CHAPTER I

INTRODUCTION

1.1 Aluminium [1-2]

1.1.1 Occurrence of aluminium

Aluminium is the third most abundant metallic element on earth, comprising some 8.1 % of the Earth's crust. It is light weight metal and passivated aluminium is remarkable for its ability to resist corrosion. These characteristic make it widely used for food and drink packing. Aluminium salts are widely used in water treatment as coagulants to reduce organic matter, colour, turbidity and microorganism levels. Such use may lead to increased concentrations of aluminium in finished water. Where residual concentrations are high, undesirable colour and turbidity may ensue.

1.1.2 Toxicology of Aluminium [3-6]

Aluminium toxicity is well recognised as an important factor in many clinical disorders. It is a known neurotoxicant which contributes to dialysis encephalopathy syndrome and may cause Alzheimer's disease and other neurodegenerative diseases.[4] It is also associated with the mobilisation of bone phosphate, and gives rise to toxicity in the hemapoietic systems in humans.[4] Most of the understanding of aluminium toxicity in humans was established as a result of disorders experienced by dialysis patients when the concentration of aluminium in the dialysis fluid exceeded 0.5 mmol L^{-1} [5]. In such patients, aluminium accumulated in various tissues, including kidney, liver, bone, and heart,[4] giving rise to pathological conditions such as dialysis encephalopathy which leads to dementia and death. Aluminium is naturally

present in the human body at approximately 35 mg. The main route of aluminium input to humans is through the food chain, water, medicines, and the use of aluminium cooking utensils [6]. On the other hand, despite aluminium being present at low levels in natural waters, significant amounts are added to water supplies as a flocculating and coagulating agent in the purification of water and, in many cases, increasing the level of aluminium in potable water. The WHO guideline for the permissible level of aluminium in drinking water is only 0.2 mg L^{-1}

The major source of aluminium shown in the following list may contribute to total human exposure to the element.

1. Drinking water
2. Residue in food
3. Cooking utensils
4. Food and beverage packaging
5. Antacid indigestion remedies
6. Antiperspirant formulations
7. Cosmetics

1.1.3 Determination of Aluminium

Determination of aluminium is generally performed using atomic spectrometry. Such techniques are very selective, but they do not provide sufficiently low determination ranges. Moreover, they can be very time consuming (especially electrothermal AAS) and expensive in terms of both purchase and operating costs. In addition, they do not allow real-time or even on-site determinations. In order to obtain better detection limits, a pre-concentration or solid-phase extraction step is often required prior to the analysis. Molecular fluorescence spectrometry is an important analytical technique for quantitative determination of trace and ultra-trace inorganic substances since it is inherently more sensitive than many other molecular spectroscopic methods. Electrothermal atomic absorption spectrometry is the most widely used method and can produce reliable results, provided the matrix effects on standardization are recognized and corrected. A brief review of aluminum determination is shown in Table 1.1.

Table 1.1 A brief review of the methods for the determination of aluminium

Method	Preconcentration	Sample	LOD ($\mu g\ L^{-1}$)	Reference
GF-AAS	Cloud point extraction (CPE) (1-phenyl-3-methyl-4-benzoyl-5-pyrozolone, (PMBP))	Biological and lake water	0.09	7
ET-AAS	Continuous flow extraction (CFE) (8-HQ)	Dialysis concentrate	0.3	8
ETV-ICP-MS	Capillary microextraction (CME) (N-(2-aminoethyl)-3 – amino propyltrimethoxysilane-silica monolithic)	Rain water and fruit juice	1.6	9
ICP-MS	Ion exchange (Chromothrope 2B immobilized on AG 1-X8 resin)	Tap water and dialysis solution	0.1	10
HPLC-UV	Super critical fluid extraction (Chromazural S immobilized on Amberlite IRA-400)	Beverages and tap water	-	11
HPLC Fluorometry	Solid phase extraction (8-HQ) (LiChrosorb RP-18)	Seawater	-	12
Fluorometry	Cloud point extraction (CPE) 8-HQS	Foodstuffs and water samples	0.79	13

Spectrophotometric methods are widely used for aluminium determination due to their simplicity, rapidity, low costs and wide applications [14-16]. Visible spectrophotometric detection is much more viable as useful technique to develop a portable, on-line or at-line system. A brief review of spectrophotometric for determination of aluminum is shown in Table 1.2

Table 1.2 Characteristic performance of some reported spectrophotometric methods for aluminium determination

Reagent	λ_{max} (nm)	ε (L mol^{-1} cm^{-1})	Linear range (µg mL^{-1})	LOD (µg mL^{-1})	Reference
Hydroxynaphthol blue	569	1.66×10^4	0.03-1.6	-	17
Chrome azurol S	625	1.34×10^5	0-0.4	5 ng/ml	18
Pyrocatechol violet	710	6.4×10^4	3-10	0.003	19
Polyvinylpyrrolidone	510	1.70×10^4	-	-	20
Bromopyrogallol red	623	-	0-0.3	0.001	21
Catechol violet	615	5.3×10^3	0.27-54	-	22
Azurol S	567.5	2.15×10^4	0- 1200	-	23
m-carboxyphenyl fluorine	561	1.70×10^5	0.003 -0.14	-	24
tetrahydroxyazon SN	479	5.46×10^4	0.005-1.079	-	25

Table 1.2 (Continue)

Reagent	λ_{max} (nm)	ε (L mol^{-1} cm^{-1})	Linear range (µg mL^{-1})	LOD (µg mL^{-1})	Reference
Morin	421	5.3×10^3	0.01-5.0	0.005	26
ECR	584	1.9×10^5	0.004- 0.4	0.14	27
2,2′,3,4,-tetrahydroxy-3,′5′-disulphoazobenzene	500	6.42×10^4	0.05-1.6	-	28
Quercetin	433	8.09×10^4	0.03-0.43	0.08	29

Nowadays, Flow injection method and Sequential injection method play important role for Al(III) determination. A number of these methods are based on various detectors such as Spectrofluorimeter and Spectophotometer. A brief review of FIA and SIA for determination of aluminum is shown in Table 1.3

Table 1.3 A brief review of the FIA and SIA methods for the determination of aluminium.

