1. INTRODUCTION

1.1 Overview of the Research

Trace heavy metals are of great environmental concern due to their serious toxicity although they are present at very low-level concentration. Heavy metals in natural water are mostly considered because they could be very easily migrated and/or transported further to the living things. The heavy metals in water appear in various forms, for examples dissolved metal ions, metals associated with organic colloids and metals sorbed on solids. Thus, reliable trace analysis methods and speciation of those forms of heavy metals are needed.

To speciate metals bound to colloidal phases, analysis techniques involving size-based element speciation appear appropriate. Field-flow fractionation (FFF) is known as an effective size-based speciation technique for the characterization of aquatic colloids (*1-9*). The FFF provides the information on particle size distribution. On-line combination of the FFF with other analytical method provides the information of metal ions attached to colloidal particles.

There are a number of analytical methods available for the determination of total concentrations of trace heavy metals. Some are for single element analysis, especially atomic absorption spectrometry (AAS). Some are multi-element analysis techniques such as inductively coupled plasma-atomic emission spectrometry (ICP-AES), inductively coupled plasma-mass spectrometry (ICP-MS), various types of electrochemical techniques including voltammetry, X-ray fluorescence (XRF), isotope dilution mass spectrometry, and neutron activation analysis (*10-12*). Among those analytical techniques, voltammetric method requires relatively low investment and running costs. Moreover, it has been used for simultaneous determination of trace metals in environmental samples due to its ability of multi-element analysis with high sensitivity, precision and accuracy (*10, 11, 13-17*).

Recently, flow-based analysis (FBA) methods such as flow injection analysis (FIA) and sequential injection analysis (SIA) have been combined with voltammetric methods for on-line determination of some trace heavy metals (*18-30*). The on-line FBA with voltammetric technique helps to reduce the consumption of reagent and sample, and thus minimizes waste production. The FBA combined on-line with voltammetry could also be used to speciate free metal ions, which are dissolved in natural water. To determine total concentration of metals in natural water samples, pretreatment method are necessary.

In this research work, the release of heavy metals from the coal mining disposal soil was of interest. There have been papers reporting that the leachates from mining areas contain high amount of heavy metals and may be very acidic (*31, 32*). The authors studied the leaching behavior of trace metals using speciation techniques such as leaching and sequential extraction methods (*33-35*). In Thailand, there have been a few studies involving the risk assessment of coal-mining disposal weathering (*36*). Development of on-line speciation analysis techniques of heavy metals, including size-based element speciation and determination of dissolved forms of trace metals, are believed to be useful for understanding the behavior of the released metals from mining area.

1.2 Speciation of Elements

Van Loon and Barefoot (1992) defined ‘speciation analysis of an element’ as *“the determination of the concentrations of the individual physico-chemical forms of the element in a sample”* (*12*). The individual physico-chemical forms of elements are gaseous, solid and dissolved forms, depending on the nature of the sample. However, there is no specific definition for ‘speciation’. Many meanings have been used by various researchers. Ure and Davidson (1995) defined broadly the term speciation in their book (*37*) as *“...Speciation may be defined as either (1) the process of identifying and quantifying the different, defined species, forms or phases present in a material, or (2) the description of the amounts and kinds of species, forms or phases are defined functionally, operationally, or as specific chemical compounds or oxidation states.”*. Functionally defined speciation includes the use of terms such as ‘biologically active’ or ‘mobile’ forms. Operationally defined speciation involves the physical or chemical fractionation process applied to the samples. An example of the operational speciation is ‘sequential extraction’, which is commonly used to separate metals associated with the water/acid soluble, exchangeable, reducible, oxidizable and residual fractions of sediments. Nevertheless, even the distinction between soluble and insoluble species in aquatic systems may by considered a form of operational speciation because it is based on the ability of a substance to pass through a 450-nm filter rather than on whether the species is actually in solution or not. The speciation of metal ions according to their oxidation states may be achieved by the analysis of water samples by separating individual species or distinguishing them by spectroscopy.

Recently, Evans (2003) (*38*) defined the term speciation analysis as "*Speciation analysis is a term used to describe the process whereby the analyte is characterized subject to a set of defined criteria, typically yielding information about: (i) the chemical form of the analyte that is functionally important (e.g. ligands on an organometallic species or oxidation state), (ii) the exact structural and chemical form of the analyte (e.g. NMR-derived structure), and (iii) the nature of the chemical species as determined from an operational point of view, dependent on the method of sample preparation or analysis used (e.g. metals extractable by acetic acid or other solvent).*" However, the International Union of Pure and Applied Chemistry (IUPAC) has defined speciation as "*the specific form of a chemical element defined according to its molecular complex, electronic or nuclear structure*" (*39*).

Therefore, speciation analysis can be considered to be the identification and quantification of the different chemical and physical forms of an element existing in a sample. The chemical form of an element will determine its toxicity, stability and transport, so this measurement is a very important factor in the wider definition of speciation. Modern speciation analysis often includes a range of analytical methodologies that are employed to explain the behavior of chemical compounds (*38*).

In natural water, there are colloidal phases and dissolved forms of heavy metals, which are toxic and can easily migrate from their sources. The speciation of trace metals in the natural water is important for the understanding of their behaviors such as their involvement in biological cycles. Species distribution studies have shown that trace element concentrations in soils and sediments vary with physical location, e.g. depth below the water surface, and with particle size (*37*). This work was interested in the colloidal particles, which may associate with trace metals. Therefore, the particle sizes of colloids are considered here. Speciation of a substance depending on its size was termed as 'size-based speciation' here, and the speciation of element distributed among different size ranges of particles was called 'size-based element speciation'. Size-based element speciation provides the element content of each size fraction by combining a size fractionation method with a trace element analysis procedure.

Speciation analysis techniques

There are a number of speciation procedures for identifying and quantifying the individual forms of heavy metals. The selection of the speciation technique depends on which individual form is of interest and what kind of sample is investigated. In this work, speciation of trace elements in contaminated water was of interest. Generally, there are dissolved metals and various kinds of particles, either organic or inorganic substances, in natural water. This work aims to study the size-based speciation of aquatic colloids and to investigate the interaction of dissolved forms metal ions with them.

Size characterization of aquatic colloids and the elements attached to them requires an appropriate method. **Table 1.1** shows some size-based element speciation methods, which have been recently used to speciate elements associated with different kinds of particles. Field-flow fractionation (FFF) is known as a powerful size-based separation technique for characterization of humic acid colloids *(1, 40-44)*. The FFF contributes the information of the size distribution of the colloidal particles.

Combining the FFF with elemental analysis methods such as ICP-MS will provide the elemental composition of colloid species.

