CHAPTER 1

INTRODUCTION

1.1 Overview of the research

Environmental contamination from heavy metals (such as Cd, Ni, Cu, Pb, Cr, As and Zn) is of concern because they have toxic effects to living things even if they are present at very low concentration. Thus, the determination of trace metals in environmental samples including water and soil, etc. is becoming increasingly important.

Various techniques have been employed to determine the heavy metals. Due to the problems of trace amounts sensitivity and interference in environmental samples, preconcentration and preseparation steps are required prior to determination of the heavy metals, especially, when using a simple detection system, such as flame atomic absorption spectrometry (FAAS) or electrochemical techniques.

Cost-effective techniques/procedures should be considered for an analysis of a number of samples in environmental studies. Flow-based analysis including flow injection analysis (FIA) and sequential injection analysis (SIA) with various techniques has attracted increasing attention because of its simplicity, high precision and high sample frequency. Moreover, it requires small volumes of reagents and samples. Due to these features, it is suitable for routine analysis with economic benefits. Hence, this research work is focused on development of flow-based techniques for the determination of some heavy metals in environmental samples.

1.2 Heavy metals

1.2.1 Lead [1-3]

Lead is one of the most toxic metals. It is mainly obtained from the sulphide ore galena, by a roasting process, making it one of the most widespread metals in terms of primary production. In addition to this, secondary smelters and processing lead metal products reflect widespread recycling of lead in electric storage batteries. Natural sources of atmospheric lead include wind-borne soil particles and volcanic emissions, forest fires, biogenic processes and sea salt sprays.

As a metal, it is often used as pipe-work for water distribution, or as containers for storing corrosive liquids (e.g. sulphuric acid). Its alloys are used in welding, printing and as anti-friction metals; and great quantities, both of the metal and its dioxide, are used in electric storage batteries. Other uses include compound in petrol, paint pigments, PVC stabilisers, pesticides, varnishes, lubricants, etc.

Lead tends to accumulate in soils and sediments, where it can remain accessible to the food chain and to human metabolism for many years. Plants and animals can accumulate lead from water, soil and sediment, with organic forms being more easily taken up than inorganic one.

The toxic effects of lead have been associated with a wide range of adverse effects. Health impacts include nervous system disorders, anaemia and decreased haemoglobin synthesis, cardiovascular disease and disorders in bone metabolism. The World Health Organization (WHO) currently recommends a limit of 10 $\mu g\ l^{-1}$ for lead in drinking water. Regarding soil contamination, the UK Department of the Environment regards a level of 0-500 mg kg^{-1} as being typical of uncontamination.

1.2.2 Copper [1, 3]

Copper is a common element that occurs naturally in the environment in different forms. Humans widely use copper, for instance, it is applied in the industries and in agriculture. Most copper compounds will settle and be bound to either water sediment or soil particles. Soluble copper compounds form the largest threat to human health. Usually water-soluble copper compounds occur in the environment after release through application in agriculture. Copper can be released into the environment by both natural sources and human activities. It is often found near mines, industrial settings, landfills and waste disposals.

When copper ends up in soil, it strongly attaches to organic matter and minerals. It can accumulate in plants and animals. On copper-rich soils, for example, near copper-disposing factories, only a limited number of plants has a chance of survival. Due to the effects upon plants, copper is a serious threat to the productions of farmlands. When the soils of farmland are polluted with copper, animals may intake the copper and would affect their health.

Copper concentrations in uncontaminated soil were reported to be 30 mg kg^{-1} (WHO, 1998).

1.2.3 Nickel [3-4]

Nickel is a compound that occurs in the environment at low levels. The most common application of nickel is the use as an ingredient of stainless steel and other metal products. It can be found in common metal products such as jewellery.

Nickel is released into the air by power plants and waste incinerators. It will then deposit or precipitate after reactions with raindrops. It usually takes a long time for nickel to be removed from air. Nickel can also end up in surface water when it is a part of wastewater streams. The larger part of all nickel compounds that are released to the environment will adsorb to sediment or soil particles and become immobile as a result. High nickel concentrations on soils can damage plants and high nickel concentrations in surface water can diminish the growth rates of algae.

Plants can accumulate nickel from soil. Small amounts of nickel are essential, but when the uptake is too high, it can be a danger to human health. An uptake of too large quantities of nickel can affect in development of lung cancer, nose cancer, larynx cancer and prostate cancer, birth defects, asthma and chronic bronchitis, heart disorders and including skin allergy.

For animals nickel is an essential foodstuff in small amount. However, it can also be dangerous when they take up high amount. This can cause various kinds of cancer on different sites within the bodies of animals, mainly of those that live near refineries.

1.2.4 Cadmium [1, 5]

Cadmium can mainly be found in the earth's crust. It always occurs in combination with zinc. It also consists in the industries as an inevitable by-product of zinc, lead and copper extraction.

Naturally a very large amount of cadmium is released into the environment. Cadmium can be released into rivers through weathering of rocks and some cadmium is released into air through forest fires and volcanoes. The rest of the cadmium is released through human activities, such as manufacturing. Cadmium waste streams from the industries mainly end up in soils. Another important source of cadmium emission to soil is the production of commercial phosphate fertilizers.

Cadmium adsorbs to organic matter in soils. When cadmium is present in soils, it can be extremely dangerous, as the uptake through food chain will increase. Soils that are acidified enhance the cadmium uptake by plants. This is a potential danger to the animals that are dependent upon the plants for survival. Cadmium can accumulate in their bodies, especially when they eat multiple plants. Earthworms and other essential soil organisms are extremely susceptive to cadmium poisoning. They can die at very low concentrations and this has consequences for the soil structure. When cadmium concentrations in soils are high, they can influence soil processes of microorganisms and threat the whole soil ecosystem.

