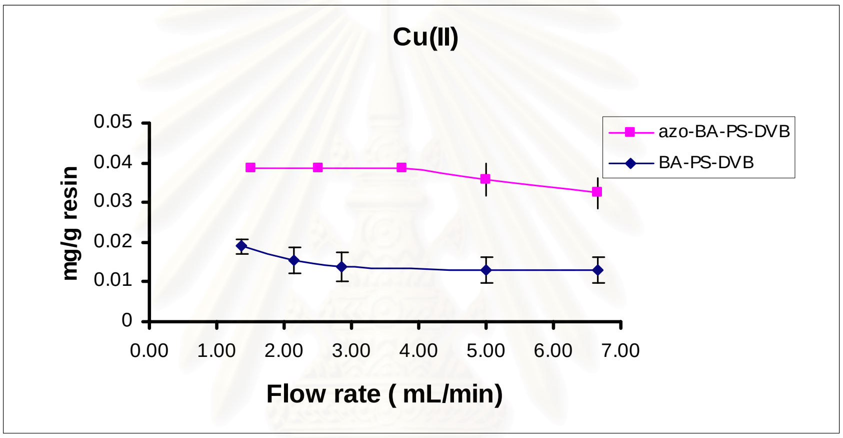
In the column procedure, the effect of the flow rate on sorption of metal ions was investigated. The studies show that the flow rate influences the sorption of metal ions. The results are illustrated in Figure 4.11.

The difference in the optimum range for each metal is due to the different kinetic properties. Metal sorption is minor at high flow rates due to increasing in the velocity of the ions that reduces the contact time between the sorbents, damaging the retention. On the other hand, low flow rates decreases sample frequencies, damaging system efficiency. The optimum flow rates for maximum loading of metal ions onto BA-PS-DVB and azo-BA-PS-DVB are in the range of 2.0-4.0 and 2.0-7.0 mL min^{-1}, respectively. Flow rates slower than 2.0 mL min^{-1} were not studied to avoid a longer time of analysis.

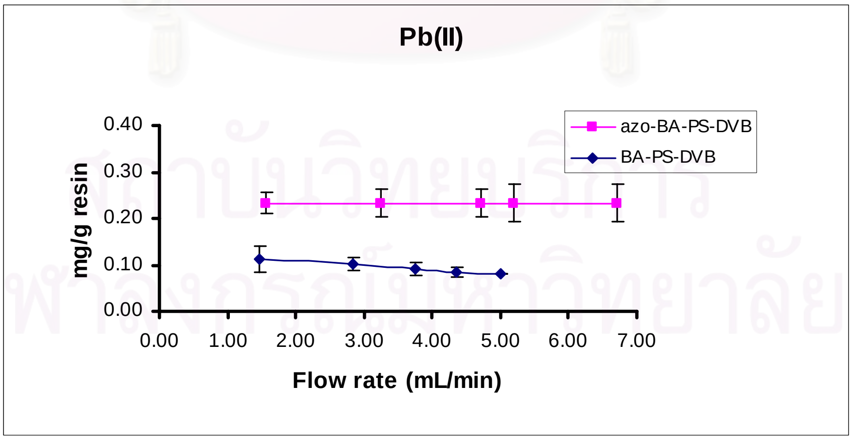
Percent extraction of Cd(II), Cu(II), and Pb(II) on BA-PS-DVB at flow rate 2.0-4.0 mL min^{-1}are 65-70, 60-83 and 60-84, respectively. If the higher flow rates were used, low percent extraction would be observed. For azo-BA-PS-DVB the flow rates did not affect the percent extraction of metal ions. At flow rate 2.0-7.0 mL min^{-1} percent extraction of Cd(II) and Pb(II) are constant at 70 and 79 but for Cu(II) it was slightly different in range 71-84. Thus, the flow rates of 3.0, 2.5 and 3.5 mL min^{-1} for BA-PS-DVB and the flow rates of 4.0 mL min^{-1} for azo-BA-PS-DVB were chosen for Cd(II), Cu(II), and Pb(II), respectively. Similarly, for desorption of metal ions with 5.0 mL of eluent, a flow rate of 1.0-2.0 mL min $^{-1}$ was found to be suitable.



