CHAPTER 1

INTRODUCTION

1.1 Introduction

There is increasing concern about the quality of foods in several parts of world. The determination of toxic elements in food has prompted studies on toxicological effects of them in food (Khansari *et al*., 2001). Lead is a toxic metal. If consumed or inhaled, it can affect nearly all systems in the body (Andrews, 1992). Lead hidden in foods is one of the main sources of lead absorbed in human body, so the determination of lead in foods is becoming increasingly important (Fang *et al*., 2001).

Lead poisoning as a health problem was recognized 2500 years ago (Mauss, 1993). Lead poisoning may be due to inorganic or organic lead (Sabry and Wahbi, 1999). In the case of inorganic lead, it is distributed to soft tissue, bones and teeth (95% in bones and teeth). Organic forms of lead are fat soluble and therefore have a particular tendency to concentrate in brain (Korn *et al.*, 2006). There are many sources of lead in our environment. Major sources are lead-based paint and contaminated soil, dust, drinking water, air, food and related products (Toscano and Guilarte, 2005).

Paint is the most common source of lead exposure for children and adults can breathe in or eat paint dust at any time (Andrews, 1992). Many compounds of lead are strongly colored and highly durable, so they have a long history of application in paints, pigments, and even cosmetics. Some lead compounds are also added to paints as drying agents. Typical lead contents are 0.1-0.5% in paints ready for use. At present, the use of lead compounds has significantly been reduced, though they are still used for a few specific applications (Thornton *et al.*, 2001).

Air is polluted with lead emitted by automobiles (from lead gasoline additives) and lead-using industries. Airborne lead levels are highest in urban areas with heavy automobile traffic and in the vicinity of major industrial sources (Edward, 1986). The combustion of leaded gasoline in the United States from 1923 until 1986 is estimated to have dispersed approximately 4 million metric tons of Pb^{2+} into the atmosphere and the soil. It is estimated by the Agency for Toxic Substances and Disease Registry (ATSDR) that the combustion of leaded gasoline has

accounted for 90% of the Pb^{2+} deposited in the atmosphere (Toscano and Guilarte, 2005). The World Health Organisation set an air quality guideline for lead of between 0.5 and 1.0 $\mu g/m^3$ as an annual average (WHO, 1987). Lead is removed from the air by rain and by particles falling to land or into surface water (ATSDR, 2005).

Soil and dust often contain high levels of lead, mostly from disposal of mine tailings, from lead industries, such as smelters and battery plants and from erosion of exterior lead painted surfaces, or indirectly as fallout from lead air pollution from industries or from vehicles using leaded fuel (Jin *et.al*., 1997). Accumulation of lead in surface soils can impact on environmental health and can affect food quality and human health (Tongtavee *et al.*, 2005). Children 2-3 years old are at greatest risk of exposure to lead-contaminated soil, due to "hand-to-mouth" activity by playing on floors and mouthing dirty objects (Edward, 1986).

Drinking water can contain significant amounts of lead if soft, acidic water is delivered through lead pipes or systems with lead soldered joints (Edward, 1986). Consequently, exposure to lead through water is generally low compared with exposure through food (WHO, 2001). This approximates the maximum concentration of lead in drinking water of 10 $\mu g\ L^{-1}$ recommended by WHO (Thornton *et al.*, 2001).

Food is a major source of lead intake for people (WHO, 2001). Lead can enter food during harvesting, and the lead added in processing or packaging (Edward, 1986). Fresh food may be contaminated by small amounts of lead from airborne fallout, particularly noted for foods such as lettuce, parsley and mint, which have a very large surface area compared to their mass. Vegetables grown in soils with high lead contents can also contain traces of soil (Thornton *et al*., 2001).

Lead-containing solders used in food cans could cause lead contamination, particularly of foods containing acidic fruits, tomatoes or similar, until their use was prohibited in the early 1980s (Thornton *et al*., 2001). The majority of food cans are sealed with a solder that is 98 % lead and 2 % tin (Edward, 1986). Tin-lead solders (a type of soft solder) are the most widely used, because they are cheaper than alternative solder alloys. A study of lead contents of foods in the UK in 1984 found canned foods, including fruit and food in tomato sauce, often contained elevated lead contents (Thornton *et al*., 2001).

Welding of the side seam of tin cans has gradually replaced the Pb-soldered side seams during the last two decades. The impact of the introduction of this new production technique cannot be overemphasized. The earlier type of tin can with Pb-soldered side seams resulted in foodstuffs often containing more than 0.1 mg of Pb kg^{-1}, not seldom exceeding 0.5 mg kg^{-1}. In cans with welded side seams, the lead content is not very different from that in the corresponding fresh food (D'Mello, 2003).

The effect of dissolution of lead from can side-seams on lead concentrations in canned food is shown in Table 1-1. The food which was put into the cans was identical, the only difference being that one batch of cans were soldered using an alloy of 98% Pb and 2% Sn whilst the other batch were welded - a process which does not use lead. The effect of container type on the lead concentration in the food is plain to see, there was no major effect on tin and iron concentrations (Sherlock, 1987).

Table 1-1 Lead, tin and iron in canned ravioli. Effect of can type (10 samples of each type of can analyzed)

Metal	Mean concentration (range) µg/kg	
	Soldered cans	Welded cans
Lead	150	30
	(70-260)	(20-50)
Tin	3400	1800
	(800-4800)	(800-4200)
Iron	2700	2900
	(1600-4800)	(1900-5700)

Source: Sherlock, 1987

Fish is widely consumed in many parts of the world by humans because it has high protein content, low saturated fat and also contains omega fatty acids known to support good health. Canned fishes in particular are well eaten in the developed world because it is convenient and affordable for most working families (Ikem and Egiebor, 2005). So their toxic metal content should be of some concern to human health (Voegborlo *et al*., 1999).