For the determination of the individual element forms in the separated fractions, sensitive, selective, reliable, accurate and precise analytical methods are used. There are a number of analysis techniques such as AAS, AES, ICP-AES, ICP-MS, anodic stripping voltammetry (ASV) and polarography (*37*). However, the ASV method is of interest due to its high sensitivity, which allows low detection limits of elements, capability of multi-elemental analysis, and relatively low costs of investment and running.

Table 1.1 Some size-based element speciation techniques

No.	Analyte and type of sample	Size fractionation method – Element speciation method	Reference
1	Trace metals complexed to soil-, compost-, sediment-derived humic and fulvic acids	Flow field-flow fractionation – ICP-MS	(*1*)
2	Mo in colloidal particles in river water	Filtration and ultrafiltration – catalytic spectrophotometry	(*45*)
3	Pu in colloidal particles in groundwater	Cross-flow ultrafiltration – thermal ionization mass spectrometry	(*46*)
4	Se, As, Cu, Cd and Zn in tissue extracts	Size exclusion chromatography – ICP-MS	(*47*)
5	V(IV) and V(V) in colloidal particles in rain water	Filtration and ultrafiltration – catalytic fluorometry	(*48*)
6	Cu, Pb and Zn in particulates in treated industrial effluents and natural water	Ultrafiltration – anodic stripping voltammetry	(*49*)
7	Cd in plant tissues	Size exclusion chromatography – ICP-MS	(*50*)
8	Metallothionein-like proteins of the mussel Mytilus Edulis from coastal regions	Size exclusion chromatography – ICP-MS	(*51*)
9	Fe on SiO_2 particles	Gravitational field-flow fractionation – ICP-MS	(*52*)
10	Heavy metal binding non-protein thiols in Agropyron elongatum	Size exclusion HPLC – ICP-MS	(*53*)

1.3 Field-flow Fractionation

Field-flow fractionation (FFF) is a flow-based, size-separation technique. It is based on an injection of a small amount of sample suspension into a carrier stream, and the size-based separated sample is detected by a detector. FFF was first proposed by Giddings in 1966 (*54*). The instrument is similar to that of liquid chromatography, except the separation part. In chromatography, analytes are separated in a separation column by interaction of the components between a stationary phase and a mobile phase. In FFF, the separation takes place in a separation channel. The FFF channel is a thin ribbon-liked channel, whose thickness may vary from 50-250 μm (*55*). The channel contains no packing materials. The separation in the FFF channel needs no stationary phase but a type of force field is used instead. The force field is applied perpendicular to the flow stream of carrier. The applied field provides the separation in the FFF channel according to molecular weight or size of the individual components.

Generally, the FFF channel (**Figure 1.1**) is cut out of a thin plastic or metal spacer. The channel is sandwiched between two walls that delimit the applied force field. A flow stream of carrier is introduced at one end of the channel and exits at the opposite end. The flow profile in the channel exhibits parabolic shape (**Figure 1.2**). The highest flow velocity is observed in the middle of the thin channel. Suspension of a mixture species of interest is introduced into the channel at the upstream of the flowing carrier. The separation in the FFF channel is achieved through the partitioning into regions of different carrier velocities with in the flow channel under the influence of an applied field. The applied force field retains the particles more gently and with more precise control than in chromatography. Thus the FFF is useful for studying macromolecules and colloidal particles, since these species can interact adversely or irreversibly with active interfaces or may undergo shear degradation during their passage through packed chromatographic columns (*56*).

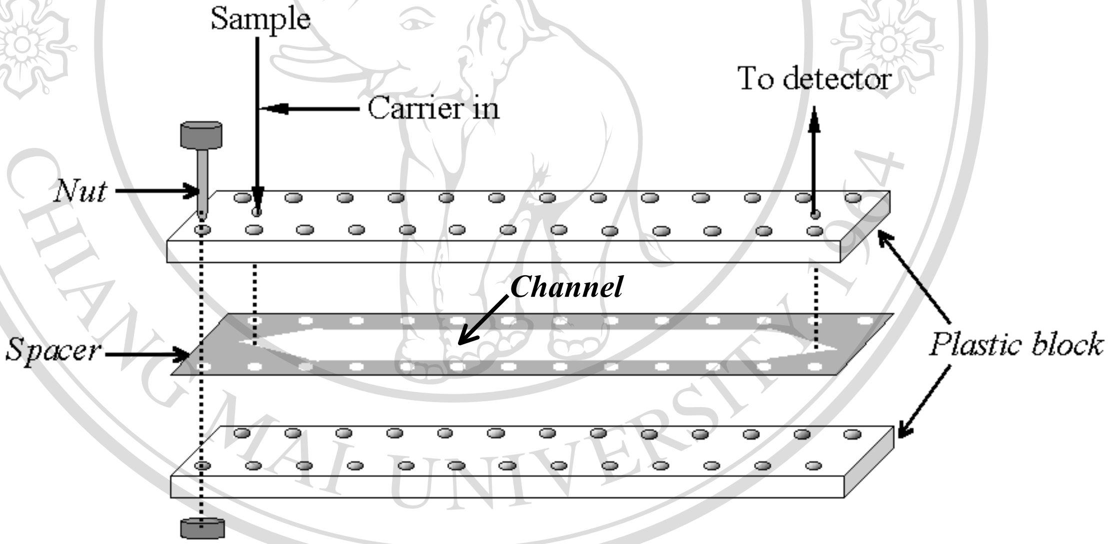

Figure 1.1 The field-flow fractionation (FFF) channel

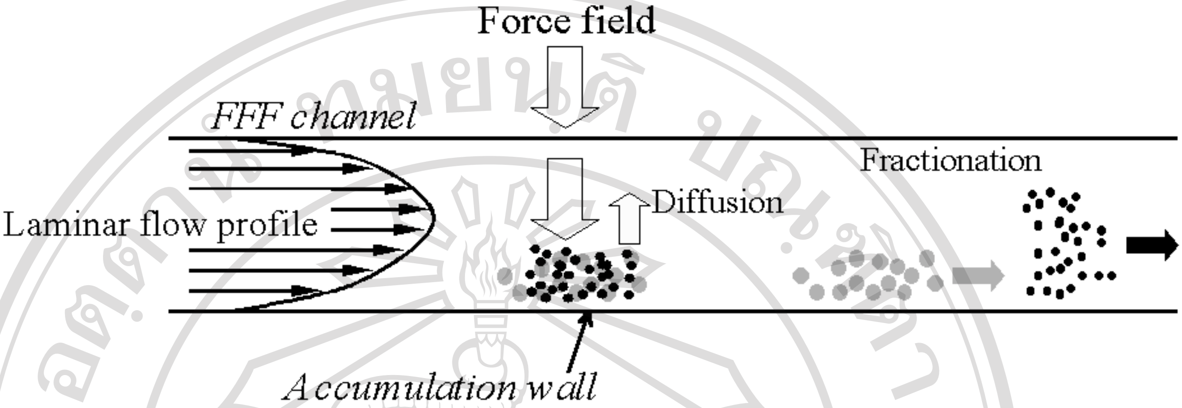

Figure 1.2 The separation in the FFF channel

The applied field in FFF can be sedimentation, gravitational, thermal, cross-flow, electrical, magnetic and dielectric fields, which provide sub-techniques of FFF as sedimentation FFF (SdFFF), gravitational FFF (GrFFF), thermal FFF (ThFFF), flow FFF (FlFFF), electrical FFF (ElFFF), magnetic FFF (MgFFF) and dielectric FFF (DlFFF), respectively. Those FFF sub-techniques are used for different purposes. In this thesis, the asymmetric FlFFF was used to characterize colloidal particles, defined as the particle size range of smaller than 100 nm (*57*).