Human uptake of cadmium takes place mainly through food. Other high exposures can occur with people who live near hazardous waste sites or factories that release cadmium into the air and people that work in the metal refinery industry. Cadmium accumulates in kidneys, where it damages filtering mechanisms. This causes a stop to re-absorption of Ca and Mg and the excretion of essential proteins and sugars from the body and further kidney damage. It takes a very long time before cadmium that has accumulated in kidneys is excreted from a human body.

Internationally maximum soil guidelines vary between 0.5-3 mg kg^{-1}.

1.2.5 Zinc [1, 3]

Zinc is commonly found in the Earth's crust. It is found as a mineral (most commonly sphalerite, zinc sulphide), often associated with the ores of other metals (e.g. copper, lead and cadmium). Natural sources of atmospheric zinc include wind-borne soil particles, emissions from forest fires and volcanoes, biogenic emissions and sea-salt sprays. It is employed as a protective coating for other metals e.g. galvanised steel, or as a component of bronze, brass and die-casting alloys. In addition, zinc salts are widely employed as wood preservatives, herbicides, catalysts, analytical reagents, vulcanisation accelerators for rubber and stabilisers in PVC.

Zinc is a nutritionally essential metal, having enzymatic, structural and regulatory roles in many biological systems. Deficiency in humans can result in severe health consequences including growth retardation, anorexia, dermatitis, depression and neuropsychiatric symptoms. At the other extreme, excessive dietary exposure, in both humans and animals, can cause gastrointestinal distress and diarrhoea, pancreatic damage and anaemia. Plant studies have shown that although an essential element for higher plants, in elevated concentrations zinc is considered phytotoxic, directly affecting crop yield and soil fertility.

The UK Department of the Environment classifies a soil level of 0-250 mg kg^{-1} as being typical of uncontamination.

1.2.6 Chromium [1]

Chromium is used in various industries, for examples, for metallurgical purposes in the production of alloys and stainless steel, for important components of refractory materials, such as glass or ceramics and for color. Large amount of waste containing of chromium can be released to environment after their processes.

The determination of chromium speciation in environmental and biological systems is increasingly attention. The important oxidation states of chromium, Cr(III) and Cr(VI) are most found in environment. Cr(III) is considered to be a trace element essential for human. But the uptake of too much Cr(III) can cause health effects as well, for instance, skin rashes. Cr(VI) has acute and chronic toxic, including carcinogenic effects in animal studies. Therefore, a detailed knowledge of each species rather than the total level is required to properly evaluated physiological and toxicological effects of chromium.

1.3 Flow-based analysis (FBA)

Flow-based analysis is the generic term which describes a group of automated sample manipulation procedures that make use of flowing streams in micro-conduits. One of the techniques is ***Flow Injection Analysis (FIA***). Another is ***Sequential Injection***

analysis (SIA), which is a variation of FIA where a selection valve is used to produce a stack of well-defined zones of reagent and sample. These zones penetrate one another as they are carried to the detector by a suitable pump.

1.3.1 Flow injection analysis (FIA) [6]

There are many definitions of FIA in history. One described as “A non-chromatographic flow analysis technique for quantitative analysis, performed by reproducibly manipulating sample and reagent zones in a flow stream under thermodynamically non-equilibrated conditions” [7].

FIA is based on combination of three principles, sample injection, controlled dispersion and reproducible timing. Sample injection provides a well-defined starting point for initial concentration and startup time. Controlled dispersion results in well-defined dilution of injected sample zone. Reproducible timing of injected sample zone to detector is obtained by applying constant flow rate of carrier.

The most important physical phenomenon in FIA is the dispersion process. A homogenised sample solution (before injection) has the original concentration $\mathbf{C^0}$, (presented as a square signal in **Figure 1.1** on the left). When the sample zone is injected into a carrier stream, it follows the movement of the carrier stream and forms a dispersed zone whose form depends on the geometry of the channel and flow velocity. Therefore, square profile of original concentration changes to a continuous concentration gradient with maximum concentration $\mathbf{C^{max}}$ at the apex of the peak (**Figure 1. 1** on the right).

Dispersion can be expressed in term of dispersion coefficient, D_p, defined as the ratio of concentrations of the sample material before and after the dispersion process has taken place in carrier stream that yields the analytical readout as shown in the **equation 1.1**:

$$\mathbf{D_p = C^0/C} \qquad \ldots\ldots\ldots\ldots\ldots\ldots \textbf{ equation 1.1}$$

where C^0 is the original concentration of the sample before dispersion and C is the concentration of the sample after dispersion.

It can also be defined as **equation 1.2** if the signal is considered as the highest concentration (i.e. readout at FI peak maximum)

$$\mathbf{D_p = C^0/C^{max}} \qquad \ldots\ldots\ldots\ldots\ldots\ldots \textbf{ equation 1.2}$$

where C^{max} is the concentration of the sample at peak maximum of the dispersed zone.

Dispersion coefficient can be classified into 3 categories. There are limited, medium and large dispersion. These adjectives refer to dispersions of 1 to 3, 3 to 10 and greater than 10, respectively. The limited-dispersion FI-techniques have been found considerable application for high-speed feeding of such detector systems as FAAS, ICP-AES, pH or conductivity measurements and so on. The medium-dispersion FI- techniques are often designed for techniques, for instance, spectrophotometry, which sample and reagent must be mixed to form a detectable product. The large-dispersion is suitable for highly concentrated samples, which must be diluted into the detection range.

Figure 1.2 presents the basic components of FIA. A typical FIA manifold is comprised of a pump, an injection valve, a detector and tubing manifold. The pump is used to aspirate carrier streams through the detector via narrow conduit (0.5-0.8 mm ID).

The injection valve is used to introduce a small volume of sample into a carrier stream. While the sample is carried to the detector, the fluid dynamics of flow through narrow bore tubing mixes sample and reagent, leading to chemical reaction to form a detectable species. These species are then measured by the detector.

