

พัฒนาเทคนิคการเพิ่มความเข้มข้นของโลหะโดยใช้คาร์บอนกัมมันต์
เพื่อหาปริมาณโลหะในเนื้อปลา



นางสาวพรลดา ดาวรัตน์ชัย

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาเคมี ภาควิชาเคมี

คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2546

ISBN 974-17-5207-5

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

**PRECONCENTRATION TECHNIQUE USING ACTIVATED CARBON
FOR DETERMINATION OF METALS IN FISH**

Miss Pornlada Daorattanachai

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

**A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Chemistry
Department of Chemistry
Faculty of Science
Chulalongkorn University
Academic Year 2003
ISBN 974-17-5207-5**

Thesis title Preconcentration technique using activated carbon for
determination of metals in fish
By Miss Pornlada Daorattanachai
Field of study Chemistry
Thesis Advisor Apichat Imyim, Ph.D.
Co-Advisor Fuangfa Unob, Ph.D.

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

.....Dean of the Faculty of Science
(Professor Piamsak Menasveta, Ph.D.)

Thesis committee

.....Chairman
(Professor Sophon Roengsumran, Ph.D.)

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

.....Co-Advisor
(Fuangfa Unob, Ph.D.)

.....Member
(Pakorn Varanusupakul, Ph.D.)

.....Member
(Assistant Professor Wilaiwan Utoomprurkorn, Ph.D.)

พรลดา ดาวรัตน์ชัย : พัฒนาเทคนิคการเพิ่มความเข้มข้นของโลหะโดยใช้คาร์บอนกัมมันต์ เพื่อหาปริมาณโลหะในเนื้อปลา. (PRECONCENTRATION TECHNIQUE USING ACTIVATED CARBON FOR DETERMINATION OF METALS IN FISH) อ.ที่ปรึกษา อ. ดร. อภิชาติ อิ่มอิม, อ.ที่ปรึกษาร่วม อ. ดร. เฟื่องฟ้า อุ๋นอบ, 76 หน้า. ISBN 974-17-5207-5

พัฒนาเทคนิคที่ใช้ในการเพิ่มความเข้มข้นของโลหะ สำหรับวิเคราะห์โลหะแคดเมียม ทองแดง นิกเกิล ตะกั่ว และสังกะสีในสารละลายด้วยเทคนิคเฟสของแข็ง โดยใช้คาร์บอนกัมมันต์ และใช้แอมโมเนียมไฟโรรีดินไดไทโอคาร์บามาต และวิเคราะห์หาปริมาณโลหะด้วยเทคนิคอะตอมมิกแอบซอร์บชันสเปกโทรเมตรี โดยศึกษาวิธีการในการเพิ่มความเข้มข้นของโลหะบนคาร์บอนกัมมันต์ที่เคลือบด้วยแอมโมเนียมไฟโรรีดินไดไทโอคาร์บามาต และการเพิ่มความเข้มข้นของโลหะ โดยการทำให้เกิดสารประกอบเชิงซ้อนกับแอมโมเนียมไฟโรรีดินไดไทโอคาร์บามาต ก่อนจะผ่าน คาร์บอนกัมมันต์ สภาวะที่เหมาะสมในการเพิ่มความเข้มข้นของโลหะจะถูกศึกษาและทดสอบกับ สารละลายตัวอย่างก่อนจะนำไปใช้กับตัวอย่างเนื้อปลา พบว่า %recovery และ %RSD ของทั้งสองวิธีให้ผลการทดลองที่ไม่แตกต่างกัน และเมื่อนำวิธีการเพิ่มความเข้มข้นของโลหะด้วยเทคนิค เฟสของแข็งทั้ง 2 วิธีมาใช้วิเคราะห์ตัวอย่างเนื้อปลาพบว่า การเพิ่มความเข้มข้นของโลหะโดยการ ทำให้เกิดสารประกอบเชิงซ้อนกับแอมโมเนียมไฟโรรีดินไดไทโอคาร์บามาต ก่อนจะผ่านคาร์บอน กัมมันต์ ให้ผลดีกว่าการเพิ่มความเข้มข้นของโลหะบนคาร์บอนกัมมันต์ที่เคลือบด้วยแอมโมเนียม ไฟโรรีดินไดไทโอคาร์บามาต โดยอิทธิพลของตัวแปรต่าง ๆ อย่างเช่น อัตราการไหล ปริมาณของ แอมโมเนียมไฟโรรีดินไดไทโอคาร์บามาต และค่าความเป็นกรด-เบส ในการเพิ่มความเข้มข้นการ เพิ่มความเข้มข้นของโลหะโดยการทำให้เกิดสารประกอบเชิงซ้อนกับแอมโมเนียมไฟโรรีดินไดไท โอคาร์บามาต ก่อนจะผ่านคาร์บอนกัมมันต์จะถูกศึกษาและทำการวาลิเดชัน พบว่า %RSD เท่ากับ 4.53, 15.28, 0.64, 5.28 และ 5.97% MDL เท่ากับ 11.70, 10.60, 13.40, 10.05 และ 12.65 $\mu\text{g kg}^{-1}$ และ %recovery เท่ากับ 97.42, 92.50, 81.87, 88.30 และ 101.06% สำหรับการ วิเคราะห์โลหะแคดเมียม ทองแดง นิกเกิล ตะกั่ว และสังกะสีตามลำดับ

ภาควิชา.....เคมี.....

สาขาวิชา.....เคมี.....

ปีการศึกษา.....2546.....

ลายมือชื่อ.....

ลายมือชื่ออาจารย์ที่ปรึกษา.....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4472342023: MAJOR CHEMISTRY

KEY WORDS: SPE, ACTIVATED CARBON, METAL, FISH,
AMMONIUM PYRROLIDINEDITHIOCARBAMATE

PORNLADA DAORATTANACHAI: PRECONCENTRATION

TECHNIQUE USING ACTIVATED CARBON FOR DETERMINATION OF
METALS IN FISH. THESIS ADVISOR: APICHAT IMYIM, Ph.D.

CO-ADVISOR: FUANGFA UNOB, Ph.D. 76 pp. ISBN 974-17-5207-5

A preconcentration procedure was developed for the determination of trace levels of Cd, Cu, Ni, Pb and Zn in aqueous solution. Ammonium pyrrolidinedithiocarbamate (APDC) and activated carbon were used for preconcentration in SPE technique following by determination by AAS. This work consisted of the study of preconcentration of the metals on an activated carbon impregnated with APDC column and preconcentration of metallic complexes on an activated carbon column. The optimum conditions were investigated and tested with model solutions. The %recovery and %RSD achieved with the two methods were similar. The methods were applied for determination of the metals in a solution obtained from digestion of fish tissue. It was found that the preconcentration of metallic complexes on activated carbon column was more efficient. The influences of various parameters such as flow rate, amount of APDC and pH in preconcentration of metallic complexes in fish tissue on activated carbon column were investigated. The present method was validated, and the good results were observed such as the %RSD were 4.53, 15.28, 0.64, 5.28, and 5.97%, the MDL were 11.70, 10.60, 13.40, 10.05, and 12.65 $\mu\text{g kg}^{-1}$, and the %recovery was 97.42, 92.50, 81.70, 88.30, and 101.06% for Cd, Cu, Ni, Pb and Zn, respectively.

Department.....Chemistry.....

Field of study.....Chemistry.....

Academic year.....2003.....

Student's signature.....

Advisor's signature.....

Co-advisor's signature.....

ACKNOWLEDGEMENT

The success of this reseach can be attributed to the extensive support and assistance from my advisor, Dr. Apichat Imyim and my co-advisor, Dr. Fuangfa Unob. In addition, I would like to thank and pay my respect to Professor Dr. Sophon Roengsumran, Dr. Pakorn Varanusupakul and Assistant Professor Dr. Wilaiwan Utoomprurkporn for their valuable suggestions as committees and thesis examiners.

This work cannot be completed without kindness and helps of many people. I would like to thank Dr. Wanlapa Aeungmaitrepiromfor for her suggestions and helps. My thanks is also extended to the Environmental Analysis Research Group of Chulalongkorn University.

Finally, I am grateful to my family for their financial support, entirely care, 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.



สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

CONTENTS

	Page
Abstract in Thai.....	iv
Abstract in English.....	v
Acknowledgement.....	vi
Contents.....	vii
List of Figures.....	xii
List of Tables.....	xiii
List of Symbols and Abbreviations.....	xv
CHAPTER I INTRODUCTION.....	1
1.1 Heavy metals in the environment.....	1
1.2 Background information of metals.....	4
1.2.1 Cadmium.....	4
1.2.2 Copper.....	4
1.2.3 Nickel.....	5
1.2.4 Lead.....	5
1.2.5 Zinc.....	6
1.3 Determination of metals in seafood.....	7
1.3.1 Mineralization of seafood samples.....	7
1.3.2 Determination of metals.....	8
1.4 Preconcentration of trace elements.....	9
1.5 Theory of solid phase extraction.....	11
1.5.1 Retention of traces elements on the sorbent.....	11
1.5.1.1 Adsorption.....	11
1.5.1.2 Chelation.....	11
1.5.1.3 Ion-paring.....	12
1.5.1.4 Ion exchange.....	12
1.5.2 Step-by-step method development guide.....	13
1.5.2.1 Selection of solid sorbent.....	13

CONTENTS (CONTINUED)

	Page
1.5.2.2 Influential parameters.....	13
1.5.2.2.1 Conditioning parameters.....	13
1.5.2.2.2 Loading parameters.....	14
1.5.2.2.3 Elution parameters.....	15
1.6 Application of SPE to the determination of trace elements.....	15
1.7 Conclusion.....	18
1.8 Aims and scope of this work.....	19
 CHAPTER II EXPERIMENT SECTION	 20
 2.1 Apparatus.....	 20
2.1.1 UV-Vis Spectrophotometer.....	20
2.1.2 Flame atomic absorption spectrometer.....	20
2.1.3 Graphite furnace atomic absorption spectrometer.....	21
2.1.4 Manifold.....	22
2.1.5 Microwave digestion unit.....	23
2.2 Chemicals.....	24
2.3 Preparation of solution.....	25
2.3.1 Working standard solutions.....	25
2.3.2 Mixed working standard solution.....	25
2.3.3 Chelating agents.....	25
2.3.4 Ammonium acetate buffer solution.....	25
2.3.5 Model solution.....	25
2.4 Preconcentration of the activated carbon SPE.....	26
2.4.1 Preparation of the activated carbon.....	26
2.4.2 Preparation of the impregnated activated carbon.....	26
2.5 Preconcentration procedure of model solution.....	27
2.5.1 Preconcentration of metals in solution on impregnated	

CONTENTS (CONTINUED)

	Page
activated carbon.....	27
2.5.1.1 Efficiency of impregnated activated carbon....	27
2.5.1.2 Desorption of metals.....	27
2.5.1.3 Effect of the sample volume on the metal sorption.....	28
2.5.1.4 Effect of the amount of activated carbon.....	28
2.5.1.5 Effect of nitrate ion.....	28
2.5.2 Preconcentration of metallic complexes in solution on activated carbon.....	28
2.5.2.1 Effect of amount of complexing agent.....	29
2.6 Preconcentration procedure of metals from fish tissues.....	29
2.6.1 Sample preparation.....	29
2.6.2 Preconcentration of metals in sample on impregnated activated carbon.....	30
2.6.2.1 Matrix effect on metals sorption	30
2.6.2.2 Effect of the digestion procedure.....	30
2.6.3 Preconcentration of metallic complex in sample on activated carbon.....	30
2.6.3.1 Effect of the flow rate on sorption.....	31
2.6.3.2 Effect of the amount of complexing agent.....	31
2.6.3.3 Influences of pH of sample solution on sorption.....	31
2.7 Method validation.....	32
2.8 Application to water and soil.....	33
2.8.1 Water.....	33
2.8.2 Soil.....	33

CONTENTS (CONTINUED)

	Page
CHAPTER III RESULTS AND DISCUSSION.....	35
3.1 Preconcentration of model solutions.....	35
3.1.1 Preconcentration of metals in model solution on impregnated activated carbon.....	35
3.1.1.1 Efficiency of impregnated activated carbon....	35
3.1.1.2 Desorption of metals.....	37
3.1.1.3 Effect of the sample volume on metal sorption.....	40
3.1.1.4 Effect of the amount of activated carbon.....	42
3.1.1.5 Effect of nitrate ion.....	48
3.1.2 Preconcentration of metallic complex in solution on activated carbon.....	49
3.1.2.1 Effect of the amount of complexing agent.....	49
3.2 Preconcentration of metals from fish tissue.....	51
3.2.1 Preconcentration of metals in sample on impregnated activated carbon.....	51
3.2.1.1 Matrix effect on metals sorption.....	52
3.2.1.2 Effect of digestion method.....	53
3.2.2 Preconcentration of metallic complex in sample on activated carbon.....	55
3.2.2.1 Effect of the flow rate on metal recoveries.....	56
3.2.2.2 Effect of the amount of complexing agent.....	57
3.2.2.3 Influence of pH on metal sorption.....	59
3.4 Method Validation.....	62
3.5 Application to water and soil samples.....	65

CONTENTS (CONTINUED)

	Page
CHAPTER IV CONCLUSION	68
REFERENCES.....	70
VITA.....	76



สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

LIST OF FIGURES

	Page
Figure 2.1 Visiprep™ SPE vacuum manifold.....	22
Figure 3.1 Effect of the amount of activated carbon on the analyte recoveries using 10 mL of 1 M nitric acid in acetone as eluent.....	42
Figure 3.2 Effect of the volume of eluent for 100 mg of activated carbon.....	43
Figure 3.3 Effect of the volume of eluent for 300 mg of activated carbon.....	44
Figure 3.4 Effect of the volume of eluent for 500 mg of activated carbon.....	45
Figure 3.5 Effect of the volume of eluent for 700 mg of activated carbon.....	46
Figure 3.6 Effect of the volume of eluent for 1000 mg of activated carbon.....	47
Figure 3.7 Effect of sodium nitrate on the metals sorption	48
Figure 3.8 Effect of amount of APDC on the analyte retention	50
Figure 3.9 Matrix effect on the analyte retention	52
Figure 3.10 Effect of hydrogen peroxide on the metals sorption	53
Figure 3.11 Effect of the amount of reagent on the analyte retention	57
Figure 3.12 Effect of pH on the sorption of metallic complex on activated carbon..	59

LIST OF TABLES

		Page
Table 1.1	List of maximum of levels of contaminants at human tolerance.....	2
Table 1.2	Results of survey of trace elements in 7 markets in Bangkok	3
Table 1.3	Major uses of cadmium	4
Table 1.4	Major uses of lead	6
Table 1.5	Typical detection limits (DL) of elemental analysis techniques.....	9
Table 2.1	FAAS conditions for the determination of elements in solutions.....	20
Table 2.2	Instrumental operating conditions for determination of cadmium, copper, lead, nickel and zinc	21
Table 2.3	THGA temperature program for the determination of Cd, Cu, Ni, Pb and Zn	22
Table 2.4	Condition for microwave digestion unit	23
Table 2.5	Chemical lists	24
Table 3.1	Percentage of metal sorption of untreated and impregnated activated carbon	36
Table 3.2	Comparative data from recent references on metals determination by SPE	37
Table 3.3	The effect of different eluents on elution of Cu	38
Table 3.4	Recoveries of Cd, Cu, Ni, Pb and Zn in model solution after preconcentration on impregnated activated carbon using 1 M nitric acid in acetone as eluent	39
Table 3.5	Effect of the sample volume on the sorption of trace metal ions on the activated carbon impregnated with APDC	40
Table 3.6	Recoveries of Cd, Cu, Ni, Pb and Zn from the model solution of different volume	41
Table 3.7	Recoveries of Cd, Cu, Ni, Pb and Zn in model solution using different amount of 1% w/v APDC	50
Table 3.8	Recoveries of Cd, Cu, Ni, Pb and Zn in sample solution	51

LIST OF TABLES (CONTINUED)

		Page
Table 3.9	Recoveries of Cd, Cu, Ni, Pb and Zn in sample solution	54
Table 3.10	Recoveries of Cd, Cu, Ni, Pb and Zn in sample solution	55
Table 3.11	Recoveries of Cd, Cu, Ni, Pb and Zn from different flow rate of sample solution	56
Table 3.12	Recoveries of Cd, Cu, Ni, Pb and Zn in sample solution from preconcentration procedure	58
Table 3.13	Recoveries of Cd, Cu, Ni, Pb and Zn in sample solution from preconcentration procedure	60
Table 3.14	Recoveries of Cd, Cu, Ni, Pb and Zn in sample solution from condition with acetone followed ammonium acetate buffer solution..	61
Table 3.15	Recovery, precision and detection limit of the proposed method	63
Table 3.16	Analyte recovery and precision at different concentration	64
Table 3.17	Concentrations of Cd, Cu, Ni, Pb and Zn in fish tissue samples	64
Table 3.18	Recoveries of Cd, Cu, Ni, Pb and Zn in water sample	65
Table 3.19	Recoveries of Cd, Cu, Ni, Pb and Zn in soil sample	67

LIST OF SYMBOLS AND ABBREVIATION

FAAS	Flame atomic absorption spectrometry
GFAAS	Graphite furnace atomic absorption spectrometry
ICP-AES	Inductively coupled plasma-atomic absorption spectrometry
ICP-MS	Inductively coupled plasma-mass spectrometry
XRF	X-ray fluorescence spectrometry
SPE	Solid phase extraction
APDC	Ammonium pyrrolidinedithiocarbamate
THGA	Transversely heated graphite atomizer
RSD	Relative standard deviation
IDL	Instrument detection limit
MDL	Method detection limit



สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER I

INTRODUCTION

Increasing industrialization has been accompanied throughout the world by the extraction and distribution of mineral substances from their natural deposits. Following concentrations, many of these have undergone chemical changes through technical processes and finally passed, and finely dispersed in solutions by ways of effluent, sewage, dumps and dust, into the water, the earth, the air and, hence into the food chain. Thus, the determination of their samples plays an important role in the monitoring of environmental analysis. The information obtained can serve as base to characterize the level of pollution of a certain area.