Technique	Reagent/ condition	Sample	Linear range ($\mu g\ L^{-1}$)	LOD ($\mu g\ L^{-1}$)	Reference
FIA Spectrophotometry	Chrome azurol S as reagent, on-line preconcentration with polyethylene powder column	Hemodialysis fluids	0 – 80	0.017	30
FIA Spectrofluorimetry	Lumogallion as a complexing agent, pH = 4, λ_{ex} = 500 nm and λ_{em} = 595 nm	Beverages	-	12	31
FIA spectrofluorimetry	8-hydroxyquinoline-5-sulphonic acid as reagent, Hexadecyltrimethylammonium chloride as Surfactant	Drinking water	10 – 500	0.5	32
FIA Spectrophotometric	Chrome azurol S as reagent, benzyldimethyltetradecylammonium chloride (BDTAC) as surfactant	Water	5.4 – 675	3.0	33
FIA Spectrofluorimetric	Salicylaldehyde picolinoylhydrazone (SAPH) as reagent, λ_{ex} = 384 nm and λ_{em} = 468 nm, pH 5.4	Drinking water	5 – 30	1.9	34

Table 1.3 Continue

Technique	Reagent/ condition	Sample	Linear range ($\mu g\ L^{-1}$)	LOD ($\mu g\ L^{-1}$)	Reference
FIA Spectrophotometric	Erichrome cyanine R (ECR) as the chromogenic reagent, pH = 6, λ_{max} = 535 nm	Anti-perpirants	150 - 900	16.1	35
FIA Spectrofluorimetric	Morin as reagent, λ_{ex} = 410 nm and λ_{em} = 497.6 nm - without surfactant - In the presence of surfactant (SDS)	Drinking water	2 – 250 2 – 50	3.1 2.8	36
FIA Spectrofluorimetric	N-*o*-vanidillidine-2-amino-*p*-cresol as reagent, pH= 4.0 , λ_{ex} = 423 nm and λ_{em} = 553 nm	River and sea waters	-	0.057	37
SIA Spectrofluorimetry	morin as reagent, Tween-20 as non-ionic surfactant, λ_{ex} = 495 nm and λ_{em} = 420 nm	Tap water	50-100	3	38
SIA Spectrofluorimetry	8-hydroxy-7-(4-sulfo-naphthylazo)-5-quinoline sulfonic acid as reagent, pH = 5.6, λ_{ex} = 357 nm and λ_{em} = 492 nm	Drinking water	100-800	4	39

1.2 Quercetin [40- 42]

Quercetin (3,3′,4′,5,7-pentahydroxy-flavone) is a flavonoid compound found in plant products. It is present in plant-related food including fruits, vegetables, oils, nuts, and herbs and in beverages such as wine, tea, coffee, and beer. It is weakly soluble in water and its solubility increases in lipids and proteins. Flavonoids exhibit a broad range of biological activity, which is thought by some researchers to be connected to their antioxidant activity, i.e. ability to scavenge free radicals, absorb UV radiation, and chelate metal ions [40]. Being suitable for metal determination, complexing reaction of flavonoids have been extensively investigate during last ten years. Quercetin is well known colorimetric reagents the spectrophotometric and also for fluorimetric for determination of aluminum(III) traces in water and in biological samples. Besides these analytical applications, the chelation of mono and polyhydroxy flavones with cations is an important factor in their bioactivity as carriers and regulators of metal concentration [41]. Moreover quercetin was the most widely used for the determination of some metals such as Cr(III), W(IV), Fe(III) and Mo(VI) [30,42]. The chemical structure of quercetin was shown in figure 1.1.

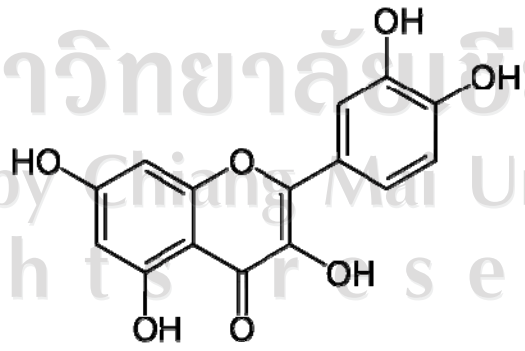

Figure 1.1 The chemical structure of quercetin [42]

1.3 Flow Injection Analysis (FIA) [43-45]

1.3.1 Introduction

Nowadays, the development of science and technology and population growths led to the ever-increasing demand for analyses in environmental, pharmaceutical, clinical, agricultural, industrial and process analytical control. Therefore, the continuous flow analysis was developed to increase analytical efficiency, reduce analysis time, reduce costs and increase accuracy and precision.