(a)



(b)



(c)

Figure 4.11 Effect of flow rate on metal sorption (n=3);

(a) Cd(II), (b) Cu(II) and (c) Pb(II)

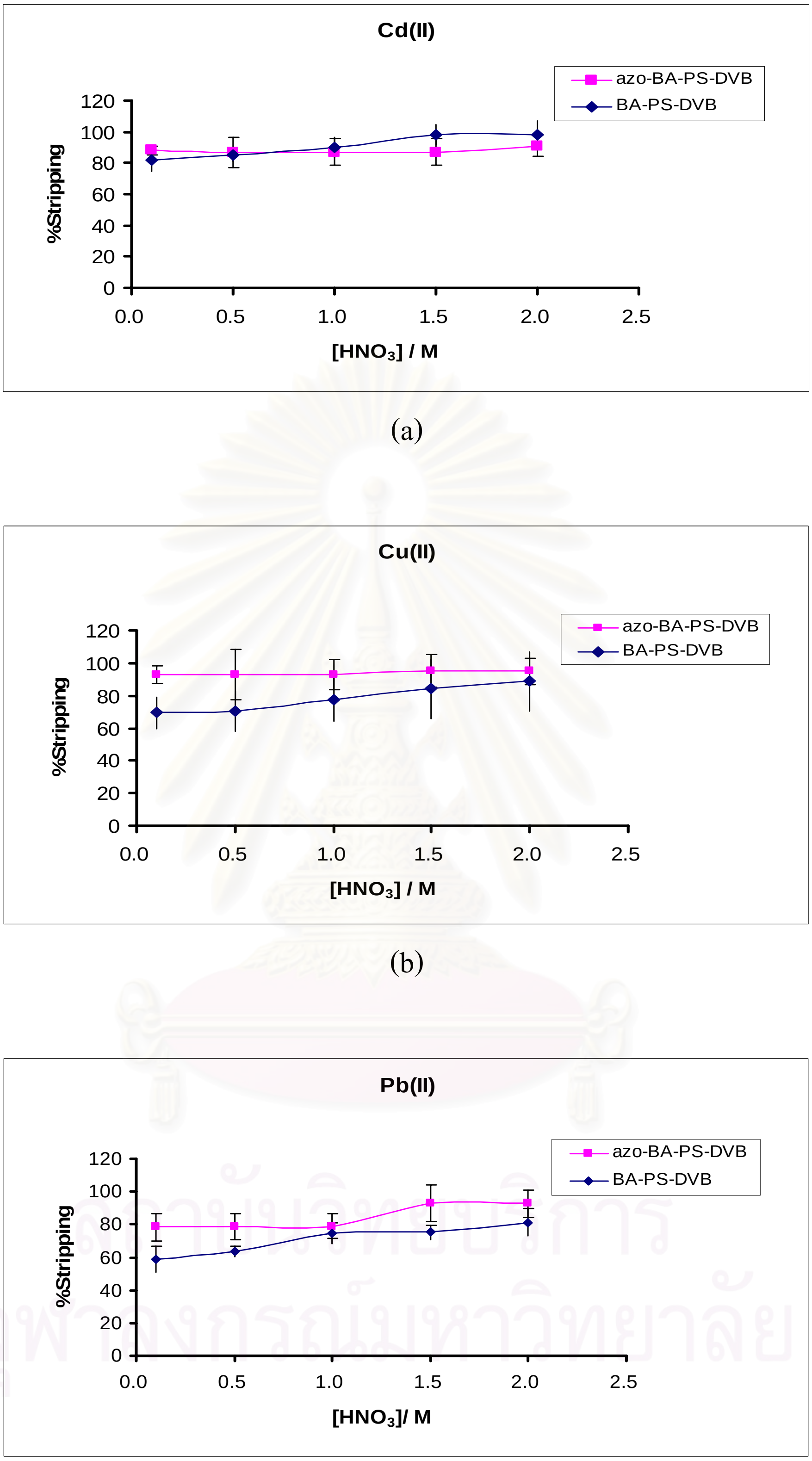
4.2.2.2 Effect of eluent

To obtain quantitative eluting, the effect of concentrations of eluent in the step of desorption of the metals from sorbent was investigated. The initial flow rates in adsorption steps were 3.0, 2.5 and 3.5 mL min^{-1} for BA-PS-DVB and the flow rates of 4.0 mL min^{-1} for azo-BA-PS-DVB were chosen for Cd(II), Cu(II), and Pb(II), respectively. The volume of eluent was 5.0 mL. For desorption of metal ions, a flow rate of 1.0-2.0 mL min^{-1} was found to be suitable. Because the smaller flow rate favorites the longer contact time giving high percent stripping.