1.1 Heavy metals in the environment

Metals are described as “heavy metals” 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. They occur as pure elements, ions and complexes. Heavy metals are 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 large tendency to form complexes, especially with nitrogen, sulphur and oxygen containing ligand of the biological milieu [2]. Toxicological effects can be explained by this interaction. As a result, changes in molecular structure of proteins, breaking of hydrogen bonds or inhibition of enzymes can take place. Acute poisoning is rarely observed usually as the result of suicide

attempts or accidents. Chronic toxicity is much more relevant, caused by repeated exposure over long periods of time. Besides the fact that mercury, cadmium and arsenic are highly toxic, some 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, standards 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 1.1. In 2002, Fisheries Department of Thailand published the results of a survey on trace elements in fish as shown in Table 1.2.

Table 1.1 List of maximum levels of contaminants at human tolerance [3]

Contaminant	Maximum level at human intake/day (mg of contaminant/ 1 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

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Table 1.2 Results of survey of trace elements in fishes from 7 markets in Bangkok [4]

Species	Range of mean metal levels (mg/kg)		
	Lead	Mercury	Cadmium
Mullet	0.268	0.011	0.022
White snapper	0.105-0.381	0.025-0.153	0.017-2.067
Red snapper	0.075-0.303	0.052-0.121	0.018-0.061
White butterfish	0.053-0.364	0.004-0.034	0.021-0.567
Black butterfish	0.111-0.374	0.008-0.051	0.038-0.166
Mackerel	0.194-0.486	0.006-0.049	0.036-0.184
Turbot	0.157-0.429	0.021-0.393	0.033-0.116

Before assessing the requirements for trace element analysis, it is necessary to identify the sources of elemental contamination and the effect of environmental pollution.

1.2 Background information of metals [1, 2]

1.2.1 Cadmium

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

Cadmium also is a frequent material in industrial waste discharges and has been introduced into water systems through mining operations. It has also been employed in metal plating, where it is used as a sacrificial coating to prevent rust on steel. Some uses of the element are summarized in Table 1.3.

Table 1.3 Major uses of cadmium

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
Fungicide	Various compounds have limited used, mostly on golf courses

1.2.2 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 metals, large amounts are toxic.

1.2.3 Nickel

Nickel is widely distributed in the environment. Nickel concentration in environment is related to the consumption of fossil fuels, emissions from nickel mining and refining, and by the incineration of wastes. Nickel dusts can rapidly damage the respiratory system and can cause occupation cancers, including lung cancer.

1.2.4 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 group 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 1.4) 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 a 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 1.4 shows some applications of lead.

Table 1.4 Major uses of lead

As metal	
Batteries	Lead-acid storage batteries are most widely used.
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
Stabilizers in plastics	Heat stabilizer in poly(vinyl chloride)
As organolead	
Gasoline additives	Generally phased out except in undeveloped nations

1.2.5 Zinc

Zinc is a nutritionally essential element. It is necessary for growth and is involved in several physiological functions. Some of zinc compounds can be hazardous. These include zinc chloride, zinc sulfate, zinc sulphide, zinc chromate and zinc oxide. Zinc oxide has been identified as the main cause of metal fume fever. Among its symptoms are headache, fever, chills, muscle aches and vomiting. The fume is irritating to the upper respiratory tract. An outdoor exposure to zinc chloride fume may also be encountered from smoke generators and smoke bombs. The ingestion of elemental zinc has been shown to cause lethargy. Eating large amount of zinc can cause stomach cramps, nausea and vomiting.

Zinc enters the environment in a number of ways. Some is released through natural process but most comes from human activities such as mining, coal burning, steel production and burning of waste.

1.3 Determination of metals in seafood

1.3.1 Mineralization of seafood samples

When measuring trace elements in solid samples as seafood products, a prior sample pre-treatment stage is mandatory. Due to the absence of insoluble substances in this sample type compared with other environmental samples such as solids, sediments or plants, wet or dry ash procedures can be successfully applied. However, the time for the sample pre-treatment exceeds the time needed for the measurement, therefore, this preliminary step of the analytical method worthies being carefully studied. The sample pre-treatment stage commonly consists of destroying organic matters in the sample. Wet ash digestion with concentrated acids and alkaline sample decomposition procedures are the most reported sample pre-treatments for the metal determination in biological materials by atomic spectrometric techniques. The analytical portion is digested with HNO_3 or a mixture of HNO_3 and HCl . Combinations of HNO_3 , HClO_4 , and H_2SO_4 have also been used for more efficient oxidation [5]. Dry ash digestion is a long, slow ashing step, usually performed overnight in a muffle furnace [6]. The AOAC Official Method 999.11 [7] suggested the use of dry ashing and flame and graphite furnace for determination of copper, iron, and zinc in fish tissue.

The large time required to perform total sample decomposition has been shortened with the use of microwave energy to speed up the digestion process. The AOAC Official Method 999.10 [8] suggested the use of microwave digestion for decomposition of fish tissue.

Regardless of the digestion procedure selected, appropriate quality assurance and quality control guidelines need to be followed. These include the use of contamination control, digestion blanks, spiked test portion, replicate analyses, an appropriate standard reference material, and recovery calculations [6].

1.3.2 Determination of metals

Selection of a 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. For these reasons only a few methods developed for seafood analysis are used for routine testing. Spectrometric techniques that commonly used are flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption spectrometry (GFAAS), 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 fish because of the ease and affordability. The AOAC Official Method 999.10 suggested the use of FAAS [8]. However, detection limits of FAAS are too high for some elements. In this case, GFAAS is the method of choice due to its higher sensitivity. But, 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 called for. Another strategy to reduce the dilution encountered in sample preparation is direct solid sampling into the graphite tube [6].

ICP-AES has also been used for routine elemental analysis of shellfish [6]. Sequential or simultaneous mode ICP-AES allows rapid analysis, dramatically improving throughput. Detection limits in ICP-AES are generally the same as those in FAAS except for elements that are difficult to be atomized.

ICP-MS have been used for the determination of metals but the cost of the required instrumentation may be prohibitive to many laboratories.

Other methods developed for trace element determination in shellfish include voltammetry, polarography, HPLC with electrochemical and spectrophotometric detection [6].

In Table 1.5, the comparison of detection limits of some metals by using various techniques is summarized.

Table 1.5 Typical detection limits (DL) of elemental analysis techniques [9]

Element	Flame AAS (ppb)	GFAAS (ppb)	ICP-AES ^a (ppb)	ICP-AES ^b (ppb)	ICP-MS (ppt)
Cd	1.5	0.02	1	0.1	0.01-0.1
Cu	3	0.07	2	0.3	0.1-1
Ni	10	0.24	6	0.4	0.1-10
Pb	10	0.04	14	1	0.01-0.1
Zn	1.0	0.0075	1	0.06	0.01-0.1

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

^a Radial

^b Axial

1.4 Preconcentration of trace elements

Various techniques have been applied to the determination of trace heavy metal in biological samples. Direct instrumental analysis of samples is difficult because of many complexes and large matrices, which always affect the capability of the instrument to obtain the correct result in normal operation [10]. In addition to the interference of matrices in the analysis, some metals have concentration in the mgL^{-1} or μgL^{-1} range, which are near or below the limit of detection of the instrument. The preconcentration step can solve the above two problems and lead to easy determination. There are many methods of preconcentration, including coprecipitation, solvent extraction and solid phase extraction.

Solvent extraction or liquid-liquid extraction (LLE) is useful for separation of analytes from interferences by partitioning the sample between two immiscible liquid phases [11]. Solvent extraction followed by FAAS is widely used for the determination of trace metal in aqueous systems. Ammonium pyrrolidinedithiocarbamate (APDC) is one of a few chelating agents that permits the separation of trace metals from solutions, in addition dithizone is a chelating agent

usually employed for preconcentration of metals in FAAS technique [12]. APDC was employed in the extraction of chromium, cadmium and lead in whole-fish samples [13], and in the extraction of cadmium in marine organism [14]. Dithizone was used for the extraction of lead in food samples [15]. The standard method for determination of trace metals in aqueous samples recommends the use of APDC for complex formation, following by extraction of the metal complex with MIBK, and subsequent determination by FAAS [11]. The disadvantages of this technique are that it requires a large amount of solvent (approximately 300-500 mL), the solvent must be evaporated to concentrate the analytes before determination, and the process is a single sample run that takes several hours or days to complete [16].

Coprecipitation is frequently used for the enrichment of trace element by generally using a milligram quantity of a carrier element to ensure complete trace recovery and to facilitate the separation of the precipitate from the mother liquor. Coprecipitation procedures including the use of organic and inorganic coprecipitants have been developed and well documented. Among inorganic coprecipitants, metal hydroxides are most popular due to good recovery and sufficient separation factors for alkaline and alkaline earth metals. Saracoglu et al. [17] used $\text{Ce}(\text{OH})_4$ as a coprecipitant to separate copper, iron, lead, cadmium, cobalt and nickel from baking powder and baking soda sample. In coprecipitation procedures, a very large amount of precipitating materials is added and such compounds are not free from impurities.

Solid phase extraction (SPE) is another approach that offers a number of important benefits. It reduces solvent usage and exposure, disposal costs and extraction time for sample preparation. Consequently, in recent years SPE has been successfully used for the separation and sensitive determination of metal ions, mainly in water samples. Basic principles and guidelines of method development of SPE are presented below.

1.5 Theory of solid phase extraction [18, 19]

The principle of SPE is similar to that of liquid/liquid extraction (LLE), involving a partitioning of solutes between two phases. However, instead of two immiscible liquid phases, as in LLE, SPE involves partitioning between a liquid (sample matrix) and a solid (sorberent) phase. This sample treatment technique enables the concentration and purification of analytes from solution by sorption on a solid sorberent. The basic approach involves passing the liquid sample through a column, a cartridge, a tube or a disk containing an adsorbent that retains the analytes. After all of the samples have been passed through the sorberent, retained analytes are subsequently recovered upon elution with an appropriate solvent.

1.5.1 Retention of traces elements on the sorberent

The adsorption of trace elements on the solid sorberent is required for preconcentration. The mechanism of retention depends on the nature of the sorberent, and may include simple adsorption, chelation, ion exchange or ion-pairing.

1.5.1.1 Adsorption: Trace elements are usually adsorbed on a solid phase through van der Waals forces or a hydrophobic interaction. The most common sorberent is octadecyl-bonded silica. More recently, reversed polymeric phases have appeared, especially the styrene-divinylbenzene copolymer that provides additional π - π interaction when π -electron are present in the analyte. However, because most trace element species are ionic, they will not be well retained by such sorberents.

1.5.1.2 Chelation: Several atoms containing in chelating agents are capable to chelate trace elements. The atoms that are most frequently present in the chelating agent 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 the functional group will give an idea of the selectivity of the ligand towards trace elements. In practice cations may be divided into 3 groups:

group I : 'hard' cations : these preferentially react via electrostatics (due to a gain in entropy caused by changes in orientation of hydration water molecules); this group includes alkaline and alkaline-earth metals that form rather weak outer-sphere complexes with only hard oxygen ligands.

group II : 'borderline' : these have an intermediate character; this group contains Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Pb^{2+} and Mn^{2+} .

group III : 'soft' cations: these tend to form covalent bonds. Hence, Cd^{2+} and Hg^{2+} possess strong affinity for intermediate (N) and soft (S) ligands.

Chelating agents may be directly added to the sample for chelating trace elements. The chelates are further retained on an appropriate chelating group into the sorbents. For that purpose, three different means are available: (1) the synthesis of new sorbents; (2) the chemical bonding of such groups on existing sorbents (functionalized sorbents); and (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 lifetime of the impregnated sorbent.

Binding of metals to the chelated functionality is dependent on several factors: (1) nature, charge and size of metal ions; (2) nature of the donor atoms in the ligand; (3) buffering conditions which favor certain metal extraction and binding to active donor or groups; and (4) nature of solid support.

1.5.1.3 Ion-pairing: When a non-polar sorbent is to be used, an ion-pair reagents can be added to the sorbent. Such reagents contain a non polar portion and a polar portion. The non-polar portion interacts with the reversed-phase non polar sorbent, while the polar portion forms an ion-pair with the ionic species in the matrix.

1.5.1.4 Ion exchange: Ion-exchange sorbents usually contain cationic or anionic functional groups that can exchange the associated counter-ions. 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 pKa. Retention on ion-exchangers depends on the distribution ratio of ion on the resin, the stability constants of the complexes in solution, the exchange kinetics and

presence of other competing ions. Even though ion-exchangers recover hydrated 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.

1.5.2 Step-by step method development guide

Development of an SPE method can be considered as a two-step procedure. First, the most appropriate sorbent for the application should be chosen. An optimization of the most influential parameters should then be undertaken. Obviously, the 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. Matrix components (such as ligand and other ions) may change the trace element retention on the sorbent, thereby recoveries of the target species may decrease.

1.5.2.1 Selection of solid sorbent

In practice, the main requirements for a solid sorbent are the possibility to extract a large number of trace elements over a wide pH range, the fast and quantitative sorption and elution, a high capacity, an easy regenerability and a good accessibility.

1.5.2.2 Influential parameters

There are three main steps for SPE application, conditioning, loading, and elution. In each steps, many parameters influence the efficiency of the SPE.

1.5.2.2.1 Conditioning parameters

a) Washing step. A washing step is highly recommended, especially when ultratraces of elements are to be 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.

1.5.2.2.2 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 prime importance for efficient retention of the trace elements on the sorbent. 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 that may not dissociate, there are 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 glycine [20]. 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 [21]. 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.

1.5.2.2.3 Elution parameters

a) Nature of the solvent. The eluting solvent should be carefully chosen to ensure efficient recovery of the retained target species, 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.

1.6 Application of SPE to the determination of trace elements

Different sorbents have been used for the preconcentration of trace metal from various media. Some natural and synthetic materials such as silica, carbon, cellulose and synthetic materials polymers have been used as it is or after modification by physical or chemical treatments.

In the last two decades, the use of silica-based ion exchangers in preconcentration and separation of trace heavy metal ions from different media has been of particular interest. Silica gel can be directly used as an adsorbent. However, for most metal ions, the interactions with the silica surface are rather weak, because of the low acidity of the silanol group as well as the less pronounced donor properties of the surface oxygen atoms. The use of chelating agents is a promising route for the activation of the surface of the silica to raise its efficiency and selectivity of extraction [18]. Substrates such as dithizone [22], 2-mercapto-5-phenylamino-1,3,4-thiadiazole [23], 1,8-Dihydroxyanthraquinone modified silica gel were used for preconcentration of lead, zinc and cadmium from underground, tap and river water samples [24]. Silica modified with N-propylsalicylaldehyde, 3-aminopropyltriethoxysilane were used to separate trace amounts of cadmium, chromium, copper, manganese and lead from natural water [25, 26].

Octadecyl-bonded silica has currently become the most popular phase used. In particular, organometallic compounds can be retained on this sorbent due to a possible

hydrophobic interaction. Bare C₁₈-silica can also retain a fraction of inorganic trace metals, probably due to the presence of silanol groups on its surface. However, in practice, because of its hydrophobic character, C₁₈-silica is not well suited for retention of trace element species, which are often polar or ionic [18]. The retention on C₁₈-silica may be improved by addition of a ligand reagent to the sample before its percolation through the sorbent. The hydrophobic part of the ligand will thus have a hydrophobic interaction with the C₁₈-silica and be retained on the sorbent, while the functional group of the ligand will ensure the chelation of the trace element. Among reagents, one can cite APDC [27] and 1,10-phenanthroline [28]. Despite their broad application to trace element preconcentration, bonded silica phases (either C-18 silica or functionalized-silica gel) present the drawback of limited range of pH that can be used, as in acidic (below 2 to 4) and basic (above 8) pHs. The hydrolysis may occur between the sorbent and the trace elements [18].