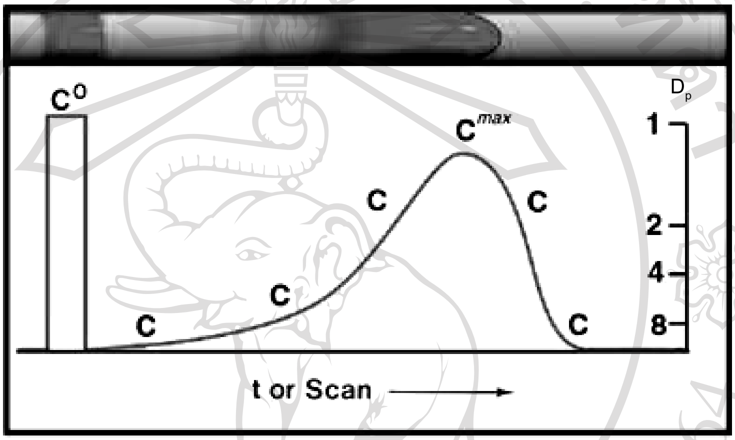

Figure 1.1 Dispersion phenomenon and peak profile of the sample zone before and after dispersion process [6]

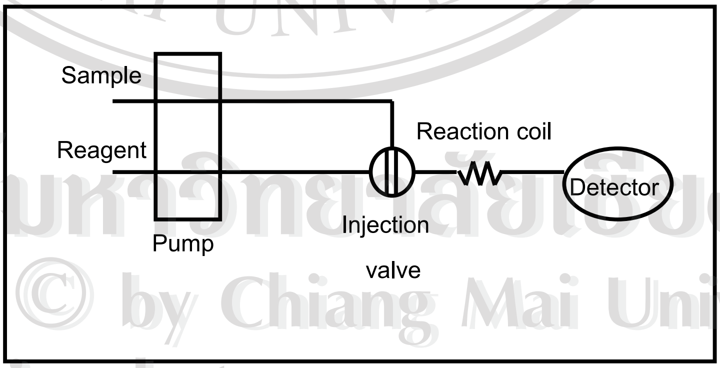

Figure 1.2 The basic components of FI system [8]

Recently, many research works involved FIA have been published in increasing numbers. This may be due to the advantages of FIA offering, for examples, low instrumentation cost, low scheduled maintenance requirement, simple to assemble, accurate and precise sample manipulation. In addition, a wide range of useful manifold components allows complex operations such as dilution, separation and preconcentration, gas permeation, dialysis and many others to be incorporated into the FI-manifold.

1.3.2 Sequential injection analysis (SIA) [8]

Sequential injection analysis (SIA) is one of a new generation of FIA. It is an automated approach to sample handling resulting in a rapid, precise, and efficient manner.

In SIA, bi-directional syringe pump is used to draw up small volumes of sample and reagents via a selection valve which replacing an injection valve in FIA. The sample and reagents are then propelled through a holding coil. The mixing of the sample and reagents forms a detectable species and the carrier transports that species from holding coil through the detector. The signal is simultaneously recorded. **Figure 1.3** demonstrates the basic components of SI-system.

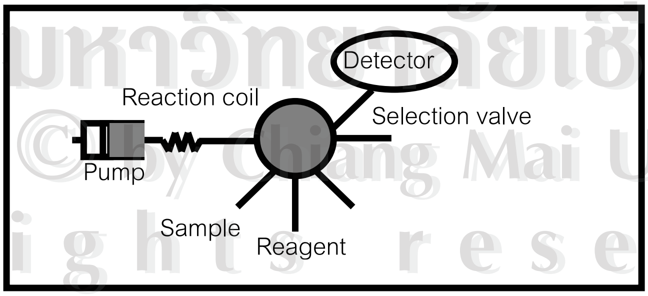

Figure 1.3 The basic components of SI system [8]

The main benefits of SIA are simple, automatic and robust system. Small volume of sample and reagents are required, so waste can be minimised. Moreover, it is suitable for miniaturisation. These features offer SIA more attractive than FIA.

1.4 Preconcentration and separation procedure for heavy metal determination

Preconcentration and separation procedure incorporating with various detection systems for heavy metals determination (e.g. Cu, Pb, Ni, Cd, Zn and Cr) in environmental samples have been reported [9-12]. The preconcentration and separation procedure is usually incorporated into the system in order to solve the problems of low concentration of heavy metals and the present of interfering components in environmental samples [13-29]. For ETAAS and ICP-AES, due to instrumental and operational cost, they are not affordable by many labs. For spectrometric technique, sometimes it suffers from sensitivity and/or selectivity. Therefore, FAAS is an alternative method for routine analysis with reasonable sensitivity and fairly low operational cost. Moreover, its instrument is available in most of laboratories.

Common preconcentration and separation methods, for instance, methods based on ion exchange resin or sorbents in packed column [13-21] and solvent extraction [22-29] were employed.

Off-line preconcentration and separation procedures have been studied [10, 30-35]. Owning to the laboriousness of operation, risk of contamination from utensils and the laboratory environments, which may induce complications in trace analysis, on-line preconcentration and separation methods were considered (**Table 1.1**). Moreover, the automation of the system would be very useful for routine analysis. Hence, these research works were focused on automated on-line preconcentration and separation procedure for heavy metal determination by using FAAS.