Fish may be contaminated by toxic elements during fish growth, transportation, and storage. Contamination may also occur during production handling and canning process (Ikem and Egiebor, 2005). Lead is found at high concentration in muscles and organs of fish. It accumulates in the human body where it replaces calcium in bones (Ashraf *et.al*., 2006).

Several agencies and organizations provide guidelines on the intake of lead by humans. The Joint Food and Agriculture Organization/World Health Organization (FAO/WHO, 1972) expert committee on food additives have suggested a tolerable intake of 3 mg Pb per week for man. The maximum concentration of Pb which is permitted in prepared foods for babies and children is 0.2 mg kg^{-1} (Ashraf *et.al*., 2006). The European communities (EU) have reached an agreement on maximum levels (MLs) for Pb in fish sample is 0.2 mg Pb kg^{-1} wet weight (Ikem and Egiebor, 2005). The Ministry of Health of Thailand assigns the heavy metal contaminated in food for the consumer safety on the announcement of Ministry of Health. Lead levels in canned food are not more than 1 µg g^{-1}(Department of Medical Science, 2000).

For trace metal determinations several techniques can be employed such as graphite furnace atomic absorption spectrometry (GFAAS) (Santos *et al*., 2002), atomic emission spectrometry (AES) generally with inductively coupled plasma (ICP-AES) (Chew *et. al*., 2000), inductively coupled plasma-mass spectrometry (ICP-MS) (Chen *et al*., 2002), neutron-activation analysis (NAA) (Fajgelj and Byrne, 1995) and X-ray fluorescence spectrometry (Golob *et.al*., 2005). However, these techniques have some disadvantages, such as complicated operation, high cost of maintenance, expensive apparatus and requiring well-controlled experimental conditions. (Hu *et.al*., 2003).

Electroanalytical techniques specially stripping analysis are well known as excellent procedures for the determination of trace chemical species. Generally these methods are only applied to the determination of single component or multi components without overlapping peaks (Shams *et al*., 2004). Electrochemical methods of analysis have been extensively employed for determination of trace metals in a wide range of matrixes of human interest, for example food, beverages, biological fluids and others (Oliveira *et.al*., 2004). The advantages of this techniques are low cost, high sensitivity, easy operation and the ability of analyzing element speciation (Hu *et.al*., 2003).

Stripping analysis is generally recognized as one of the most suitable methods for trace metal determination. Its remarkable sensitivity is attributed to the combination of an effective preconcentration step with advanced measurement procedures that generate an extremely favorable signal-to-background ratio. Since the metals are preconcentrated into the electrode by factors of 100 to 1000 (Wang, 2000). A comparison of stripping analysis methods with other modern analytical methods (Table 1-2) used in trace analysis, shows that the electroanalytical methods may be successfully used (Abu Zuhri and Voelter, 1998).

Table 1-2 Detection limits of modern analytical methods

Method	Detection limit (mol L^{-1})
Potentiometry	5×10^{-6}
Direct current polarography	5×10^{-6}
Atomic-emission spectroscopy	5×10^{-6}
Spectrophotometry	5×10^{-6}
Atomic-absorption spectroscopy	5×10^{-7}
Atomic-fluorescence spectroscopy	5×10^{-8}
Differential pulse and square-wave polarography	5×10^{-8}
Mass spectrometry	10^{-9}
Stripping analysis with HMDE and with DPP	10^{-9}
Neutron-activation analysis	10^{-10}

Source: Abu Zuhri and Voelter, 1998

Stripping analysis is a two-step technique. The first, or deposition step, involves the electrolytic deposition of a small portion of the metal ions in solution into the surface of an electrode (usually mercury) to preconcentrate the metals. In the second step, or the stripping step, the deposited analyte is removed from the electrode by a potential scan and the resulting current peaks are used to determine the concentration of each analyte species in the sample. Different versions of stripping analysis can be employed, depending upon the nature of the deposition and measurement steps (Wang, 2000).

A frequently used electroanalytical procedure for metal trace determination is anodic stripping voltammetry (ASV); reported detection limits (DL) are in the ppb ($\mu g\ L^{-1}$) range for various metals (Oliveira *et.al*., 2004). It is a two step technique:

The deposition step or preconcentration step: In this case, the metals are preconcentrated by electrodeposition into a small-volume mercury electrode (a thin mercury film or a hanging mercury drop). The preconcentration is done by cathodic deposition at a controlled time and potential. The deposition potential is usually 0.3-0.5 V more negative than E° for the least easily reduced metal ion to be determined. The metal ions reach the mercury electrode by diffusion and convection, where they are reduced and concentrated as amalgams (Wang, 2000).

$$M^{n+} + Hg + ne- \rightarrow M(Hg)$$

The stripping step: After the solution is allowed to become quiescent, the concentration of metal in the amalgam becomes more uniform. During the stripping step, an excitation waveform is applied to be reoxidized the analyte back into the solution and generate a current signal. The stripping current due to oxidation of each analyte is proportional to the concentration of the analyte on or in the electrode (Supapan, 2005).

$$M(Hg) \rightarrow M^{n+} + Hg + ne-$$

The performance of the voltammetric procedure is strongly influenced by the material of the working electrode (Wang, 2000). Mercury is the often the best electrode material for the trace analysis of metals. This is because many metals of interest have fast electrode kinetics at mercury and form amalgams. Amalgam formation provides an excellent approach for the preconcentration of metals into the working electrode of the cell prior to the determination of the metals by their stripping (removal) from the mercury phase. However, the toxicity of mercury has led to a growing interest in the search for new electrode materials (e.g., carbon, platinum or gold), which offer a similar or better performance for various electroanalytical applications (Herzog and Arrigan, 2005).