As a consequence, polymeric sorbents may be preferred. The polymeric sorbents have been, by far, the most used for trace element preconcentration having the advantage over bonded silica in that they can be used over the entire pH range. Their disadvantage is that the conditioning step is more time consuming as they require extensive cleaning before use [18]. Amberlite XAD-2 (polystyrene-divinylbenzene polymer) is used in preconcentration procedures due to its good physical and chemical properties such as porosity, high surface area, durability and purity. Ferreira et al. used Amberlite XAD-2 modified by 2-(2-benzothiazolylazo)-2-p-cresol (BTAC) as a sorbent in on-line preconcentration system for determination of lead [29] and nickel [30] in biological reference materials. The calmagite reagent (1-(1-Hydroxy-4-methyl-2-phenylazo)-2-naphthol-4-sulfonic acid) [31] was used for determination of copper in seawater and biological samples. Melo et al. [32] used Amberlite XAD-2 loaded with TAM reagent for determination of cadmium in lobster hepatopancreas (TORT-1) standard reference materials.

Polytetrafluoroethylene polymers (PTFE) can retain trace elements after addition of a chelating reagent to the sample such as APDC, DDTC or dithizone. PTFE may also be percolated with a suitable ligand, like 2-methyl-8-hydroxyquinoline [33]. The sorbent can be used as PTFE turnings [34] or as a PTFE turning in a knotted reactor [35] for determination of metals in fish tissue.

An activated carbon has widely been used for many purposes both in laboratory and industrial settings, due to its ability to adsorb organic compounds and

organic metal complexes. Since its introduction in analytical chemistry, enrichment of trace metals using activated has been favorably performed with vary high concentration factors in different matrices [36].

Generally, sorption of dissolved metal ions on activated carbon can be improved in the presence of chelating or precipitating agents [37]. The mechanism of sorption is still under investigation and the adsorption of heavy metals on activated carbon could be explained using Langmuir and Freundlich equations. The major advantage of these models is their simplicity, however, the models fail to accurately describe the adsorption equilibrium under varying conditions, such as pH and ionic strength. The adsorption equilibrium studies have revealed that pH is the dominant parameter controlling the adsorption [38].

There are general approaches to metal preconcentration using activated carbon, namely from aqueous solutions by simply adjusting the pH to an adequate value and by using chelating agent [18]. Some examples of the use of activated carbon in water analysis are described below. Narin et al. [39] determined copper, manganese, copper, cadmium, lead, nickel and chromium in natural water by FAAS after complexation using pyrocatechol violet and adsorption with activated carbon. Yunes et al. [40] used column of activated carbon without complexing agent for adsorption and determination of nickel. Santelli et al. [41] used APDC and activated carbon for preconcentration and determination of copper. Cerutti et al. [42] determined cadmium after complexation using 8-hydroxyquinoline. Quinaia et al. [43] used *o,o*-diethyl-dithiophosphate and activated carbon for preconcentration and determination of lead.

Giacomelli et al. [44] determined molybdenum and bismuth in steels by GFAAS after complexation with ammonium salt of dithiophosphoric acid *O,O*-diethyl ester and sorption on activated carbon. APDC was employed as complexing agent for determination of traces of silver and bismuth from cobalt and nickel and their nitrates [45]. De. Pena et al. [46] determined lead in minerals and skim milk after complexation with APDC and dithizone and sorption onto activated carbon. Dithizone was employed as complexing agent for determination of traces of cadmium in biological samples [47].

1.7 Conclusion

Solid phase extraction is an effective method of preconcentration for trace heavy metal ions. Many sorbents such as silica gel, activated carbon, Amberlite XAD resins, and other sorbents have been used for separation and preconcentration of trace amounts of heavy metal ions from various matrices.

An activated carbon has widely been used for many purposes both in laboratory and industrial settings. The activated carbon can be directly used as an adsorbent. However, metal chelates may also be retained on this sorbent after addition of proper chelating agent to the sample solution. The chelating agent should be chosen to avoid a strong interaction with the sorbent. APDC have been widely used as chelating agent for the separation and preconcentration of traces metals because of their thermodynamically stable metal complexes. However, the activated carbon has been extensively used in the extraction of trace metals from water samples, but its use for biota samples is still rare.

The determination of heavy trace metals in seafood is important for human health. The level of heavy metal ions in seafood is generally very low. Also seafood has complex matrix for the determination of trace metal ions by atomic absorption spectrometry. In atomic absorption spectrometric determinations of heavy metal ions, even by using GFAAS, low metal concentration is another problem. These two problems (low metal concentrations and matrix interference) are generally improved by using various separation/preconcentration methods such as solvent extraction, evaporation, ion exchange, electroanalytical techniques and solid phase extraction.

Thus, in this work we combined the advantages of activated carbon and APDC to simultaneous preconcentration of metals (Cd, Cu, Ni, Pb, and Zn) in aqueous solution, and subsequent AAS determination. The optimized method was applied for determination of the metal in a solution obtained from the acid digestion of fish tissue.

1.8 Aims and scope of this work

This thesis focused on development method for determination of trace levels of Cd, Cu, Ni, Pb and Zn in aqueous solution using activated carbon as adsorbent in SPE technique following by the determination by AAS.

Initially, the method was tested with model solutions. The experiments are divided mainly into two parts, (i) preconcentration of metals on impregnated activated carbon, (ii) preconcentration of metallic complex on activated carbon. The optimum conditions for preconcentration of Cd, Cu, Ni, Pb and Zn on activated carbon prior to analysis were investigated.

Consequently, the optimized condition from model solution was used in preconcentration of the metals in fish tissue. The parameters e.g. desorption of metals, flow rate, sample volume, amount of activated carbon, amount of APDC and pH for sample preparation/preconcentration of metals in samples were investigated and optimized. Finally, the optimum method for preconcentration of metals in fish tissue was validated and applied to the determination of metals in water and soil samples.

CHAPTER II

EXPERIMENTAL SECTION

2.1 Apparatus

2.1.1 UV-Vis spectrophotometer

UV-Vis Spectrophotometer model HP 8453 (Hewlett Packard) was used for the determination of APDC concentration.

2.1.2 Flame atomic absorption spectrometer

Atomic absorption spectrometer model AAnalyst 100 (Perkin-Elmer) was used to determine metal concentrations. The instrumental parameters are listed in Table 2.1.

Table 2.1 FAAS conditions for the determination of elements in solutions

Operating conditions	Cd	Cu	Ni	Pb	Zn
Wavelength (nm)	228.8	324.8	232.0	283.3	213.9
Slit width	0.70	0.70	0.20	0.70	0.70
Lamp type	HCL ^a	HCL ^a	HCL ^a	HCL ^a	HCL ^a
Lamp current (mA)	4	15	25	10	15
C ₂ H ₂ flow-rate (min ⁻¹)	3	3	3	3	3
Air flow-rate (min ⁻¹)	10	10	10	10	10

^a Hollow cathode lamp

2.1.3 Graphite furnace atomic absorption spectrometer

Graphite furnace atomic absorption spectrometer model 4110ZL (Perkin-Elmer) with a longitudinal Zeeman-effect background correction system, furnished with a transversely heated graphite atomizer (THGA) and an AS-71 autosampler were used. The operating conditions are shown in Table 2.2 and 2.3.

Table 2.2 Instrumental operating conditions for determination of cadmium, copper, lead, nickel and zinc

	Cd	Cu	Ni	Pb	Zn
Wavelength (nm)	228.2	324.8	232.0	283.3	213.9
Slit width (nm)	0.7	0.7	0.2	0.7	0.7
Sample volume (μL)	20	20	20	20	20
Modifier volume (μL)	5 ^a	5 ^b	-	5 ^a	5 ^c

^a chemical modifier ; 0.05 mg $\text{NH}_4\text{H}_2\text{PO}_4$ + 0.003 mg $\text{Mg}(\text{NO}_3)_2$

^b chemical modifier ; 0.005 mg Pd + 0.003 mg $\text{Mg}(\text{NO}_3)_2$

^c chemical modifier ; 0.005 mg $\text{Mg}(\text{NO}_3)_2$

Table 2.3 THGA temperature program for the determination of Cd, Cu, Ni, Pb and Zn

Step ^a	Cd	Cu	Pb	Ni	Zn
Drying 1	110/1/30	110/1/30	110/1/30	110/1/30	110/1/30
Drying 2	130/15/30	130/15/30	130/15/30	130/15/30	130/15/30
Ashing	500/10/20	1200/10/20	850/10/20	1100/10/20	700/10/20
Atomizing	1500/0/5	2000/0/5	1600/0/5	2300/0/5	1800/0/5
Clean	2450/1/3	2450/1/3	2450/1/3	2450/1/3	2450/1/3

^a Temp (°C) / ramp/ hold (s)

The argon flow rate is 250 mL min⁻¹, except for atomization step (no gas flow)

Injection temperature: 20 °C

2.1.4 Manifold

The solid phase extraction was performed using VisiprepTM Vacuum Manifold (Supelco) connected to a vacuum pump. The hardware and accessories are show in figure 2.1.



Figure 2.1 VisiprepTM SPE vacuum manifold model.

2.1.5 Microwave digestion unit

The digestion experiments were carried out using microwave model Paar Physica (Perkin-Elmer). The instrumental parameters are listed in Table 2.4.

Table 2.4 Condition for microwave digestion unit

Step	Power (watts)	Time (min)
1	100	5.00
2	600	5.00
3	1000	5.00
4	0	15.00

2.1.6 pH Meter

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

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

2.2 Chemicals

All chemicals were ACS grade and were presented in Table 2.5.

Table 2.5 Chemical lists

Chemicals	Supplier
Activated carbon, No. 2186	Merck
Acetic acid	Fisher Scientific
Acetone	Fisher Scientific
Ammonium acetate	Carlo Erba Reagenti
Ammonium hydroxide, 30%	Carlo Erba Reagenti
Ammonium pyrrolidinedithiocarbamate	Sigma
Nitric acid	Fisher Scientific
Nitric acid, Suprapure grade	Merck
Hydrogen peroxide, 30%	Carlo Elba Reagenti
Methyl isobutyl ketone	Aldrich
Single standard solution for of Cd, Cu, Ni, Pb and Zn (1000 mg L ⁻¹)	BDH

2.3 Preparation of solution

All solutions were prepared using de-ionized water.

2.3.1 Working standard solutions

Working standard solutions of Cd, Cu, Ni, Pb and Zn were prepared by stepwise dilution of 1000 mg L⁻¹ stock standard solution to the required concentrations.

2.3.2 Mixed working standard solution

A standard solution containing 10-100 mg L⁻¹ of Cd, Cu, Ni, Pb and Zn was prepared by stepwise dilution of 1000 mg L⁻¹ stock standard solution of each element to the required concentrations.

2.3.3 Chelating agents

APDC solution was prepared daily by dissolving the appropriate amount of APDC in de-ionized water.

2.3.4 Ammonium acetate buffer solution (0.2 M)

Ammonium acetate buffer solutions were prepared by adding an appropriate amount of acetic acid to ammonium acetate solutions of pH 5.

2.3.5 Model solution

- *Model solution A (4 ppm of metals, pH 5)*

10 mL of the model solution A contain 40 µg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II) and 2 mL of 0.2 M ammonium acetate buffer (pH 5).

- *Model solution B (0.8 ppm of metals, pH5)*

50 mL of the model solution B contain 40 µg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II) and 10 mL of 0.2 M ammonium acetate buffer (pH 5).

-Model solution C (0.4 ppm of metals, pH 5)

100 mL of the model solution C contain 40 µg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II) and 20 mL of 0.2 M ammonium acetate buffer (pH 5).

-Model solution D (0.4 ppm of metals, pH 5)

50 mL of the model solution D contain 20 µg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II) and 10 mL of 0.2 M ammonium acetate buffer (pH 5).

2.4 Preparation of the activated carbon SPE

2.4.1 Preparation of the activated carbon

Activated carbon powder used as adsorbent in the preconcentration of metal ions was treated with 20% nitric acid, washed with de-ionized water and dried at 110 °C. SPE tubes and frits were soaked in 1% nitric acid overnight and then washed with water. The cleaned frit was placed on the bottom of the SPE tube. The column was then filled with washed activated carbon and ready for SPE experiments.

2.4.2 Preparation of the impregnated activated carbon

The activated carbon was impregnated with APDC by percolating 10 mL of 1% w/v APDC solution through the column of activated carbon at a flow rate of 1 mL min⁻¹. The filtrate was collected and analyzed for unadsorbed APDC with UV-vis spectrophotometer at $\lambda = 277$ nm. The column was then washed with 5 mL water and the filtrate was also analyzed for the washed APDC.

2.5 Preconcentration procedure of model solution

In this procedure, the procedure is divided into two parts, (i) preconcentration of model solutions, (ii) preconcentration of fish tissue. In the preconcentration procedure of model solutions is divided mainly into two parts, (i) preconcentration of metals in solution on impregnated activated carbon, (ii) preconcentration of metallic complex in solution on activated carbon. Methods from model solution were applied to the analysis of metals in fish tissue.

2.5.1 Preconcentration of metals in solution on impregnated activated carbon

The model solutions were passed through the impregnated activated carbon column at flow rate 1 mL min^{-1} . The filtrate was collected and analyzed for unsorbed metals. The column was then washed with 5 mL water and eluted with five successive portions of 10 mL eluting agents at flow rate of 0.6 mL min^{-1} . Each portion of eluate was further evaporation to about 1 mL using heating gun and diluted with 1% nitric acid to 10 mL in a volumetric flask. The metal concentrations in this solution were determined by FAAS.

2.5.1.1 Efficiency of impregnated activated carbon

The efficiency of impregnated activated carbon SPE column on the sorption of metals was studied by using 0.5 g of impregnated activated carbon and 0.5 g of activated carbon for preconcentration of metal in model solution A and comparing percentage of metal sorption calculated by the difference between initial and remaining amount of metals in solution. The experiment was carried out as mentioned in 2.5.1.

2.5.1.2 Desorption of metals

The desorption of retained metal from the column containing 0.5 g activated carbon was investigated using 50 mL of various eluting agents. A model solution for preconcentration was 0.8 ppm Cu(II) solution (pH 5, 50 mL). 1 M nitric acid, 5 M

nitric acid, acetone, 1 M nitric acid in acetone and MIBK were used as extractants for elution of the adsorbed metal from the column.

Afterwards, the best eluent was chosen and used in the preconcentration of Cd (II), Cu(II), Ni(II), Pb(II) and Zn(II) in 50 mL of solution A.

2.5.1.3 Effect of the sample volume on the metal sorption

The effect of the sample solution volume on the sorption of metals was studied by passing 10, 50 and 100 mL of model solution A, B and C into a column containing 0.5 g activated carbon and comparing the percentage of metal sorption.

2.5.1.4 Effect of the amount of activated carbon

The effect of the amount of activated carbon on the sorption of metal ions was examined by using 50 mL of model solution D and 10 mL of 1 M nitric acid in acetone as eluting agent. The amount of activated carbon use was varied in the range of 100-1000 mg.

2.5.1.5 Effect of nitrate ion

The effect of nitrate ions on the sorption of metals was studied by adding of sodium nitrate in the range of 0-1.5 M into model solution D. Preconcentration procedure was the same as mentioned in 2.5.1.

2.5.2 Preconcentration of metallic complexes in solution on activated carbon

1% w/v APDC was added to the model solution D in order to form metal-APDC complexes prior to solid phase extraction. The column containing 0.1 g of activated carbon was conditioned with the 0.2 M ammonium acetate buffer solution (pH 5). The metal-APDC solution was passed through the column at a flow rate of 1 mL min⁻¹. The metal-APDC complexes retained on the column were eluted with 10 mL of 1 M nitric acid in acetone at flow rate of 0.6 mL min⁻¹. The eluate was

evaporated to about 1 mL and then made up to 10 mL in a volumetric flask with 1% nitric acid. The metals concentration in this solution was determined by FAAS.

2.5.2.1 Effect of amount of complexing agent

The amount of 1% w/v APDC solution added to the metals solution prior to solid phase extraction was varied from 1 to 5 mL. The experiments were performed as mentioned earlier.