Table 1.1 The on-line preconcentration/separation methods for heavy metal determination

Analytes	**Techniques**	**Preconcentration/ separation methods**	**Analytical characteristics**	**Samples**	**Ref**
Pb	FI-FAAS	-IX (Resin 122)	CE= 16 min^{-1}, %RSD= 1.5-4.1	Tap water, polluted water and sea water	36
Pb	FI-FAAS	-SPE -Using Octadecyl silica membrane disk -Acetic acid for elution	DL= 50 ng l^{-1} , EF=300	Soil and water samples	37
Cu^{2+}, Co^{2+} Cd^{2+},Ni^{2+}, Pb^{2+} and Zn^{2+}	FI-FAAS	-Metals sorbed on 8-quinolinol immobilised column and eluted with acid	EF=500 at 25 min	Tap water	21
Cd^{2+}, Co^{2+}, Cr^{6+}, Cu^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}	FI-FAAS	-Solvent extraction -Dibenzyldithio-carbamates as a complexing agent, -MIBK as organic phase	LR/µg l^{-1}: 1.4-40 Cd^{2+}, 2.8-120 Co^{2+}, 8.2-240 Cr^{6+}, 5.3-160 Cu^{2+}, 4.7-180 Ni^{2+}, 7.3-430 Pb^{2+} and 5.1-51 Zn^{2+} DL/ µg l^{-1}: 1.4 Cd^{2+}, 3.1 Co^{2+}, 8.5 Cr^{6+}, 5.6 Cu^{2+}, 4.9 Ni^{2+}, 3.7 Pb^{2+} and 5.2 Zn^{2+}	Synthetic samples	38

Table 1.1 (Continued)

Analytes	Techniques	Preconcentration/ separation methods	Analytical characteristics	Samples	Ref
Cu	FI-FAAS	-SPE -Cu-APDC sorbed on PTFE packed column and eluted with MIBK	EF= 340, DL= 0.05 $\mu g\ l^{-1}$ %RSD= 1.5 f= 40 h^{-1}	Water samples	39
Pb	FI-FAAS	-SPE -Sr. Spec^{TM} as extractant -0.005 M EDTA for elution	LR= 0.1-5.0 μg Pb CE= 11 min^{-1}, DL= 0.09 μg %RSD= 5.2	Leachate of tin tailing samples and certified reference material	28
Pb	FI-FAAS	-SPE -Sr. Spec^{TM} as extractant -Ammonium oxalate for elution	CE= 6 min^{-1}, DL= 0.08 μg %RSD= 4.1 f= 17 h^{-1}	Soil samples	17
Ni	FI-ETAAS	-Solvent extraction -APDC as complexing agent and MIBK for organic phase	EF= 25, DL= 4 ng l^{-1} %RSD= 1.5	Body fluid and other biological samples	40
Pb	ETAAS	-SPE -DDC as a complexing agent -Ethanol as eluent	CE= 10.4 min^{-1}, DL= 0.003 $\mu g l^{-1}$ %RSD= 1.9 f= 24 h^{-1}	Soil samples	41
Pb	FI-Spectro-photometry	-Solvent extraction -Dicyclohexyl-18-crown-6 as complexing agent -Chloroform as organic phase	LR= 50-2,000 $\mu g\ l^{-1}$ DL= 5 $\mu g\ l^{-1}$ %RSD= 0.5 f= 45 h^{-1}	Alloys, soil leachate, sea water	42
Pb	FI-Spectro-photometry	-Extraction of dithizone complex into chloroform and Anex EDE-10P anion exchange	DL= 2 $\mu g\ l^{-1}$	Water, soil and food	43
Pb	FI-Spectro-photometry	-Extraction of quinolin-8-ol into chloroform	DL= 1 $\mu g\ l^{-1}$ %RSD= 5.2	Drinking water	44
Zn	FI-Spectro-photometry	-IX (Dowex 1-X8)	LR= 0.00- 2.00 mg l^{-1} %RSD <2 f= 45 h^{-1}	Plants	45

Table 1.1 (Continued)

Analytes	**Techniques**	**Preconcentration/ separation methods**	**Analytical characteristics**	**Samples**	**Ref**
Cd	FI-ICP-AES	-Extraction of Cd-DDC complex into tetrachloride	LR= up to 300 μg l^{-1} %RSD= 1.5 DL= 0.4 μg l^{-1}	Biological reference material	46
Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn	FI-FAAS	-Using mini-column with poly (aminophosphonic acid) chelating resin -3.0 M HCl for elution	DL= 0.5, 5.0, 1.5, 1.6, 3.5, 0.6, 3.2, 3.1 and 0.4 μg l^{-1} for Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn, respectively f= 48 h^{-1}	Natural water	47
Cd, Pb, Cu and Ni	ETAAS	-Formation of metal-DDC complex and sorption on the RP-C18 -Ethanol for elution	LR= 0.1-0.3 μg l^{-1} Pb, 0.01-0.03 μg l^{-1} Cd, 0.2-0.6 μg l^{-1} Ni, 0.1-0.3 μg l^{-1} Cu	Sea water	48
Mn, Co, Ni, Cu, Zn, Cd and Pb	FI-ICP-MS	-8-hydroxyquinoline immobilised on silicone tubing (Sil-8HQ)	DL = in ng l^{-1} range	Sea water	49
Cr^{6+}	SI-ETAAS	-Solvent extraction -APDC as a complexing agent -MIBK for organic phase	EF= 18, DL= 3.3 ng l^{-1} f= 24.2 h^{-1}	Reference material, synthetic seawater and waste water	50
Cd	SI-ETAAS	-Solvent extraction -APDC as a complexing agent -MIBK for organic phase	EF= 21.4, DL= 2.7 ng l^{-1} %RSD= 1.8 f= 13 h^{-1}	Certified reference material	51

(EF= enrichment factor, CE= concentration enrichment, DL= detection limit, LR= linear range, IX= ion exchange and f= sample frequency)

1.4.1 Solvent extraction

The solvent extraction is one of the most frequently used for preconcentration and separation method in the analytical laboratories. It takes an advantage of the relative solubilities of solutes in immiscible solvents. The solute dissolves more readily and becomes more concentrated in the solvent in which it has a higher solubility. A partial separation occurs when a number of solutes have different relative solubilities in the two solvents used. The use of ligands in solvent extraction allows various metal species to be separate from the other components following by selective measurement techniques. Common ligands, for instance, dibenzyl-dithiocarbamate [38], diethyl-dithiocarbamate (DDC) [52-56], ammonium pyrrolidine-dithiocarbamate (APDC) [31,50,57] and 8-hydroxyquinoline [58] were employed. For this research work, DDC was used as complexing agent because it has high binding affinity for many of the metals of interest, and the metal complexes with DDC may be extracted under an acidic condition [28-29].