The carbon paste electrode (CPE) was introduced by Adams in 1958 and was initially used for analysis by Jacobs in 1963 (Pauliukaite *et al*., 2002). Carbon paste electrode generally consists of Teflon well into which is inserted a platinum, copper, steel or graphite

contact (Ibrahim, 2005). It is prepared by mixing graphite powder and water-immiscible organic binder (pasting liquids) such as mineral oil, paraffin oil, silicone grease and bromonaphthalene (Wang, 2000). CPEs have several advantages such as inexpensive, non-toxic, low background current, wide range of used potential, rapid renewal, and easy fabrication (Mousavi *et al*., 2001). By adding modifier materials in paste can improve the electrode selectivity and sensitivity (Ibrahim, 2005).

Chemically modified carbon paste electrodes (CMCPEs) were developed to improve the selectivity and sensitivity of the electrochemical measurements by preconcentrating the target analyte from a dilute solution on the electrode surface. (Degefa *et.al*., 1999). The mechanism can be described as following:

M^{2+} = Metal ion

L = Ligand

$(M^{2+})_{solution} + (L)_{surface} \rightarrow (M^{2+}\text{-}L)_{adsorption}$ (accumulation stage)

$(M^{2+}\text{-}L)_{adsorption} + 2e^- \rightarrow (M^{0}\text{-}L)_{adsorption}$ (reduction stage)

$(M^{0}\text{-}L)_{adsorption} \rightarrow (M^{2+})_{solution} + (L)_{surface} + 2e^-$ (stripping stage)

(Hu *et al*., 2003)

A number of studies on the use of CMCPEs for voltammetric determination of Pb(II) have been reported. These are CPEs modified with diphenylthiocarbazone (Molina-Holgado *et. al*., 1995), tributyl phosphate (Huang *et. al*., 1998), *N-p*-chlorophenylcinnamo-hydroxamic acid (Degefa *et. al*., 1999), 1,4-bis(prop-2′-enyloxy)-9,10-anthraquinone (Mousavi *et. al*., 2001), and diacetyldioxime (Hu *et. al*., 2003).

Xanthones, a kind of polyphenolic compounds that commonly occur in plants such *Calophyllum* sp. and *Garcinia* sp., have widely been synthesized (Jiang *et al*., 2003). Xanthones have many pharmacological effects such as antitumor activity, cytotoxicity, antibacterial activity, antifungal activity, anti-inflammatory properties, antioxidant activity and tuberculoatatic activity (Bo and Liu, 2004). Xanthone have been used to modifier in carbon paste electrode for determination of silver. It was found that xanthone can be used to increase the sensitivity of the determination of silver (Photicunapat, 2005). Group of xanthone compounds

(xanthone, xanthene, thioxanthone and acridone) are interesting modifier for determination of toxic heavy metals**.** The structures are shown in Figure1-1.

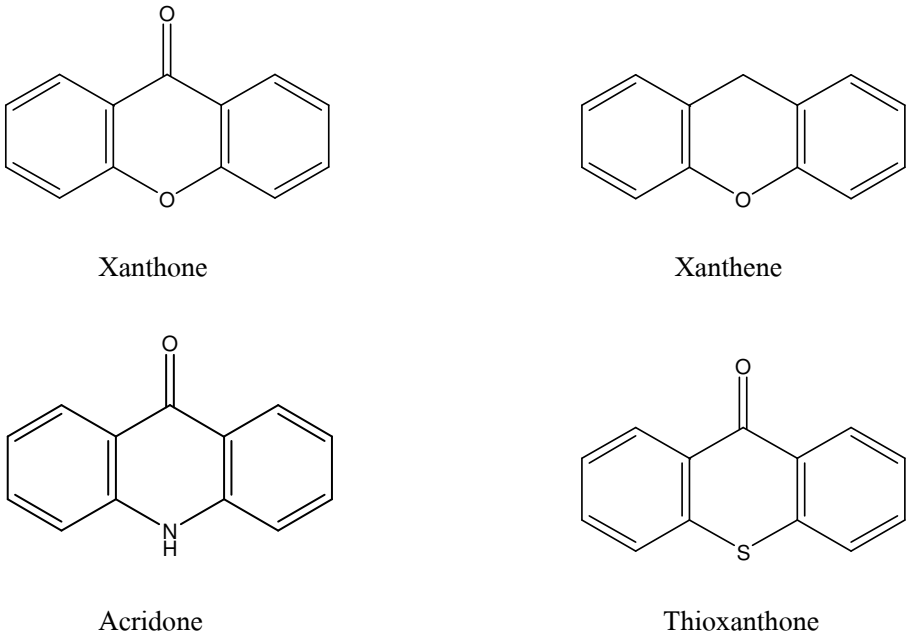

Figure 1-1 The structures of group of xanthone compounds

Adsorptive cathodic stripping voltammetry (AdCSV) is becoming increasingly popular for the determination of trace and ultratrace levels of metal ions and organics (Abollino *et al*., 1999). The technique is based upon adsorptive accumulation of the metal ion complex with a suitable ligand at the electrode scanning in the negative direction (Shemirani1 *et al.,* 2005). In AdCSV, a ligand of M^{n+} is added to form a complex, which is preconcentrated by adsorption at the electrode surface (Buffle and Tercier-Waeber, 2005). The reduction step, with a negative-going potential scan or constant cathodic current, can be employed for measuring the adsorbed complex (Figure 1-2). The adsorptive accumulation approach results in a very effective preconcentration with short adsorption times (1–5 min) and extremely sensitive or selectivity trace metal measurements (Wang, 2000). The sensitivity in AdCSV is often greater than in ASV, because the metal is not dissolved in the mercury, but rather forms a monomolecular complex layer on, e.g., a mercury film electrode surface (Abu Zuhri and Voelter, 1998). The limit of detection of AdCSV for trace metals is typically on the order of 10^{-9}-10^{-11} M (Achterberg and Braungardt, 1999). Most AdCSV procedures utilize the hanging mercury drop electrode (HMDE)

for measuring reducible species, which offers the advantages of self-cleaning, reproducible surface area, and automatic control (Abu Zuhri and Voelter, 1998).

