CHAPTER 1

INTRODUCTION

1.1 Introduction

Food is the basic necessary for human living and growth. The relation between food and human is started from the consumption of food. The necessary nutrients help the human body to growth and control the body system to be normal operation. The important nutrients for human body are carbohydrate, lipid, protein and vitamins (Suwanpinij, 2004).

The main food resources come from agriculture, farm and fishery. All of these sources are important for producing the foods for human consumption in our country. Especially the fishery products have important role in the exportation. The marine product manufactures from fishery in each year about 1.8 to 2.0 million tons are frozen for inside country consumption. The frozen seafood products are exported to the foreign country such as prawns, mussels, crabs, fishes and squids (Katenil, 2005). They are more important exporting products that make profit for our country. Nevertheless, the foreign countries which import our frozen seafood products have been declined in sometimes. Because the imported countries find the over limit of heavy metal contaminations in frozen seafood products. This problem affects to the exportation of our country (Suthatham, 2005).

The standard levels heavy metals contaminated in foods of each country depend on the agreement of the committee in various countries. For example, the cadmium and lead contamination in seafood for Russia specify as follow; cadmium levels are not more than 0.05 μ g.g^{-1} and lead levels are not more than 0.5 μg g^{-1} (Suthatham, 2005).

Australian food safety authority specify the heavy metal contamination in seafood are as follow; cadmium and lead levels are not more than 2 μg g^{-1} (Australian New Zealand Food Authority, 1998).

The contamination of cadmium and lead in seafood not only have affected to exportation but also affected to human health due to the highly toxic of these metals (Katenil, 2005).

Cadmium and lead are well recognized to be highly toxic elements to human being. They are widely dispersed in the environment, and exposure element can increase the

number of adverse health effects due to their toxicity after accumulation in multiple organs in human body (Wang *et al.,* 2004). Food is a constant source for cadmium and lead accumulation in human body (Correia *et al.,* 2000).

The contamination of heavy metals in food is interested owing to the consumption of these metals has affected to human health. The cadmium and lead contamination in octopuses and cuttlefishes from Samuthprakran, Samuthsakorn, Rayong, Songkhla and Nakhornsrithammarach provinces were studies. The results found that the contamination of cadmium in octopuses and cuttlefishes were in between 0.12-0.48 μg g^{-1} and 0.06-0.09 μg g^{-1} respectively and the lead contaminations in octopuses and cuttlefishes were in between 0.06-0.31 μg g^{-1} and 0.05-0.22 μg g^{-1} respectively (Kungsuwan *et al*., 1997).

The cadmium and lead levels in fishes and prawn from Songkhla Lake were determined. The cadmium contamination in fishes and prawns were found in between 0-0.010 μg g^{-1} and 0-0.380 μg g^{-1} respectively and lead levels in fishes and prawns were found in between 0.163-1.985 μg g^{-1} and 0-2.625 μg g^{-1} respectively (Meesuk and Benjakul, 1998). The contamination of cadmium in seafood samples from the eastern seashore of the Gulf of Thailand was found in between 0.01-3.52 μg g^{-1} (dry weight) (Kan-atireklap *et al*., 1999). The lead contamination in shrimps and fishes were in range 0.83-1.721 μg g^{-1} (dry weight) and 0.88-1.34 μ g g^{-1} (dry weight) (Lemos and Ferreira, 2001). Moreover, the contamination of cadmium and lead in fishes were monitored. The contamination were found in between 0.09-0.48 μg g^{-1} (dry weight) and 0.22 – 0.85 μg g^{-1} (dry weight) respectively (Tuzen, 2003).

The concentration of cadmium and lead in food are usually found at the trace levels and the matrix effect has interference to the analysis. Then the sensitive instrument technique and sample preparation techniques are important (Wang *et al*., 2004).

The sample preparation step is an important for trace element analysis due to it can avoid the organic matrix problem and make the good accuracy and precision analysis results. The sample preparation methods using for food samples have variously such as dry ashing, wet digestion (acid digestion) and microwave digestion (Hamepatawee and Krongkrot, 2001).

Dry ashing is the most commonly used preparative technique in the determination of metal contents of foods. With dry ashing, organic matter is destroyed by high temperature oxidation in range 400-750 ^{o}C. Dry ashing offers method simplicity, require little

attention, and is suited for large numbers of samples. Ashing acid may be used to assist in the oxidation of organic matter. Drawbacks of dry ashing include volatized losses of some metals at high temperature and some analyte is adsorbed onto the crucible (Jeon *et al*., 1995).

Wet digestion is used typically for sample preparation. Various acids are used to oxidize the organic materials of the food matrix. The acidic materials most frequently used are nitric acid, sulphuric acid, hydrochloric acid or perchloric acid. For foods of biological origin the digestion reagents often used are nitric acid. The organic matrix in sample is destroyed by using acid and heating. The wet digestion performs on the hot plate in the temperature range 60-100 ^{o}C at the atmospheric pressure. This method is called "Hot plate digestion". The disadvantages of this method are time consuming, more acid volume, labor intensive and work carefully. Wet digestion method can reduce the sample loss and sample destruction due to the method performs in low temperature (Hamepatawee and Krongkrot, 2001).

Microwave digestion is one of the acid digestion methods which perform by pressurized dissolution. Solution are heated so efficiently that reaction time scales are dramatically reduced and process control offered by microwave heating is better than any other heating method (Buldini *et al*., 2002). Microwave digestions perform in the close system which is very safety. The advantages of this method are safety, minimized contamination, rapid, automation and many samples can digest together in one time (Shulikawit *et al*., 2002).