Asymmetric flow field-flow fractionation

In asymmetric FlFFF (asym. FlFFF or AF^4), a cross-flow stream is used as the force field. This cross-flow is applied through a permeable channel wall. This type of the force field is the most universal technique since it does not require specific properties of the analyte (e.g. charge or magnetic properties). Particles from macromolecules, with molecular weight as low as 1000 g/mol, to the materials as large as 100 μm, can be separated by AF^4 (*58*).

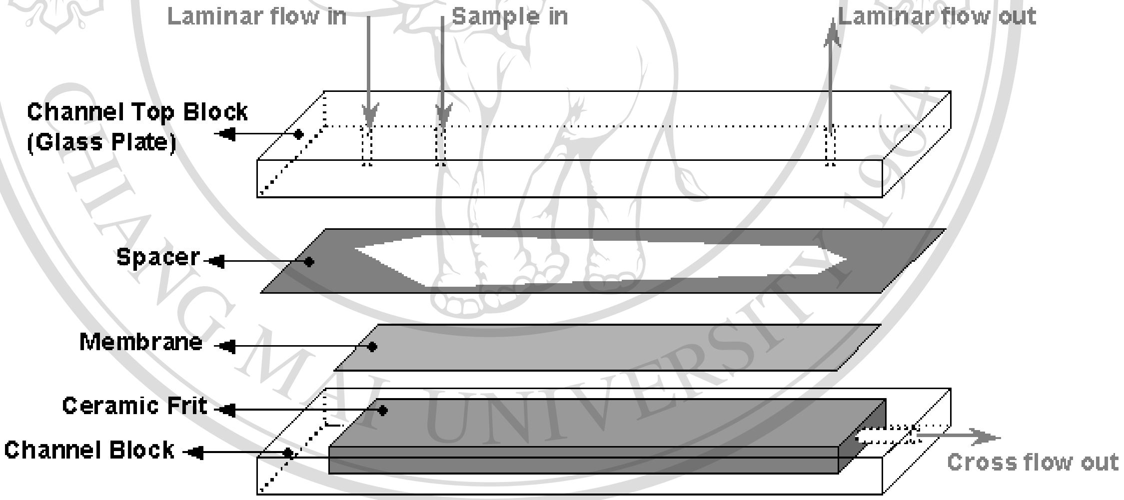

Figure 1.3 Asymmetric flow field-flow fractionation (AF^4) channel setup

An asym. FlFFF channel (**Figure 1.3**) consists of one wall of a glass plate or plexiglass and another wall represented by a frit that supports the accumulation wall membrane. Between the two blocks, a spacer and a membrane are sandwiched. A carrier stream is introduced at one end of the channel, and exits at the other end. The cross-flow stream (the force field) is collected at one end of the ceramic frit. The AF^4 system needs two pumps; one for the channel or carrier flow and the other for sample introduction. Since the cross-flow is created spontaneously in the channel by the pressure drop across the channel, thus there is no need to have a pump for creating the cross-flow. Adjustment the flow rates of the cross-flow and channel flow can be made by regulating the cross-flow and channel flow outlet, respectively. Three- and four-way switching valves are used to select the flow stream for injection, focusing or relaxing, elution and washing.

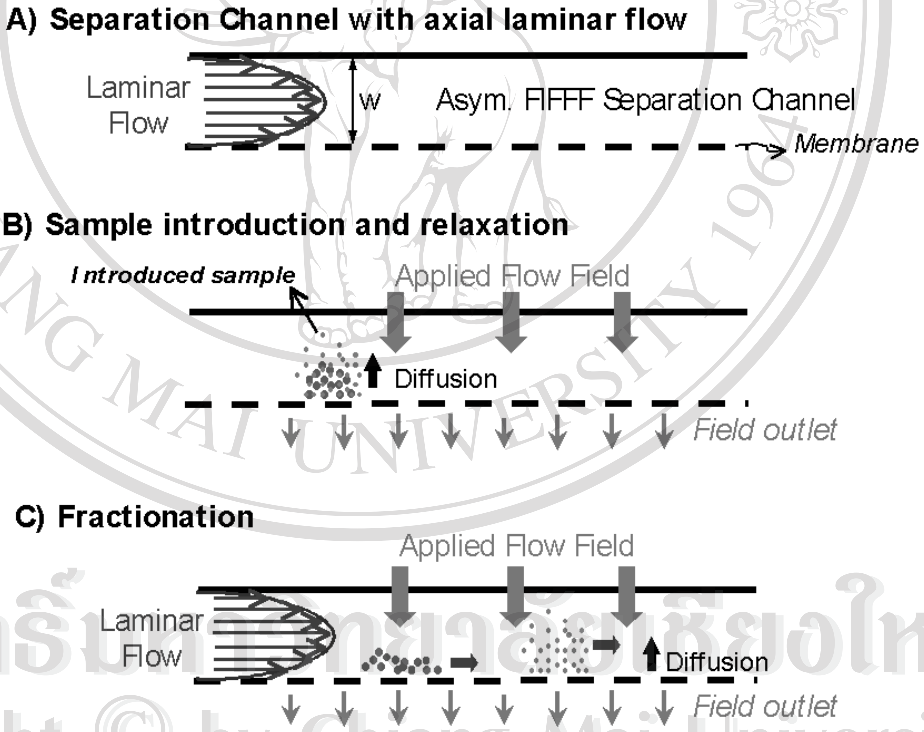

Figure 1.4 Separation in the asymmetric FlFFF channel, w: channel width (thickness)