In 1957, Skeggs introduced the "Segmented Flow Analysis" (SFA) to overcome the sample throughput problem. It was based on the aspiration of samples into a continuous flowing stream of reagents. The stream was segmented by air-bubbles to avoid interaction between the samples by dispersion and mass-transfer between successive samples. The reaction mixture was monitored downstream after removal of the air bubbles. However, the efficiency of this technique required steady state conditions.

In the mid 1970s, Ruzicka and Hansen in Denmark and Stewart *et al*. in the United States independently introduced the non-segmented flow analysis, which abandoned the principle of steady state readout. In 1975, Ruzicka and Hansen named this technique as "Flow Injection Analysis" (FIA).

Flow injection analysis (FIA) is based on the optimal combinations of fundamental principles, the three comer stones, which are: sample injection, controlled dispersion of the injected sample, are reproducible timing of its movement from the injection point toward and into the detector. Under these conditions physical and chemical equilibrium are not required to achieve reproducible results at any point at any given time. Further, the controllable reaction time enables the FI technique to

carry out kinetic discrimination of interferences that are especially dependent on time, improving selectivity. Another important characteristic of FIA is that it is a closed system that completely removes or significantly reduces the contamination risks. A closed system is especially desirable when applying liquid-liquid extraction to avoid the drawbacks of annoying odor and sample pretreatment, the versatility of FIA is further illustrated by its ability to cooperate with any detector. The development of new and interesting procedures is only limited by the imagination.

1.3.2 Principle of FIA

The three basic principles of FIA are the sample injection, reproducible timing and controlled dispersion. This technique is based on a well-defined volume of a sample being injected into a non-segmented carrier stream, which is continuously pushed down the narrow tube by the pump with a constant flow rate. The reagent stream is continuously pumped down another tube and mixed with the carrier stream containing the sample at the junction. The mixing occurs together in the mixing reactor with a controlled dispersion process. The steady state conditions are not a necessary requirement. The injected sample forms a zone which is transported toward a detector that continuously records the absorbance, electrode potential, or other physical parameter as it continuously changes due to the passage of the sample material through the detector and further recorded (Figure 1.1). Usually change must be proportional to the analyte concentration if controlled experimental conditions as usual and kept equal for both samples and standard

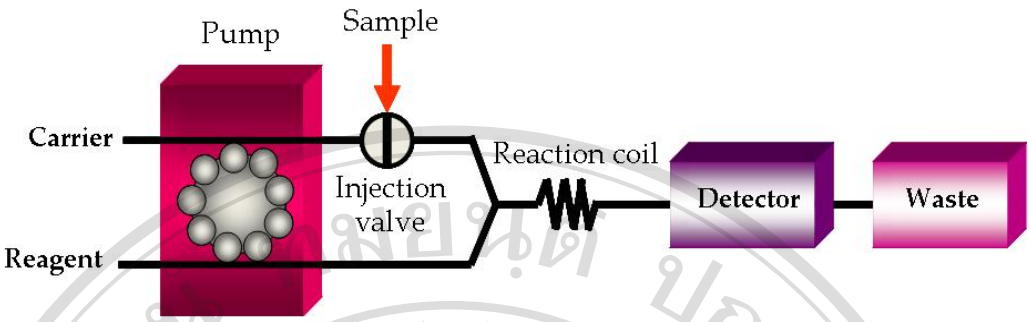

Figure 1.2 The basic components of the FIA system

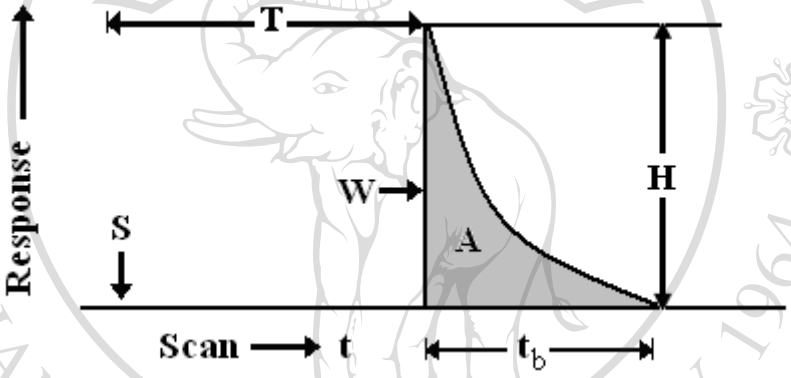

Figure 1.3 The analog output has the form of a FIA peak, the recording starting at S (time of injection), H is the peak height, W is the peak width at a selected level, A is a peak area, T is the residence time corresponding to the peak height measurement and t_b is the peak width at the base line [46].

A typical recorder output has the form of a peak and subsequently recorded as a function of time (Figure 1.2). The peak height (H), width (W) or area (A) which is related to the concentration of the analyte. The time spent between the sample injection (S) and the peak maximum (peak height) is the residence time (T) during which the chemical reaction takes place.

1.3.3 Dispersion [44-46]

The most common physical phenomenon in manipulation of sample zone in the FIA system is dispersion. The shape of the resulting zone is determined by two main processes: convective transport and diffusion transport. Convective transport occurs from mechanical flow driven by a propelling system. It consists of two processes: turbulent and laminar flows (Figure 1.4a). The turbulent flow occurs in transporting of liquid with air-segmentation. The laminar flow occurs for non-segmented liquids in narrow tubing. In FIA, laminar flow is predominant and causes the sample zone to spread in a parabolic due to higher velocity at the center of tubing (about 2 times the average velocity).