AdCSV makes use of a specific added ligand (L), which is added to the water sample and forms an adsorptive complex with the trace metal.

$$y\mathrm{M}^{n+} + z\mathrm{L}^{m-} \leftrightarrow \mathrm{M}_y(\mathrm{L})_z^{(yn-zm)}$$

A pH buffer is used to control the pH of the sample, as the formation of the metal–AdCSV ligand complex is pH dependent. A minute fraction of the metal–ligand complex is adsorbed on the surface of the Hg drop and a potential scan is carried out.

$$\mathrm{M}_y(\mathrm{L})_z^{(yn-zm)} \leftrightarrow \mathrm{M}_y(\mathrm{L})_{z\ \mathrm{adsorbed}}^{(yn-zm)}$$

The metal is then released form the complex by reduction. The scan direction is towards more negative potentials and the resulting current is measured.

$$\mathrm{M}_y(\mathrm{L})_{z\ \mathrm{adsorbed}}^{(yn-zm)} \leftrightarrow y\mathrm{M}^{(n-1)+} + z\mathrm{L}^{m-}$$

(Achterberg and Braungardt, 1999)

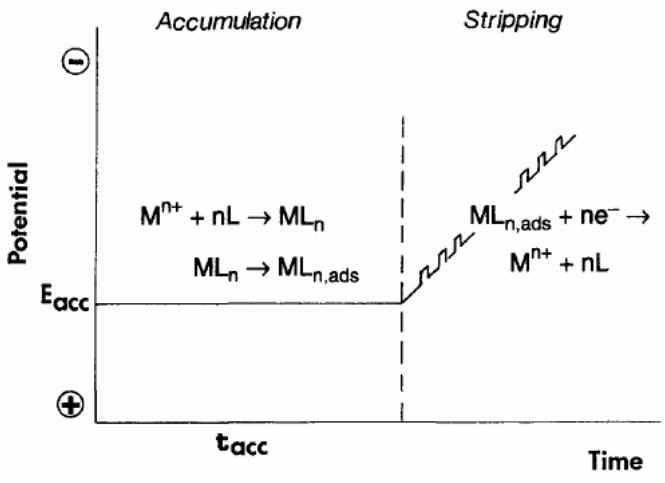

Figure 1-2 Accumulation and stripping stripping in adsorptive stripping measurement of a metal ion (M^{n+}) in presence of an appropriate chelating agent (L)

Because of the great sensitivity enhancement obtained with AdCSV methods, several complexing agents have been studied for the adsorptive collection of complexes with Pb(II) on the hanging mercury drop electrode (HMDE). It has been previously described the use of 8-hydroxyquinoline (Van Den Berg, 1986), Xylenol Orange (Wu and Batley, 1995), Calcein Blue (Yokoi *et al*., 1995), Morin (Shams *et al*., 2004), and Thymolphthalexone (Babaei *et al*., 2006) as complexing agents for the voltammetric determination of metals.

8-Hydroxyquinoline (oxine) is a well known complexing agent for the analytical determination of cations of transition metals (Garay and Solis, 1999). 8-Hydroxyquinoline molecules are adsorbed on mercury, and this property is used as a preconcentration step for labile and non-labile complexes in electroanalytical procedures (Arancibia *et al*., 2004).

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 1-3. 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) (Anil *et al*., 1970).

Figure 1-3 The structure of 8-hydroxyquinoline (a) and metal oxinate complex (b)

Food is a complex non-homogeneous mixture of a staggering range of chemical substances that makes it hard to isolate and determine analytes of interest. After sampling, it is necessary to prepare the sample for the determination of analytes (Buldini *et al*., 2002). Some of the major functions of sample preparation are:

- To degrade and solubilize the matrix, to release all metals for analysis.

- To extract metals from the sample matrix into a solvent more suited to the analytical method to be used.
- To concentrate metals present at very low levels to bring them into a concentration range suitable for analysis.
- To separate a single analyte or group of analytes from other species that might interfere in the analysis.
- To dilute the matrix sufficiently so that the effect of the matrix on the analysis will be constant and measurable.
- To separate different chemical forms of the analytes for individual determination of the species present (Mitra, 2003).

In 1993, FDA conducts routine food analysis using traditional dry ash or acid digestion procedures (Dolan and Capar, 2002). Microwave digestion has been gaining in popularity in the past few years (Loska and Wiechula, 2006).

Dry ashing is useful for moist samples, such as food or botanical samples, because it destroys large amounts of wet organic matter easily and quickly (Mitra, 2003). Dry ashing temperatures commonly admitted for trace element analysis range between 450 and 500 °C (Hoenig, 2001). The associated mineral part of the matrix is transformed into carbonates or oxides, which are dissolved in an appropriate acid (Hoenig and Kersabiec, 1996). The drawbacks of the method are the possible loss of some elements by volatilization or adsorption of elements on the walls of the furnace, such that As, Cr and Pb may be lost at ashing temperatures of 500.-550 °C (Hseu, 2004).

Wet digestion with oxidizing acid is the most common sample preparation procedure. The acidic materials most frequently used are commonly used are nitric acid, sulfuric acid, perchloric acid, hydrochloric and hydrofluoric acid (Hoenig and Kersabiec, 1996). Nitric acid is commonly used, because there is no chance of forming insoluble salts as might happen with HCl or H_2SO_4. Hydrogen peroxide may be added to increase the oxidizing power of the digestion solution. The organic matrix in sample is destroyed by using acid and heating (Mitra, 2003). Wet digestion may be carried out in open or closed systems. The digestion in open systems, this method is called “hot plate digestion”, is wide spread in element analysis (Maichin *et al*., 2003). It is limited, however, by the low decomposition temperatures which cannot exceed the

boiling point of a given acid or mixture of acids under the conditions of atmospheric pressure. Other disadvantages are the risk of sample contamination, the necessity to use large amounts of acids and finally the loss of the assayed analytes due to their volatility. The digestion in closed systems prevents sample contamination and losses caused by the volatility of elements (Loska and Wiechula, 2006).