2.6 Preconcentration procedure of metals from fish tissues

2.6.1 Sample preparation

- Method A (microwave digestion)

A portion of 1.0 g homogenized fresh tissue sample was precisely weighted into quartz tubes and 5 mL of concentrated nitric acid were added. The tubes were placed in a six-position pressurized bomb and the mixtures were digested under pressure. The solution obtained was spiked with a known amount of metals. The pH of solution was then adjusted to the desired pH with concentrated ammonium hydroxide prior to making up volume to 50 mL with water.

- Method B (wet digestion)

A portion of 1.0 g homogenized fresh tissue sample was precisely weighted into a flask. Sample was treated with 5.0 mL of concentrated nitric acid and kept overnight in flask. Add 10 mL concentrated nitric acid to the flask and, within 60 min, heat gently in hood until 3-5 mL of sample was left. Add 0, 5 or 10 mL of 30% hydrogen peroxide, and heat gently within 30 min until 2-3 mL of sample remains. Filter the residue, adjust pH and spike a known amount of each analyte to the sample. The final volume was made up to 50 mL.

- Method C (microwave digestion)

2 mL of concentrated nitric acid and 2 mL of 30% hydrogen peroxide were used as reagent for digestion. The rest of the experiment was performed as mentioned in method A.

2.6.2 Preconcentration of metals in sample on impregnated activated carbon

The sample solution was passed through the column containing 0.1 g of impregnated activated carbon at flow rate 1 mL min^{-1} . The filtrate was collected and analyzed for unsorbed metals. The column was then washed with 5 mL water and eluted with 10 mL eluting agent at flow rate of 0.6 mL min^{-1} . Eluate was further evaporated to about 1 mL using heating gun and diluted with 1% nitric acid to 10 mL in a volumetric flask. The metals concentration in this solution was determined by FAAS.

2.6.2.1 Matrix effect on metals sorption

Three portion of 1.0 g of fish tissue were digested following method A. The solutions obtained were diluted to 25, 50 and 100 mL with water. The preconcentration procedure was the same as mentioned in 2.6.2.

2.6.2.2 Effect of the digestion procedure

Sample was prepared following method B. Different volume of hydrogen peroxide (0-10 mL) was added to sample solution. Preconcentration procedure was the same as mentioned at 2.6.2.

2.6.3 Preconcentration of metallic complex in sample on activated carbon

APDC was added to the sample solution in order to form complex with metal prior to solid phase extraction. The column containing 0.1 g of activated carbon was conditioned with 0.2 M ammonium acetate buffer solution (pH 5). The metal-APDC solution was passed through the conditioned column at a flow rate of 1 mL min^{-1} . The metal-APDC complexes retained on the column were eluted with 10 mL of 1 M nitric acid in acetone at flow rate of 0.6 mL min^{-1} . After eluting, the solution obtained was evaporated to about 1 mL and then made up to 10 mL in a volumetric flask with 1% nitric acid. The metals concentration in this solution was determined by FAAS.

2.6.3.1 Effect of the flow rate on sorption

The evaluation of the effect of loading flow rate on percentage of metal sorption was performed using: 50 mL of sample solution (method C) spiked with 20 µg of each of elements before the digestion and adjusted to pH 5. 1% w/v APDC were then mixed with the sample solution to form the metal complexes. The mixture obtained was passed through the minicolumn packed with 0.1 g activated carbon at flow rates between 1 and 10 mL min⁻¹. Finally, the retained metal complexes were eluted with 1 M nitric acid in acetone. The eluate was evaporated to about 1 mL and then made up to 10 mL in a volumetric flask with 1% nitric acid. The metal concentration in this solution was determined by FAAS.

2.6.3.2 Effect of the amount of complexing agent

To investigate the influence of the concentration of the complexing agent, APDC of different concentrations (0.05-1.0% w/v) were added to sample solution. Samples were prepared following method C (spiked with 20 µg of each of elements before the digestion). The metal-APDC solution was passed through the column at the flow rate of 10 mL min⁻¹ and eluted with 1 M nitric acid in acetone with the flow rate of 0.6 mL min⁻¹. The eluate obtained was evaporated to about 1 mL and then made up to 10 mL in a volumetric flask with 1% nitric acid. The metals concentration in this solution was determined by FAAS. Compare the percentage of metal sorption.

2.6.3.3 Influences of pH of sample solution on sorption

The effect of pH on the metal sorption was investigated in the pH ranging from 0.7 to 5. The pH of sample solutions obtained from method C (50 mL) spiked with 1 µg of each of elements before the digestion were adjusted to pH 0.7, 2 and 5 with ammonium hydroxide solution prior to APDC addition. The metal-APDC solutions were passed through the 0.1 g of activated carbon column at flow rate of 10 mL min⁻¹ and eluted with 10 mL of 1 M nitric acid in acetone at flow rate of 0.6 mL min⁻¹. The eluate obtained was evaporated to about 1 mL and then made up to 10 mL in a

volumetric flask with 1% nitric acid. The metals concentration in this solution was determined by GFAAS.

2.7 Method validation

In order to demonstrate the validity of this method, the precision of the method was calculated as the relative standard deviation (RSD). Recoveries were investigated by using the sample solutions containing spiked metals.

The optimum method for preconcentration of metal in fish tissue was validated as follows:

Sample preparation step: A portion of 1.0 g fresh tissue sample was precisely weighted into quartz tubes. 1 μg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II) were spiked into fish tissue. Then, 2 mL of concentrated nitric acid and 30% hydrogen peroxide were added. The tubes were placed in a six-position pressurized bomb and the mixtures were digested under pressure. The final volume was diluted with de-ionized water to 50 mL after adjusting the pH to 5 with ammonium hydroxide.

Preconcentration step: 1 mL of 0.1 % w/v APDC was added to the 50 mL of sample solution in order to form complex with metal prior to solid phase extraction. The column containing 0.1 g of activated carbon was conditioned by passing 5 mL of acetone followed by 5 mL of 0.2 M ammonium acetate buffer solution (pH 5). The metal-APDC solution was passed through the column at a flow rate of 10 mL min^{-1} . The metal-APDC complexes retained on the column were eluted with 10 mL of 1 M nitric acid in acetone at flow rate of 0.6 mL min^{-1} . The eluate obtained was evaporated to about 1 mL and then made up to 5 mL in a volumetric flask with 1% nitric acid. The metals concentration in this solution was determined by GFAAS.

2.8 Application to water and soil

The validated method was applied to the determination of Cd, Cu, Ni, Pb and Zn in water and soil samples.

2.8.1 Water

The order to quantity dissolved metal ions, a water sample was collected from Chulalongkorn University Pond. Particulates in the water sample were removed by filtration and divided in five portions of 100 mL each. The sample was spiked with 1 μg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II) and adjusted to pH 5 with acetic acid. Preconcentration procedure was the same as mentioned in section 2.7.

2.8.2 Soil

Sample preparation step (followed by EPA Method 3050B [48]);

1. A portion of 1.0 g soil sample was precisely weighed into a flask
2. Spiked with 1 μg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II) before the digestion
3. Add 10 mL of 1:1 nitric acid, mix the slurry, and cover with a watch glass
4. Heat the sample to $95\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$ and reflux for 15 minutes without boiling
5. Allow the sample to cool, add 5 mL of concentrated nitric acid, replace the cover, and reflux for 30 minutes
6. Allow the solution to evaporate to approximately 5 mL
7. After sample has cooled down, add 2 mL of water and 3 mL of 30% H_2O_2 . Cover the flask with a watch glass and return the covered flask to the heat source for warming
8. Add 2 mL of 30% H_2O_2 in 1 mL aliquots with warming
9. Cover the sample with watch glass and continue heating until the volume was reduced to approximately 5 mL
10. Allow the sample to cool, dilute to 50 mL in the volumetric flask with water. Particulates in the sample were removed by filtration

11. Preconcentration procedure was the same as in mentioned in section 2.7.



สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER III

RESULTS AND DISCUSSION

In this chapter, the results are divided into two parts, (i) preconcentration of the model solutions, (ii) preconcentration of the metals in fish tissue. Finally, the method was validated and applied for determination of the metals in water and sediment sample.

3.1 Preconcentration of model solutions

The study is divided mainly into two parts, (i) preconcentration of metals in solution on impregnated activated carbon, (ii) preconcentration of metallic complexes in solution on the activated carbon.

3.1.1 Preconcentration of metals in model solution on impregnated activated carbon

3.1.1.1 Efficiency of impregnated activated carbon

The metal retention efficiency onto the impregnated activated carbon was investigated and compared with untreated activated carbon. Starting with 40 µg of metals in solution, the quantity of unretained metals was determined in the filtrated solutions by FAAS. The percentage sorption of the metals retained on the activated carbon was calculated from the difference between the starting amount of each metal (N_{starting}) and the amount of metal left in the filtrate (N_{filtrate}) as equation 3.1.

$$\boxed{\% \text{Sorption} = [N_{\text{starting}} - N_{\text{filtrate}} / N_{\text{starting}}] \times 100} \dots\dots\dots (3.1)$$

The results are shown in Table 3.1. The activated carbon impregnated with APDC can retain all metals (Cd, Cu, Ni, Pb and Zn) while the untreated activated carbon can not quantitatively retain Cd, Ni and Zn.

Evidently, the preconcentration of the metals with the untreated activated carbon is not suitable for Cd, Ni and Zn. Therefore, the impregnated activated carbon seems to be a better sorbent in simultaneous sorption of these five elements.

Table 3.1 Percentage of metal sorption of untreated and impregnated activated carbon (40 µg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II))

Element	Sorption, ^a %	
	Activated carbon	Impregnated activated carbon
Cd	14.82 ± 0.53	> 99.40
Cu	> 99.69	> 99.69
Ni	29.08 ± 0.23	> 99.85
Pb	> 99.96	> 99.96
Zn	12.16 ± 1.08	> 99.84

^a Mean value ± SD, n=3

As shown in table 3.1, the quasi-total sorption of the metals on the APDC-activated carbon was achieved.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

3.1.1.2 Desorption of metals

An appropriate eluent for metal desorption was selected by taking into account the following considerations; (i) the eluent should desorb the metals or chelate complexes, (ii) the eluent should not destroy the sorbent and (iii) the eluent should be suitable for subsequent determination technique [49]. Organic solvents and inorganic acids have been found to meet the requirements. The examples of eluents are showed in Table 3.2.

Table 3.2 Comparative data from recent references on metals determination by SPE

Elements	Chelating agent	Sorbent	Eluent	Analysis method	Ref.
	Cd(II), Cu(II),	none	APDC-coated-	4 N HNO ₃	ICP-OES [50]
Ni(II), Pb(II), Zn(II)		XAD 4 and pipDTC-coated -XAD4			
Cu(II), Cd(II)	APDC	Silica C-18	Methanol	GFAAS	[36]
Cu(II), Fe(II), Pb(II), Cd(II),	APDC	Chromosorb- 102	Acetone	FAAS/ GFAAS	[17]
Co(II), Ni(II) [51]	HMA-	Amberlite		1 M HCl	FAAS
Cu(II), Pb(II), Ni(II), Cd(II)	HMDTC	XAD 16	in acetone		
Mn(II), Fe(II) [33] Pb(II)	APDC	PTFE	MIBK		FAAS

PepDTC: piperidine dithiocarbamate

HMA-HMDTC: hexamethylenammonium-hexamethylenedithiocarbamate

In this work, the eluents chosen were MIBK, 1 M nitric acid, 5 M nitric acid, acetone and 1 M nitric acid in acetone. The effectiveness of metal desorption from impregnated activated carbon was evaluated from the %recovery of copper. The %recovery was calculated from the amount of metals in starting solution (N_{starting}) and the amount of metals eluted from the column (N_{final}) as equation 3.2.

..... (3.2)

$$\% \text{Recovery} = [N_{\text{final}} / N_{\text{starting}}] \times 100$$

The experimental results are summarized in Table 3.3. MIBK could not elute the metals, while the others could elute the metal with the various %recovery ranging from 7 to 97%. It was obviously found that 1 M nitric acid in acetone was the best eluent. 97 %recovery and 7.94% RSD were obtained when 1 M nitric acid in acetone was used. Thus, it was chosen for desorption of all heavy metals studied in further experiments.

Table 3.3 The effect of different eluents on elution of Cu (40 µg)

Eluent	Recovery ^a , %	RSD, %
MIBK	-	-
Acetone	20.24	-
1 M nitric acid	10.82 ± 1.17	10.78
5 M nitric acid	7.45 ± 3.30	7.32
1 M nitric acid in acetone	97.07 ± 8.17	7.94

^a Mean value ± SD, n=3

The recoveries of Cd, Cu, Ni, Pb and Zn using 1 M nitric acid in acetone as eluent are shown in Table 3.4. The results showed that the recoveries of higher than 95% could be obtained in the case of Cd, Cu, Ni and Zn. However, it could not desorb Pb from the impregnated activated carbon.

Table 3.4 Recoveries of Cd, Cu, Ni, Pb and Zn in model solution after preconcentration on impregnated activated carbon using 1 M nitric acid in acetone as eluent (40 µg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II))

Element	Recovery ^a , %	RSD, %
Cd	96.89 ± 4.38	4.52
Cu	101.72 ± 2.45	2.40
Ni	99.47 ± 4.89	4.70
Pb	-	-
Zn	104.76 ± 4.68	4.47

^a Mean value ± SD, n=3



สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

3.1.1.3 Effect of the sample volume on metal sorption

For analysis of a real sample using preconcentration, the sample volume is one of the most important parameters for obtaining high preconcentration factors. In this study, the effect of sample volume on the sorption behavior of the analytes was investigated by varying the sample volume from 10 to 100 mL. The results are summarized in Table 3.5. It was found that the sorption of the metal ions on 0.5 g activated carbon impregnated with APDC was not affected by sample volume between 10-100 mL. The recoveries of desorption are summarized in Table 3.6. The volume of the eluent was 50 mL with the flow rate of 0.6 mL min⁻¹.

Thus, in the further experiments, the sample volume used is in the range of 10-100 mL.

Table 3.5 Effect of the sample volume on the sorption of trace metal ions on the activated carbon impregnated with APDC (40 µg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II))

Elements	Sorption ^a , %		
	10 mL	50 mL	100 mL
Cd	> 99.54	> 99.54	> 99.54
Cu	> 99.81	> 99.81	> 99.81
Ni	> 99.89	> 99.89	> 99.89
Pb	> 99.97	> 99.97	> 99.97
Zn	> 99.27	> 99.27	> 99.27

^a Mean value, n = 4

Table 3.6 Recoveries of Cd, Cu, Ni, Pb and Zn from the model solution of different volume (40 μg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II))

Elements	Recovery ^a , %		
	10 mL	50 mL	100 mL
Cd	94.90 \pm 4.06	96.32 \pm 2.86	97.02 \pm 2.23
Cu	95.40 \pm 2.70	96.85 \pm 0.59	100.3 \pm 3.6
Ni	97.27 \pm 0.29	97.50 \pm 1.85	96.55 \pm 0.81
Pb	-	-	-
Zn	106.4 \pm 3.7	106.4 \pm 6.3	103.6 \pm 5.7

^a Mean value \pm SD, n = 4

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

3.1.1.4 Effect of the amount of activated carbon

The quantitative retention of metals is not practically obtained by using activated carbon in the smaller amount than the optimum one. On the other hand, the excess amount of activated carbon also prevents the quantitative elution of the retained metals by a small volume of eluent [39]. For this reason, the activated carbon amount and eluent volume were optimized. To investigate the effect of the activated carbon amounts for quantitative retention of analytes, the activated carbon within the range of 100-1000 mg were packed into the column. The elution was performed using 10 mL of 1 M nitric acid in acetone at the flow rate of 0.6 mL min^{-1} . The recoveries are shown in Figure 3.1.