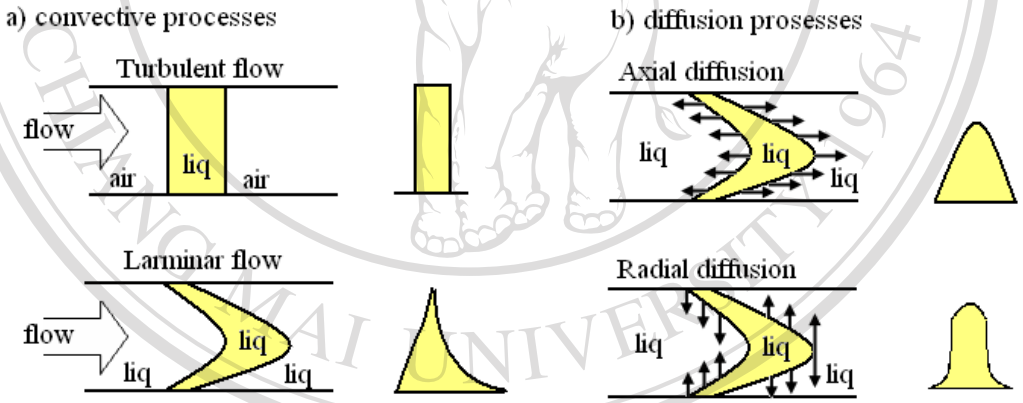

Figure 1.4 General types of transport in closed tubes and the recorded profiles at the detector [45].

Diffusion transport is caused by concentration gradients. There are two types of diffusion processes: axial and radial, as shown in Figure 1.4b. Axial diffusion is insignificant compared to convective flow, but the radial diffusion contributes more significantly to sample dispersion. This process, termed “secondary flow”, results in a washout effect accounting for the low mutual contamination of samples

successively injected into the carrier stream and also serves to limit band spreading. At low flow rate it may even be the major mechanism for dispersion. In fact, flow injection analyses usually performed under conditions in which dispersion by both convective process and radical diffusion occurs as shown in Figure 1.5c.

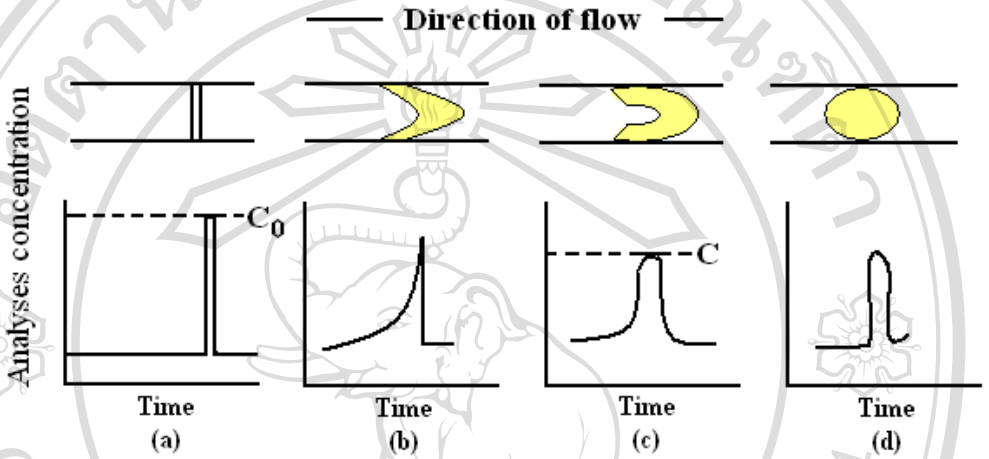

Figure 1.5 Effects of convection and diffusion on concentration profiles of analyses at the detector: (a) no dispersion; (b) dispersion by convective process; (c) dispersion by convective process and radical diffusion; (d) dispersion by diffusion [47].

A simple dispersion experiment is used to pursue dispersion by measure dispersion by means of the dispersion coefficient as shown in Figure 1.6. A sample solution is homogeneous and has the original concentration C^0 that would yield a square signal. The height of square signal would be proportional to the sample concentration (Figure 1.6, left). When the sample zone is injected, it forms a dispersed zone whose form depends on the geometry of the channel and flow velocity. Therefore, the response curve has the shape of a peak reflecting a continuum of concentrations (Figure 1.6, right), which composed of a certain concentration of individual elements of fluid.

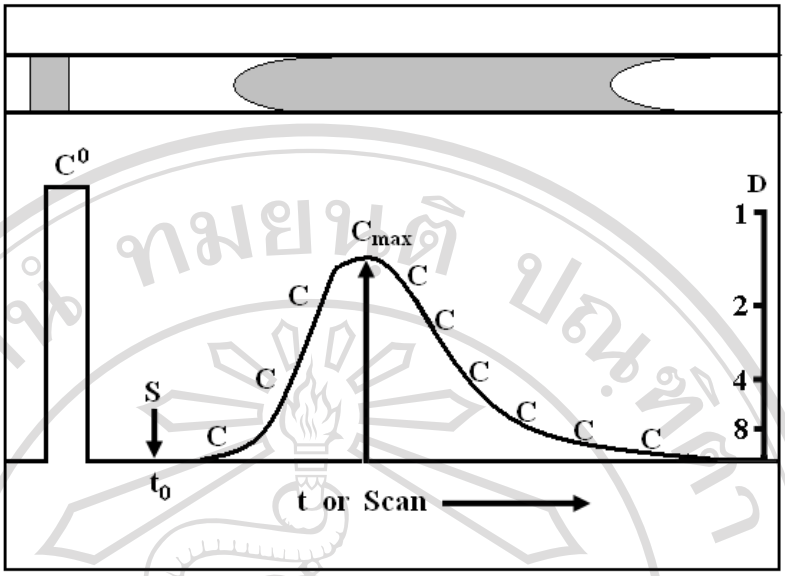

Figure 1.6 Dispersed sample zone in flow system; an original homogeneous sample zone (top left) disperses during its movement through a tubular reactor (top center), thus changing from an original square profile (bottom left) of original concentration C^0 to a continuous concentration gradient with maximum concentration C_{max} at the apex of the peak [45].