The concentration of cadmium and lead are found at the trace levels then the high sensitivity and accuracy method are important. Moreover, the interferic effects sourced from the matrix of real samples are main difficulties in these metals analysis. These problems could generally be prevented by using preconcentration and separation methods.

The preconcentration techniques have been used such as liquid-liquid extraction (Solvent extraction) and solid phase extraction (Narin and Soylak, 2003).

Liquid-liquid extraction (LLE) of different metal ions has been widely employed in chemistry and industry for many years. However, the use of classical extraction methods are usually time-consuming, labor-intensive and require relative large volumes of high purity solvents (Hashemi *et al*., 2001).

Solid phase extraction technique involves the use of solid adsorbent to trap analytes and separate them from the bulk of the matrix. As the sample solution passes through

the sorbent, analytes concentrate on its surface, while the other sample components pass through the sorbent (Buldini *et al*., 2002).

Solid phase extraction is an attractive technique that reduces solvent usage and exposure which can create a severe environmental problem and health effect, disposal costs and extraction time for sample preparation. This technique has been successfully used for the preconcentration, separation and sensitive determination of trace metals ions (Shamsipur *et al*., 2001). Moreover, the solid phase extraction has many advantages. First; they are more ecofriendly and second, it is easier to reused the solid sorbent (Goswami and Singh, 2002).

1.1.1 Solid phase extraction (SPE)

The principle of solid phase extraction is similar to that of liquid-liquid extraction (LLE), involving a partitioning of solutes between two phases. Liquid-liquid extraction (LLE) is the partition between two immiscible liquid phases. Solid phase extraction involves partitioning between a liquid (sample matrix) and solid (sorbent) phase. This sample treatment technique enables the concentrate and purification of analytes from solution by sorption on a solid sorbent (Liska, 2000; Poole, 2000 (a)).

1.1.1.1 Solid phase extraction procedure

The solid phase extraction method always consists of three successive steps are as follows; First, the solid sorbent should be conditioned using an appropriate solvent. This step is crucial, as it enables the wetting of the packing material and the solvation of the functional group. In addition, it removes possible impurities initially contained in the sorbent or the packaging. The nature of the conditioning solvent depends on the nature of the solid sorbent. Typically, for reversed phase sorbent such as octadecyl-bonded silica, methanol and ethanol are frequently used. The second step is the percolation of the sample through the solid sorbent. The sample may be applied to the column by gravity, pumping, aspirated by vacuum or by an automated system. The analytes are concentrated on the sorbent. The third step consists of the elution of the analytes of interest by an appropriate solvent, without removing retained matrix components (Camel, 2003).

1.1.1.2 Adsorption of metals

Adsorption of trace elements on the solid sorbent is required for preconcentration. The mechanism of retention depends on the nature of the sorbent, and may include simple adsorption such as chelation or ion-exchange. Trace elements are usually adsorbed on solid phase through van der waals force or hydrophobic interaction. Hydrophobic interaction occurs when the solid sorbent is highly non-polar (reverse phase). The most common sorbent of this type is octadecyl-bonded silica (C18-silica) (Lundgren and Schilt, 1977).

1.1.1.3 Chelation

Several functional groups are capable of chelating trace elements. The atoms most frequently used are nitrogen (e.g. N present in amine, azo group, amides, nitriles), oxygen (e.g. O present in carboxylic, hydroxyl, phenolic, ether, carbonyl, phosphoryl groups), 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, inorganic cations may be divided into 3 groups.

Group I: “Hard cations” these preferentially react via electrostatic interactions, this group includes alkaline and alkaline-earth metals such as Ca^{2+}, Mg^{2+} and Na^{+} that form rather weak outer-sphere complexes with only hard oxygen ligands.

Group II: “Borderline cations” these have an intermediate character, this group contains Fe^{2+}, Co^{2+}, Ni^{2+},Cu^{2+}, Zn^{2+}, Pb^{2+} and Mn^{2+} . They possess affinity for both hard and soft ligands.

Group III: “Soft cations”: These tend to form covalent bonds. Hence, Cd^{2+} and Hg^{2+}possess strong affinity for intermediated (Nitrogen; N) and soft (sulfur; S) ligands (Camel, 2003).

The chelating agent uses for complexation should form the high stability metal complex. Generally, the frequency use molecules are ion-exchange group or chelating functions depend on the interested metals. The chelation techniques are pointed on the various parameters such as amount of chelating ligand, optimum pH and adsorption and flow rate.

The complex formation; ligands attach to the metal by coordinate covalent bonds and the electrons of the bond is entirely derived from the ligand. The ligand is a lewis base and acts as an electron pair donor. The metal is the electron acceptor and lewis acid.

The ligands that attach at a single point are monodentate and many points are multidentate ligands. The stability of complex increase with the number of attachment points so multiligand complexes are more stable than monodentate, called the chelate effect.

The complexes containing 5 or 6 member rings are the most stable, while 4 or 7 membered rings are less stable (Abdollahi and Zeinali, 2004).

The stability of complex is presented on the formation constant. The complexation equation of metal (M) and ligand (L) are illustrated as follows.

$$nL + M \; <====> \; ML_n$$

Formation constant (β_n)

$$\beta_n = [ML_n] \; / \; [M]\,[L]^n \qquad (1)$$

The stability constant or formation constant is large indicate stable complexes (Strahmann and Kock, 1978). Chelating agents may be directly added to the sample for chelating trace elements, the chelates being further retained on an appropriate sorbent. An alternative is introduced the functional chelating group into the sorbent. For that purpose, three differences means are available:

(1) The synthesis of new sorbents containing such groups (new sorbents).