Separation in the AF^4 channel is illustrated in **Figure 1.4**. Firstly, a sample is injected into the channel (**Figure 1.4B)**). Then the sample is focused by a cross-flow stream (the channel axial flow is stopped.). At this stage, the particles are responding to the cross-flow depending on their diffusion coefficient (D). The relation of the diffusion coefficient and the hydrodynamic diameter (d) of the macromolecule can be defined as,

$$D = \frac{kT}{3\pi\eta d} \qquad \textit{Equation (1.1)}$$

where k is Boltzmann's constant, T is temperature (K), and η is the carrier liquid viscosity (*58*). Therefore, the separation of macromolecules and colloids occurs by the different size of the macromolecules. The smaller the particle size, the higher the diffusion of the macromolecules. It means that the smaller particles diffuse closer towards the middle layer of the laminar axial flow in the channel than the larger ones. When the laminar flow is re-started (elution or fractionation step, **Figure 1.4C)**), the smaller particles are transported forward with the high flow rate of the axial flow in the middle layer of the thin channel. Thus, the smaller macromolecules are eluted to the detector before the larger ones. In the elution step, the cross-flow is applied along the channel. Then, in the washing step, the non-eluted fraction is washed out from the channel by the axial flow without cross-flow stream. UV detector is usually used as the detector for particle mass in the AF^4 system. The signal obtained from FFF is termed as fractogram, which is a plot of measured signal, against elution time or elution volume. The elution volume (V_R) is related to the diffusion coefficient, represented by the Stokes equation, and thus is related to the hydrodynamic diameter (d) of the particle. The simplified equation is illustrated:

$$\left(V_R - \frac{V_o}{3}\right) = \frac{\upsilon_c w^2}{6D} = \frac{\pi\eta w^2 \upsilon_c}{kT} \cdot \frac{d}{2} \qquad \textit{Equation (1.2)}$$

where V_o is the void volume of the channel, v_c is the volumetric cross-flow rate, w is the channel width or thickness (*42*). As illustrated in the equation above, the AF^4 is capable to separate particles based on their sizes.

The AF^4 has been coupled with many detection systems such as multiangle laser light scattering (MALLS) detector (*59, 60*), thermal lens spectroscopy (*61*) and total-reflexion X-ray fluorescence analysis (TXRF) (*62*) and thus it is possible to gain information beyond the pure size distribution. Inductively coupled plasma-mass spectrometry (ICP-MS) is known as a technique for multi-elemental analysis with high sensitivity. Hence the AF^4 provides the information of the particle size distribution and the ICP-MS contributes element concentrations. This leads to some information of metal speciation. Combination of the AF^4 and the ICP-MS is a promising on-line technique for size-based element speciation.

1.4 Anodic Stripping Voltammetry (ASV)

Voltammetry is an electrochemical method, where a current is measured while voltage is applied to an electrochemical cell containing (1) electroactive analyte(s), (2) a supporting electrolyte solution, (3) a reference electrode (RE), (4) an indicator or working electrode (WE) and (5) an auxiliary electrode (AE) (**Figure 1.5**). A current vs. voltage curve is a so called *voltammogram*.

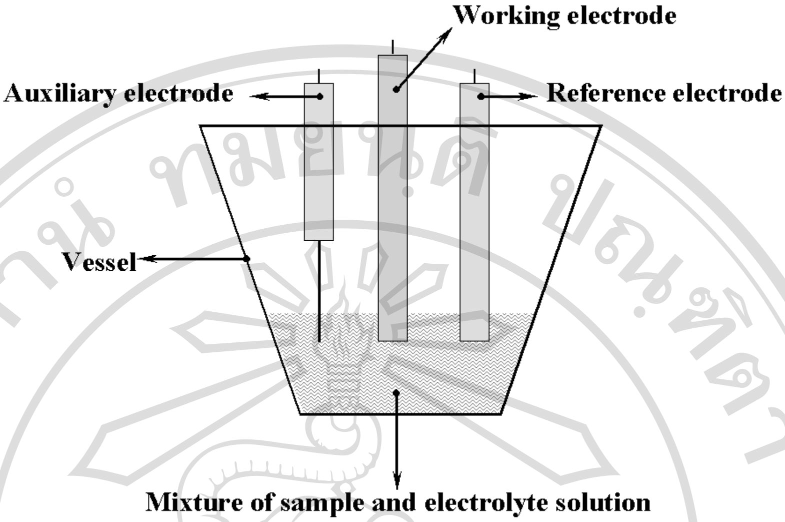

Figure 1.5 Diagram of an electrochemical cell for a voltammetric system

The diffusion current (i_d) produced by the desired reaction is proportional to the concentration of analyte (C_A) under ideal conditions,

$$i_d = k \cdot C_A \qquad \text{Equation (1.3)}$$

where k is a constant (*14*). Thus, the plot of i_d versus concentration can be used for calibration.

Stripping voltammetry (SV) is one of various types of voltammetric method. The SV technique has been widely used for determination of trace metal ions because there is an in-situ preconcentration step included. In principle, the SV has 2 steps of operation (**Figure 1.6**). The first step is a preconcentration or deposition step. In the first step, cationic metal ions are accumulated by reduction at the surface of the WE. The period of preconcentration is the so called deposition time, t_d. In the SV technique, hanging mercury drop electrode (HMDE) is widely used as the WE because mercury is chemically inert in most aqueous solutions, and hydrogen is generated by electrolysis only at quite negative potentials (*14*). This provides the possibility to reduce many electrochemically active species at the mercury electrode. Moreover, the HMDE always provides a new WE surface, which help to reduce the interference from the previous measurement. In the first step of the stripping voltammetric method using HMDE, metal ions are preconcentrated within the mercury electrode as an amalgam. The second step is the stripping step. By the second step, the accumulated metal ions are oxidized and stripped out off the mercury electrode by a potential sweep (*Scan E*) into the anodic direction. The current is then measured during the stripping step. Since the current is measured while the potential is scanned into the anodic direction, this SV method for cation determination is thus called *anodic stripping voltammetry (ASV)*.