The dispersion coefficient (D) is defined as the ratio of the analyte concentration before and after the dispersion takes place:

$$D = C^0 / C_{max}$$

Where C^0 is the original concentration of injected sample solution and C_{max} is the concentration of dispersed sample solution.

Dispersion may be considered in terms of the three general categories:

(1) Low dispersion systems (D < 2) are used whenever one intends to prevent the original concentration of the analyte in the injected fluid zone being diluted by the carrier.

(2) Medium dispersion systems (2 < D < 10) are also used in single channel FI systems, where reagents are used as carrier streams, to attain adequate mixing of sample and reagent.

(3) Large dispersion (D > 10) and medium systems are used to achieve sample dilutions, usually to bring the analyte concentration into an appropriate range for readout.

The FI experimental parameters which may influence the dispersion including sample volume, carrier flow rate, flow rate ratio between sample carrier and merging reagent and geometrical dimensions and configurations of manifold components. Varying the values of these parameters confers a significant degree of control over the dispersion characteristics and facilitates optimization of a flow injection system for many diverse applications.

1.3.4 FIA Instrumentation [43-46]

The basic components of a simple FI manifold typically consist of a propulsion system, an injection or insertion system, a transport and reaction systems and a detection system.

(a) Propulsion system

The liquid propulsion system is a basic unit in all flow analysis systems. FIA is a technique based on highly reproducible timing, a feature that demands pulse less and reproducible flow rate in liquid propulsion. The high versatility of FIA also demands easily manageable propulsion devices, which will not depreciate the flexibility of the technique. For FIA system various pump types have been used. The peristaltic pump is a highly versatile propulsion device, which is no doubt used most frequently, not only in FIA but also in other continuous flow analysis system, because it may accommodate several channels whereby, according to individual tube diameters, equal or different pumping rates may be obtained. The peristaltic pump consists of a motor-driven wheel with peripherally placed rollers and a compression cam (or band) which is squeezed against the rollers. One or several pump tubes are affixed so that they rest on a minimum of the rollers at all times (Figure 1.7)

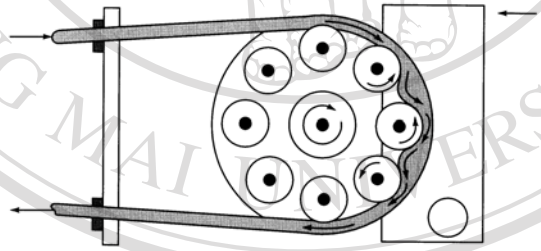

Figure 1.7 Relationship between the rollers of a peristaltic pump and pump tubes [47]

(b) Injection or Insertion System

The injectors employed in FIA are similar in kind to those used in HPLC, but it is not necessary for FI valves to withstand extremely high pressures as for HPLC. For a successful analysis, it is vital that the sample solution is injected rapidly as a pulse or plug of liquid; in addition, the injections must not disturb the flow of the carrier stream. The earliest injection system employed in FIA was as simple as a

syringe and hypodermic needle. Currently, the injection systems most frequently used are the rotary valve, proportional injector and multi-injection system.

(c) Transport and Reaction System

The transport system is an integral component of any flow analysis system. The function of transport system is to provide connections between the different components of the system. Normally, the transport system consists of small-bore tube of I.D. such as PTFE tubing of 0.35-1.0 mm I.D.

The purpose of using the connectors in FIA system is to join the tubes to one another and to other parts of the system. In FIA, there is a wide range of connectors, but basically there are either dual (linear or V-shaped) or triple (T-, Y- or W-shaped) ways or pieces.

The reactor is a major component of the transport system. The main function of reactor is to promote the reproducible radial mixing of two or more components merged through the system. The reactor is usually made of PTFE tubing. There are many types of the reactors such as straight open tube, coiled tube, mixing chamber, single-bead string reactor (s.b.s.r.) and knitted or 3-D reactor.

(d) Detection System

The detection system is the sensing part of the FI manifolds, which allows continuous monitoring of a given property of the sample or its reaction product and provide qualitative and quantitative information of the analyte. In theory, any detection system, which could be adapted for flow through detection may be used as detectors for FIA. These include the spectrophotometer (visible and UV), atomic

absorption and inductively coupled plasma spectrometers, nephelometer, fluorimeter, radiometric and various electrochemical detectors.