(2) The chemical bonding of such group on existing sorbents (functionalized sorbents).

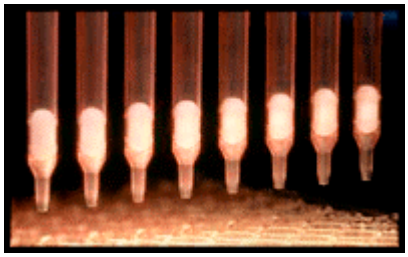
(3) The physical binding of the groups on the sorbent by impregnation the solid matrix with a solution containing the chelating ligand (impregnated, coated or loaded sorbents).

Different ligands immobilized on a variety of solid matrices have been

successfully used for the preconcentration, separation and determination of trace metal ions. Chelating agents with hydrophobic group are retained on hydrophobic sorbents (C18-silica) (Kilian and Pyrzynska, 2001).

1.1.1.4 Sorbent formats

The sorbent may be packaged in different formats: filled micro-columns, cartridges, syringe barrels and disk. The disposable sorbent containers are illustrated in Figure 1-1.



(a) Micro-column

(b) Syringe barrel

(c) Cartridges

(d) Disks

Figure 1-1 Disposable sorbent containers; (a) Micro column, (b) Syringe barrel (c) Cartridges, (d) Disks

- Micro-column

The use of a micro-column is a common procedure for extraction of trace metals from various samples. The size of the column may be adapted to the sample volume. In particular, it allows larger sample volumes, thus enabling the preconcentration of metal ions at very low concentration levels. However, such columns must be reused, so that careful blank washing should be conducted to avoid cross-contamination. In addition, columns with a narrow internal diameter limit usable flow rates to a range 1-10 ml min^{-1} that necessitates long trace-enrichment times for large sample volumes (Kumar *et al*., 2000).

- Cartridges and syringe barrels

The most frequently used design in solid phase extraction is the cartridge or the syringe barrel. They are usually made of polypropylene or polyethylene and filled with packing material having different functional groups. They afford great selectivity due to the bond types of sorbents contained in commercially available systems with different column volume available. In addition, their disposable character prevents possible cross contamination. The major disadvantages of cartridges and syringe barrels are slow sample-processing rates and a low tolerance to blockage by particles and adsorbed matrix components, due to their small cross-sectional area. Channeling reduces the capacity of the cartridge to retain analytes and results in contamination of the isolated analytes with impurities originating of the manufacturing and packing process (Junk *et al*., 1988).

- Disks

The use of flat disks with a high cross-sectional area may largely prevent all the problem encountered with columns, cartridges and syringe such as back pressure and low flow rate (Poole (a), 2000). The packing material is usually embedded in an inert matrix of polytetrafluoroethylene (PTFE) microfibrils (Hagen *et al*., 1990). The disks are available in different diameters, the size most frequently used being 47 mm. They are designed to be used in conjunction with a filtration apparatus connected to a water aspirator. In order to remove potential interferences and to ensure optimal extraction of the analyte of interest, disk cleaning and conditioning should be done before its use (Thurman *et al*., 2000).

Disks present the advantage of reducing solvent volumes for both the conditioning and elution steps. Additionally, the back-pressure encountered with these devices enables the use of high flow rates. The disks are primarily dedicated to biological samples (Rossi *et al*., 2003).

- Octadecyl silica membrane disks

Octadecyl silica membrane disk is comprised of polytetrafluoroethylene (PTFE) polymer and chemically modified silica particles tightly bound in a web of micro-PTFE fibrils. The particles are individually suspended so that the surface of each is free for maximum interaction with the sample during separation. Since the particles are mechanically held by the PTFE matrix, no chemical binders are necessary. The chemical structure of octadecyl silica membrane disk is shown in Figure 1-2 and scanning electron microscope (SEM) of PTFE polymer in Figure 1-3 (3M Company, 2004).

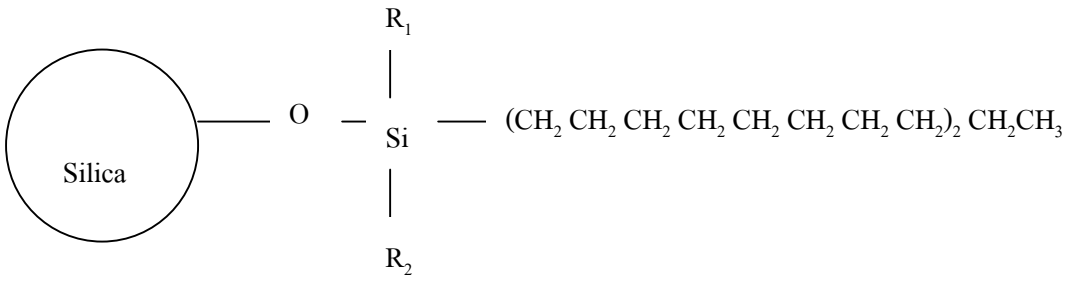
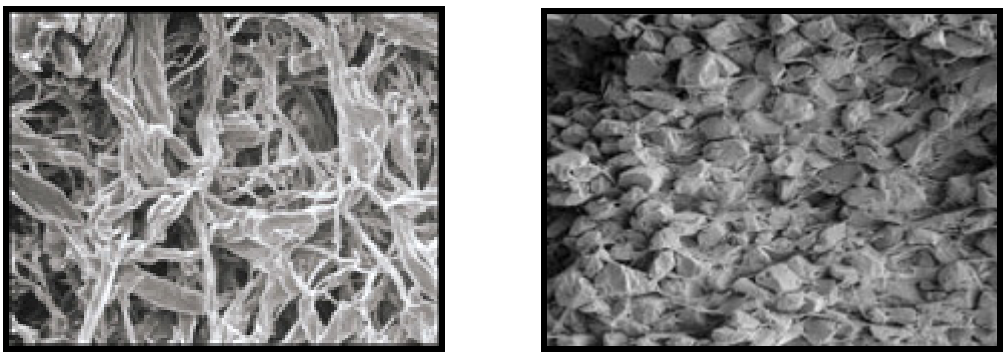