As mentioned before that solvent extraction procedure can solve the problem relating to sensitivity and selectivity, there are other advantages when it was coupled with FAAS. The organic solvent, which was fed to the FAAS, will enhance the sensitivity due to the property of low viscosity for high efficiency of nebulisation. The lower viscosity than water will increase nebulisation rate resulting in smaller droplet formation and higher atomisation efficiency [59].

1.4.1.1 Extraction of some heavy metal ions with diethyl-dithiocarbamate (DDC) into methy-isobutyl ketone (MIBK)

Some heavy metal ions can form complexes with DDC. The metal complexes can be extracted into MIBK.

Following Bajo and Wyttenbach 's description [29] on the extraction equilibrium of a divalent metal (M) with excess DDC between an aqueous phase (aq) and an organic phase (org), they could be as following:

Considering an extraction constant, K_{ex}

$$2\ HDDC_{org} + M_{aq}^{2+} \rightleftharpoons M(DDC)_{2,org} + 2\ H_{aq}^{+} \quad \textbf{.........equation 1.3}$$

$$\text{where } K_{ex} = [M(DDC)_2]_{org}[H^+]^2_{aq}/[M^{2+}]_{aq}[HDDC]^2_{org}$$

The extraction into MIBK and the acidity of the ligand itself (K_s) can be described as shown in **equations 1.4** and **1.5**.

$$HDDC_{org} \rightleftharpoons HDDC_{aq} \quad \textbf{.........equation 1.4}$$

$$\text{with } K_{H,ex} = [HDDC]_{aq}/[HDDC]_{org} = 10^{-2.85}$$

$$HDDC_{aq} \rightleftharpoons H^+_{aq} + DDC^-_{aq} \quad \textbf{.........equation 1.5}$$

$$\text{with } Ks = [H^+]_{aq}[DDC^-]_{aq}/[HDDC]_{aq} = 10^{-3.36}$$

The ratio between DDC complexed metal in the MIBK phase and metal in the aqueous phase is calculated for 10 metals of interest with extraction constants (K_{ex})

presented in **Table 1.2**. Moreover, it shows the pH at which 1% of the metal is left in the aqueous phase (the ratio $[M(DDC)_2]_{org}/[M^{2+}]_{aq}=100$).

The data suggest that quantitative extraction may take place in rather acidic environment (pH<0.3) for the metals with highest K_{ex}. Such low pH is often encountered in acidic digest, even after proper dilution. Therefore, it is able to extract metals without addition of neutralising or buffering agents. This can be also benefit to minimise tedious work and contamination from neutralising or buffering agents. Moreover, co-extraction of interfering iron at low pH is minimised.

The acid form HDDC is unstable in water. This instability is not occurred in the continuous flow extraction system. The HDDC is quickly transferred to the organic phase as assumed from **Equation 1.4** ($(HDDC)_{org}/(HDDC)_{aq} \approx 500$) protecting the ligand against acid hydrolysis.

In this work, on-line solvent extraction using DDC reagent for sample pretreatment before FAAS detection is to be developed for some heavy metal ions (Cu(II), Pb(II), Ni(II), Cd(II) and Zn(II))

1.4.1.2 Cr(III) and Cr(VI) speciation

A brief summary of Cr(III) and Cr(VI) speciation is presented in **Table 1.3**. Speciation for Cr(III) and Cr(VI) by using on-line solvent extraction as a DDC complex with FAAS detection is to be investigated.

Table 1.2 Theoretical values for extraction equilibriums for metal-DDC's into MIBK [28-29]

Metals	**Extraction constant** K_{ex}	$[M(DDC)_2]_{org}/[M^{2+}]_{aq}$ **for pH=0.3**	**pH for** $[M(DDC)_2]_{org}/[M^{2+}]_{aq}$ **=100**
Mn	$3.8x10^5$	$<10^{-6}$	5.2
Fe	$31.6x10^1$	0.006	2.5
Co	$2.1x10^2$	0.08	1.8
Zn	$9.1x10^2$	0.36	1.5
Cd	$2.6x10^5$	103	0.2
Pb	$5.9x10^7$	23000	-0.9
Ag	$3.3x10^{11}$	$>10^6$	< -2
Ni	$3.8x10^{11}$	$>10^6$	< -2
Cu	$5.0x10^{13}$	$>10^6$	< -2
Hg	$8.7x10^{31}$	$>10^6$	< -2