1.3.5 Reverse Flow Injection Analysis [48-49]

Reversed flow injection analysis (rFIA) was first suggested by Johnson and Petty. The role of sample and reagent are reversed that the conventional FIA operational procedure involve inserting the sample in a carrier or carrier-reagent stream and recording a transient signal on passage through the detector. The rFIA method being based on injection of reagent into a carrier stream of sample. The most salient difference is that reversed FIA uses very large sample volumes, so it must be excluded in those cases where sample availability is severely limited. On the other hand, it uses reagents sparingly, so it is especially appropriate for dualing with expensive and/or hazardous reagents. Sample changeovers in reversed FIA entail lengthy flushing of sample solution to wash not only the sample loop in the injection valve as in normal FIA, but also the whole assembly. This increases sample consumption and detracts from throughput.

In many applications the reversed FIA mode increases the sensitivity of measurement, partly by widening the dynamic concentration range attainable as compared to that achieved by conventional FIA, and partly by providing a lower range of concentration to be reached, a feature that is of ultimate importance in micro trace analysis. This technique is very suitable for using expensive reagents and for determining of the sample which is plentiful and inexpensive.

1.4 Sequential Injection Analysis

Sequential injection analysis (SIA) was first reported by Ruzicka and Marshall at the University of Washington in 1990. The principles upon which SIA is based are similar to those of FIA, namely controlled partial dispersion and reproducible sample handling. Comparing SIA and FIA for this simple sample manipulation, the following points can be made [50].

1. SIA makes use of a simpler, more robust single channel manifold even with multi-component chemical systems. In FIA, additional flow channels are required for each reagent.

2. In SIA, the multi-channel peristaltic pumps commonly used in FIA are replaced by more accurate, robust syringe pumps.

3. With SIA, the sample and reagent consumptions are drastically reduced.

4. The single-channel operation of SIA enables the use of the same manifold to implement a wide range of assays.

5. In SIA, the selection valve provides a means for performing convenient automated calibration

6. In SIA, accurate handling of sample and reagent zones necessitates computer control, so automation becomes essential.

1.4.1 Programmable Flow of SIA

Sequential injection uses programmable, bi-directional discontinuous flow, precisely choreographed by means of computer control. Sample and reagents are injected sequentially, by means of a multiposition valve, into a carrier stream using a single syringe pump placed upstream of the valve. Shown here are sample and reagent zones, at the interface where a detectable product is formed. Flow reversal (D, E) transports the reaction mixture into the detector (Fig. 1.8). Each step can be described as follows: A = The sample was loaded into the holding coil, B = The reagent was loaded into the holding coil, C = The stack zone was aspirated into the holding coil to improve mixing and dispersion, D = The product was produced and was propelled to the detector and E = The product was monitored by the detector and the signal was recorded.

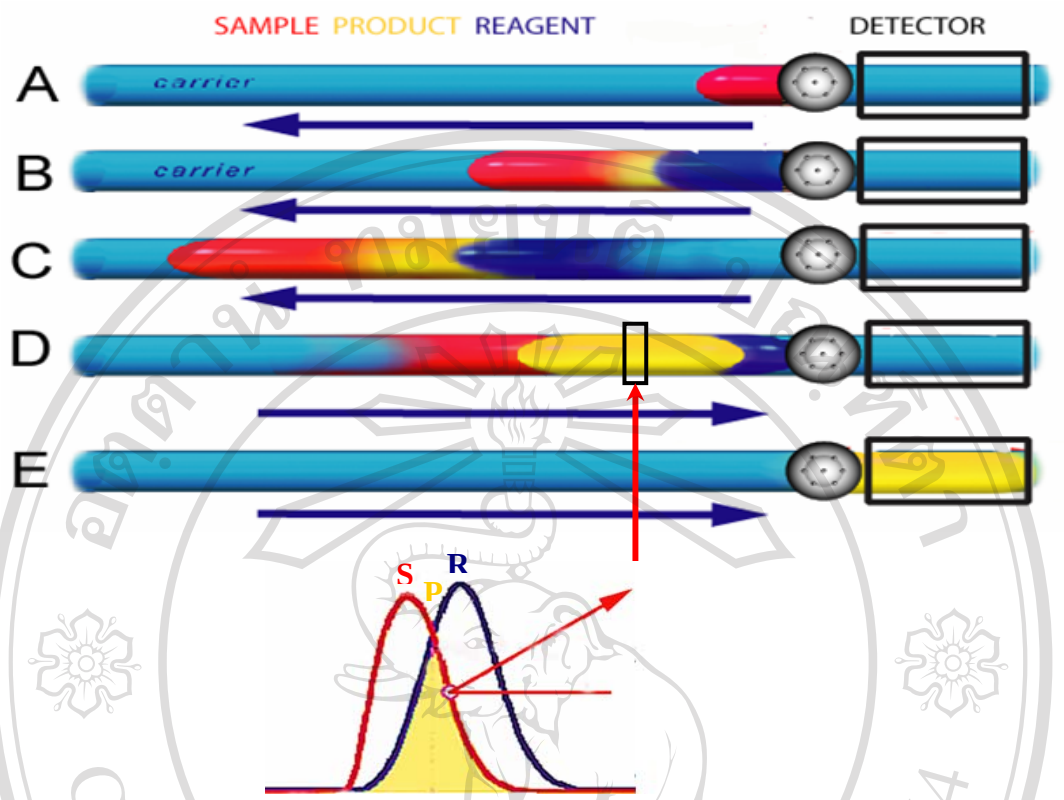

Figure 1.8 Structure of injected zones and concentration profiles as seen by the detector; R-reagent; S-sample; P- composite region where the analyte is transformed into a detector product [51]

1.4.2 Sequential Injection Analyzer

A general schematic flow diagram of a sequential injection analyzer is depicted in Fig. 1.9. The versatility of the technique is centered on a selection valve (SV) where each port of the valve allows a different operation to be performed.