Figure 1-2 Chemical structure of C18 silica membrane disks.



(a) PTFE fibril (b) Bonded silica

Figure 1-3 Scanning Electron Microscope (SEM) of the membrane disk materials

(a) Polytetrafluoroethylene (PTFE) fibril

(b) Boned silica

The sorbent that using for solid phase extraction should have good properties such as negligible swelling capacity to resist swelling and shrinking with change in pH or solvent conditions, strong mechanical stability to withstand high flow rate and high chemical resistibility to endure harsh conditions (Wang *et al*., 2004).

Polytetrafluoroethylene (PTFE) is very attractive as the solid support owing to it has good properties such as chemical inertness, elevated resistibility, low friction coefficient and good swelling resistance (Wang *et al*., 2004).

Silica is a polymer of silic acid, consisting of inter-linked SiO_4 in tetrahedral structure, the active silica surface with large specific surface area is the great importance in adsorption. Silica has good properties such as good selectivity, no swelling and good chemical stability then it is attractive material to functionalize the surface using as solid sorbent (Jal *et al*., 2004).

- 8-Hydroxyquinoline

8-hydroxyquinoline (Oxine or 8-Quinolinol; HOx) is a well characterized chelating organic ligand. It can form covalent compounds with various metal ions under controlled pH conditions. It has been immobilized or modified on a variety of solid support using for solid phase extraction (Wen and Shan, 2002).

8-hydroxyquinoline has a hydrogen atom that is replaceable by a metal, and a heterocyclic nitrogen atom which forms with this metal a five member ring. The structures are illustrated in Figure 1-4.

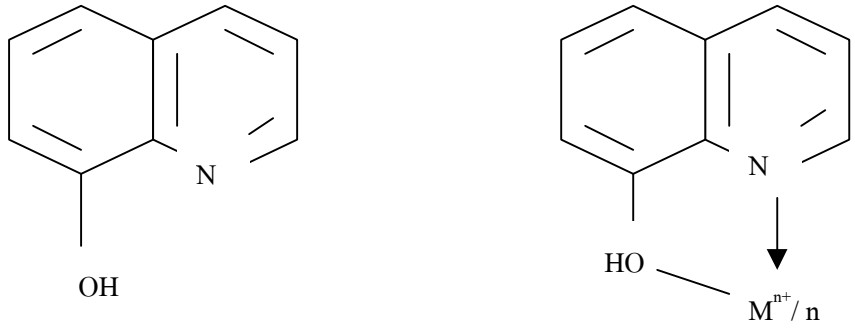


Figure 1-4 The structure of 8-hydroxyquinoline (a) and metal oxinate complex (b).

8-hydroxyquinoline is a white crystalline compound, molecular weigh (M.W.) 145.16, melting point (M.P.) 76 ^{o}C. It is insoluble in water but dissolved readily in most organic solvents such as ether, alcohols, aromatic hydrocarbon and chloroform. It is amphoteric (protonation of the nitrogen atom; dissociation of the hydrogen group). The complexes of metals and 8-hydroxyquinoline (oxine) are called "Metal oxinates" (Anil *et al*., 1970).

The use of a solid adsorbent material to extract analytes from a solution was developed and now widely applied to many matrices, including food samples. A sorbent with strong affinity towards some target analytes will retain and concentrate these compounds from the sample solution (Buldini *et al*., 2002).

Solid phase extraction (SPE) using macrocyclic molecules modified octadecyl silica membrane disks is an attractive technique for separation and preconcentration of trace metals prior to determination by GFAAS (Shamsipure *et al*., 2000).

The purpose of this research aims to study the efficient simple and rapid method for analysis of trace cadmium and lead in seafood samples. The purpose method emphasized on the sample preparation method by using solid phase extraction technique which performed by using 8-hydroxyquinoline modified octadecyl silica membrane disk to preconcentrate trace cadmium and lead before analysis by GFAAS.

1.2 Review of literatures

1.2.1 Physical and chemical properties of Cd and Pb

Heavy metals are the elements that have the specific gravity more than 5 g cm^{-3} such as cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), and zinc (Zn). The high levels of heavy metal accumulate in human become toxic. Generally, the trace heavy metals are found in the environment (Suwannarath, 1995).

- Cadmium

Cadmium is the heavy metal which classified in group II B of the element periodic. It is a soft, bluish white metal. It is quit ductile and more feasible and volatile than zinc, water dissoluble and soluble in nitric acid (Chappuis *et al*., 1995). Cadmium is not usually present in the environment as a pure metal but as a mineral combined with other elements such as oxygen (cadmium oxide), chlorine (cadmium chloride), or sulfur (cadmium sulfate, cadmium sulfide) (ATSDR, 2005). A summary of the physical properties of cadmium are given in Table 1-1.