For BA-PS-DVB, it was observed that 2.0 M nitric acid was able quantitatively elute Cd(II), Cu(II) and Pb(II) from the resin with the percent stripping of 98, 89 and 81, respectively. Percent extraction of 67, 77 and 89 and percent recovery of 66, 64 and 71 were achieved for Cd(II), Cu(II) and Pb(II), respectively.

Thus for, azo-BA-PS-DVB, it was observed that 0.5-2.0 M nitric acid was appropriate to elute Cd(II) and Cu(II) and Pb(II) from the resin with the percent stripping of 87, 95 and 93, respectively. Percent extraction of 70, 84 and 82 and percent recovery of 63, 80 and 71 were achieved for Cd(II), Cu(II) and Pb(II), respectively.

The results are shown in Figure 4.12.



(a)

(b)

(c)

Figure 4.12 Effect of nitric acid concentration on metal desorption (n=3);

(a) Cd(II), (b) Cu(II) and (c) Pb(II)

4.2.2.3 Effect of sample volume

In order to deal with real samples containing very low concentrations of trace metal ions, the maximum applicable sample must be considered. Therefore, the effect of sample volume on the recoveries was investigated. It was studied by a procedure using an increasing volume of metal solution, and keeping the total amount of loaded metal ions constant. The effect of sample volume on recoveries was shown in Table 4.4.

The recovery (%) in this section was calculated by the following equation 4.4.

$$\text{Recovery (\%)} = \frac{N_f}{N_s} \times 100 \quad \text{...} \quad (4.4)$$

where N_s = amount of metal ions (mg) in starting solution

N_f = amount of metal ions (mg) eluted from column

Table 4.4 Effect of sample volume on retention of metal-sorbent

Sample volume (mL)	**Recovery[a] (%)**					
	BA-PS-DVB			**azo-BA-PS-DVB**		
	Cd(II)	**Cu(II)**	**Pb(II)**	**Cd(II)**	**Cu(II)**	**Pb(II)**
25	20 ± 1.0	60 ± 7.5	13 ± 3.2	13 ± 3.1	82 ± 2.9	14 ± 2.9
50	20 ± 1.8	71 ± 5.7	13 ± 3.2	35 ± 2.4	82 ± 2.9	12 ± 0.22
100	33 ± 4.4	70 ± 5.7	13 ± 3.2	66 ± 3.4	86 ± 2.9	12 ± 0.22
250	40 ± 4.6	40 ± 6.9	13 ± 3.2	61 ± 1.8	86 ± 2.9	15 ± 2.9
500	26 ± 2.7	20 ± 2.9	13 ± 3.2	52 ± 1.0	57 ± 3.8	24 ± 2.9

[a] Mean value ± SD, (n=3)

The acceptable values of the recovery and RSD for trace analysis are summarized in Table 4.5. According to Table 4.4, it was found that the recoveries of Cu(II) ion on 0.1 g of BA-PS-DVB was not effected by sample volume between 25 to 100 mL. Conversely, the recoveries of Cd(II) and Pb(II) were effected by the sample volume, but the recoveries were fairly low. In addition, the recoveries of Cd(II) and Cu(II) on 0.05 g of azo-BA-PS-DVB was not obviously effected by the sample volume between 25 to 250 mL. In this study, the low recovery of Pb(II) was obtained. It probably not suitable for the preconcentration of Pb(II). Thus, a sample volume in this range could be used in our procedures.

In conclusion for column method, the BA-PS-DVB was only suitable for the preconcentration of Cu(II) and the azo-BA-PS-DVB was adequate for both Cd(II) and Cu(II). Thus, Cd(II) and Cu(II) were considered for further experiments.

Table 4.5 Analyte recovery and precision at different concentration [51]

Analyte, %	Analyte ratio	Unit	Mean recovery, %	RSD, %
100	1	100%	98-102	1.3
10	10^{-1}	10%	98-102	2.8
1	10^{-2}	1%	97-103	2.7
0.1	10^{-3}	0.1%	95-105	3.7
0.01	10^{-4}	100 ppm	90-107	5.3
0.001	10^{-5}	10 ppm	80-110	7.3
0.0001	10^{-6}	1 ppm	80-110	11
0.00001	10^{-7}	100 ppb	80-110	15
0.000001	10^{-8}	10 ppb	60-115	21
0.0000001	10^{-9}	1 ppb	40-120	30

4.3 Method validation

In order to demonstrate the validity of this method, the accuracy and precision of the method were investigated by using the sample solutions containing spiked metal.