Microwave digestion is widely applied and has become the procedure of choice for decomposition of wide variety of sample matrices. Microwaves have been reported as giving a rate of decomposition of organic materials 20-60 times faster than conventional methods. The difference in time is due to the different heat transfer mechanism employed. The fact that closed vessels are utilized enables higher temperatures to be reached through increased pressure, which is extremely beneficial for samples with difficult matrices (Sandroni and Smith, 2002). Microwave digestions are usually performed with nitric acid in a closed high-pressure polytetrafluoroethylene (PTFE) lined vessel at temperatures above the boiling point of nitric acid. Microwave digestion is usually complete within 1 h. (Dolan and Capar, 2002). In addition to the reduction in analysis time, other advantages of microwave digestion include reduced contamination, lower reagent and sample usage, reduced loss of volatile species and enhanced operator safety (Sandroni and Smith, 2002).

This research aims to determine lead in canned fish samples by stripping voltammetry.

1.2 Review of literatures

1.2.1 Physical and chemical properties of lead

Lead (Pb) has atomic number 82, atomic weight 207.19, and a specific gravity of 11.34 g cm^{-3}. It is a bluish or silvery-grey metal with a melting point of 327.5 °C a boiling point at atmospheric pressure of 1740 °C and electron configuration [Xe] $4f^{14}$ $5d^{10}$ $6s^2$ $6P^2$ (WHO, 2001). Lead primary form in the natural state is galena (PbS). Lead occurs mainly as Pb^{2+}, although its oxidation state, +4, is also known, and it forms other several minerals which are quite insoluble in natural waters (Martinez-Villegas *et al*., 2004).

1.2.2 Uses of lead

Lead occurs in variety of organic and inorganic compounds with a multitude of additional uses. Lead acetate is used in dyeing, manufacture of pesticides, antifouling paints and hair dyes. Lead nitrate is used in matches and explosives, as a mordant in dyeing and textile printing. Lead oxide (red) is used in storage batteries, varnishes, coloring rubber, matches, pigment in printing inks, and paints. Organic lead compounds include tetramethyl and tetraethyl lead was used heavily as a gasoline additive to increase octane rating (Johnson, 1998). However, their use was phased out in the United States in the 1980s, and lead was banned for use in gasoline for motor vehicles beginning January 1, 1996 (ATSDR, 2005).

1.2.3 Toxicity and Health Effect of lead

Lead toxicity may be caused by organic or inorganic lead poisoning. Lead poisoning in humans usually results from inorganic lead, but organic lead poisoning may occur in those who inhale gasoline recreationally (Graeme and Pollack, 1998). The accumulation of Pb in fish is dependent on both uptake and elimination rates. It has been shown that uptake is dependent on locality, species, sex, age, the specific organs studied and the state of maturity. Lead occurs in aquatic environments in the form of many speciations, both inorganic and organic (Celik *et al*., 2004).

The toxicity of lead may largely be explained by its interference with different enzyme systems: lead inactivates these enzymes by binding to SH-groups of its proteins or by displacing other essential metal ions (WHO, 2001). For this reason many organ systems are potential targets for lead including the central and peripheral nervous systems, the red blood cells, the kidneys, the cardiovascular system, and the male and female reproductive organs (Landrigan *et al*., 2000).

Lead may be absorbed through inhalation or by ingestion. In adults, 10% of lead that passes through the gastrointestinal (G1) tract is absorbed; in children, up to 50% of lead may be absorbed. Some salts of lead are absorbed more quickly and more completely, leading to a more rapid, acute disease. Iron deficiency increases the risk of lead toxicity (Graeme, and Pollack, 1998).

The toxicity of lead after exposes the high level of lead. Symptoms are occurred such as anorexia, stomach pain, headache, diarrhea, vomiting, collapse and coma (Thornton *et al.,* 2001). The main target for lead toxicity is the nervous system, both in adults and children. Long-term exposure of adults to lead at work has resulted in decreased performance in some tests that measure functions of the nervous system. Lead exposure may also cause weakness in fingers, wrists, or ankles and anemia. EPA has determined that lead is human carcinogenic (ATSDR, 2005).

1.2.4 Anodic stripping voltammetry using chemically modified carbon paste electrodes

Stripping voltammetry (SV) comprises a variety of electrochemical approaches, having a step of preconcentration onto the electrode surface prior to the voltammetric measurement. In trace analysis of heavy metal ions, anodic stripping voltammetry (ASV) is the most popular stripping voltammetric technique (Abu Zuhri and Voelter, 1998). The chemically modified carbon paste electrode (CMCPE) has been developed for voltammetric analysis because it provides several advantages such as non-toxicity, low background current and wide range of deposition potentials over the mercury electrode (Huang *et al.,* 1998). One of the main reasons for modification is to improve the selectivity and sensitivity of the electrochemical measurement by preconcentrating the target analyte from a dilute solution on the electrode surface (Degefa *et al*., 1999). Different modifiers for carbon paste have been reported in the last years for the electrochemical stripping analysis of Pb(II) can be summarized as follows:

Molina-Holgado *et al*., (1995) developed chemically modified prepared carbon paste electrode modified with diphenylthiocarbazone was employed for quantification of Pb(II) from aqueous solutions by differential pulse anodic stripping voltammetry. The ratio of diphenylthiocarbazone to graphite powder in the paste was set at 15%. Optimal analytical conditions were found to be a 0.1 M HCl as supporting electrolyte and reduced at -1.0 V (vs. Ag/AgCl). For 6 min preconcentration time, the calibration graph is linear from 20.7 ng mL^{-1} to 207.2 ng mL^{-1} and from 207.2 ng mL^{-1} to 518.0 ng mL^{-1}. The detection limit was about 16.7 ng mL^{-1} and the response could be reproduced with a 5.8% relative standard deviation (RSD) at the concentration level of 31.1 ng mL^{-1}.