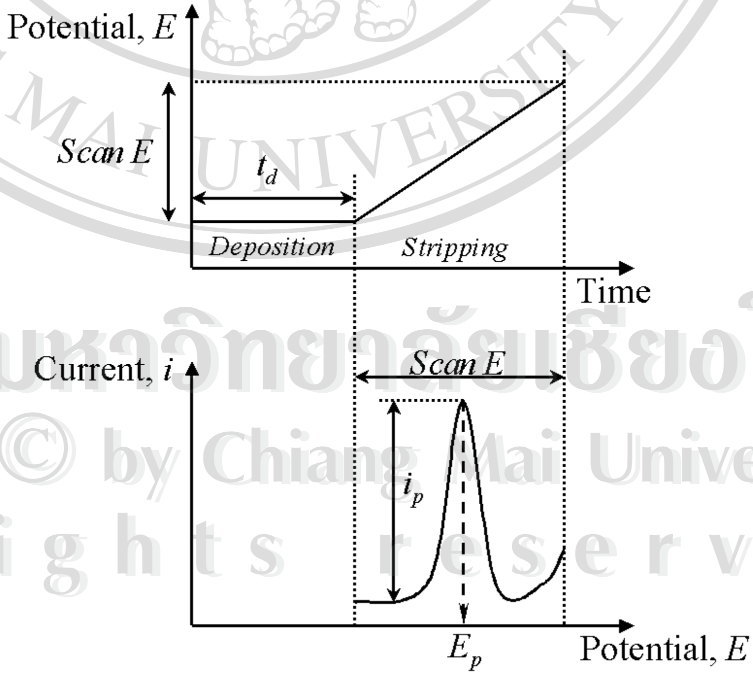

Figure 1.6 Operating steps in stripping voltammetric method

Since the ASV can in-situ preconcentrate trace metal ions, the detection limit of this technique is very low, down to 10^{-12} mol/l (*10, 14*). It can be used for the analysis of various metal ions because of the wide redox working range of the mercury electrode.

Square wave voltammetry (SWV) was first described by Barker (*63*) and then was applied and further developed use in a number of related areas by Osteryoung (*64*). The SWV waveform is described as a square wave modulation imposed on a stair case waveform (**Figure 1.7**) (*64*). Since the potential sweep is discontinuous rather than linear, the tops of the pulses remain flat, even at rapid sweep rates. Because the double layer charging current is proportional to $e^{-t/RC}$, where t is time, R is the solution resistance and C is the double layer capacitance, and the Faradaic current is approximately proportional to $t^{-1/2}$, the charging current decays much more rapidly than the Faradaic current (**Figure 1.8**) (*65*). This phenomenon allows measurements to be made at a time when the charging current can be considered negligible. Therefore, the current is measured at the end of the flat part of the pulse where the charging current is considered negligible. The final current (or net current) is the differential sum of the current measured at the end of forward pulse (i_f) and that of reverse pulse (i_r). This provides the advantage of excellent sensitivity and rejection of background currents.

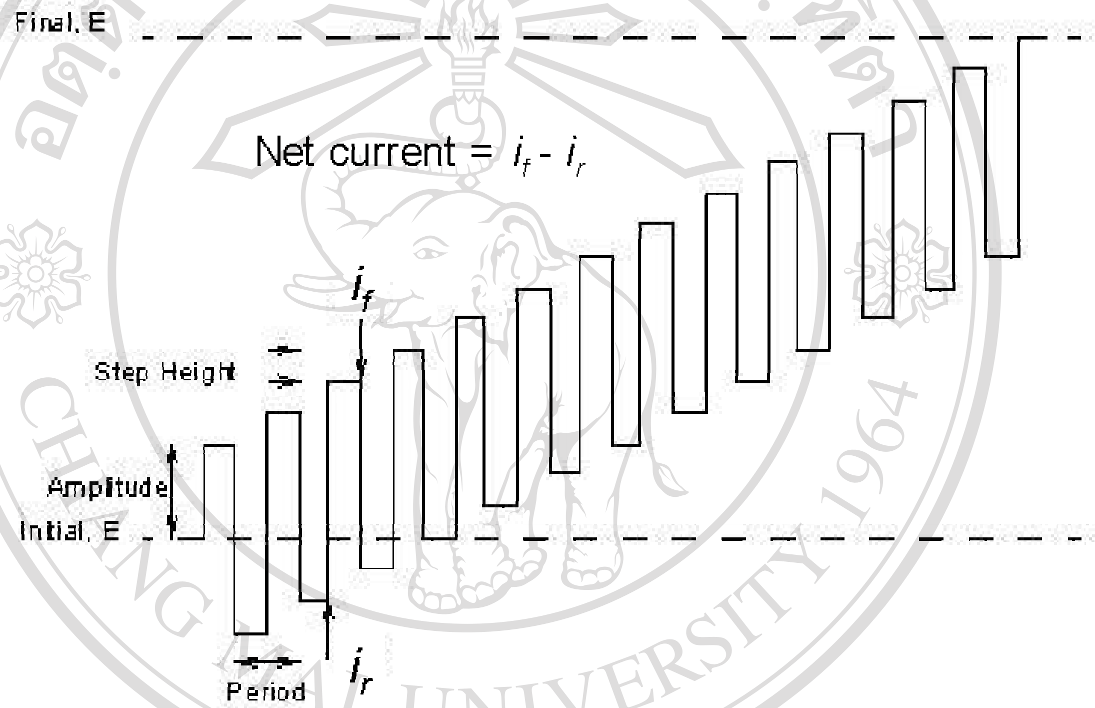

Figure 1.7 Square wave waveform

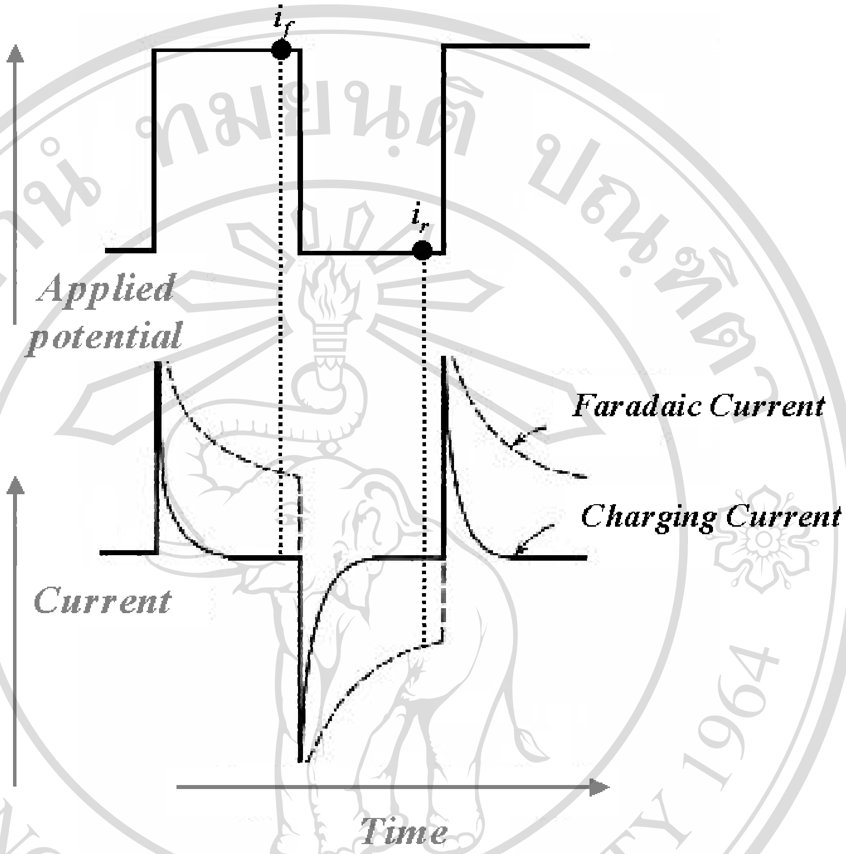

Figure 1.8 Measuring current in SWV

Combining the advantage of low detection limits of the anodic stripping voltammetry and that of low, or considered negligible, background currents of square wave voltammetry is very promising. Square wave anodic stripping voltammetry (SWASV) has been widely used to determine ultra-trace metal ions in seawater and marine samples (*66-76*). However, using batch ASV consumes high amounts of reagents. In this work, the development of flow-based analysis (FBA) techniques with

SWASV, as the detection system, for the on-line determination of some trace metal ions was carried out.