An important advantage of SIA is the versatility that the multi-position valve provides [52–55]. Each port of the valve is dedicated to a specific purpose and the combinations of sample, standards, reagents and detectors around the valve are easily modified to suit a particular analysis. The basic components of the system are a pump with only one carrier stream, a single selection valve, a single channel and a detector.

The concept is based on the sequential injection of a sample zone and a reaction zone(s) into a channel [56–60]. In this way, a stack of well-defined zones adjacent to each other is obtained in a holding coil. After the valve has been selected to the detector position, the flow in the carrier stream is reversed and the zones mutually disperse and penetrate each other as they passed through a reaction coil to the detector. The flow reversal as a result of the injection step, therefore, creates a composite zone in which sample and reagent zone penetrate each other due to combined axial and radial dispersion. Controlled dispersion and reproducible sample handling [61–69] are integral and indispensable prerequisite for the success of SIA. Computer control of the SIA system is, therefore, an essential prerequisite [61–76] because an analytical procedure often requires a complex and high reproducible flow patterns. Some of the prerequisites of process analyzers are that the system should be simple and robust, reliable with a low frequency of maintenance and that the consumption of reagents should be very low

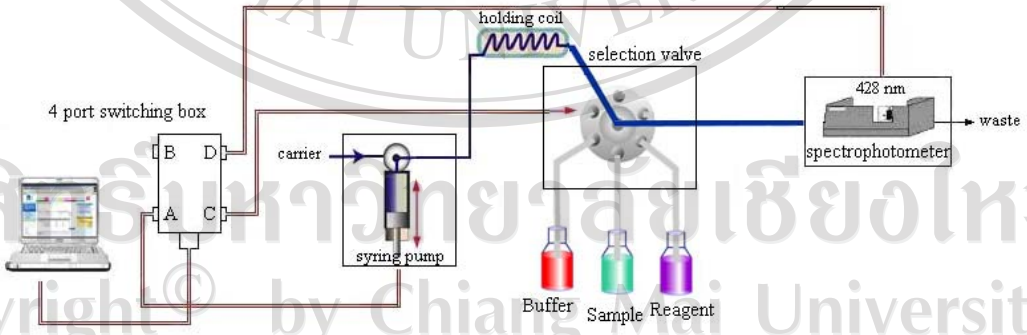

Figure 1.9 Schematic flow diagram of a sequential injection analyzer

The core elements of the SI network were [67]

(a) A selection valve (SV) was furnished with central communication channel that can be made to address each of the peripheral port.

(b) A syringe pump (SP) was used as liquid driver that allows the manipulation of sample and reagent volumes at the low µl level with high precision and reproducibly permits flow reversals and exploitation of stopped flow schemes.

1.4.2.1 Essential Compartments of SIA [78]

The SIA assembly includes the following essential parts:

(a) Pump

Syringe pumps have been most widely used to aspirate zones and propel the stack of zones through the detector. Some researcher have used peristaltic pump. The requirements for the pump are that it is precise, reproducible, bi-directional, and able to measure mall volumes. Computer control is imperative. However, it is relatively expensive requires priming before using and has a limited reservoir volumes.

(b) Selection Valve

The selection valve must allow random access of the ports. Small dead volume and zero cross contamination between ports are essential features of good selection valve. The common port is connected to the pump through the holding coil. Other ports are connected to reagent solutions, samples and the detector flow cell. The 10 port multi-position valve is by far the most widely used.

(c) Connectors and Reactors

While an i.d. of 0.5 to 0.8 mm tubing is a typical how line for a majority of SI system, there are also many tubing materials available for reactor coils and connection lines. Teflon and PEEK are the most frequently used polymers. Stainless steel is another material that has the advantages of heat conductivity, gas impermeability, and surface properties that minimize protein adsorption. A majority of polymer tubing is transparent and is often color coded, so that tubing i.d. can be identified at glance. Connectors made of color-coded polymers are fitted with ferrules that are designed to grip tubing while the connector nut is being tightened. Since all SI systems operate at low pressure, it is not necessary to use connectors designed for HPLC. It is, however, very important to use nuts, ferrules and fittings from a single manufacturer, as products from different sources are often incompatible, resulting in leaking.

(d) Detector

The wide ranges of detectors that are employed for FIA are suitable for SIA. Almost detectors are inserted with suitable flow cell.

(e) Software

The important of SIA is the SIA program. This sequence of events results in the assembly of the stack of zones in holding coil and subsequent transport to the detector flow-cell. Microprocessor control is imperative. Several packages have been written to achieve this. Some software are used for SIA such as AnalySIA, Flow TEK^{TM}, Lab VIEW, and FIAlab.

1.4.3 SIA Dispersion Zones [44, 50, 78]

The sequential injection technique, sample injection, controlled dispersion and reproducible timing is the same as those of on which flow injection is based. The difference is that SI uses programmable flow to control these parameters. The key parameters in SIA are zone sequencing and the mutual dispersion of the zones. Fig. 1.10 is shown the sample and reagent injection provides the initial input, serving as a starting point for the initial concentration (Cº) of analyte (red) and reagent (blue).