Table 1.3 A brief summary for Cr(III)/Cr(VI) speciation procedures

Analytes	Techniques	Reagents/conditions	Analytical characteristics	Samples	Ref
Cr(III)/ Cr(VI)	ETAAS	-Solvent extraction -APDC-MIBK system -Specific condition for Cr(III)/Cr(VI) without the need to convert Cr(III) to Cr(VI)	DL=0.3 $\mu g\ l^{-1}$ for Cr(VI)	Natural and drinking water samples	31
Cr(III)/ total Cr	FI-FAAS	-Using mini-column with poly (aminophosphonic acid) chelating resin -0.5 M HCl for elution -Convert Cr(VI) to Cr(III) by ascorbic acid	DL= 0.2 $\mu g\ l^{-1}$, %RSD=1.2-5.9 for Cr(III) and 1.2-5.7 % for total Cr	Natural water; tap, mineral and river waters	60
Cr(III)/ Cr(VI)	FAAS	-Adsorption of Cr(VI) on melamine-formaldehyde resin -0.1 M NaAc solution for elution -Convert Cr(VI) to Cr(III) by hydrogen peroxide	DL=1.1 $\mu g\ l^{-1}$ for Cr(VI)	Lake water	61
Cr(III)/ total Cr	FI-FAAS	-Cr(VI) was measured by corlorimetry (with 1,5-diphenyl carbohydrazide) -Total Cr by FAAS	DL= 0.005 mg l^{-1} %RSD= <1%	Welding dust	62
Cr(III)/ Cr(VI)	SI-Spectro-photometry	-Wetting film extraction -Cr(VI)-1,5 diphenylcarbazide extracted into MIBK-octanol wetting film -Acetronitrile as eluent -Convert Cr(III) to Cr(VI) by Ce(IV) solution	LR= up to 100 $\mu g\ l^{-1}$ for Cr(VI) EF= 25 DL= 2.0 $\mu g\ l^{-1}$ f= 17 h^{-1} %RSD= 2.8 and 2.0 for 25 and 100 $\mu g\ l^{-1}$Cr(VI) ,respectively	Tap water, lake water and Seattle area sea water samples	63

Table 1.3 (Continued)

Analytes	Techniques	Reagents/conditions	Analytical characteristics	Samples	Ref
Cr(III)/ Cr(VI)	FI-Fluorimetry	-Fluorescent product from 2-(α-pyridyl) thioquinaldinamide and Cr(VI) -On-line oxidation of Cr(III) to Cr(VI) by metaperiodate	LR= 0.1-10 mg l^{-1} for Cr(VI) , 0.1-1.0 mg l^{-1} for Cr(III) %RSD= 0.1-2 f= 30 h^{-1}	Environmental water, food sample and synthetic mixture	64
Cr(III)/ Cr(VI)	FI-Spectrometry	-Cr(VI)-1,5-diphenylcarbazide complex, detected at 548 nm -On-line oxidation of Cr(III) to Cr(VI) by hydrogen peroxide	LR= mg l^{-1} range DL= 6 mg l^{-1} %RSD= <5	Waste water	65
Cr(III)/ Cr(VI)	FI-HPLC-FAAS	-Cr(VI)-APDC complex sorbed on inner surface of sorption loop -MIBK as eluent -Cr(III)-KH naphthalate bound on C18 column and elute with methanol	CE= 6 min^{-1}, DL= 0.92 μg l^{-1} for Cr(III) and 0.54 μg l^{-1} for Cr(VI) %RSD= 3.8, 2.9 for 50 μg l^{-1} Cr(III) and Cr(VI), respectively	Cigarette samples	66
Cr(III)/ Cr(VI)	FAAS	-Solvent extraction -Complex of Cr(VI) and HCl extract into MIBK -Convert Cr(III) to Cr(VI) by 0.005 M $KMnO_4$	DL= 4 μg kg^{-1} Cr(VI) and 13 μg kg^{-1} Cr(III)	Foods	67
Cr(III)/ Cr(VI)	SI-FAAS	-Retention of Cr(III)/ Cr(VI) on alumina mini- columns	DL= 42 μg l^{-1} for Cr(VI) and 81 μg l^{-1} for Cr(III) %RSD= <10	Sewage waters	68

(EF= enrichment factor, CE= concentration enrichment, DL= detection limit, LR= linear range and f= sample frequency)

1.4.2. Solid phase extraction (SPE)

The preconcentration and separation procedure based on solid phase extraction (SPE) has been used extensively for enhancing the selectivity and sensitivity of analytical method. SPE is a specific type of sample preparation in which an analyte, contained in a liquid phase, comes in contact with a solid phase (e.g. particles in a column) and is selectively adsorbed onto the surface of that solid phase. All other materials not adsorbed remain in the liquid phase and pass through the sorbent particle material. Generally, a wash solution is then passed through the sorbent to remove possible adsorbed contaminants from the sample matrix while the analyte of interest is still remained on the solid phase. Finally, an eluting solution is added to the sorbent which disrupts the attraction between analyte and solid phase, causing desorption from the sorbent. The analyte in eluting solution was then measured with specific detector.

Many research works involving on-line-SPE for heavy metal determination have been published recently [13-21]. The general merit of on-line-SPE is inherently easy to operate. On-line operation often dramatically decreases sample consumption and minimise the risk of contamination. The equipment is robust. An additional benefit is its extremely high versatility, owning to the availability of a broad range of choice for different sorbents, complexing system and eluents.

1.4.2.1 FI on-line preconcentration for lead determination

SPE procedures have been developed for the determination of lead [69-71]. The most commonly used procedure involves lead complexation with DDC, followed by retention on C-18 silica and Amberlite XAD-7 [70-72]. Various other sorbents, for instance, alumina [73] and cellulose [74] have been used. However, these extractants have the disadvantage of being nonselective, and retain more elements than only lead. The preconcentration of lead would be more efficient with a more selective resin.

Sr.spec^{TM} resin is a selective extraction chromatographic material produced by EIChroM Industries. Its structure is shown in **Figure 1.4**. Sr.spec^{TM} resin or crown ether (4,4$^{/}$,(5$^{/}$)-bis-(t-butyl-cyclohexano)-18 crown-6 sorbed on polymeric support such as amberlite (80-100 μm of grain diameter)), which provides selectivity by retaining only those cations which can fit within the macrocycle cavity [75] was used as packing material. It's based on complex formation. Pb(II) forms with crown ether as a chelate. Pb(II) ion has specific ionic radius fitting well with 18-crown-6 cavity. So Pb(II) can be retained on this resin very strong. Other ions having similar ionic radii such as Po and Pu which may not present in the sample of interest. So there should be no problem about interference effect.