1.5 Flow-based Analysis

Flow-based analysis (FBA) refers to any on-line techniques involving a flow of fluid stream with either chromatographic or non-chromatographic process. In this work, FBA includes flow injection analysis (FIA), sequential injection analysis (SIA) and some hyphenated techniques.

1.5.1 Flow injection analysis (FIA)

Flow injection analysis (FIA) was introduced by Ruzicka and Hansen in 1975 (*77*). They defined FIA as "*A method based on injection of a liquid sample into a moving unsegmented continuous stream of a suitable liquid. The injected sample forms a zone, which is then transported toward a detector that continuously records the signal, as it continuously changes as a result of the passage of sample material through the flow cell.*" (*78*). After that there has been many definitions of the FIA from other researchers. However, the main point is that FIA is an analytical method involving injection of a sample into a flowing stream of reagent and the signal is continuously monitored. The main components of the FIA system are a solution delivery device, an injection valve, a mixing reactor and a detector (**Figure 1.9**).

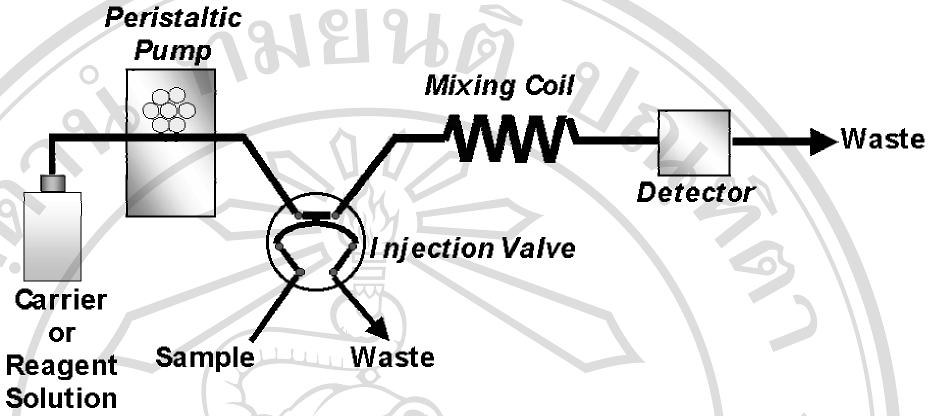

Figure 1.9 Diagram of a simple FIA

The most important physical phenomenon in FIA is the dispersion of the injected zone into the continuous stream or the carrier stream. The dispersion is composed of molecular diffusion and convection (*78*). However, convection is more dominant than diffusion due to the differences in the flow velocity of fluid elements located at the different points along the radial axis of a tubing. The mixing through convection can be made so reproducible that the longitudinal distribution profiles of analyte or reagent concentration in the coil may be precisely overlapped. That makes the dispersion of the injected sample reproducible and controllable. Thus, in FIA, either physical or chemical equilibrium is unnecessary for a reproducible signal.

The controllable dispersion in FIA system can be observed in a quantitative way by determining the dispersion coefficient (*D*) as proposed by Ruzicka and Hansen (*77*). The dispersion coefficient is a concentration ratio of an analyte concentration before and after dispersion has taken place, expressed by

$$D = \frac{C^o}{C} \qquad \text{Equation (1.4)}$$

where C^o is the original concentration of the analyte in the sample and C is the concentration of the dispersed solution zone (see **Figure 1.10**).

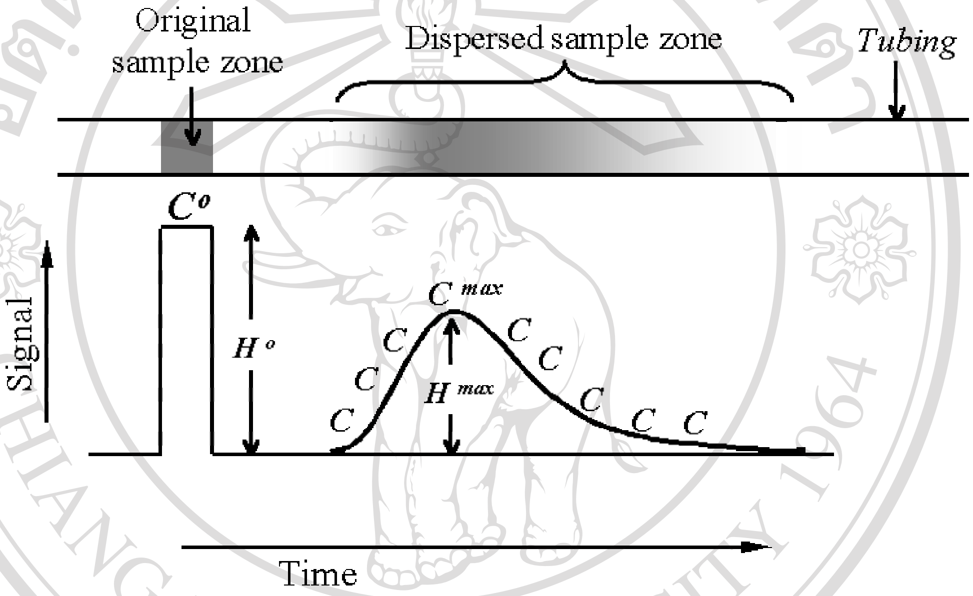

Figure 1.10 Dispersion in FIA

At the maximum of the analyte concentration distribution in the flow, the dispersion coefficient can be expressed as

$$D = \frac{C^o}{C^{\max}} \qquad \text{Equation (1.5)}$$

where C^{max} is the concentration of the injected solution at peak maximum of the dispersed zone. Since the peak height is proportional to the concentration of the injected sample, the dispersion coefficient can be defined as,

$$D = \frac{H^{o}}{H^{\max}} \qquad \textit{Equation (1.6)}$$

where H^{o} is the analytical signal peak height obtained at the original concentration of the analyte in the sample solution, and H^{max} is the maximum peak height of the dispersed zone in the injected solution.