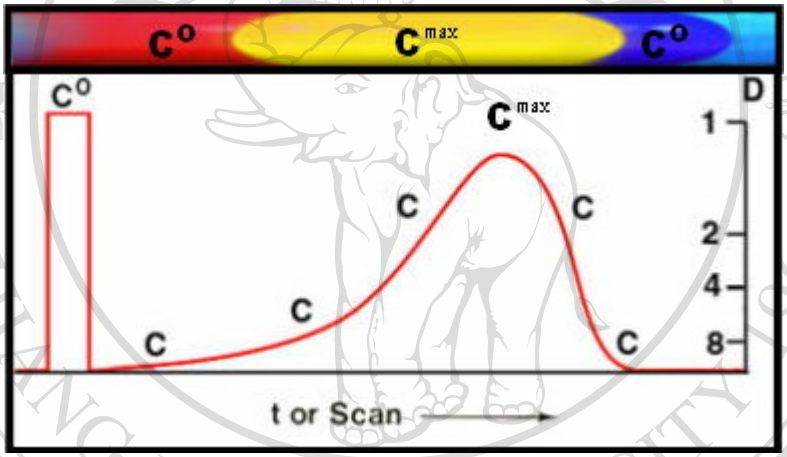

Figure 1.10 Dispersed sample zones of SIA system []

The dispersion coefficient (D) has been defined as the ratio of concentrations of sample material before (Cº) and after (C) the dispersion process has taken place in that element of fluid that yields the analytical readout that is:

$$D = \frac{C^0}{C} \qquad (1.2)$$

Where Cº is the original concentration of the constituent of interest in the solution to be injected, and C the concentration of that fluid element of the dispersed

solution zone, which is under consideration. When the fluid element with the highest concentration is concerned (i.e. readout at SI peak maximum), equation 1.2 is expressed as:

Which, for $C = C^{max}$

$$D = \frac{C^0}{C^{max}} \quad (0<D<\alpha) \qquad (1.3)$$

The dispersion of the sample zone has to be adjusted to suit the requirement of the intended measurement. Thus, for direct measurements (e.g. pH, ICP, AAS, conductivity, potentiometry) limited dispersion (D = 1-2) is required. For reagent-based chemistries such as colorimetry, fluorescence or chemiluminescence, sample and reagent zones must mix in a suitable proportion and a medium dispersion (D = 2-10) has to be achieved. And for extensive sample dilution a large dispersion (D = 10-10000) may be necessary.

Controlled dispersion takes place as stacked zones move upstream into the holding coil and then move back through the valve into a detector. This process forms a well-defined concentration gradient that is seen as a continuum of elements with varying concentrations of analyte, product and reagent. To produce a readout that is proportional to the initial concentration of the analyte, it is essential to achieve complete overlap of sample by reagent zones. The overlap is evaluated by measuring the dispersion coefficient of the sample ($D = C^o / C^{max}$) as it yields a degree of sample dilution. Reagent zones will be less diluted as they are stacked in the holding coil after the sample, where they travel a shorter path and are dispersed to a lesser degree.

Reproducible timing in a SI system is achieved through repeatability of all events of the measurement cycle. This includes sequencing of sample and reagent into

the holding coil, transport of stacked zones to the detector and length of the stop flow period. Therefore T is the time elapsed from the moment of injection (Tº) to the moment of peak maximum readout (T^{max}) or to the end of the stop flow period.

1.4.3.1 Factors Influencing Dispersion [45]

The SIA experimental parameters or factors which may influence dispersion include:

1. Sample volume
2. Flow rate ratio between sample and merging reagent
3. Geometrical dimensions and configurations of manifold components
4. Viscosity of the fluids
5. Temperature

Under normal conditions, the last two factors have very limited effect on the dispersion, and in most cases may be neglected.

1.4.4 Mixing and Zone Overlap of SIA

Since the reaction product (yellow) (Fig. 1.11) is formed at the interface between the sample and reagent zones, it is essential to maximize zone overlap by increasing the amplitude of the forward flow. As the stacked zones are pushed into the holding coil (HC), axial dispersion is promoted, since the center of the stream travels at twice the mean flow velocity.

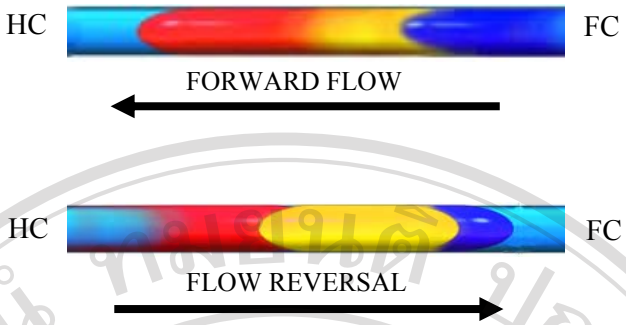

Figure 1.11 Forward and reversal flow of SIA system

The resulting parabolic profile telescopes the trailing zone toward the leading edge of the sample zone, and the radial dispersion promotes mixing of adjacent parallel layers of sample and reagent. Upon flow reversal, the flow velocity profile is suddenly inversed. First, radial mixing is caused by local turbulence, and then axial dispersion and zone overlap are increased when the stacked zones travel downstream toward the flow cell (FC). Combined volumes of sample and reagents define the amplitude of flow reversal. When a spacer zone of carrier solution is injected, zone overlap and mixing are further promoted.

1.5 Research Aims

The aims of this research can be summarized as follows:

1. To design and construct a flow injection and sequential injection with spectrophotometric detection system for the determination of aluminum

2. To investigate the optimum conditions for the determination of aluminum by flow injection and sequential injection spectrophotometric method

3. To apply the proposed method to the determination of aluminum in water samples