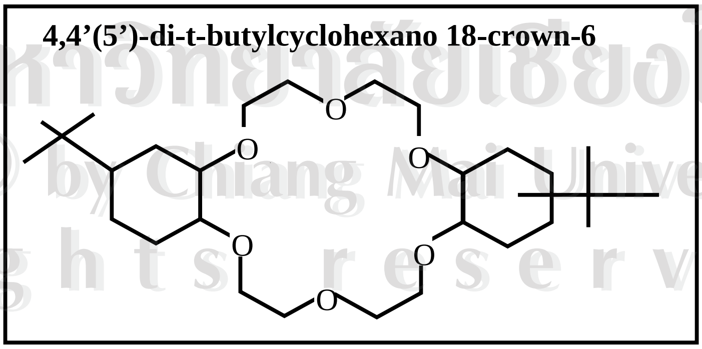

Figure 1.4 Chemical structure of the crown ether [75]

FI on-line preconcentration for lead determination by using Sr.spec™ has been reported [18]. In this work, the attempt to improve detection limit for such system has been made. The method was applied for Pb determination in water samples discharged from industrial estate.

1.5 Anodic stripping voltammetry (ASV) for arsenic speciation

1.5.1 Anodic stripping voltammetry (ASV)

Voltammetry is a group of electrochemical techniques where current is measured as a response to potential. These techniques have a broad range of application in modern chemistry. The voltammetric cell (as presented in **Figure 1.5**) consists of supporting electrolyte solution, a working electrode (WE), a reference electrode (RE) and an auxiliary electrode (AE). A current against voltage graph is called a ***voltammogram***.

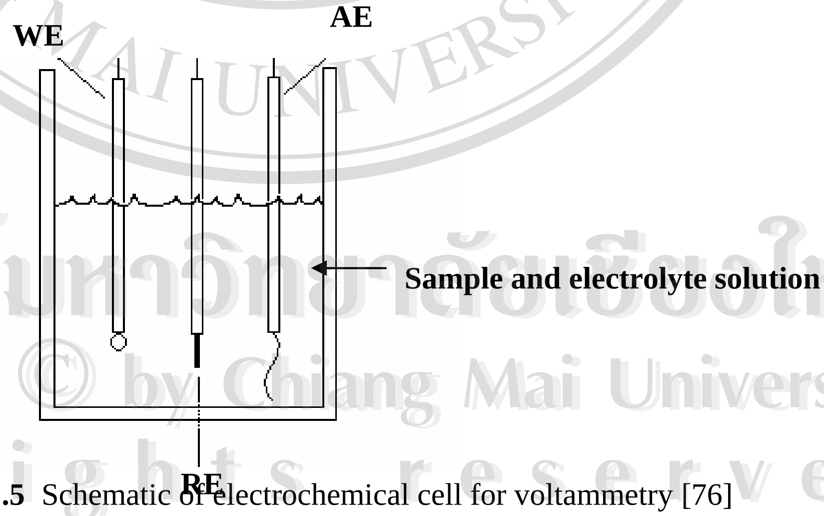

Figure 1.5 Schematic of electrochemical cell for voltammetry [76]

Stripping voltammetry is one of voltammetric technique. It is a very sensitive method for the trace analysis. There are 3 important steps in a stripping voltammetry: deposition, quiet time and stripping. During the deposition step, the potential applied to the working electrode is held at a value (deposition potential) at which the metal ions are reduced to metal for a time period (deposition time). After the deposition step, the stirring is stopped and the system is allowed to reach equilibrium. This is the quiet time, which is typically 10 - 15 s. During the stripping step, the applied potential is scanned in a positive direction and the metal in the working electrode is oxidized back to metal ions in the solution. This is called ***anodic stripping voltammetry***. If the potential of electrode is held to cause analyte species oxidise and accumulate at electrode surface before potential is scanned to more negative direction, this technique is called ***cathodic stripping voltammetry***. The potential at which the stripping occurs is related to the redox potential of the analyte and hence the potential of the current peak on the stripping step can be used to identify the analyte. The magnitude of the current of the stripping peak is proportional to the concentration of the analyte in the working electrode. Since the concentration of the analyte in the electrode is related to its concentration in solution, the stripping peak current is therefore proportional to the analyte concentration in solution.

A number of different waveforms have been used for the stripping step including linear sweep, differential pulse and square wave. The differential pulse and square wave are more commonly used. For differential pulse, the potential waveform is depicted in **Figure 1.6**. It consists of small pulses (of constant amplitude) superimposed upon a staircase waveform. The current is sampled twice in each pulse period (before and at the end of the pulse) and the difference between these two current values is recorded and displayed. The differential pulse has the advantages of sensitive detection limits and discrimination against background currents.

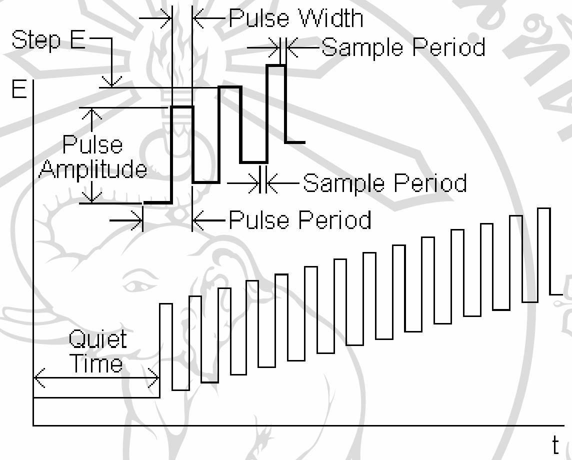

Figure 1.6 Differential pulse waveform [77]

1.5.2 Arsenic speciation

Arsenic is a naturally occurring element present in the environment in both organic and inorganic forms. Inorganic arsenic is considered to be the most toxic form of the element, and it is found in groundwater and surface water as well as in many foods. The presence of arsenic in natural water is of concern because of its toxicity and possible carcinogenic activity [78]. The toxicity of As(III) is much greater than As(V). Therefore, the speciation analysis of arsenic is more important than total arsenic determination.