The factors influencing the dispersion in the FIA system are sample volume, carrier or reagent flow rate, geometrical dimensions and configurations of manifold components, viscosity of the liquids and temperature. The last two factors may be negligible under normal conditions due to their limited effects.

FIA has become popular since it was first introduction by Ruzicka and Hansen (*79*) due to its rapidness, precise and versatility. Many detection systems including anodic stripping voltammetry (ASV) have been used with FIA for trace metal determination, as shown in **Table 1.2**.

Table 1.2 Some examples of flow-based analysis (FBA) with voltammetric detection for trace metal determination

No.	Analyte	Sample	Method	Reference
1	Cr, Ni, Zn, Cd, Pb, Cu	Wastewater stream	On-line system with DPASV	(*21*)
2	Zn, Cd, Pb, Cu	Fresh water	In-flow SWASV	(*23*)
3	Cd, Pb	-	FIA-multiple differential pulse voltammetry (DPV)	(*24*)
4	Pb	Blood	FIA-ASV using a wall-jet with mercury film electrode (MFE)	(*25*)
5	Cu, Pb, Cd, Zn	River sediment extracts	SIA-ASV with MFE	(*27*)
6	Cd, Pb, Cu, Zn	-	FIA-DPASV using glassy carbon electrode	(*28*)
7	Zn, Cd, Pb, Cu	Drinking water	FIA-ASV and SIA-ASV using on-line preplated MFE	(*29*)
8	Zn, Cd, Pb, Cu	Drinking water and waste water	FIA-SWASV using a cross-flow cell with MFE	(*30*)

Table 1.2 (Continued)

No.	Analyte	Sample	Method	Reference
9	Zn, Cd, Pb, Cu	Natural water	FIA-SWASV with gel-integrated Hg-plated-Ir-microelectrode array (GIME)	(*67*)
10	Cu, Pb, Cd, Zn	Rainwater, sea water	FIA-DPASV with mercury microelectrode	(*80*)
11	Cd, Pb	Lake water	FIA-ASV using MFE	(*81*)
12	Zn, Cd, Pb, Ni	Soil extracts	FIA-SWASV and FIA-SWCSV (square wave-cathodic stripping voltammetry)	(*82*)
13	U(VI)	-	FIA-adsorptive stripping voltammetry using 8-hydroxyquinoline as a complexing agent	(*83*)
14	Cr(VI)	-	On-line system with Adsorptive CSV detection	(*84*)
15	V(V)	-	On-line adsorptive stripping voltammetry using MFE	(*85*)
16	Thallium	Tap water, river water	FIA-DPASV using a wall-jet with mercury film electrode (MFE)	(*86*)

Table 1.2 (Continued)

No.	Analyte	Sample	Method	Reference
17	Cd, Pb, Cu	Tears	Capillary flow injection-ASV using a mercury-coated platinum microdisc microelectrode	(*87*)
18	Cd, Pb	-	Micro flow system with fast-scan ASV	(*88*)

1.5.2 Sequential injection analysis (SIA)

Sequential injection analysis (SIA), first developed and published by Ruzicka and Marshall in 1990 (*89*), is a second generation of FIA, which is a computer-controlled, single-line injection technique. The principle of SIA is based on sequentially introducing fluids into a carrier stream. This technique simplifies the FI manifold and is more robust for operation. The injected zones are in microliter level so that the sample or reagents consumption, and thus waste production, are reduced.

The common instrumental components of SIA are a reversible pump, a multiposition selection valve (SV), a holding coil (HC), a reaction coil (RC) and a detector (D) (**Figure 1.11**). The reversible pump and the selection valve are controlled by a computer program. The pump has to have 2 directions of pumping: forward and reverse to dispense and aspirate solution zones, respectively. The most widely used pumping unit in SIA system is syringe pump or piston pump (*11*) because it can precisely aspirate solution in microliter volumes. A multiport selection valve is used for sample introduction, comparable to an injection valve in FIA system. The different ports of the selection valve are connected to sample, reagents, standard solutions, waste and detector. A holding coil is put between the pump and valve to prevent the aspirated solution from entering the pump.

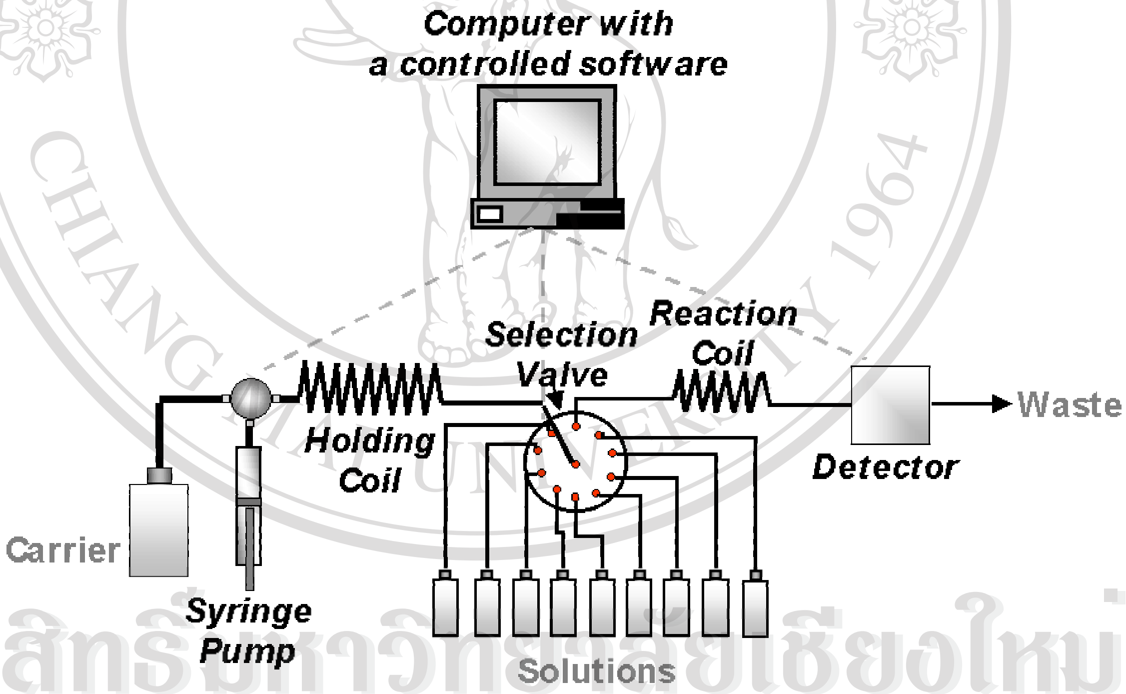

Figure 1.11 A simple SIA system

To operate the system, first, the pump is filled with carrier. Then the carrier is dispensed through the tubings from the pump to the detector until the waste. Before beginning the analysis sequence, the sample, reagent and standard tubings at the selection valve are filled with those solutions. After that, the selection valve is switched to the sample, reagent and standard solutions sequentially to be aspirated into the holding coil. The sequence of aspiration of those solutions depends on the desired reaction or product. Finally, the selection valve is switched to the detector, and the pump propels the product zone through the reaction coil and detector.