Huang *et. al*., (1998) developed chemically modified carbon paste electrode with tributyl phosphate (TBP). In the presence of 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone (PMBP), trace Pb(II) and Cd(II) in acetic acid-acetate medium can be preconcentrated on the electrode. The paste composition (graphite:paraffin:glycerol:modifier) was 45:20:20:15%. The preconcentration potential was -0.80 V and the preconcentration time was 6 min. The linear ranges were 1.0×10^{-8} to 8.0×10^{-7} mol.L^{-1} Pb(II) and for 5.0×10^{-8} to 2.0×10^{-6} mol.L^{-1} Cd(II). The detection limits of Pb(II) and Cd(II) were 4.6×10^{-9} mol.$L^{-1}$and 1.4×10^{-8} mol.L^{-1}, respectively. The electrode was verified by the determination of trace Pb(II) and Cd(II) in waste water and human hair samples.

Degefa *et al*., (1999) prepared chemically carbon paste electrode modified with *N-p*-Chlorophenylcinnamohydroxamic acid for the selective preconcentration and quantitation of Pb(II) by differential pulse anodic stripping voltammetry. The ratio between the modifier and carbon powder in carbon paste is 15% w/w. Two linear ranges were obtained in the concentration ranges 1.00×10^{-6} to 2.40×10^{-5} mol L^{-1} Pb(II) with 2 min preconcentration time 2.00×10^{-8} to 1.00×10^{-6} mol L^{-1} lead(II) with 6 min preconcentration time. The detection limit was found to be 1.00×10^{-8} mol L^{-1} with 6 min preconcentration time. The relative standard deviations (RSD) were 5.3% and 4.9% at 4×10^{-6} mol L^{-1} and 8×10^{-8} mol L^{-1} Pb(II), respectively. The method was applied to Pb(II) determination in potable water.

Ijeri and Srivastava, (2001) prepared chemically carbon paste electrode modified with crown ethers for Pb(II) determination. The crown ethers studied and compared were 18-crown-6 and dibenzo-18-crown-6. The result shown that carbon paste electrode modified with 5% 18-crown-6 carbon paste electrode could be determination of Pb(II) at sub-mg L^{-1} levels by differential stripping voltammetry with a detection limit of 0.02 mg L^{-1}. A linear calibration curve was obtained over the range 10 to 100 μg L^{-1} (coefficient of correlation = 0.9995) for 1 min deposition period and over the range 1 to 100 μg L^{-1} (coefficient of correlation = 0.9993) for 2 min deposition period. Detection limit was 0.2 μg L^{-1} for deposition period 4 min. This method applied to the determination of lead in commercial samples of gin, vodka, and country liquor.

Mousavi *et al*., (2001) developed chemically modified carbon paste electrode modified with 1,4-Bis(prop-2′-enyloxy)-9,10-anthraquinone (AQ) for the determination of Pb(II)

at trace levels by differential pulse anodic stripping voltammetry. The optimum parameters were 7.5% w/w ratio of 1,4-Bis(prop-2′-enyloxy)-9,10-anthraquinone to the carbon powder, 0.3 M HNO_3 as supporting electrolyte and reduction potential was -1.5 V. A linear calibration graph was obtained in the concentration range 2.00×10^{-9} to 1.06×10^{-5} M Pb(II) with 30 s preconcentration time. The detection limit was 1×10^{-9} M. The method was applied to lead determination in waste waters.

Hu *et al*., (2003) developed chemically modified carbon paste electrode modified with diacetyldioxime for the simultaneous determination of Pb(II) and Cd(II) by using differential pulse stripping voltammetry. The best ratio between the modifier and carbon powder in carbon paste is 20% w/w., NaH_2PO_4 0.1 mol L^{-1} was suitable for the supporting electrolyte. Pb(II) and Cd(II) were preconcentrated on the surface of the modified electrode by complexing with diacetyldioxime and reduced at -1.10 V. Calibration graphs were linear in the concentration ranges of 1.0×10^{-7} to 1.5×10^{-5} mol L^{-1} Pb (II) and 2.5×10^{-7} to 2.5×10^{-5} mol L^{-1} Cd(II) respectively. Detection limits were 1×10^{-8} mol L^{-1} Pb (II) and 4×10^{-8} mol L^{-1} Cd(II) for 5 min preconcentration . The relative standard deviations (RSD) were 2.9% Pb(II) and 3.2% Cd(II), respectively. The method was applied to the simultaneous determination of Pb(II) and Cd(II) in water samples and average recoveries of 100% Pb(II) and 101% Cd(II) were obtained.

1.2.5 Adsorptive cathodic stripping voltammetry

Adsorptive cathodic stripping voltammetry (AdCSV) is a useful method to determine trace elements since it combines excellent sensitivity, selectivity, accuracy and precision with low cost of instrumentation and maintenance. This technique is based on adsorptive accumulation of a complex of the element with an added specific ligand on a hanging mercury drop electrode followed by electrochemical reduction of either the element or the ligand in the complex (Li *et al*., 2005). Several ligands have been used for the determination of Pb(II) can be summarized as follows:

Van Den Berg, (1986) presented to determine simultaneous Cu(II), Pb(II), and Cd(II) in seawater by differential pulse cathodic stripping voltammetry preceded by adsorptive collection of complexes with 8-hydroxyquinoline onto a hanging mercury drop electrode

(HMDE). The optimal analytical conditions were found to be 8-hydroxyquinoline concentration of between 0.8 and 2.0 × 10^{-5} mol L^{-1}, pH between 7.5 and 8.0, the adsorption potential at -1.1 V., and the initial scanning potential at -0.3 V. The detection limits for a 1 min stirred adsorption time are 0.12 nmol L^{-1} Cd(II), 0.30 nmol L^{-1} Pb(II) and 0.24 nmol L^{-1} Cu(II). These limits are reduced 10x by increasing the adsorption time to 10 min.