Table1-1 Some physical properties of cadmium (Chappuis and Pineau, 1995)

Physical properties of cadmium	
Element symbol	Cd
Relative atomic mass	112.41
Atomic number	48
Oxidation state	2
Melting point (s.t.p.)	320.9 ^{o}C
Boiling point (s.t.p.)	765 ^{o}C
Density(20 ^{o}C)	8.65 g cm^{-3}
Electron configuration	[Kr] 4d^{10} 5s^{2}

- Lead

Lead is the heavy metal which classified in group IV A of the element periodic. It is a soft, white gray metal. It is easy to beat, press or straighten and soluble in nitric acid (Hill, 1995). A summary of the physical properties of lead are given in Table 1-2.

Table 1-2 Some physical properties of lead (Hill, 1995)

Physical properties of cadmium	
Element symbol	Pb
Relative atomic mass	207.2
Atomic number	82

Oxidation state	0, 2, 4
Melting point (s.t.p.)	327.4 ^{o}C
Boiling point (s.t.p.)	1755 ^{o}C
Density(20 ^{o}C)	11.35 g cm^{-3}
Electron configuration	[Xe] 4f^{14} 5d^{10} 6s^{2} 6P^{2}

1.2.2 Uses of Cd and Pb

Cadmium in the environment is found as the trace level comparing with other elements. Generally, it is found in the form of cadmium sulfide (CdS). Cadmium is the important material to use in various industries such as battery, painting, coating, lubricants, polymer, plastic and cosmetic industry (NRCC, 1979).

Lead is applied to use in many industry such as painting, battery, coating, printing, rubber, polymer, pesticide and cosmetic industry (UNEP, 1991).

1.2.3 Sources and potential exposure of Cd and Pb

Cadmium enters the environment from the natural weathering of minerals, forest fires and volcanic emission, but most is released by human activities such as mining and smelting operations, and fuel combustion (ATSDR, 2005). Sometimes cadmium can occurred from the bacteria activity that require sulfur from cadmium sulfide besides the human activity bring the contamination of cadmium such as metal industry, pigment, printing, painting and coating industry. The wastes from the industries are released to the environment (NRCC, 1979).

Cadmium can introduce to human body in many ways such as exposing the dust of cadmium to the respiratory system and consuming the contaminated food (Khaositiwong, 1995). Human exposure to cadmium can result from consumption of food. The exposured cadmium will accumulate in kidneys and liver. Cadmium can bind with metallothionein protein to be Cd-metallothionein complex and distribute to kidneys when the complex pass to the glomeruli and can also bind with heamoglobin, plasma and albumin. Cadmium accumulates in liver can bind with metallothionein protein. The complex is broken by lysosomes and free cadmium occurs (Lauwery and Hoet, 1993).

Lead can occur from the natural variation such as the explosion of volcanoes and the erosion of soil and rock. The human activities bring to the contamination of lead in the environment such as coating, batteries, painting, cosmetic, plastic and printing industries.

Lead can introduce to human body by exposing into respiratory system, gastrointestinal tract. The consumption of lead contaminated food brings to the lead accumulation in human body. The exposed lead will dissolve in the stomach by hydrochloric acid and some lead chloride can dissolve with water. The dissolved lead will adsorb in Duodenum (5-10 %) and some lead will release to large intestine. The exposed lead will distribute and accumulate in the body. The organic lead can not change its form by oxidation, reduction and hydrolysis but it can change the form under the metabolism and binding with glucuronic acid or sulfate. The alkyl leads will oxidize in the liver. The lead adsorb from intestine will distribute to the liver. The exposed lead will accumulate in liver, kidneys, bone, teeth, brain, lung and spleen. Lead will release with bile, urine and sweat (ATSDR, 1993).

1.2.4 Toxicity and Health Effect of Cd and Pb

The consumption of cadmium contaminated food brings to the various symptoms such as nauseate, vomit, diarrhea, cramp and maybe shock (Khaositiwong, 1995). The effect of cadmium toxicity on the body system is interference to the enzyme activity by substituting zinc in enzyme and brings to the inhibition of enzyme. The kidneys have the severe effect from cadmium toxic. They loss protein glucose and some amino acid with urine and kidney dysfunction occurs, leading to protein urea. The urinary loss of amino acids, glucose, calcium and phosphorus can cause disturbances in bone metabolism ostemalacia and formation of kidney stones (Chappuis et al., 1995). Moreover, toxic of cadmium bring to high blood pressure and cardiogenic schock. The accumulation of cadmium in body is the cause of Itai-itai disease. It can destroy central nervous system (CNS), immune system and Deoxyribonucleic acid (DNA) and it can cause of cancer (Franzblu, 1994). The toxic levels of cadmium in human body are shown in Table 1-3.

Table 1-3 Toxic levels of cadmium in human body (Daecharat, 2002).

Exposed level (mg)	Health effect
3-90	Vomit
15	Severe vomit
10-325	Severe toxic
350-3,500	Severe toxic and shock
1,530-8,900	Shock and death

The toxicity of lead after exposes the high level of lead. Many symptoms are occurred such as appetite, nauseated, vomit, thirsty, feel the sweet taste in the mouth, headache, stomach-ache, diarrhea, tired and unconscious. Long-term exposure of lead decrease performance function of the nervous system. It may also cause weakness in fingers, wrists, or ankles. Lead exposure also causes small increase in blood pressure, anemia, severely damage the brain and kidney. EPA has determined that lead is human carcinogenic (ATSDR, 2005). The lead level in blood and human health effect are shown in Table 1-4.