There have been some reports on development of analytical method for arsenic determination (**Table 1.4**), for instance, hydride generation techniques in conjunction with atomic absorption spectrometry (AAS), high performance liquid chromatography (HPLC) and inductively coupled plasma-mass spectrometry (ICP-MS) [79-82]. But these involve relatively expensive and complicate instruments, so that cost effective and reliable method with enough sensitivity is still needed. Electrochemical methods can distinguish between the different oxidation states of arsenic and have a great sensitivity. The instrumentation required is relatively simple and low cost than those techniques. Arsenic determination have been made using classical dc polarography [83], differential pulse polarography [84], cathodic stripping voltammetry [85-87] and anodic stripping voltammetry [88-89]. On-line electrochemical techniques such as stripping voltammetry has been performed for arsenic determination [90].

Different electrode materials have been used for arsenic determination by stripping voltammetric techniques for example, hanging mercury drop electrode [85-86], co-deposition with copper on a rotating platinum electrode [90-91]. Gold is the most suitable electrode material for arsenic determination because of high hydrogen over voltage and better reversibility of the electrode reaction in both the plating and stripping steps [92]. However, the response of gold electrode is very strongly dependent on the past history, pretreatment and the oxide films formation. To overcome this problem renewable gold plating on glassy carbon electrode was used as working electrode.

In this work, it was focused on SI-system coupling with ASV for As(III) determination by using gold electrode as a working electrode. The SI system provides the benefit of automation. As(V) was reduced to As(III) by off-line method. Then As(V) can be measured as As(III).

Table 1.4 A brief review of arsenic determination

Analytes	Techniques	Reagents/conditions	Analytical characteristics	Ref
As(III)/As(V)	Uv-vis-Spectro-photometry	-Silver- diethyldithio-carbamate complex -λ_{anal}= 525 nm	DL= 2-4 μg As	93
As(III), As(V), methylarsonate and dimethylarsinate	HG-AAS	-Cryogenic trap -λ_{anal}= 193.7 nm	DL= 3-10 μg	94
Total As	HG-AAS ICP-AES	-Hydride reagent, sodium tetrahydroborate 3% (w/v) -λ_{anal}= 193.7 nm	DL= 0.017 μg g^{-1}	95
As(III), As(V), monomethyl-arsonic acid (MMA) and dimethylarsenic acid (DMA)	LC-HG-AAS	-Mobile phase, phosphate buffer pH 4.5, NaBH4 2.0%, -λ_{anal}=193.7 nm with band pass 0.7 nm	DL= 0.49, 0.44, 0.92 and 0.40 μg l^{-1} for As(III), As(V), MMA and DMA in serum, respectively	96
As(III), As(V) and PO_4^{3-}	FI-Spectro-photometry	-Molybdenum blue method	LR= 600-18,700 μg l^{-1} for As(III) and As(V), 1,900-4,750 μg l^{-1} for PO_4^{3-}	97
As(III)/As(V)	FI-ETAAS	-Reducing agent (As(V) to As(III) by 2 % KI) -λ_{anal}= 193.7 nm with slit width 0.7 nm	DL= 0.1 μg l^{-1}	98
As(III)/As(V)	FI-HG-AAS	-Reducing agent (As(V) to As(III) by 0.50% ascorbic acid and 1.0% KI in 4 M HCl) -λ_{anal}= 193.7 nm with slit width 0.7 nm	LR= 0.1-10.0 μg l^{-1} DL= 0.037 μg l^{-1} As(III) and 0.033 μg l^{-1} As(V)	99

Table 1.4 (Continued)

Analytes	**Techniques**	**Reagents/conditions**	**Analytical characteristics**	**Ref**
As(III)/As(V)	FI-ETAAS	-Sorption on mini- column -Complexing agent 0.1% (w/v) ammonium dithiophospate, sorbent C18 bonded silica gel -Reducing agent (As(V) to As(III) by ascorbic acid and KI) -λ_{anal}=193.7 nm with slit width 0.7 nm	LR=0.3-3.0 μg l^{-1} As	100
As(III)/As(V)	Cathodic-stripping voltammetry	-HDME as working electrode	DL= 0.2 μg l^{-1} As(III) %RSD= 6	101
Total As	Flow current stripping analysis	-Gold fiber electrode as working electrode - Iodide as reducing agent As(V) to As(III)	DL=0.15 μg l^{-1}	102
As(III)/As(V)	Constant current stripping analysis	-Gold-plated carbon paste electrode as working electrode -Cysteine as reducing agent As(V) to As(III)	DL=3 μg l^{-1} As(III), 0.5 μg l^{-1} As(V)	103
As(III)/As(V)	Anodic stripping voltammetry	-Gold-film electrode as working electrode -Sulphur dioxide as reducing agent As(V) to As(III)	DL= 0.19 μg l^{-1} at 4 min deposition time %RSD= 2-0.6	90
Total As	Differential pulse stripping voltammetry	-Gold-film electrode as working electrode -Wall jet cell	DL= 0.1 mg l^{-1} at 1 min deposition time	104

(DL= detection limit and LR= linear range)

1.6 Research aims

The main research is development of flow-based analysis systems with sample preconcentration and preseparation procedures for the determination of some heavy metals in environmental samples. The aims of this research are as following:

1.6.1. To develop an automated on-line solvent extraction devices coupled with FAAS for some heavy metal ions determination (Cu(II), Pb(II), Cd(II), Ni(II) and Zn(II)) and Cr (III)/Cr(VI) speciation

1.6.2. To investigate on-line preconcentration and preseparation by using FI and SI with a column packed with resins coupled with FAAS for lead and iron determination, respectively

1.6.3. To study preliminary SI-ASV for arsenic determination