Wu and Batley, (1995) presented method for the determination of Pb(II) in sea water by adsorptive cathodic stripping voltammetry with ligand competition using xylenol orange (3,3′-bis[*N,N*-bis(carboxymethyl)aminomethyl]-*o*-cresolsulfophthalein, X0) on to a HMDE. Optimal analytical conditions were found to be xylenol orange concentration of 1 × 10^{-5} M, pH = 5, and adsorption potential at -1.2 V with stripping from - 0.2 V. The limit of detection for 180 s stirred adsorption time is 6 ng L^{-1}.

Yokoi *et al*., (1995) developed cathodic stripping voltammetric method for the determination of Pb(II), with adsorptive collection of complexes with Calcein Blue (8-[*N,N*-bis(carboxymethyl)aminomethyl]-4-methylumbelliferone) on to a HMDE. After accumulation of the complex, the electrode potential is scanned in a negative direction with differential pulse modulation and the reduction current for the lead complex is measured at -0.5 V. Optimum conditions were 300 nM Calcein Blue, pH = 6.5 and adsorption potential at -0.15 V. Under the optimum conditions the calibration graph is linear up to 100 nM of Pb(II) with 1 min adsorption The limit of detection 0.04 nM after 1 min collection was obtained. The method is successfully applied to standard river water SLRS-2 and various fresh water samples.

Limson and Nyokong (1997) determined Cu(II), Cd(II), Pb(II) and Bi(II) by adsorptive cathodic stripping voltammetry (AdCSV) on a mercury film glassy carbon electrode, using catechol, 4-methylcatechol, 4-t-butylcatechol and resorcinol as complexing ligands. Complexes of Pb(II) , Cu(II) and Bi(II) bismuth with resorcinol showed the largest increase in current with increase in metal concentration, whereas complexes of these metals with 4-t-butylcatechol showed the lowest current response. The four metals could be determined simultaneously in the presence of resorcinol, although considerable interference was observed between Bi(II) and Cu(II).

Shams *et al*., (2004) developed method for the simultaneous determination of Cu(II), Zn(II) and Pb(II) is presented. The method is based on the adsorptive accumulation of 2′,3,4′,5,7-pentahydroxyflavone (Morin) complexes of these elements onto a HMDE, followed by reduction of adsorbed species by voltammetric scan using differential pulse modulation. Optimal analytical conditions were found to be Morin concentration of 2.0 μmol L^{-1}, pH = 4.0 and an adsorption potential at -0.5 V vs Ag/AgCl. With an accumulation time of 60 s, linear calibration graph were obtained in the concentration range of 1-60, 0.3-80 and 1-70 ng mL^{-1} for Cu(II), Pb(II) and Zn(II) , respectively. The detection limits of 0.06, 0.08 and 0.06 ng mL^{-1} were obtained for Cu(II), Pb(II) and Zn(II), respectively.

Babaei *et al*., (2006) presented adsorptive stripping procedure for simultaneous determination of Cu(II), Bi(II) and Pb(II) is presented. The method is based on the adsorptive accumulation of thymolphthalexone (TPN) complexes of these elements onto a HMDE. The optimum analytical conditions were found to be TPN concentration of 4.0 μM, pH of 9.0, and accumulation potential at -0.80 V vs. Ag/AgCl with an accumulation time of 80 s. The linear calibration graph were obtained in the concentration range of 0.4 - 300, 1 - 200 and 1 - 100 ng mL^{-1} for Cu(II), Bi(II) and Pb(II), respectively. The detection limits of 0.4, 0.8 and 0.7 ng mL^{-1} for Cu(II), Bi(II) and Pb(II), respectively. The procedure was applied to the simultaneous determination of Cu(II), Bi(II) and Pb(II) in the tap water and some synthetic samples with satisfactory results.

1.2.6 Sample digestion method

Many analytical detection techniques, like atomic absorption spectrometry, atomic emission spectrometry, voltammetry, and spectrophotometry, require a sample (analyte) to be present in a dissolved form. Matrix dissolution is a method of converting the components of the sample into simple chemical forms (Novozamsky *et al*., 1995). The digestion method must be selected to suit the type of sample, the metals being determined, and finally, the analytical method (Mitra, 2003). In 1993 FDA conducted routine food analysis using traditional dry ash or acid digestion procedures (Dolan and Capar, 2002). Digestion methods were used in preparation of food samples and can be summarized as follows:

Dry ashing consists of the incineration of organic matter at 450 – 800 °C in open vessels, but its use is limited by the volatility of the bonds of certain elements. Addition of compounds such as MgO or $Mg(NO_3)_2$ prevents elements from becoming volatile and makes sample decomposition faster (Loska and Wiechula, 2006).

Erdoğrul and Erbilir, (2006) applied the dry ashing method to prepare the fish samples for Pb(II) determination. A sample (ca. 4 – 5 g wet weight) was placed in a high form porcelain crucible. The furnace temperature was slowly increased from room temperature to 100 °C in 2 h. The sample was ashed for about 450 °C for one night until a white or grey ash residue was obtained. The residue was dissolved in 4 ml of HNO_3 and 8 ml $HClO_3$ mixture. The mixture was filtered by Whatman No 42 filter paper. The analysis of lead was carried out by using graphite furnace atomic absorption spectrometry (GFAAS). The digestion and analytical procedures were checked by analysis of standard reference material (DORM-2: Dogfish Muscle).

Juresa and Blanusa, (2003) studied the Pb(II) contamination in fish and shellfish. The sample preparation method was performed by using dry ashing method. Samples of about 5 g of fresh tissue were dry-ashed in quartz crucibles in a muffle furnace overnight at 450 °C. After ashing, the samples were dissolved in concentrated HNO_3 and adjusted to 10 mL with distilled water to form a 2% HNO_3 solution. The Pb(II) levels were analyzed by electrothermal atomic absorption spectrometry (ETAAS). The accuracy of method was obtained by analysis of certified reference samples (DORM-2: Dogfish muscle). The precession (RSD) and accuracy of the method were 38% and 129%, respectively.