Table 1-4 Lead level in blood and human health effect (Daecharat, 2002).

Lead level in blood ($\mu g\ dL^{-1}$)	Health effect
25	- Inhibition of ALAD enzyme
40	- Zinc protoporphyrin(zpp) level high; nervous and renal system dysfunction.
50	- Hemoglobin produces system dysfunction.
60	
70	- Severe stomach-ache(colic pain)
80	- Anemia
100	- Duramater inflammation in children. - Duramater inflammation in adult.

The study of cadmium and lead contamination in food is an intensive point due to the highly toxic of both metals. Because even low concentration of cadmium and lead can

cause serious toxic effects to human then the analysis method become important. The distribution of these metals starts from the releasing from industrial waste and accumulates in biological and introduced to food chain, accumulated in biological species and distribute to human body. The awareness of the cadmium and lead toxicity has been emphasized.

The ministry of health of Thailand assigns the heavy metal contaminated in food for the consumer safety on the announcement of Ministry of Health. The contaminated levels are illustrated in Table 1-5.

Table 1-5 The acceptance of heavy metals contamination in food (Department of Medical Science, 2000).

Metals	Level ($\mu g\ g^{-1}$)
Cadmium (Cd)	1.00
Copper (Cu)	20
Lead (Pb)	1.00
Mercury (Hg)	0.02
Tin (Sn)	250
Zinc (Zn)	100

1.2.5 Instrumentation Analysis method

Cadmium and lead are potentially accumulated in marine organism (fishes, squids, Octopuses and prawns) and subsequently transferred to human through the food chain (Tuzen, 2003). Due to the highly toxic of these metals, it is important to know the concentration of cadmium and lead in seafood samples. The contamination of cadmium and lead in food are found at the trace levels so the sensitive instrument techniques are required. Electrothermal atomic absorption spectrometry or inductively coupled plasma mass spectrometry usually have enough sensitivity to allow the determination of this element in these samples (Ferreira *et al*., 2001)

Graphite furnace atomic absorption spectrometry (GFAAS) is one of the most suitable methods for the determination of cadmium and lead at low concentrations in biological samples owing to its inherent high sensitivity, specificity and favorable detection limits, minimum need for sample preparation and the possibility of automation (Acar, 2001).

In this research, Graphite furnace atomic absorption spectrometry (GFAAS); Perkin Elmer AAnalyst 800 with Zeeman Effect background correlation, Transverse Heated - graphite tube (THGA) and Auto sampler AS800 were used for cadmium and lead determination.

1.2.6 Sample digestion method

In analytical procedures involving elimination of the organic matrix, the chemical sample pretreatment is frequently considered. For the preparation of biological samples, dry ashing or wet digestion methods are commonly used (Nascentes *et al*., 2001). Many digestion methods were used in preparation of biological samples and can be summarized as follows.

Monteiro – Neto *et al.,* (2003) studied the cadmium and lead contamination in fishes. The sample preparation method was performed by using dry ashing method. Samples for cadmium and lead determination were dried at 80 ^{o}C for 24 h. Dried samples were weighed (0.3

g) and burned to ashed at 450 ^{o}C for 24 h. The ashes were dissolved in 3:1 nitric acid (HNO_3) and hydrochloric acid (HCl) solution and evaporated on a hot plate until dryness. The residue was again dissolved in 1.0 M HCl to a 5.0 mL final volume and filtered. The extract was taken for reading on atomic absorption spectrophotometer. Lead levels were lower than 0.1 µg g^{-1} and cadmium levels were averaged in 0.8 µg g^{-1}.

Tuzen, (2003) applied the dry ashing method prepare the fish samples for cadmium and lead determination. A sample (1.0 g dry weight) was placed in a high form porcelain crucible. The furnace temperature was slowly increased from room temperature to 450 ^{o}C in 1 h. The samples were ashed for about 4 h. until a white or grey ash residue was obtained. The residue was dissolved in 5 mL of nitric acid (25% v/v). The solution was heated slowly to dissolve the residue. The cadmium and lead levels were analyzed by graphite furnace atomic absorption spectrometry (GFAAS). The concentration of cadmium varied from 0.09 to 0.48 µg g^{-1} and for lead it ranged from 0.22 to 0.85 µg g^{-1}. Detection limit, precision and accuracy of analyses were determined by reported analyses of certified reference materials (NIES-6 and NBS-1566). The recoveries were quantitative for study (more than 95%) and relative standard deviation (RSD) were less than 7%.

Wet digestion or acid digestion is the common method which uses to decompose the sample before analysis. The hot plate digestion method is a kind of wet digestion which performed by using acid and heated on the hot plate in temperature range 70-100 ^{o}C (Hamepatawee and Krongkrot, 2001).

Kungsuwan *et al*., (1997) applied the digestion method to prepare octopuses and cuttlefishes for cadmium and lead determination. The sample (1.0 g wet weight) was placed in Teflon vessel and 6 mL of concentrated nitric acid was added. The Teflon vessel was placed in the stainless vessel and covered by spiral cap. The vessel was place in the oven at 170 ^{o}C for 40 min or until clear solution. The analysis of cadmium and lead was carried out by using FAAS. The average contents of cadmium and lead in octopuses and cuttlefishes were respectively as

follows; Cd 0.12-0.48 μg g^{-1} and 0.06-0.09 μg g^{-1}; Pb 0.06-0.31 μg g^{-1} and 0.05-0.22 μg g^{-1} respectively.