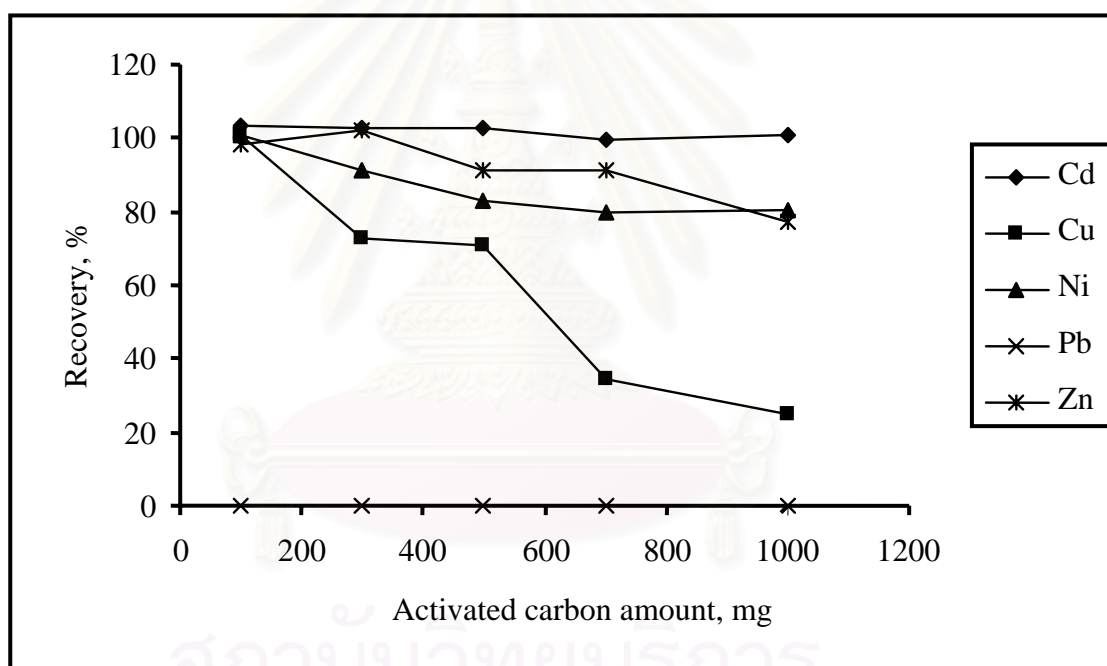


Figure 3.1 Effect of the amount of activated carbon on the analyte recoveries using 10 mL of 1 M nitric acid in acetone as eluent ($20 \mu\text{g}$ each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II), $n=2$).

As can be seen in Figure 3.1, when 100 mg of impregnated activated carbon was used, 10 mL of 1 M nitric acid in acetone was sufficient for the quantitative recoveries of Cd, Cu, Ni and Zn ($\sim 100\%$). In case of Cd, there was no effect of the amount of activated carbon used on the recovery. For Zn, above 300 mg of sorbent,

the recoveries decreased slightly. For Ni and Cu, above 100 mg of sorbent, the recoveries were below 95% with 10 mL of the eluent. Above 100 mg of impregnated activated carbon, above 10 mL of 1 M nitric acid in acetone was sufficient for quantitative recoveries of four metals. The results are shown in Figure 3.2-3.6.

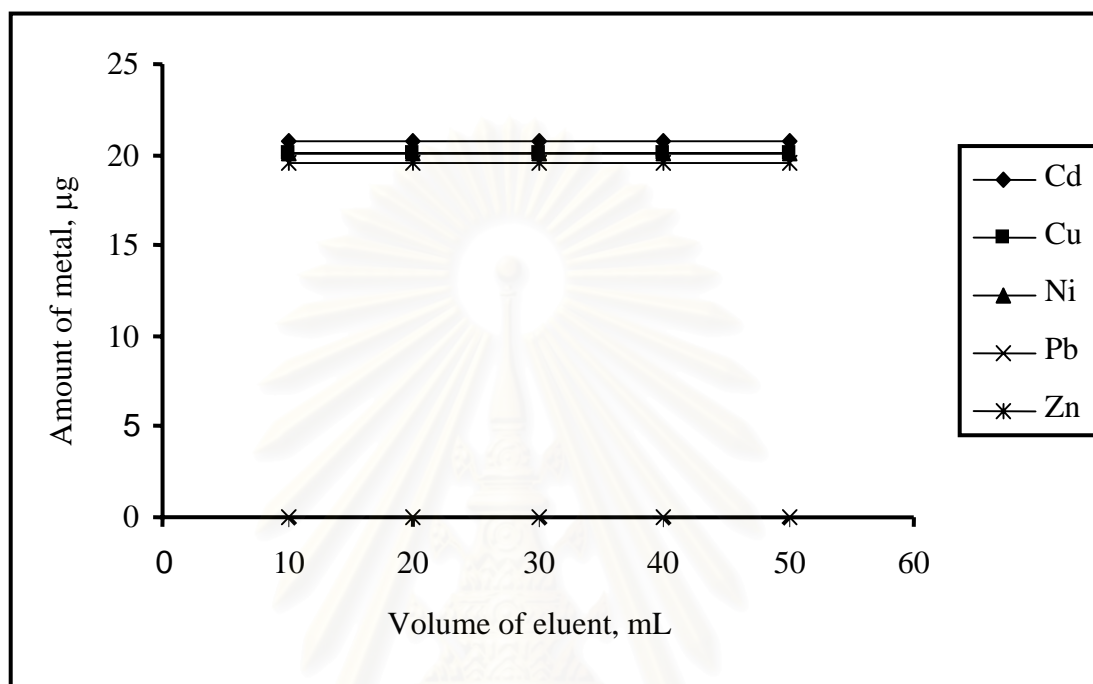


Figure 3.2 Effect of the volume of eluent for 100 mg of activated carbon (20 µg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II), n=2)

In case 100 mg of activated carbon, the volume of eluent from 10-50 mL could not affect the amount of metals (Cd, Cu, Ni, Pb and Zn) in the eluent. Thus, 10 mL of eluent with 100 mg of activated carbon was recommended for further experiments.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

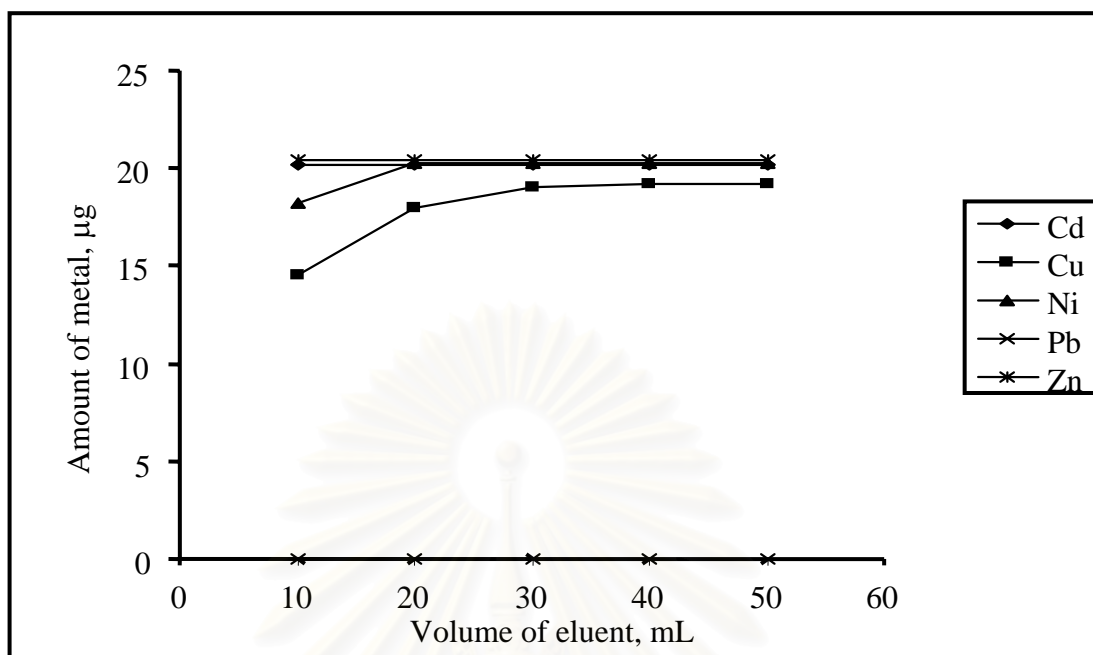


Figure 3.3 Effect of the volume of eluent for 300 mg of activated carbon (20 μg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II), n=2)

In case 300 mg of activated carbon, the amount of Cu and Ni in the eluate were affected by the volume of eluent. The amount of Cu and Ni in the eluate increased with the increasing volume of eluent. However, the volume of eluent could not affect the desorbed amount of Cd, Pb and Zn.

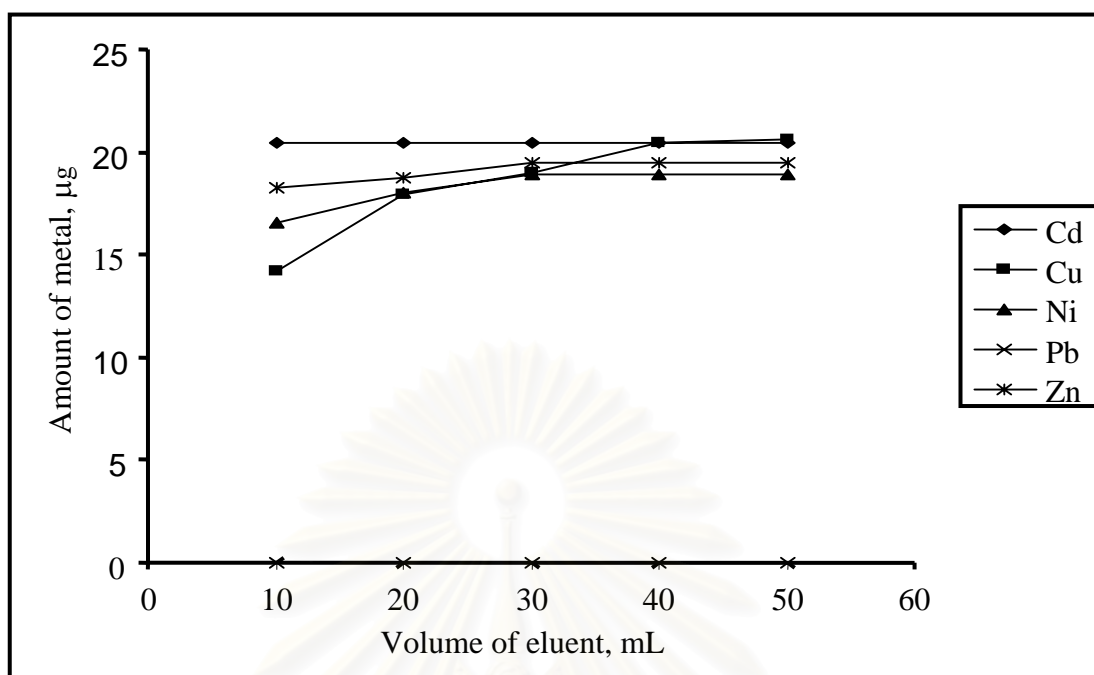


Figure 3.4 Effect of the the volume of eluent for 500 mg of activated carbon (20 µg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II), n=2)

From Figure 3.4, the same results as Figure 3.3 were obtained. 500 mg of activated carbon, the amount of Cu, Ni and Zn in eluates were affected by the volume of eluent. However, the volume of eluent could not affect the amount of Cd, Pb and Zn in the eluates.

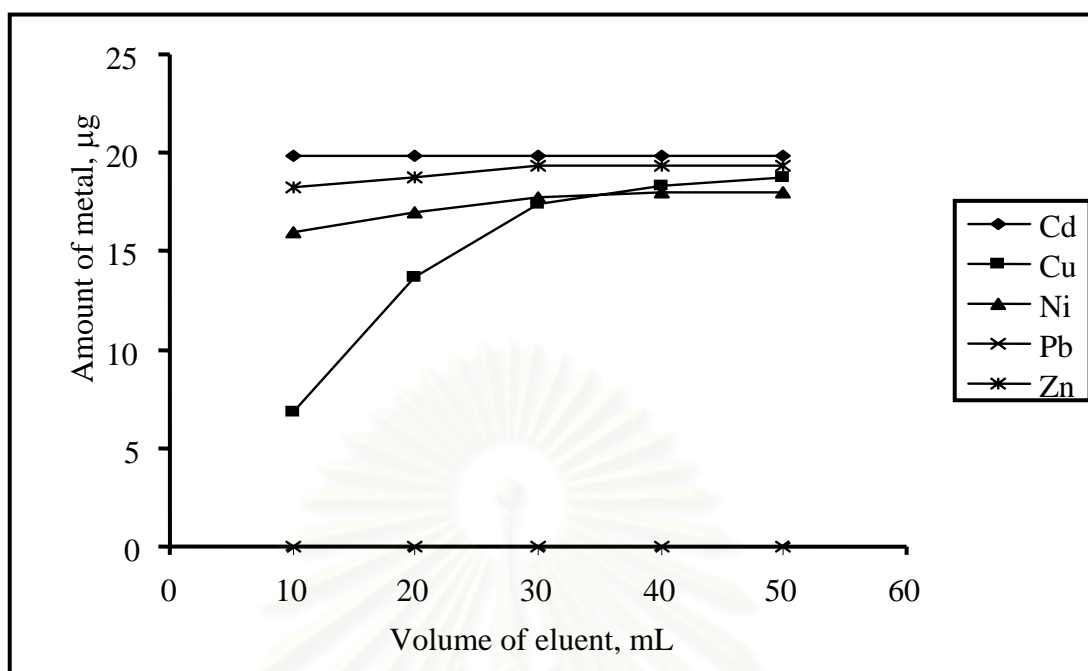


Figure 3.5 Effect of the volume of eluent for 700 mg of activated carbon (20 µg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II), n=2)

The results were similar to the case of 500 mg of activated carbon but the amount of Cu in the eluant increased more than the previous case with increasing volume of the eluent.

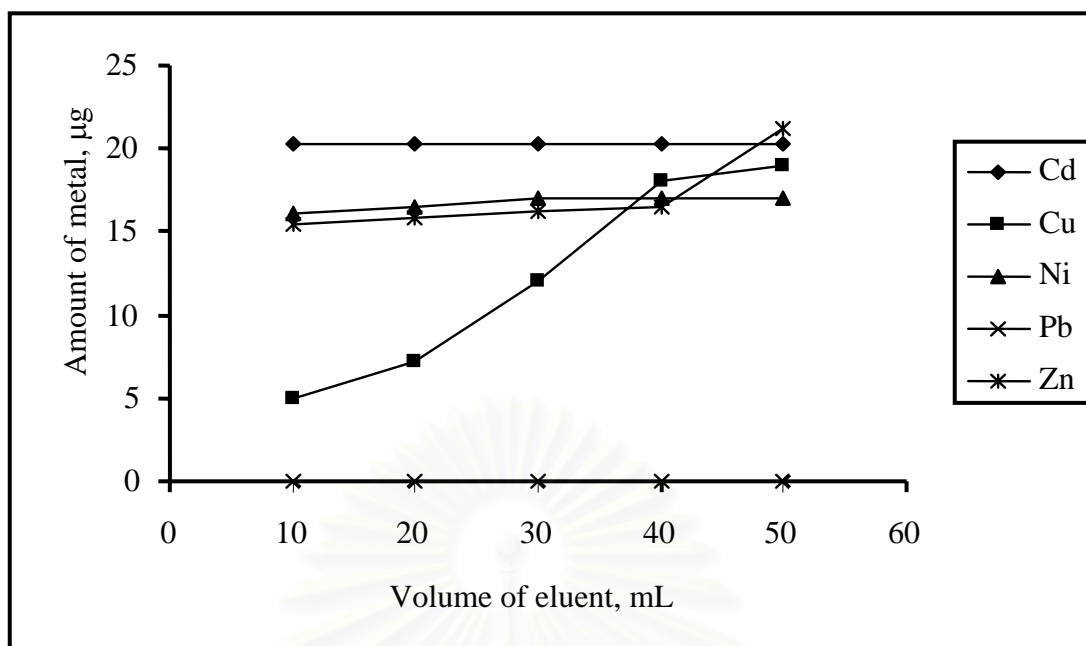


Figure 3.6 Effect of the volume of eluent for 1000 mg of activated carbon (20 µg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II), n=2)

The results showed the similarity as the case of 500 mg of activated carbon but in this case (Figure 3.6), the amounts of Cu, Ni and Zn in eluates were strongly affected by the volume of eluent. The amount of metal in eluates increased with increasing volume of eluent especially in case of Cu. However, the volume of eluent could not affect the amount of Cd and Pb in the eluate.

In conclusion, the effects of the amount of activated carbon and the volume of eluent on the desorption of metal ions were examined in the range of 100-1000 mg of sorbent and 10-50 mL of eluent. The results demonstrated the quantitative recoveries (~100%) of the working elements were observed while using 100 mg of activated carbon when 10-50 mL of eluent was used. For further experiments, 100 mg of activated carbon and 10 mL of eluent with a 0.6 mL min^{-1} flow rate are used.

3.1.1.5 Effect of nitrate ion

The effect of nitrate ions on the sorption efficiency of Cd, Cu, Ni, Pb and Zn on impregnated activated carbon was studied using the optimum conditions from model solution. Sodium nitrate was added to the model solutions to obtain the nitrate concentration between 0-1.5 M. The results are shown in Figure 3.7. When the amount of nitrate ion increased, the % sorption of all metal decreased. Goswami and Singh [52] also demonstrated that sodium nitrate with concentration higher than 0.15 M had effect on sorption efficiency of metals (Cu, Pb, Fe, Zn, Co, Ni and Cd) in model solution on modified silica gel.