Wet digestion decomposes organic matter using oxidizing concentrated mineral acids. Nitric, sulphuric and perchloric acids are the most common acids. Wet digestion may be carried out in open or closed systems (Loska and Wiechula, 2006).

Khansari *et al.*, (2005) presented the wet digestion method for Pb(II) determination in canned tuna fish. Sample was homogenized thoroughly in a food blender with stainless steel cutters. Homogenized sample (2 ± 0.001 g) was placed into a 200 ml beaker and 10 ml of conc. HNO_3 were added. The beaker was covered with a watch glass and after most of the

sample had dissolved by standing overnight, heated on a hot plate with boiling until any vigorous reaction had subsided. The analyte was determined by GFAAS. The recovery of Pb(II) was found in range 93 - 103 %.

Tarley *et al*., (2001) applied wet digestion with acid method to prepare sardines canned for Pb(II) determination. The sardine samples were dried at 60 °C for 48 h. Dried samples (2.0000 ± 0.0001 g) placed in a digestion tube were pre-digested using 10 mL concentrated HNO_3 at 135 °C until the liquor was clear. Next, 10 mL of HNO_3 and 1 mL of $HClO_4$ were added and the temperature was maintained at 135 °C for 1 h. The digested liquors were filtered through a Whatman 1 filter paper. The sample solution was clear and determined Pb(II) levels by atomic absorption spectrometry (AAS). The accuracy was confirmed by analysis of certified reference materials (DORM-2: Dogfish Muscle). The average contents of Pb(II) in sardines were 0.77 - 2.15 $\mu g\ g^{-1}$.

Ashraf *et al*., (2006) studied the contamination of Pb(II) in canned salmon, sardine and tuna fish by using GFAAS. Samples (5 g wet weight) were digested in quartz Erlenmeyer flask with 15 ml of HNO_3:$HClO_3$:H_2SO_4 (25 + 25 + 1 v:v:v) mixture, using a hot plate at 150 °C. Further aliquots of HNO_3 were added until a complete colorless solution occurred. The residue was dissolved in 10 mL of water with 1ml of conc. HCl at 100 °C and finally diluted to 25 mL with water. The recovery of lead was found in range 90 - 104%. The average contents of Pb(II) in samples were 0.313 $\mu g\ g^{-1}$ for salmon; 0.233 $\mu g\ g^{-1}$ for tuna and 0.835 $\mu g\ g^{-1}$ for sardines.

Voegborlo *et al*., (1999) studied the contamination of Pb(II) in canned tuna fish. The sample preparation method was performed by using wet digestion method. The sample (10.0 ± 0.01 g wet weight) was placed in beaker which covered by a watch glass and 10 mL of 1:1(v/v), H_2O_2 (30%) : HNO_3 (conc.) was added per gram of sample, slowly, in portions. After most of the sample had dissolved, sample was heated on a hot plate until the solution was clear. Heating was continued until the volume was reduced to about 5 ml. Lead levels were determined by flame atomic absorption spectrometry (FAAS). The recovery of Pb(II) was 99.8%. The contents of Pb(II) in canned tuna fish were 0.18 to 0.40 $\mu g\ g^{-1}$.

In the past few years, microwave digestion has been gaining popularity. The application of microwaves considerably streamlines sample preparation, making it faster and enabling full digestion of the material. It also reduces losses of elements and sample contamination compared to other digestion methods (Loska and Wiechula, 2006).

Ikem and Egiebor, (2005) applied the microwave digestion method to prepare canned fishes (mackerel, tuna, salmon, sardines and herrings) for Pb(II) determination. For each canned fish sample, the fish sauce was drained and a portion of the muscle in minimal contact with the sauce was weighed (between 2-4 g). Sample was placed in a Teflon digestion vessel with 15 mL of concentrated HNO_3. Microwave program were applied 25 - 96 °C for 20 min at 1000 W, hold 96 °C for 30 min, 180 °C for 10 min at 1000 W and cooled to room temperature, respectively. Then 2 mL of 30% H_2O_2 was added in the vessel for breakdown organic matter that may be undigested during the concentrated HNO_3,digestion and the mixture and heated 180 °C for 10 min at 1000 W. Pb(II) was determined by inductively coupled plasma-optical emission spectrometer (ICP-OES). The accuracy of method was obtained by analysis of certified reference samples (DORM-2: Dogfish muscle and TORT-2: lobster hepatopancreas). The ranges obtained for Pb(II) analyzed were 0.0 - 0.03 $\mu g\ g^{-1}$ (wet weight).

Tuzen and Soylak, (2007) studied the Pb(II) contamination in canned fish. The sample preparation method was performed by using microwave digestion. Sample (1 g wet weight) was digested with 6 mL of concentrated HNO_3 and 2 mL of concentrated H_2O_2 in a microwave digestion system. Conditions for microwave system applied were: 2 min for 250 W, 2 min for 0 W, 6 min for 250 W, 5 min for 400 W, 8 min for 550 W, vent: 8 min, respectively. The analysis of lead was carried out by using GFAAS. Certified reference materials (NRCC-DORM-2: Dogfish Muscle) were analyzed to check the accuracy and the precision of the method. The recovery of Pb(II) was 95%. The contents of investigated lead in canned fish samples were found to be in the range 0.09 - 0.40 $\mu g\ g^{-1}$.

1.3 Objectives

1.3.1 To study carbon paste electrodes modified with group of xanthone compounds for determination of metals by differential pulse anodic stripping voltammetry.

1.3.2 To optimized method for lead determination by adsorptive cathodic stripping votammetry with 8-hydroxyquinoline.

1.3.3 To apply optimized method for lead determination in canned food samples.