Acar, (2001) studied the decomposition method for cadmium and lead determination. The method was performed by using 6 mL of 7 M nitric acid heated on the hot plate at 120 ^{o}C. The digestion was carried out in the beaker which covered by PTFE cover. Cadmium and lead levels were determined by GFAAS. The accuracy of the method was conducted by analyzing certified reference material (IAEA-A-13). The recoveries of cadmium and lead were 102 % and 106 % respectively. The detection limit of cadmium and lead were found to be 0.04 and 0.92 μg L^{-1} respectively.

Tuzen, (2003) presented the wet digestion method for cadmium and lead determination in fishes by using high-pressure decomposition vessels, commonly known as a digestion bomb. A sample (1.0 g dry weight) was placed in to Teflon container and 5 mL of concentrated nitric acid was added. The system was heated to 130 ^{o}C for 90 minutes and finally diluted to 25 mL with de-ionized water. The sample solution was clear and determined the metal levels by GFAAS. The recoveries of cadmium and lead were more than 95% and relative standard deviation was less than 7%.

Ferreira *et al*., (2001) studied the sample preparation method for lead determination in lobster. Sample was weighed and treated with 50% (v/v) nitric acid over night in the Teflon vessel. Afterwards, the Teflon vessel was closed and placed into a stove at 130 ^{o}C for 16 h. The clear sample solution was preconcentrated by using 2-(2-benzothiazolylazo)-2-*p*-cresol (BTAC) modified Amberlite XAD-2. The metals were determined by flame atomic absorption spectrometry (FAAS). The accuracy was assured by the analysis of biological reference materials (TORT-1). The limit of detection was 3.7 μg L^{-1} with relative standard deviation (RSD) in range 4.4 – 2.3%.

Lemos and Ferreira, (2001) studied the contamination of lead in various seafood sample such as shrimp, oyster, crab, fish and mussel. The samples were triturated, homogenized

and dried overnight at 110 ^{o}C and were kept in dry ambient. Seafood sample (0.4 g) was treated with 4.0 mL of 1:1 (v/v) nitric acid solution and keep overnight in Teflon vessel. Afterwards the Teflon vessel was closed and put into a pressurized digestion system. The thermal heating was carried out in a stove at 170 ^{o}C for 16 h. The sample solution was preconcentrated with polyurethane foam modified by 2-(2-benzothiazolylazo)-2-*p*-cresol (BTAC). The analytes were determined by FAAS. The accuracy was confirmed by analysis of certified reference materials (IAEA; fish tissue, NRCC TORT-1; lobster hepatopancreas). The recoveries of several seafood samples were quantitative (90-107%) and the detection limit was 1.0 μg L^{-1} and RSD was in range 0.7-6.0%.

The wet digestion method, which is performed in the closed decomposition systems, have been found better than open ones due to reducing systematic errors and leading to correct analytical results with a higher accuracy (Buldini *et al*., 2002).

Microwave digestion is one of the methods that perform in closed vessel. The decomposition is carried out by acid and heating with microwave energy at high temperature and pressure. This method can be applied to digest various seafood samples (Buldini *et al*., 2002).

Sheppard *et al*., (2004) studied the digestion method for determination of cadmium and lead in seafood samples such as tunafish, salmon, prawn, mussel and lobster. The decomposition of sample was performed in microwave oven. Sample (1.0 g) was weighed into a Teflon digestion vessel and 5 mL of concentrated nitric acid were added. The sample vessels were placed in microwave oven and digest at pressure 138 kPa. The clear sample solution was analyzed by ICP-MS and ICP-AES. Certified reference materials (DORM-1; dogfish muscle, TORT-1; Lobster hepatopancreas marine reference material) were analyzed to check the accuracy of the method. Recoveries of cadmium and lead were found in range 75-117% and relative standard deviation (RSD) were found in range 2.5-5.5%.

Saavedra *et al*., (2004) studied the microwave digestion method for cadmium and lead monitoring in mussel samples. The microwave oven was used to decompose the sample before analysis by GFAAS. The digestion performed by using sample (0.5 g dry weight) and

concentrated nitric acid and subjected to microwave heating for 20 minutes at 180 psi. The accuracy of the method was obtained by analysis of certified reference materials (SRM 1566b; oyster tissue, CRM 278R; mussel tissue). The recoveries of cadmium and lead were found in range 100-107 % and 100-109 % respectively with relative standard deviation (RSD) 2-5%.

1.2.7 Solid phase extraction

Solid phase extraction (SPE) is a method for preconcentrating metal ions and matrix removal. It is an approach that offers a number of important benefits. It reduces solvent usage and exposure, disposal costs and extraction time for sample preparation. Consequencetly, in recent years solid phase extraction has been successfully used for the separation and sensitive determination of metal ions in various samples (Camel. 2003). Many researches involving the sample preparation by using solid phase extraction for trace cadmium and lead determination can be summarized as follows.

Sturgeon *et al*., (1981) studied the sample preparation method by using solid phase extraction. The method was performed by using 8-hydroxyquinoline immobilized on silica gel. Cadmium and lead were preconcentrated on the prepared sorbent. The optimum of sample pH was found at 8.0 ± 0.2. The retained cadmium and lead ions were eluted by 1 M hydrochloric acid and 0.1 M nitric acid prior to determination by GFAAS. The recoveries of cadmium and lead were 94 ± 5% and 92 ± 10% respectively with relative standard deviation (RSD) 6%. The method is simple, rapid, good precision and accuracy for cadmium and lead determination in seawater.