In sample solution, nitrate is present in high amount coming from concentrated nitric acid used in the digestion step. This may reduce the soption efficiency of metal on impregnated activated carbon. In further use, the matrix like nitrate has to be removed.

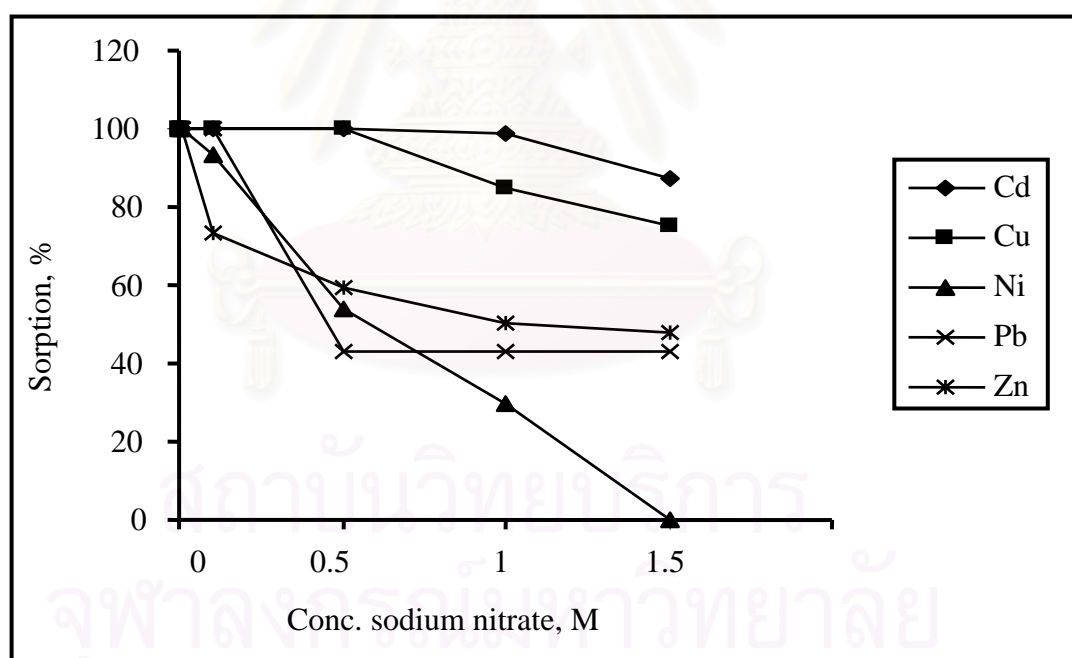


Figure 3.7 Effect of sodium nitrate on the metals sorption (20 μ g each of Cd(II), Cu (II), Ni(II), Pb(II) and Zn(II), n=2)

3.1.2 Preconcentration of metallic complex in solution on activated carbon

3.1.2.1 Effect of the amount of complexing agent

In this part, APDC was added to form complex with the metals in solution prior to preconcentration on activated carbon. The amount of APDC plays an important role in the quantitative retention of metal ions. In its absence, the activated carbon does not retain or partial retains the metal ions (Table 3.1). However, the excess amount of APDC may prevent the retention of metal ions due to sorption competitions between metal-APDC complex and free APDC on the activated carbon. Therefore, the influence of APDC amount on the retention and recovery of the metal ions was investigated using the model solution and varying volume of 1% w/v APDC solution from 0 to 5 mL. The results obtained are presented in Figure 3.8.

From Figure 3.8, the percentage of metal sorption increased with the increasing amount of APDC added and reached 100% sorption with at least 1 mL of 1% w/v APDC. This amount of APDC was then chosen for further studies. It was expected that this amount of APDC was enough for the preconcentration of metal ion found in trace to ultratrace levels found in fish sample.

The results of the recoveries were shown in Table 3.7. The recoveries of Cd, Cu, Ni and Zn using 1, 3 and 5 mL of 1% w/v APDC solution were higher than 95%. While the untreated activated carbon (0 mL of 1% w/v APDC) could not quantitatively retain Cd, Ni and Zn.

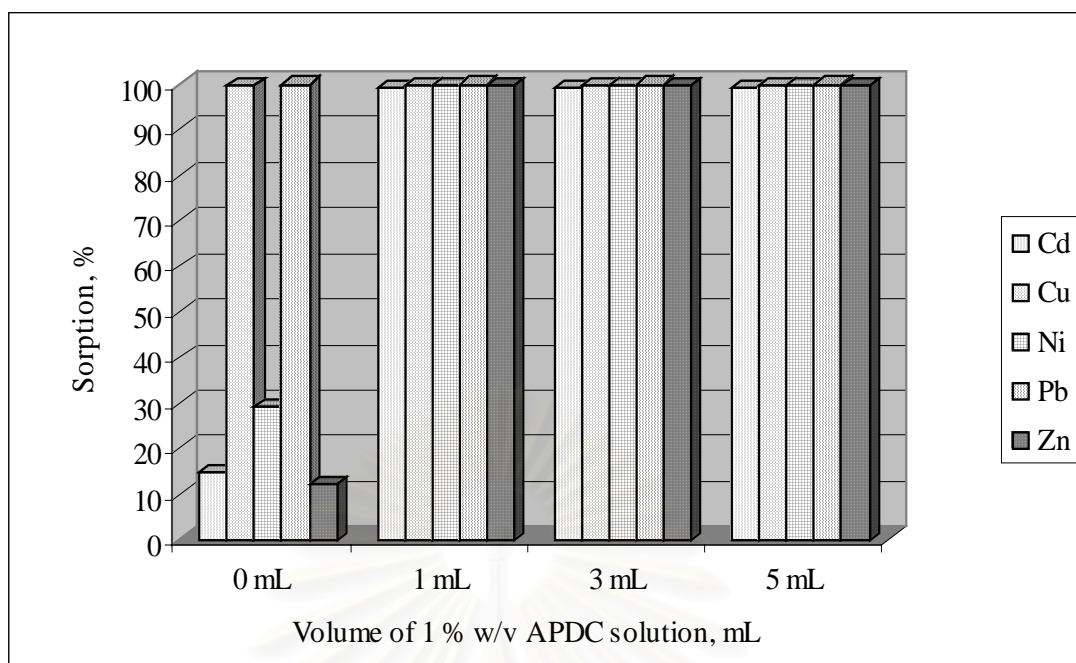


Figure 3.8 Effect of amount of APDC on the analyte retention (20 μg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II), $n=2$)

Table 3.7 Recoveries of Cd, Cu, Ni, Pb and Zn in model solution using different amount of 1% w/v APDC (20 μg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II))

Elements	Recovery ^a , %			
	0 mL	1 mL	3 mL	5 mL
Cd	-	103.45(8.45)	98.74(2.85)	97.85(5.84)
Cu	97.81(2.59)	106.01(4.87)	98.11(6.10)	97.41(2.74)
Ni	-	98.45(5.81)	104.25(1.06)	89.85(5.14)
Pb	-	-	-	-
Zn	-	95.74(2.75)	105.47(2.96)	99.84(4.12)

^a Mean value(range), $n = 2$

3.2 Preconcentration of metals from fish tissue

3.2.1. Preconcentration of metals in sample on impregnated activated carbon

The optimum method investigated from the model solutions was applied to the analysis of metals in fish tissue using activated carbon impregnated with APDC. Sample solutions were prepared following the method A as mentioned in section 2.5.1. The results are shown in Table 3.8. Only copper could be retained and recovered quantitatively from activated carbon impregnated with APDC. The results were much different from what was obtained when model solutions were used. The sample solution differed from model solution in the amount of nitrate ion resulting from concentrated nitric acid used in the digestion step. This may play a role in the sorption process of Cd, Ni, Pb and Zn. The difference in matrix may also cause the change in metal sorption.

Table 3.8 Recoveries of Cd, Cu, Ni, Pb and Zn in sample solution (20 µg each of Cd (II), Cu(II), Ni(II), Pb(II) and Zn(II))

Elements	Sorption ^a , %	Recovery ^a , %
Cd	0.00 ± 0.03	-
Cu	> 99.81	96.33 ± 2.23
Ni	0.00 ± 0.11	-
Pb	0.00 ± 0.01	-
Zn	0.00 ± 0.15	-

^a Mean value ± SD, n = 4

3.2.1.1 Matrix effect on metals sorption

To test the matrix effect on metals sorption, three portion of 1.0 g of fish tissue were digested and diluted to different volume. The results are summarized in Figure 3.9. For copper, more diluted sample gave higher recoveries and the percentage of metal sorption could be as high as 100 %. The recoveries of Cd, Ni, Pb and Zn seem not to be affected by the sample dilution.

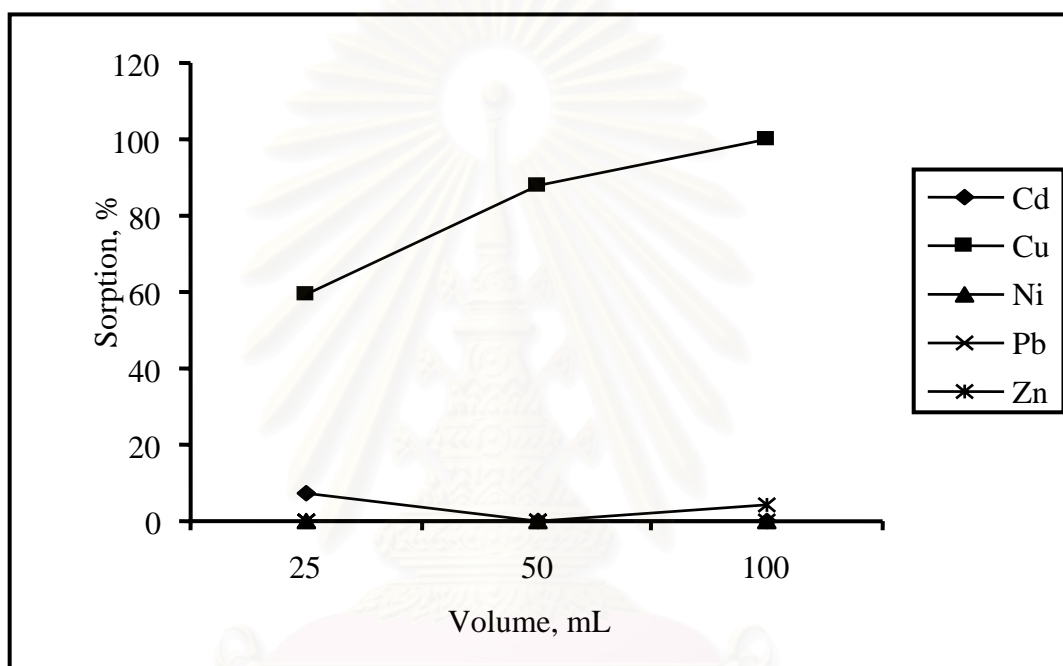


Figure 3.9 Matrix effect on the analyte retention (20 μg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II), n=2)

3.2.1.2 Effect of digestion method

It was well-known that organic matter and nitrate ion can be oxidized by hydrogen peroxide. To further investigate the effect of nitrate ion and matrix effect, hydrogen peroxide was added to the sample during wet digestion (sample preparation method B). Different volumes of hydrogen peroxide solution were used. The results are shown in Figure 3.10 and Table 3.9.

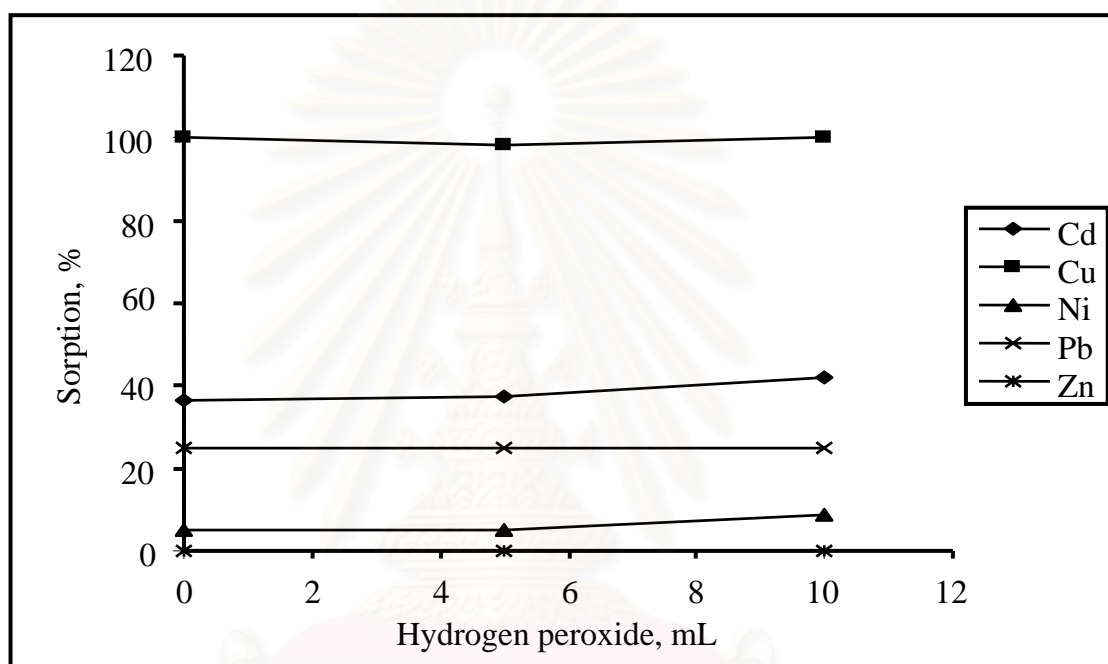


Figure 3.10 Effect of hydrogen peroxide on the sorption (20 μg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II), $n=2$)

Table 3.9 Recoveries of Cd, Cu, Ni, Pb and Zn in sample solution (20 µg each of Cd (II), Cu(II), Ni(II), Pb(II) and Zn(II))

Elements	Recovery ^a , %		
	0 mL	5 mL	10 mL
Cd	30.12 (30.09-30.15)	37.60 (35.98-39.22)	41.91 (40.11-43.71)
Cu	102.24 (100.58-103.90)	97.85 (98.70-97.00)	98.91 (98.14-99.68)
Ni	-	-	-
Pb	-	-	-
Zn	-	-	-

^a Mean value (range), n = 2

It can be seen that hydrogen peroxide did not affect metal sorption. Evidently, preconcentration of metal with impregnated activated carbon is not suitable for the analysis of metals in fish sample. Therefore, the performing of chelate before sorption may be a better method.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

3.2.2. Preconcentration of metallic complex in sample on activated carbon

The optimized method from model solution was applied to the analysis of metals in fish tissue. APDC was added to the sample solution to form complex before passing into activated carbon column. Sample was prepared using sample preparation with microwave digestion (method C). 20 µg of each metal were spiked to the sample solution. The %sorption and %recovery of metals are summarized in Table 3.10.

Table 3.10 Recoveries of Cd, Cu, Ni, Pb and Zn in sample solution (20 µg each of Cd (II), Cu(II), Ni(II), Pb(II) and Zn(II))

Elements	Sorption, %	Recovery ^a , %	RSD, %
Cd	> 99.54	106.57 ± 4.69	4.40
Cu	> 99.81	99.89 ± 8.38	8.39
Ni	> 99.89	99.99 ± 3.86	3.86
Pb	> 99.97	32.60 ± 2.37	7.29
Zn	> 99.27	97.15 ± 7.40	7.62

^a Mean value ± SD, n=11

From the results, the metal chelates were quantitatively sorbed on a column packed with activated carbon. The results are in accordance with the good sorption of metal complexes of dithiocarbamate on non-polar surface such as polyurethane foam [53] and PTFE [54].

The percentage sorption was over 99% and the %recoveries precisely observed were satisfied with 97-106% for Cd, Cu, Ni and Zn. However, the %recovery of Pb was still poor.

3.2.2.1 Effect of the flow rate on metal recoveries

The influences of flow rate on the sorption of trace metals were studied. The flow rate in the range of 1-10 mL min⁻¹ had no significant effect on the recoveries of the investigated elements. As can be seen in Table 3.11, the recoveries of the metals were generally constant in the range of 95-104%. There was no effect of the loading flow rate of the sample solution on recoveries of Cd, Cu, Ni and Zn. The maximum flow rate was recommended in order to reduce the operation time. All subsequent experiments were performed at a 10 mL min⁻¹ flow rate. The results are shown in Table 3.11.