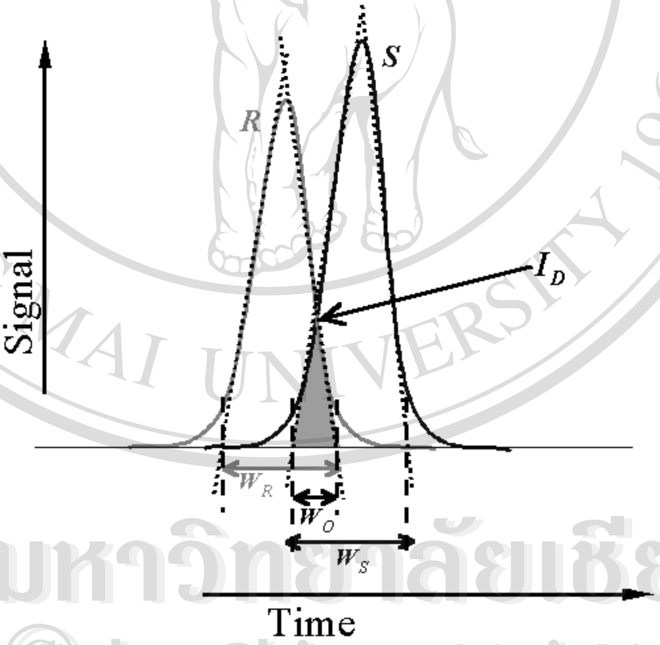

Figure 1.12 Zone penetration in SIA system

The important parameter in SIA is the degree of penetration or overlap of the aspirated zones (see **Figure 1.12**). The factors influencing the zone penetration are the aspirated volume and geometrical configurations of the system. The zone penetration, *p*, can be defined as (*11*),

$$p = \frac{2w_o}{w_s + w_r} \qquad \textit{Equation (1.7)}$$

Where w_o is base line width of the zone overlap, w_s and w_r are the baseline widths of the sample and reagent, respectively, and I_D is the isodispersion point. Normally, there is partial overlap, which the degree of penetration is lying between 0 and unity. In SIA system, a dispersion coefficient of at least 2 is needed to get a high enough degree of penetration.

The advantages of the SIA are reduced reagent and sample consumption, easy and fast operation, and capability of automation. Moreover, the instrumental units of the SIA are more versatile than the FIA system. Many detection systems have been used with SIA system for trace metal determination, summarized in **Table 1.1**. Ivaska et al. (*26*) reported that SIA could be used with ASV to enhance the sensitivity and selectivity of the method. Combination of SIA and ASV would thus be a powerful system for simultaneous determination of some trace metal ions.

1.6 Leaching of Metals from Coal-mining Disposal Soil

Size-based speciation especially in an on-line way can provide useful information on metal ion behavior in natural water. In this work, on-line size-based speciation was used for studying an environmental issue in Thailand.

In Thailand, approximately 20% of the total electricity is generated by coal burning (*90*). Most of the coal mines are the open-pit mine, where surface soil is removed to access to the coal. The overburden surface soil is disposed around the mining area. It is known that the soil in mining area contains various kinds of minerals and thus high amounts of heavy metals (*31, 91, 92*). The heavy metals in the disposal soil can be released to the environment by rainwater and surface water leaching, or so called weathering process. There have been many research studies in many countries related to the release of metals from coal mine spoils, but only a few in Thailand. Those reports concerned the weathering process of the disposal soils using leaching experiment and sequential extraction (*33-35, 92*). They mostly concentrated on how the metals are leached out. However, the leached heavy metals can migrate further to the environment by surface water. The metals can be associated with organic colloids in surface water and form colloids (*93-95*). It is considered important to understand the interaction of the leached metal ions and colloids in surface water. This research is the first study on the interaction of the metal ions leached from Thai coal-mining disposal soil and organic colloids, i.e. humic acid colloids, in surface water using on-line speciation analysis. The formation of the colloid borne metal species may lead to understand the release of heavy metals from the coal-mining area to natural water. Knowledge on such migration of the metal species to the environment is important for environmental management.

1.7 Research Aims

In this thesis, development of on-line analysis of some heavy metals involving speciation was attempted. This involved:

(1) on-line size-based element speciation using asymmetric flow field-flow fractionation with inductively coupled plasma-mass spectrometry (AF^4-ICP-MS) for the investigation of the interaction of metal ions leached from the Thai coal-mining overburden with organic colloids.

(2) development of on-line analyses with anodic stripping voltammetry (ASV) of some dissolved elements by employing

  (2.1) flow injection analysis (FIA)

  (2.2) sequential injection analysis (SIA)

  (2.3) performance studies on the developed speciation techniques tested by using model samples that contained less or none of interference.

From the above investigation, insight into the behavior of physico-chemical forms of the released elements from a mining disposal area in Thailand is explored. The combination of the ASV with FBA is tested as an appropriate tool for the routine analysis of trace heavy metals. Such a combination provides high sensitivity, precision and accuracy at a comparatively low consumption of chemicals and a high cost-effectiveness.

1.8 The Relevance of the Research Work to Thailand

This research attempts to investigate the release of heavy metals in a Thai coal-mining disposal soil to the environment, especially to natural water. Since the mining disposal soil contains high amounts of some heavy metals, which are seriously toxic even they are present in trace amount, it is important to understand their releasing behavior to the environment. This work investigated the leaching of some metals and the colloid formation of the leached metals by using an on-line size-based element speciation method; asymmetric flow field-flow fractionation-inductively coupled plasma-mass spectrometry (AF^4). This on-line technique provides the useful information of metal contents associated with colloidal particles that are present in surface water. In Thailand, this work was the first study about the formation of metal ion-organic colloids by adopting basic science research.

However, there are not only metal ions in the colloidal phases present in natural water but also in dissolved forms. Thus, the development of cost-effective flow injection analysis (FIA) and sequential injection analysis (SIA) systems with anodic stripping voltammetry (ASV) for metal speciation was made. The developed systems provided the reduction of reagent consumption and therefore reduced the production of waste. The proposed cost-effective systems would be economic for developing countries such as Thailand and elsewhere.