Leepipatpiboon, (1995) presented the cadmium and lead preconcentration technique by using solid phase extraction with 4-(2-pyridylazo) resorcinol (PAR) trapped on C18-SPE cartridge. Cadmium and lead ions can be chelated with PAR in pH range 7.0 -8.0 and the retained metal ions were eluted with 4.0 mL of 1 M nitric acid at flow rate 6.0 mL min^{-1}. The cadmium and lead levels were determined by FAAS. The results obtained the precision in 1-5 % (RSD) and recovery in range 81-100%. The detection limits for cadmium and lead were 0.03 mg

L^{-1} and 0.30 mg L^{-1}respectively. The method is an efficient sample preparation for enhancing trace cadmium and lead in water.

Ma *et al*., (1996) applied octadecyl functional group (C18) bonded to silica gel as sorbent and dialkyldithiophosphate $(RO)_2P(S)S^{-1}$ as the chelating agent for cadmium and lead extraction. The optimum pH for cadmium and lead extraction was 8.0 and the adsorbed cadmium and lead ions were eluted by using methanol before analysis by FAAS. The detection limits of cadmium and lead were 0.8 and 10.0 ug L^{-1} respectively. The capacity and accuracy can be demonstrated by analysis of certified reference material (CRM 141; loan soil, CRM280; lake sediment). The analytical results were in good agreement with the certified values at the 95% confidence interval and the precision was better than 2% RSD.

Willie *et al*., (1998) presented the preconcentration of trace cadmium and lead by using 8-hydroxyquinoline immobilized onto silicone tubing. The optimum pH for cadmium and lead extraction was 8.5 and the trace metals were eluted with 1 mL of 10% (v/w) hydrochloric acid and 1% (v/w) nitric acid before analysis by ICP-MS. Recoveries were found in between 85-95%. The accuracy of this procedure was evaluated by the analysis of certified reference materials (NASS-4; open ocean seawater, CASS-3; coastal seawater). The relative standard deviation (RSD) was found to be between 3 and 5% for the interested elements.

Shamsipur *et al*., (2000) developed the solid phase extraction technique by using octadecyl silica membrane disk modified by bis[1-hydroxy-9,10 antraquinone-2-methyl]sulfide (BAS) for trace lead preconcentration before determination by FAAS. The optimum pH for lead ions extraction was 7.0 on the 15 mg BAS modified C18-silica membrane disk. The retained lead ion was eluted by 5 mL of 1 M nitric acid. The recovery was 100% and the detection limit was 50 ng Pb^{2+} per 1,000 mL. The method was successfully applied to determination of lead in soil and water samples.

Ekinci and Koklu, (2000) applied 3-aminopropyltriethoxysilane modified silica-gel using for separation and preconcentration of lead prior to determination by GFAAS. The

extraction of lead ions was quantitatively at pH 8.0. The adsorbed ion was eluted by 25 mL of 0.2 M hydrochloric acid. The recovery was more than 90% with relative standard deviation (RSD) 1.4%.

Hashemi *et al*., (2001) presented the solid phase extraction method by using a new S-containing Schiff' base [Ethane amine, N, N' – bis (2-thienyl methylene)] modified octadecyl silica membrane disk. Lead (II) ions could be retained quantitatively by the modified membrane disk in the pH range 2.0-8.0. The optimum of ligand for the extraction was quantitative above 5 mg. The adsorbed lead (II) ion was eluted by 10 mL of 0.5 M nitric acid prior to determination by FAAS. The recoveries were found more than 95% with relative standard deviation (RSD) in between 0.3 to 5.2 for trace lead determination in water samples. The limit of detection of the method is 16.7 ng mL^{-1}.

Goswami and Singh, (2002) prepared solid sorbent using 1,8-dihydroxyanthraquinone (DHAQ) immobilized on silica gel for trace lead and cadmium preconcentration before determination by FAAS. The optimum pH ranges for quantitative sorption are 6.0-7.5 and 6.0-8.0 for lead and cadmium respectively. The metal ions can be desorbed with 10 mL of 2 M nitric acid. The recoveries were found more than 98% and relative standard deviation (RSD) less than 6.3%.

Wen and Shan, (2002) developed the solid phase extraction technique by using 8-hydroxyquinoline immobilized on polyacrylonitrile fiber for preconcentration of trace cadmium and lead before analysis by ICP-MS. The optimum pH for cadmium and lead extraction on the sorbent was 6.0. The adsorbed metals were eluted with 5 mL of 2 M hydrochloric acid and 0.1 M nitric acid. The method was verified by analysis of certified reference materials (NASS-5, CASS-4, SLEW-3; seawater and SLR-4; river water). The detection limits for cadmium and lead were 6.4 ng L^{-1} and 13.6 ng L^{-1} respectively.

Gurnani *et al*., (2003) prepared the solid sorbent for cadmium and lead preconcentration prior to determination by FAAS. The sorbent was prepared by using 8-

hydroxyquinoline immobilized on cellulose. The optimum pH for cadmium and lead extraction were found in between 4.2-9.0. The retained cadmium and lead ions were eluted by 20 mL of 1 M nitric acid. The recoveries were found more than 97% and relative standard deviation (RSD) less than 7.4%.

1.3 Objectives

1.3.1 To study the appropriate sample preparation technique for cadmium and lead determination in seafood samples

1.3.2 To optimized Graphite Furnace Atomic Absorption Spectrometry conditions for cadmium and lead analysis

1.3.3 To apply this method for cadmium and lead determination in seafood samples