Table 3.11 Recoveries of Cd, Cu, Ni, Pb and Zn from different flow rate of sample solution (20 µg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II))

Elements	Recovery ^a , %		
	1 mL min ⁻¹	5 mL min ⁻¹	10 mL min ⁻¹
Cd	97.50 ± 3.56	95.42 ± 2.45	101.21 ± 2.68
Cu	97.20 ± 2.45	101.50 ± 3.12	96.50 ± 4.10
Ni	97.54 ± 3.51	96.87 ± 2.45	98.25 ± 1.87
Pb	36.54 ± 1.25	35.47 ± 2.65	30.25 ± 2.87
Zn	103.25 ± 4.10	104.56 ± 2.56	102.54 ± 3.45

^a Mean value ± SD, n = 3

3.2.2.2 Effect of the amount of complexing agent

In the method optimized by using model solution, 1 mL of 1% w/v APDC solution was added to 50 mL solution to form complex with metals. The excess of APDC does not give any better results. Thus, it seemed to be worth investigating the appropriate amount of APDC needed to be added to the sample. The effect of the amount of APDC on the extraction of metals was studied by varying concentration of APDC from 0.05-1% w/v APDC. Samples were prepared following method D (spiked with 20 μg of each of element, pH 5) and determined with GFAAS. The results are summarized in Figure 3.11 and Table 3.12. Starting with 20 μg metals spiked to sample solutions, it could be seen that only Zn could not be retained when the APDC concentration decreased to 0.05% w/v. Therefore, all subsequent studies were carried out with 0.1% w/v APDC solution. The metals were desorbed with 10 mL of 1 M nitric acid in acetone at flow rate 0.6 mL min^{-1} . The recoveries of Cd, Cu, Ni and Zn with 0.1% w/v APDC were above 97%. However, the recovery of Pb was 42%.

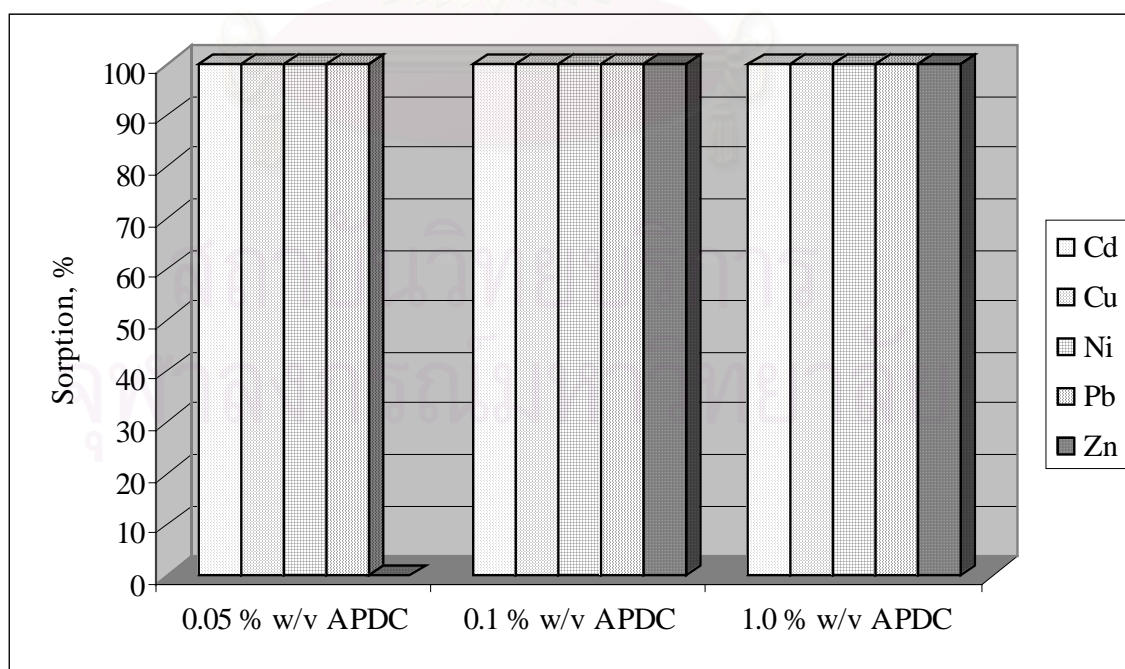


Figure 3.11 Effect of the amount of reagent on the analyte retention (20 μg each of Cd (II), Cu(II), Ni(II), Pb(II) and Zn(II), n=2)

Table 3.12 Recoveries of Cd, Cu, Ni, Pb and Zn in sample solution from preconcentration procedure (20 μg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II))

Elements	Recovery ^a , %		
	0.05% w/v APDC	0.1% w/v APDC	1.0% w/v APDC
Cd	104.12(3.12)	97.56(1.94)	96.54(2.56)
Cu	96.32(5.28)	100.78(1.32)	97.58(2.08)
Ni	98.65(2.18)	98.47(6.00)	99.85(2.68)
Pb	40.23(2.00)	42.05(0.08)	42.14(2.12)
Zn	-	103.25(4.04)	105.63(2.00)

^a Mean value(range), n=2

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

3.2.2.3 Influence of pH on metal sorption

In the method optimized by using model solution, pH of solutions was adjusted to 5 with 0.2 M ammonium acetate solution. It is controversial about pH of the complexation. From the previous works, sample solutions were adjusted to a pH of 1 [46], 2 [54] or 5 [36]. Preconcentration at the lower pH was preferable and the sample preparation is more convenient. At low pH, less amount of reagent was used to adjust the pH and this could reduce the contamination from the reagents. Thus, the pH of sample solution was studied within the range of 0.7-5 by adjusting the sample digestion solution with ammonium hydroxide solution. The results are summarized in Figure 3.12. At pH 0.7, all metals cannot be retained on the activated carbon. At pH 2, only Cu was retained. All elements were retained on activated carbon at pH 5.

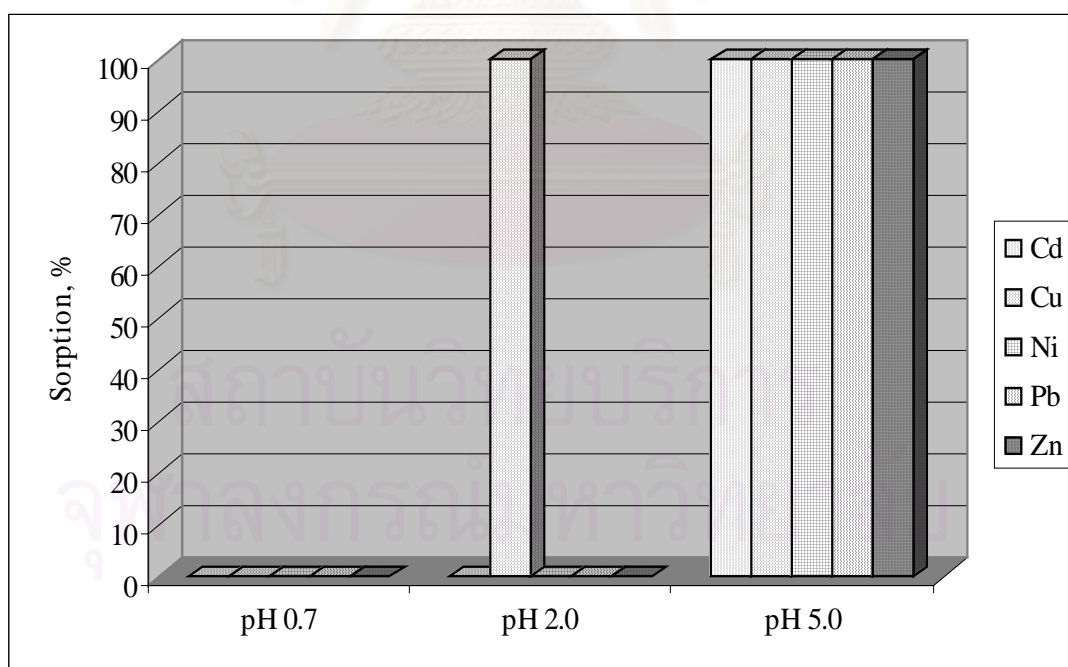


Figure 3.12 Effect of pH on the sorption of metallic complex on activated carbon(1 μg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II), $n=2$)

To study the elution of metals after sorption at pH 5, 1 M nitric acid in acetone was used to elute the sorbed metals. The spike amount of metals in this study was 1 μ g. The recoveries are shown in Table 3.13. The recoveries of Cd, Cu, Ni and Zn at pH 5 were the range of 84.25-111.15%. However, the recovery of Pb was lower (56%).

Table 3.13 Recoveries of Cd, Cu, Ni, Pb and Zn in sample solution from preconcentration procedure (1 μ g each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II))

Elements	Recovery ^a , %
Cd	100.62(6.25)
Cu	84.25(7.50)
Ni	93.52(0.25)
Pb	56.00(6.00)
Zn	111.15(0.78)

^a Mean value(range), n = 2

Even though some sorbents have been used without a conditioning step, it is not recommended [18]. Additionally, this step is crucial for successful retention of the analytes. The nature of the conditioning solvent must be appropriate to the nature of the solid sorbent to ensure a good wettability of functional groups. For example, with hydrophobic supports such as C18-silica or polystyrene divinylbenzene, quite polar organic solvents such as methanol should be used. 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 [19].

In this study, the low recovery of lead was obtained. The conditioning step is probably not suitable for the preconcentration of lead. Thus, changing the conditioning step was tried. Acetone was chosen for conditioning activated carbon and increasing wettability of the activated carbon. When the activated carbon was conditioned with acetone, followed by ammonium acetate buffer solution in subsequent, the results obtained are shown in Table 3.14. Acetone seems to be a good conditioning solvent,

giving %recoveries of Pb up to 88. Thus, it was chosen to condition the activated carbon for other metals (Cd, Cu, Ni and Zn).

Table 3.14 Recoveries of Cd, Cu, Ni, Pb and Zn in sample solution from condition with acetone followed ammonium acetate buffer solution (1 µg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II))

Elements	Recovery ^a , %	RSD, %
Cd	97.42 ± 4.42	4.53
Cu	92.50 ± 14.14	15.28
Ni	81.87 ± 0.53	0.64
Pb	88.30 ± 4.66	5.28
Zn	101.06 ± 6.03	5.97

^a Mean value ± SD, n = 4

From Table 3.14, the recoveries of Cd, Cu, Ni, Pb and Zn obtained for preconcentration of metals in fish tissue were within the range of 81-101.06%. The %RSD for Cd, Cu, Ni, Pb and Zn determination in fish tissues ranged between 0.64-15.28%. The %recoveries and %RSD obtained from the proposed method showed the performance of the method in preconcentration of the working elements in fish samples. Thus, method validation was investigated. The proposed method for preconcentration of metal in fish tissue was validated as follows:

Sample preparation step: A portion of 1.0 g fresh tissue sample was precisely weighted into quartz tubes. 1 µg each of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II) were spiked into fish tissue. Then, 2 mL of concentrated nitric acid and 30 % hydrogen peroxide were added. The tubes were placed in a six-position pressurized bomb and the mixtures were digested under pressure. The final volume was diluted with de-ionized water to 50 mL after adjusting the pH to 5 with ammonium hydroxide.

Preconcentration step: 1 mL of 0.1% w/v APDC was added to the 50 mL of sample solution in order to form complex with metal prior to solid phase extraction. The column containing 0.1 g of activated carbon was conditioned by passing 5 mL of acetone followed by 5 mL of 0.2 M ammonium acetate buffer solution (pH 5). The metal-APDC solution was passed through the column at a flow rate of 10 mL min⁻¹. The metal-APDC complexes retained on the column were eluted with 10 mL of 1 M nitric acid in acetone at flow rate of 0.6 mL min⁻¹. The eluate obtained was evaporated to about 1 mL and then made up to 5 mL in a volumetric flask with 1% nitric acid. The metals concentration in this solution was determined by GFAAS.

3.4 Method validation

In order to demonstrate the validity of this method, the precision and the accuracy of the method were investigated by using the sample solutions containing spiked metals. 1 µg of metal was spiked into 1 g of sample prior to microwave digestion and preconcentration on activated carbon. The precision and accuracy of the proposed method are presented as %RSD and %recovery respectively. The detection limit (DL) is commonly defined as analyte concentration giving a signal equal to three times of standard deviation of blank signals. The method blank was analyzed 10 times. The results of the instrument detection limits (IDL), method detection limits (MDL), the precision and recoveries are shown in Table 3.15. The IDL is calculated by the following equation 3.3.

$$\text{IDL} = 3 \times \text{SD}_{\text{method blank}} \dots\dots\dots(3.3)$$

where $\text{SD}_{\text{method blank}}$ = standard deviation of the concentration (ppb) of the method blank.

The MDL is estimated by equation 3.4.

$$\text{MDL} = \text{IDL}(\text{ppb}) \times 5.00 \text{ mL} / \text{weight of sample} \dots\dots\dots(3.4)$$

The acceptable values of %RSD and %recovery for trace analysis are summarized in Table 3.16. The recoveries of Cd, Cu, Ni, Pb and Zn obtained for preconcentration of metals in fish tissue were 97.42, 92.50, 81.87, 88.30, and

101.06%, respectively, showing the performance and the accuracy of the method in preconcentration of the working elements in fish samples.

The relative standard deviation (% RSD) for the five element determination in the preconcentration of sample was 4.53, 15.28, 0.64, 5.28, and 5.97% for Cd, Cu, Ni, Pb and Zn, respectively. The %RSD for Cd, Ni, Pb and Zn determination in fish tissues ranged between 0.64-5.97%, showing a very good precision of the method. But %RSD of Cu (n = 4) is higher than acceptable values (more than 11) from Table 3.16. The Q-test for 95% confidence limit was also applied in the results of Cu for the suspect measurement to be retained or rejected. It demonstrated also that there was no significant difference among the four values. Thus, the suspect value was still retained. Therefore, to improve the precision of Cu, it is recommended to perform a larger number of replicates.

Table 3.15 Recovery, precision and detection limit of the proposed method

Elements	Recovery ^a , %	RSD, %	IDL ($\mu\text{g L}^{-1}$)	MDL ($\mu\text{g g}^{-1}$)
Cd	97.42 \pm 4.42	4.53	2.34	11.70
Cu	92.50 \pm 14.14	15.28	2.12	10.60
Ni	81.87 \pm 0.53	0.64	2.68	13.40
Pb	88.30 \pm 4.66	5.28	2.01	10.05
Zn	101.06 \pm 6.03	5.97	2.53	12.65

^a Mean value \pm SD, n = 4

Table 3.16 Analyte recovery and precision at different concentration [55]

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

Finally, the method was applied to the analysis of fish tissue samples. The results are given in Table 3.17. In the fish tissue sample, the amount of Zn was found at 5.50 $\mu\text{g g}^{-1}$. However, Cd, Cu, Pb and Ni were not found because the concentration level of these metals were below the detection limit.

Table 3.17 Concentrations of Cd, Cu, Ni, Pb and Zn in fish tissue samples

Metal	Concentration ($\mu\text{g g}^{-1}$)
Cd	ND
Cu	ND
Ni	ND
Pb	ND
Zn	5.50 \pm 0.02

accuracy of the method in preconcentration of water samples. But recoveries of Cd and Pb are lower than acceptable values (less than 80%).

%RSD of Cu, Ni and Zn are within the range 3.87-7.27%, showing a good precision of the method.

The present method is suitable for Cu, Ni and Zn in water sample because given % recovery and % RSD related with acceptable values (Table 3.16). But this method is not suitable for determination Pb and Cd due to lower % recoveries.

The results of water samples were much different from what was obtained when model solutions were used. The sample solution differs from model solutions in matrix. In natural waters, colloidal or organic compounds, e.g. humic material and abundant inorganic compounds, may exhibit strong complexation properties for trace metals ions. Little attention has been paid analytically to the influence of such interactions on the preconcentration techniques which are generally developed for trace metal ions and are tested only on synthetic solution. When, in natural waters, an important fraction of the trace metals in presence of strong organic complexes that are difficult to decompose, or is adsorbed on colloids, the behaviors of the metals during the preconcentration step will not be the same as in synthetic solutions and the collection efficiency may vary significantly.

The observed results are consistent with the literature reported. Smits et al [56] demonstrated that the influences of the water matrix had effect on %recoveries of metals (Mn, Co, Zn and Eu) from chelation by 8-quinolinol followed by adsorption on activated carbon. From the results, for distilled water the recoveries are rather high in distilled water doped with humic material and natural waters of five different types. The recoveries of Zn and Mn in estuarine water decreased from 100 in distilled water to 84 and 89 respectively.

It seems that the proposed method might be applied for drinking water analysis or tap water.

Table 3.19 Recoveries of Cd, Cu, Ni, Pb and Zn in soil sample

Element	Added	Found ^a	Recovery, ^b %	RSD, %
Cu	0	ND	-	-
	1	0.79 ± 0.08	79.34	10.13

^a Mean value ± SD, n = 4

^b average recovery of metals added in each sample

ND = not detectable

The present method was applied to the determination of Cd, Cu, Ni, Pb and Zn in soil. The present method is not suitable for metal ion in soil. Only copper can totally sorbs on activated carbon. Cd, Ni, Pb and Zn could not be sorbed on activated carbon. During an experiment, it was noticed that iron was precipitated as hydroxide form during adjust pH to 5 with ammonium hydroxide. Because colloidal precipitated cannot be retained on activated carbon column. Thus, metal may be sorbed on the surface of this colloidal that could not be retained on the activated carbon column.