For Cd(II) solution, 250 µL of 10 mg L^{-1} of Cd(II) was spiked into a solution of 250 mL (concentration of this solution was 10 µg L^{-1}) and the metal solution was passed through the column packed with 0.05 g of azo-BA-PS-DVB (under the optimum conditions), the metal was then eluted from the column by nitric acid 1.5 M (5.0 mL).

For Cu(II) solution, 250 µL of 10 mg L^{-1} of Cu(II) was spiked into a solution of 100 mL and 250 mL (concentrations of this solution were 25 and 10 µg L^{-1}, respectively) and the metal solution was passed through the column packed with 0.10 g of BA-PS-DVB or 0.05 g of azo-BA-PS-DVB (under the optimum conditions), the metal was eluted from the column by 2.0 M and 1.5 M (5.0 mL) nitric acid, respectively.

The accuracy and precision of the proposed method are present as recovery (%) and RSD (%), respectively. The limit of detection (LOD) is commonly defined as analyte concentration giving a signal equivalent to three times of standard deviation of blank signals (analyzed 10 times).

Limit of detection (LOD) is calculated by the following equation 4.5.

$$\mathrm{LOD}_{(\mathrm{mg/L})} = y_{(\mathrm{mg/L})} + 3\mathrm{SD}_{(\mathrm{mg/L})} \quad \text{...} \quad (4.5)$$

where $y_{(\mathrm{mg/L})}$; concentration of blank signal

$\mathrm{SD}_{(\mathrm{mg/L})}$; standard deviation of the concentration of the blank

Limit of quantitation (LOQ), which is regarded as the lower limit for precise quantitative measurements, as opposed to qualitative detection. The LOQ is calculated by the following equation 4.6.

$$\mathrm{LOQ}_{(\mathrm{mg/L})} = y_{(\mathrm{mg/L})} + 10\mathrm{SD}_{(\mathrm{mg/L})} \quad \text{...} \ (4.6)$$

Method detection limit (MDL) is estimated by equation 4.7.

$$\mathrm{MDL} = \frac{\mathrm{LOD}}{\mathrm{P.F.}} \quad \text{...} \ (4.7)$$

where P.F. is the preconcentration factor which is the ratio of the initial volume of sample used and the final volume.

The results of the recoveries, precision, the limit of detection (LOD), limit of quantitation (LOQ) and method detection limits (MDL) are shown in Table 4.6.

Table 4.6 Recovery, precision and detection limit of the proposed method

Elements	**P.F.**	**Recovery** [a]**, %**	**RSD** [a] **, %**	**LOD** [b] **(μg L^{-1})**	**LOQ** [b] **(μg L^{-1})**	**MDL**[b] **(μg L^{-1})**
Cd(II)[d]	50	71.2	7.26	9.00	30.0	0.18
Cu(II)[c]	20	74.0	9.99	18.0	60.0	0.90
Cu(II)[d]	50	74.0	7.05	18.0	60.0	0.36

P.F. is the preconcentration factor

[a] Mean value (n=15)

[b] Mean value (n=10)

[c] BA-PS-DVB

[d] azo-BA-PS-DVB

The recoveries of Cd(II) on azo-BA-PS-DVB, Cu(II) on BA-PS-DVB and azo-BA-PS-DVB obtained for the preconcentration of metals in solutions were 71.2, 74.0 and 74.0%, respectively, showing the performance and the accuracy of the method in preconcentration of trace levels of metal ions in solutions.

The relative standard deviation (%RSD) for the determination of metal ions in the preconcentration less than 10%, showing a very good precision of the method.

The preconcentration factor of Cd(II) on azo-BA-PS-DVB was up to 50 (in 5.0 mL of eluent). Therefore, the preconcentration factors of Cu(II) on BA-PS-DVB and azo-BA-PS-DVB were up to 20 and 50, respectively.

CHAPTER V

CONCLUSION

A solid-phase extraction (SPE) procedure was developed using polystyrene-divinylbenzene (PS-DVB) resin modified with benzothiazole derivative and then evaluated for its extraction and preconcentration of Cd(II), Cu(II) and Pb(II) in aqueous solution before their determinations by flame atomic absorption spectrometry (FAAS).

The preparation of the chelating resin is easier than that of other solid sorbents used for similar purpose. Synthesis of chelating agent, ethyl 2-benzothiazolyacetate (BA), was simpler in one step and coupled with amino-PS-DVB through amide and azo linkage, so called BA-PS-DVB and azo-BA-PS-DVB, respectively. The chelating agent was characterized by ^{1}H-NMR spectroscopy and UV-Visible spectrometry, the results showed that the cheating agent was successfully synthesized. The modified resins in each step were characterized by elemental analysis (EA) and infrared spectroscopy (IR). The elemental analysis results showed the difference of percent C, H and N in each stage. The IR results showed the benzothiazole derivative resin based on PS-DVB polymer bead was successfully synthesized.

For batch experiments, the initial pH of the solution affected the sorption behavior of all metal ions. In case of BA-PS-DVB, the highest sorption capacity for Cd(II) was observed with pH value 6.0-8.0. For Cu(II) and Pb(II), the optimum pH were 7.0 and 6.0, respectively. In case of azo-BA-PS-DVB, the highest sorption capacity of Cd(II) was observed with pH value 7.0-8.0. For Cu(II) and Pb(II), the optimum pH were 7.0 and 6.0, respectively. The new chelating resins showed a good stability at every pH of aqueous solution. The equilibration time for BA-PS-DVB and azo-BA-PS-DVB was observed around 10 minutes for Cd(II) and Pb(II) and 20 minutes for Cu(II). Adsorption behavior of all metal ions obeyed Langmuir adsorption model. The maximum sorption capacity on

BA-PS-DVB determined from the Langmuir plot were 44.7, 64.2 and 204 mg g^{-1} for Cd(II), Cu(II), and Pb(II), respectively. In the same way, the maximum sorption capacities on azo-BA-PS-DVB were 116, 361 and 321 mg g^{-1}. This results confirmed that azo-BA-PS-DVB was more efficient than BA-PS-DVB. The results showed it is much higher than those previously reported. The results are summarized in Table 5.1.

Table 5.1 Comparison of sorption capacities

Immobilized ligand	**Sorption capacity (mg g^{-1})**		
	Cd(II)	**Cu(II)**	**Pb(II)**
Thiosalocylic acid [53]	22.2	13.6	
o-aminophenol [48]	3.48	3.37	3.32
Chromotropic acid [54]	9.33	8.52	
2-(Methylthio)aniline [49]	23.7	8.83	
Ethyl 2-benzothiazolyacetate (amide linkage)	44.7	64.2	204
Ethyl 2-benzothiazolyacetate (azo linkage)	116	361	321

The selectivity order of metal ions on BA-PS-DVB and azo-BA-PS-DVB were observed that Cu(II)>Pb(II)>Cd(II). Thus, the effect of interference ions, $NaSO_4$ KNO_3, $CaCl_2$, $Ca(NO_3)_2$ and $NaNO_3$, on the metal sorption was investigated. The results showed that the interference ions affected for Cd(II) sorption on BA-PS-DVB and azo-BA-PS-DVB, which were decreased the sorption capacity of Cd(II). The interference ions were not significantly affected for the sorption of Cu(II) on BA-PS-DVB, but they slightly resulted in decreasing its sorption on azo-BA-PS-DVB. In case of Pb(II), the interference ions enhanced the sorption on BA-PS-DVB, but they quenched its sorption on azo-BA-PS-DVB.

In column method, the optimum flow rates for maximum loading of metal ions onto BA-PS-DVB and azo-BA-PS-DVB were in the ranges of 2.0-4.0 and 2.0-7.0 mL min^{-1}. Metal ions sorped onto BA-PS-DVB column were eluted by 2.0 M nitric acid with the percent stripping of 98, 89 and 81 for Cd(II), Cu(II) and Pb(II), respectively. Thus, for azo-BA-PS-DVB column, it was observed that 0.5-2.0 M nitric acid was appropriate to elute Cd(II), Cu(II) and Pb(II) from the resin with the percent stripping of 87, 95 and 93, respectively.

The preconcentration factors of Cd(II) and Cu(II) on azo-BA-PS-DVB and Cu(II) on BA-PS-DVB were 50, 50, and 20, respectively. The recoveries of Cu(II) on BA-PS-DVB and Cd(II) and Cu(II) on azo-BA-PS-DVB were 71.2, 74.0 and 74.0%, respectively, showing the performance and the accuracy of the method in preconcentration of trace level metal ions in aqueous solutions. The overall relative standard deviation (RSD) less than 10% (n=15), showing a good precision of method.

Potential Applications and Suggestions for Future Work

The present method should be applied to the determination of all metal ions in real sample such as, tap water, CU pond water and waste water from industrial. The effect of major cations and anions in sample solution should be studied further.

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