The sample preparation used is the EPA method 3050B. This method is recommended for the determination of metals in solid sample by ICP-OES after mineralization without preconcentration step. As the results of the proposed preconcentration method, it is surely possible that the sample preparation is not suitable. The adequate mineralization procedure should be studies for further work, such as by using an acid-leaching technique [57].

CHAPTER IV

CONCLUSION

This thesis attempted to develop a method for the determination of very low levels of metals in aqueous solution using preconcentration method by activated carbon and ammonium pyrrolidinedithiocarbamate (APDC) following by the determination by AAS. A simultaneous preconcentration of Cd, Cu, Ni, Pb and Zn on an activated carbon impregnated with APDC column and preconcentration of metallic complexes on an activated carbon column was developed. The conditions for preconcentration of metals on activated carbon column were optimized using model solutions. The results achieved (%recovery and %RSD) with the two methods are similar. The recoveries of higher than 95% can be obtained in the case of Cd, Cu, Ni and Zn. However, the recovery of Pb is lower.

The optimized conditions from model solutions were applied to the analysis of the metals in fish tissue using activated carbon impregnated with APDC. Only copper could be retained and recovered quantitatively from activated carbon impregnated with APDC. The results were much different from what was obtained when model solutions were used. The difference in matrix may also cause the change in metal sorption. Thus, the preconcentration of metal with impregnated activated carbon is not suitable for the analysis of metals in fish tissue.

The second method, forming metal-APDC complexes before passing into activated carbon was applied to the analysis of metals in fish tissue. The metal chelates were quantitatively sorbed on the activated carbon. All metals chelates were efficiently sorbed onto 0.1 g of activated carbon at pH 5. APDC with the concentration of 0.1% w/v was used for the complexation of metals and 1 M nitric acid in acetone was used as eluent. The relative standard deviation (RSD) was 4.53, 15.28, 0.64, 5.28, and 5.97%, the method detection limit (MDL) was 11.70, 10.60, 13.40, 10.05, and 12.65 $\mu\text{g kg}^{-1}$, and recovery was 97.42, 92.50, 81.30, and 101.06% for Cd, Cu, Ni, Pb and Zn, respectively.

The method was applied to determination of metal in water and soil samples. The present method is suitable for Cu, Ni and Zn in water sample because given a good %recovery. But this method is not suitable for determination Pb and Cd because

of a lower %recovery. The present method is not suitable for metal ion in soil. Only copper can totally sorbs on activated carbon. Cd, Ni, Pb and Zn could not be sorbed in activated carbon.

Potential applications and suggestions for future works:

The present method will be applied to determination of metals in other samples such as drinking water and another kind of food. The effect of major cations and anions or other dissolved substances in sample solution on the sorption/desorption of working metal ions should be investigated.



สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

REFERENCES

1. Bailey, R.A.; Clark, H.M.; Ferris, J.P.; Krause, S. and Strong, R.L., *Chemistry of environment* 2nd ed. London: Academic Press, 2002.
2. Vanloon, G.W. and Duffy, S.J., *Environmental chemistry a global perspective* New York: Oxford University Press Inc., 2001.
3. CAL/ FAL 4-1978 *List of Maximum Levels Recommended for Contaminants by the Joint FAO/ WHO Codex Alimentarius Commission, Third series* Rome: Food and Agricultural Organization of the United Nations and World Health Organization, 1978.
4. พูลทรัพย์ วิรุพหกุล, โสภะหนัก: สถานะภาพการปนเปื้อนในสัตว์น้ำและผลิตภัณฑ์ของไทย กรมประมง, 2003.
5. Watson, C., "Sample preparation for the analysis of heavy metals in foods", *Trends Anal. Chem.*, **1984**, 3, 25-28.
6. U.S. Food and Drug Administration, *Guidance documents for trace elements in seafood* Washington D.C: U.S. Food and Drug Administration, 1993.
7. AOAC Official Method of Analysis, *AOAC Official Method 999.11* Arlington: AOAC International, 2000.
8. AOAC Official Method of Analysis, *AOAC Official Method 999.10* Arlington: AOAC International, 2000.
9. Skoog, D.A., *Principles of instrumental analysis* 3rd ed. Philadelphia: Saunders College Publishing, 1985.
10. Balcerzak, M., "Sample digestion methods for the determination of traces of precious metals by spectrometric techniques", *Anal. Sciences*, **2002**, 18, 737-750.
11. Fritz, J.S. *Analytical solid-phase extraction* USA: John Wiley & Sons, 1999.
12. Sandell, E.B. *Colorimetric Determination of Traces of Metals* Vol.3 USA: Interscience Publishers, Inc., 1959.
13. Okuno, I.; Whitehead, J.A. and White, R.E., "Flameless atomic absorption spectroscopic determination of heavy metals in whole-fish samples", *J. Assoc. Off. Anal. Chem.*, **1978**, 61, 664-667.

14. Sperling, K.R., "Determination of heavy metals in seawater and in marine organisms by flameless atomic absorption spectrophotometry", *Fresenius J. Anal. Chem.*, **1978**, 292, 113-119.
15. AOAC Official Method of Analysis, *AOAC Official Method 934.07* Arlington: AOAC International, 2000.
16. Salari, H. and Steffenrud, S., "Comparative study of solid phase extraction techniques for isolation of Leukotrienes from plasma", *J. Chromatogr.*, **1986**, 378, 35-44.
17. Saracoglu, S.; Divrikli, U.; Soylak, M. and Elci, L., "Determination of copper, iron, lead, cadmium, cobalt and nickel by atomic absorption spectrometry in baking powder and baking soda samples after preconcentration and separation", *J. Food Drug Anal.*, **2002**, 10, 188-194.
18. Camel, V., "Review solid phase extraction of trace elements", *Spectrochim. Acta Part B*, **2003**, 58, 1177-1233.
19. Simpson, N.J.K., *Solid phase extraction; Principles, techniques and applications* USA: Marcel Dekker, Inc., 2000.
20. Pesavento, M. and Baldini, E., "Study of sorption of copper(II) on complexing resin columns by solid phase extraction", *Anal. Chim. Acta*, **1999**, 389, 59-62.
21. Yamini, Y.; Hassan, J.; Mohandesi, R. and Bahramifar, N., "Preconcentration of trace amounts of beryllium in water sample on octadecyl silica cartridges modified by quinalizarine and its determination with atomic absorption spectrometry", *Talanta*, **2002**, 56, 375-380.
22. Zaporozhets, O.; Petruniok, N. and Sukhan, V., "Determination of Ag(I), Hg(II) and Pb(II) by using silica gel loaded with dithizone and zinc dithizone", *Talanta*, **1999**, 50, 865-873.
23. Roman, L.; Florean, E.; Sandulescu, R. and S. Mirel., "Preconcentration of Pb(II), Cd(II), Cu(II) and Hg(II) with 2-mercapto-5-phenylamino-1,3,4-thiadiazole impregnated on silica gel", *J. Pharm. Ana.*, **1996**, 14, 1003-1006.
24. Goswami, A. and Singh, A.K., "1,8-Dihydroxyanthraquinone anchored on silica gel: synthesis and application as solid phase extraction for lead(II), zinc(II) and cadmium(II) prior to their determination by flame atomic absorption spectrometry", *Talanta*, **2002**, 58, 669-678.
25. Abou-El-Sherbini, K.; Kenawy, I.M.M.; Hamed, M.A.; Issa, R.M. and Elmorsi, R., "Separation and preconcentration in a batch mode of Cd(II), Cr(III, VI), Cu(II),

- Mn(II, VII) and Pb(II) by solid-phase extraction by using of Silica modified with N-propylsalicylaldimine”, *Talanta*, **2002**, 58, 289-300.
26. Tokman, N.; Akman, S. and Ozcan, M. “Solid phase extraction of bismuth, lead and nickel from seawater using silica gel modified with 3-aminopropyltrithoxysilane filled in a syringe prior to their determination by graphite furnace atomic absorption spectrometry”, *Talanta*, **2003**, 59, 201-205.
 27. Hutchinson, S.; Kearney, G.A., Horne, E.; Lynch, B. and Glennon, M.A.; McKervey, M.A. and Harris, S.J., “Solid phase extraction of metal ions using immobilized chelating calixarene tetrahydroxamates”, *Anal. Chim. Acta*, **1994**, 291, 269.
 28. Ali, A.; Yin, X.; Shen, H.; Ye, Y. and Gu, X., “1,10-Phenanthroline as complexing agent for on-line sorbent extraction/preconcentration for flow injection-flame atomic absorption spectrometry”, *Anal. Chim. Acta*, **1999**, 392, 283-289.
 29. Ferreira, S.L.C.; Lemos, V.A.; Santelli, R.E.; Ganzarolli, E. and Curtius, A.J., “An automated on-line flow system for the preconcentration and determination of lead by flame atomic absorption spectrometry”, *Microchem. J.*, **2001**, 68, 41-46.
 30. Ferreira, S.L.C.; Santos, W.N.L.D. and Lemos, V.A., “On-line preconcentration system for nickel determination in food samples by flame atomic absorption spectrometry”, *Anal. Chem. Acta*, **2001**, 445, 145-151.
 31. Ferreira, S.L.C.; Lemos, V.A.; Moreira, B.C.; Costa, A.C.S. and Santelli, R.E., “An On-line continuous flow system for copper enrichment and determination by flame atomic absorption spectroscopy”, *Anal. Chim. Acta*, **2000**, 403, 259-264.
 32. Melo, M.H.A.; Ferreira, S.L.C. and Santelli, R.E., “Determination of cadmium by FAAS after on-line enrichment using a mini column packed with Amberlite XAD-2 loaded with TAM”, *Microchem. J.*, **2000**, 65, 59-65.
 33. Zachariadis, G.A.; Anthemidis, A.N.; Bettas, P.G. and Stratis, J.A., “Determination of lead by on-line solid phase extraction using a PTFE micro-column and flame atomic absorption spectrometry”, *Talanta*, **2002**, 57, 919-927.
 34. Fang, Z.; Xu, S.; Dong, L. and Li, W., “Determination of cadmium in biological materials by flame atomic absorption spectrometry with flow-injection on-line sorption preconcentration”, *Talanta*, **1994**, 41, 2165-2172.
 35. Matisova, E. and Skrabakova, S., “Carbon sorbents and their utilization for the preconcentration of organic pollutants in environmental samples”, *J. Chromatogr. A*, **1995**, 707, 145-179.

36. Liu, Z. and Huang, S., "Determination of copper in sea water by preconcentration and electrothermal atomic absorption spectrometry", *Anal. Chim. Acta*, **1992**, 267, 31-37.
37. Yunes, N.; Moyano, S.; Cerutti, S., Gasquez, J.A. and Martinez, L.D., "On-line preconcentration and determination of nickel in natural water samples by flow injection-inductively coupled plasma optical emission spectrometry", *Talanta*, **2003**, 59, 943-947.
38. Piperki, E.; Berndt, H. and Jackwerth, E., "Investigations on the sorption of metal chelates on activated carbon", *Anal. Chim. Acta*, **1978**, 100, 589-596.
39. Narin, I.; Soylak, M.; Elci, L. and Dogan, M., "Determination of trace metal ions by AAS in natural water samples after preconcentration of pyrocatechol violet complexes on an activated carbon", *Talanta*, **2000**, 52, 1041-1046.
40. Yunes, N.; Moyano, S.; Cerutti, S.; Gasquez, J.A. and Martinez, L.D., "On-line preconcentration and determination of nickel in natural water samples by flow injection-inductively coupled plasma optical emission spectrometry", *Talanta*, 59, **2003**, 934-949.
41. Santelli, R.E.; Gallego, M. and Valcarcel, M., "Preconcentration and atomic absorption determination of copper traces in waters by on-line adsorption-elution on an activated carbon minicolumn", *Talanta*, **1994**, 41, 817-823.
42. Cerutti, S.; Silva, M.F.; Gasquez, J.A.; Olsina, R.A. and Martinez, L.D., "On-line preconcentration/determination of cadmium in drinking water on activated carbon using 8-hydroxyquinoline in a flow injection system coupled to an inductively coupled plasma optical emission spectrometer", *Spectrochim. Acta Part B*, **2003**, 58, 43-50.
43. Quinaia, S.P.; De Silva, J.B.B.; Rollemberg, M.D.C.E. and Curtius, A.J., "Preconcentration of lead complexed with *O,O*-dithiophosphate by column solid-phase extraction using different sorbents in a flow injection system coupled to a flame atomic absorption spectrometer", *Talanta*, **2001**, 54, 687-696.
44. Giacomelli, M.B.O.; De Silva, J.B.B. and Curtius, A.J., "Determination of Mo and Bi in steels by electrothermal atomic absorption spectrometry after complexation and sorption on activated carbon", *Talanta*, **1998**, 47, 877-881.

45. Kimura, M. and Kawanami, K., "Preconcentration of traces of silver and bismuth from cobalt and nickel and their nitrates, with activated carbon", *Talanta*, **1979**, 26, 901-903.
46. De Pena, Y.P.; Gallego, M. and Valcarcel, M., "On-line sorbent extraction, preconcentration and determination of lead by atomic absorption spectrometry", *Talanta*, **1995**, 42, 211-218.
47. De Pena, Y.P.; Gallego, M. and Valcarcel, M., "Flame atomic absorption spectrometric determination of cadmium in biological samples using a preconcentration flow system with an activated carbon column and dithizone as a chelating agent", *Anal. At. Spectrom.*, **1994**, 9, 691-696.
48. Kimbrough, D.E., and Wakakuwa, J.R., "Acid digestion for sediments, sludges, soil, and solid wastes. A proposed alternative to EPA SW 846 Method 3050", *Environ. Sci. Technol.*, **1989**, 23, 898-902.
49. Atanassova, D.; Stefanova, V. and Russeva, E., "Preconcentration of trace elements on a support impregnated with sodium diethyldithiocarbamate prior to their determination by inductively coupled plasma-atomic emission spectrometry", *Talanta*, **1998**, 45, 857-864.
50. Ramesh, A.; Mohan. K.R. and Seshaiyah, K., "Preconcentration of trace metals on Amberlite XAD-4 resin coated with dithiocarbamates and determination by inductively coupled plasma-atomic emission spectrometry in saline matrices", *Talanta*, **2002**, 57, 243-252.
51. Tokalioglu, S.; Kartal, S. and Elci, L., "Determination of trace metals in waters by FAAS after enrichment as metal-HMDTC complexes using solid phase extraction", *Bull. Korean Chem. Soc.*, **2002**, 23, 693-698.
52. Goswami, A and Singh, A.K., "Solica gel functionalized with resacetophenone: synthesis of a new chelating matrix its application as metal ion collector for their flame atomic absorption spectrometric determination", *Anal. Chim. Acta*, **2002**, 454, 229-240.
53. Anthemisdis, A.N.; Zachariadis, G.A. and Stratis, J.A., "On-line preconcentration and determination of copper, lead and chromium (VI) using unloaded polyurethane foam packed column by flame atomic absorption spectrometry in natural waters and biological samples", *Talanta*, **2002**, 58, 831-840.
54. Anthemisdis, A.N.; Zachariadis, G.A. and Stratis, J.A., "On-line solid phase extraction system using PTFE packed column for the flame atomic absorption

spectrometric determination of copper in water samples”, *Talanta*, **2001**, *54*, 935-942.

55. Huber, L., *Validation and qualification in analytical laboratories* Illinois: Interpharm Press, Inc., 1999.

56. Smits, J.; Nelissen, J. and Grieken, R.V., “Comparison of preconcentration procedures for traces metals in natural waters”, *Anal. Chim. Acta*, **1979**, *111*, 215-226.

57. Sastre, J.; Sahuquillo, A; Vidal, M. and Rauret, G., “Determination of Cd, Cu, Pb and Zn in Environmental samples: microwave-assisted total digestion versus aqua regia and nitric acid extraction”, *Anal. Chim. Acta*, **2002**, *462*, 59-72.



สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

VITA

Miss Pornlada Daorattanachai was born on April 16, 1979 in Ubonratchatani, Thailand. She received her Bachelor, degree of Science in Chemistry from Srinakharinwirot University in 2000. Since 2001, she has been a graduate student at the Department of Chemistry, Chulalongkorn University and become a member of Environmental Analysis Research Group. She finished her postgraduate study in leading of Master's degree of Science in 2003.



สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย