CHAPTER 1

INTRODUCTION

1.1 Flow Injection Analysis (FIA) [1-4]

The ever-increasing demand for analyses in pharmaceutical, clinical, agricultural, industrial and process analytical control led to the development of ***flow injection analysis (FIA)*** in the mid 1970s. The inception of FIA in turns was the result of a long search for better laboratory techniques in solution manipulation. The important stages of development are shown in Figure 1.1, which also shows the relation between the various techniques for automated solution analysis and the scheme for their classification.

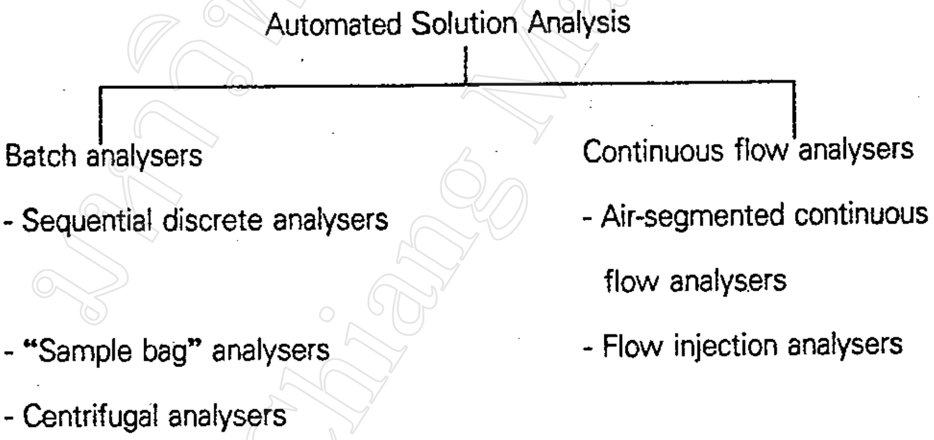

Figure 1.1 : Stages of development and classification of automated solution analysis [5].

Since its development, FIA has become a routine laboratory technique and a versatile tool for the enhancement of instrumental analysis. In addition, FIA increasingly has found its use in a multitude of areas as diverse as oceanography, clinical chemistry [6-10], agricultural [11-12], pharmaceutical [13-18] and environmental [19-22] analysis, for monitoring of industrial processes, and, most recently, in the bioanalytical field (Table 1.1).

Table 1.1 : Process FI applications.

Area	Analyte	Reference
Waste water monitoring	Phosphate, ammonia, and nitrate	23
	Total phosphorus	24
Treated water monitoring	Fluoride	25
	Aluminium and iron	26
Chemical production	Azo dyes	27
	Morphine	28
Metal production	Iron (II and III)	29
	Aluminium in Steel	30
Paper production	Calcium	31
Fish farming	Ammonia and nitrite	32
Hydroponic cultivation	Nitrate	33
Biotechnology	L-Phenylalanine	34
	Glucose, lactic acid, and protein	35
	Ammonium, glucose, and protein	36
	Acetate and phosphate	37
	β-Galactosidase	38
	Penicillin V	39

1.1.1 Principle of FIA [40-42]

FIA is a kind of continuous flow analysis which is based on the injection of the small volume of sample into a continuously moving, nonsegmented carrier stream of a suitable reagent (liquid) (Figure 1.2). The injected sample thereby forms a well defined zone which is then transported toward a detector. During this transport the sample solution is mixed with the carrier stream and possibly also with other reagents added sequentially further downstream and reacts with its component(s) to form a species which can be measured in a flow-through detector (absorbance, electrode potential, etc.), the output being a peak which its shape and magnitude reflected the concentration of the injected analyte and provided kinetic information on the chemical reactions taking place in the flowing stream.

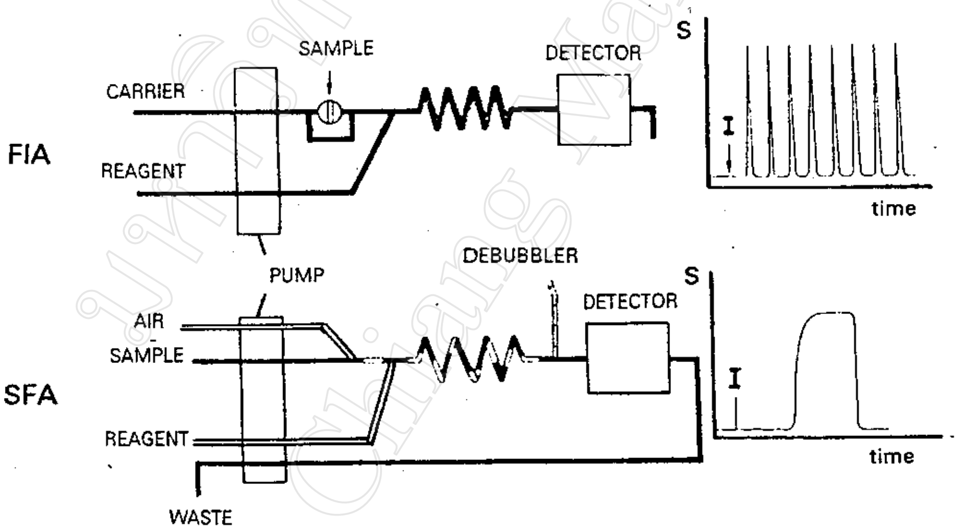

Figure 1.2 : Comparison between FIA and segmented continuous analysis [43].

The simplest and most widely adopted continuous flow injection mode can be illustrated by a diagram [Figure 1.3(a)] showing the flow system and the resulting readout. A sample(s) injected into a continuously moving carrier stream of reagent disperses into a zone as it moves through a conduit on the way to the detector. Chemical reaction takes place in a microreactor [shown as straight open tube, coiled tube, mixing chamber, single-bead string reactor (s.b.s.r.), and knitted reactor] from where the dispersed and reacted zone proceeds through a detector (D) to waste (W). A typical recorder output exhibited a single peak for each injection and subsequently recorded as a function of time [Figure 1.3(b)]. The height (H), width (W) or area (A) reflected the analyte concentration.

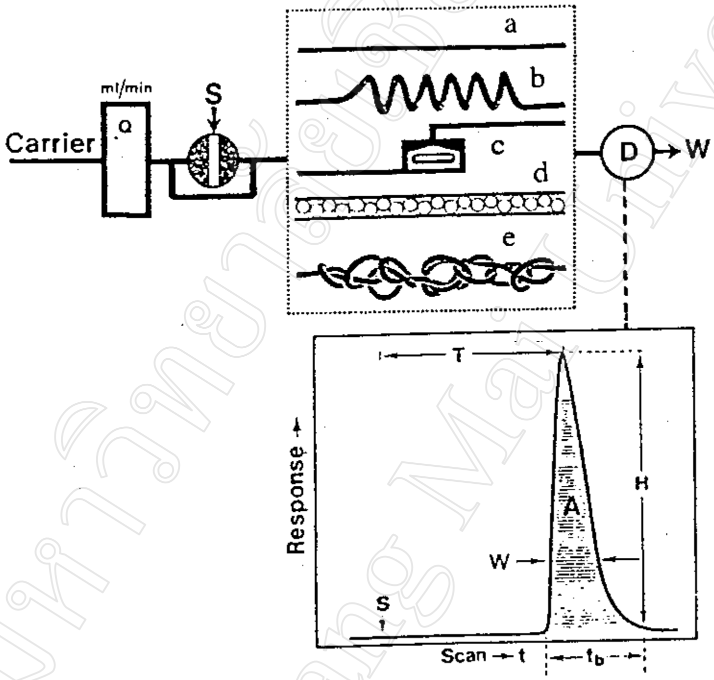

Figure 1.3 : Single-line FIA manifold showing the various components; S is the injection port, D is the flow-through cell, and W is the waste. The microreactor geometries (a) straight open tube; (b) coiled tube; (c) mixing chamber; (d) single-bead string reactor (s.b.s.r.), and (e) knitted or 3-D reactor [41].

As mentioned above, it is important to realize that FIA is founded on a combination of the following three principles [3,44]:

- sample injection
- reproducible timing and
- controlled dispersion

1.1.2 FIA Instrumentation [1,42]

The basic components of a simple FI manifold (Figure 1.3) typically consists of a propulsion system, an injection or insertion system, a transport and reaction system, and a detection system (Table 1.2).

(a) Propulsion system

A propulsion system is a basic unit in all flow analysis system. However, certain characteristics of FIA call for specific requirements in its propulsion systems. FI systems are in essence low pressure system. On the other hand, FIA is a technique based on highly reproducible timing, a feature which demands pulseless and reproducible flow-rates in liquid delivery. The high versatility of FIA also demands easily manageable propulsion devices which will not depreciate the flexibility of the technique. For these requirements various pump types have been used, including syringe, peristaltic, progressive cavity, single and dual piston pumps, and aquarium pump.

(b) Injection or insertion system

The injection or insertion system is intended to introduce a well-defined sample plug into a flowing carrier or reagent 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, solenoid valve and multi-injection systems.

Table 1.2 : Options for a FI manifold [1].

Component	**Options**	**Description**
Propulsion system	Peristaltic pump	Set a rollers on a revolving drum that squeezes flexible tubing to produce a constant, pulsing flow
	Sinusoidal flow pump	Cam-driven piston that produces a variable pulseless flow
	Gas pressure reservoir	Pressurized inert gas vessel connected by a flow regulator to each reagent or carrier reservoir, producing pulseless flow
Injection system	Rotary valve	Six-port unit with sample loop; electronic or pneumatic operation
	Hydrodynamic injection	Selective stopping and starting of both sample and reagent pumps, sample enters reagent stream while the reagent pump is stopped and is then transported to the detector when it is restarted
	Syringe	Manual injection through septum
Detection system	Optical	UV-VIS, diode array, and IR spectrophotometry; solid-state photometry (LED source, photodiode detector); fluorometry; chemiluminescence; atomic spectrometry
	Electrochemical	Potentiometry (ion-selective and pH electrodes), conductimetry, amperometry, coulometry, voltammetry

(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 tubes of I.D. such as PTFE tubing of 0.35 - 1.0 mm I.D.

The connectors used in an FIA system serve the purpose of joining the tubes to one another and to the other parts of the system. In FIA, there is a wide range of connectors, but basically there are either dual (linear or V-shaped), triple (T-, Y-, or W- shaped) or quadrupole (usually in the shape of an arrowhead).

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 reactor 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 a sensing part of the FI manifold which allows continuous monitoring of a given property of the sample or its reaction product and provide quanlitative 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 spectrometer, chemiluminescence detector, nephelometer, fluorimeter, radiometric detector and various electrochemical detectors.

1.1.3 Dispersion of Sample and Reagent Zones [7,45]

The most important physical phenomenon in the manipulation of sample zones in the FIA system is the ***dispersion***. Let us consider, in a simple dispersion experiment. A sample solution is homogeneous and has the original concentration (C^o) that would yield a square signal the height of which would be proportional to the sample concentration (Figure 1.4). When a sample zone is injected into the carrier and then formed a dispersed zone during through the system. Therefore, the response curve has the shape of a peak reflecting a continuum of concentrations which composed of a certain concentration (C) of individual elements of fluid. In order to design an FIA system rationally, the ***dispersion coefficient (D)*** has been defined as the ratio of the concentration of sample material before and after the dispersion has taken place. This is represented mathematically by:

$$D = C^o / C \qquad \text{..............................(1)}$$

where C^o = the concentration of sample solution before the dispersion process has taken place

C = the concentration of sample solution after the dispersion process has taken place.

When the sample solution with the heighest concentration was concerned (i.e. readout at FI peak maximum), equation (1) was expressed by:

$$D = C^o / C^{max} \qquad \text{............................(2)}$$

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

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

(1) **Limited dispersion** (D=1-3) can be attained to feed such detectors as electrodes and atomic absorption spectrometers to effect high analytical rates.

(2) **Medium dispersion** (D=3-10) can be applied to attain a wide variety of reaction configurations to develop some detectable entity such as colour, fluorescence or an electroreactive product.

(3) **Large dispersion** (D>10) can be utilized to give a substantial degree of mixing between sample and carrier stream to form a well-developed concentration gradient, as is necessaary when performing continuous flow titrations or for investigating the chemistry at the sample - stream interface.

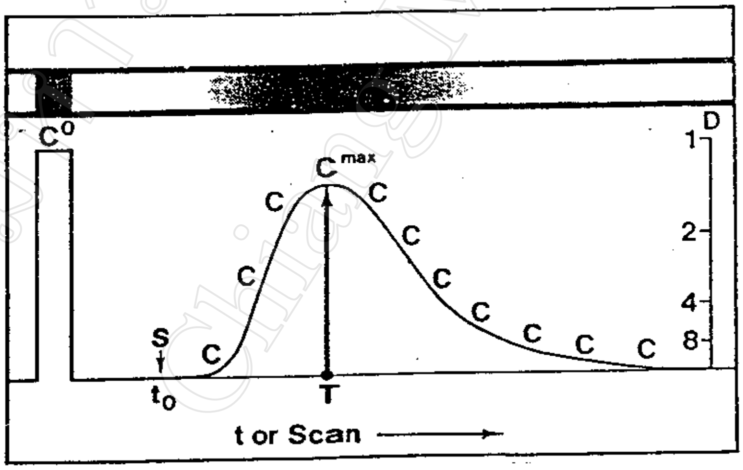

Figure 1.4 : An originally homogeneous sample zone. (C^o = original concentration, C^{max} = concentration gradient with maximum concentration)

The magnitude of the dispersion is dependent on the operating parameters applied to the system, including sample volume, tubing bore size, tubing length, flow rate, sample volume and, possibly, coil diameter. 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.

1.2 Aluminium [46-50]

1.2.1 Occurrence of Aluminium

Aluminium is a silver white metallic element in group III of the periodic table. It has a valence of +3 in all compounds, with the exception of a few high temperature monovalent and divalent gaseous species. Aluminium in combination constitutes almost 8% by weight of the earth's crust and is the most abundant of metals. It is found in many common and rare minerals and ores including: feldspars; micas; kaolin, $H_4Al_2Si_2O_9$; magnesium silicates (e.g., talc, asbestos, meer-schaum); cryolite, $Na_3(AlF_6)$; bauxite, $Al_2O_3.H_2O$. The latter are resulted from the weathering and the decay of aluminium-bearing rocks under tropical conditions and may be residual deposit replacing the original rock, or it may be transported from its place of origin and from deposits elsewhere.

The most important aluminium hydroxide are $Al(OH)_3$ and $AlO(OH)$. Three forms of the former are known: gibbsite (also known as hydrargillite or α-$Al(OH)_3$; bayerite or β-$Al(OH)_3$; and nordstrandite. There are two modifications of $AlO(OH)$, boechmite and diaspore.

1.2.2 Application of Aluminium

The reflecting power of the metal, coupled with the lightness and resistance to corrosion of aluminium, bring the application of aluminium alloys as a roofing material, aircraft frames, car engines and components, bridges, building structures, superstructures on ships, scaffolding and ladders. Similarly it is used in the linings of cold-weather clothing (e.g., anoraks) and in survival bags for emergency use on mountain. High electrical conductance and low density give aluminium a great advantage over copper when used for overhead electrical transmission lines. High thermal conductance and resistance to corrosion lead to the application of aluminium in kitchen utensils and the food chemistry.

1.2.3 Hazard Evaluation and Limiting Concentration

Environmental contact with aluminium compounds cannot be avoided since it is the third common element in the earth's crust. However, the potential toxicity to healthy living organisms is normally low except for some plants, some animals, and for human patients with chronic renal failure (which may reduce elimination in the urine). Drinking water for animals should contain less than 5 mg/l Al(III), and for humans less than 0.2 mg/l. Water used in hemodialysis should have aluminium concentrations lower than 30 μg/l. Aluminium concentration in human's blood should not exceed 10 μg/l plasma.

1.2.4 Determination of Aluminium

Several methods have been developed for the determination of the trace amounts of aluminium in environmental materials but none of them is fully satisfactory. Graphite furnace atomic absorption spectrometry (GFAAS) provides high sensitivity [51] but frequently serious contamination problems arise, which increase the detection limit. Other atomic spectrometric methods such as inductively coupled plasma atomic emission spectrometry and flame AAS are usually combined with sample preconcentration [52] because of their poor sensitivity for aluminium. Molecular fluorimetric methods are very sensitive , but usually need extraction of the fluorescent complex into an organic medium [53]. Moreover, the kinetics of this reaction are too slow for automation. 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 [50]. A brief review of aluminium(III) determination is shown in Table 1.3.

Table 1.3 : A brief review of aluminium determination.

Techniques	Reagent	Condition	Range of Al conc.	Reference
1. Graphite furnace atomic absorption spectrometry	-	λ = 309.3 nm	0 - 100 μg/l	51
2. Inductively coupled plasma atomic-emission spectrometry	Pyrocatechol violet (PV)	preconcentrated through formation Al(III)-PV complex λ = 396.15 nm	5 - 10 ng/ml	52
3. Spectrofluorimetry	3,5,7,2',4'-pentahydroxy flavone	IBMK-ethanol-water system, pH 3.7-3.8 (HCl-KCl buffer), λ_{ex}=418 nm, λ_{em}=495 nm	up to 40 ppb	53
4. Spectrofluorimetry	Lumogallion	Acetate buffer pH 5.2 λ_{ex}=500 nm, λ_{em}=595 nm	Detection limit 3.7 nM	54

Table 1.3 : (continued)

Techniques	Reagent	Condition	Range of Al conc.	Reference
5. Spectrofluorimetry	Eriochrome Red B	H_2MTA^+-HMTA or $HOAc$-OAc^- buffer λ_{ex}=525 nm, λ_{em}=595 nm	Detection limit 0.3 ng/ml	55
6. Spectrofluorimetry	N-(3-Hydroxy-2-pyridyl) salicylaldimine	pH 4-6 in an aqueous N,N-dimethylformamide medium	3.5 - 400 ng/ml	56
7. Vis-spectrophotometry	Eriochrome Cyanine R	$HOAc$-OAc^- buffer λ_{anal}=535 nm,	0.06 - 0.80 µg/ml	57
8. High performance Liquid Chromatography	Nucleosil 10 C_{18} pre-column	Mobile phase A=water-acetonitrile (90+10) Mobile phase B = acetonitrile-acetate buffer	5 ppb - 10 ppm	58
9. Differential pulse adsorptive stripping voltammetry	1,2-dihydroxy anthraquinone-3-sulfonic acid	pH 7.5±0.1 adsorbed on hanging mercury drop electrode at -0.85 V	Detection limit 0.2 µg/l	59
10. Neutron activation analysis	-	anion exchange eluent 1 M HNO_3	Detection limit 0.080 µg of Al	60

1.3 Europium

1.3.1 Occurrence of Europium

Europium was discovered and isolated by Eugene-Antole Demarcay in 1896 and 1901, respectively [49]. Europium is a soft silvery white metal which is the most reactive of the rare earth metals, its atomic number is 63 and atomic weight is 151.96. It is ranked thirteenth on the abundance chart for rare earths found in the earth's crust. Europium can exist in two oxidation states, Eu(II) and Eu(III), but its most stable oxidation state is Eu(III).

Most europium is obtained from monazite sand, which is a mixture of phosphates of calcium, thorium, cerium, and most of the other rare earths. Most of the unwanted metals can be removed magnetically or by flotation processes. the most difficult part of the processs, however, is separating the rare earths from one another. Like most rare earth metals, europium can be separated from the others by an ion-exchange displacement process. The result is an europium ion that reacts with oxygen ions to form europium oxide, Eu_2O_3. Europium is reduced from europium oxide by mixing it with powdered lanthanum metal in a tantalum crucible.

1.3.2 Application of Europium

There are few practical applications of europium metal but the oxide, for example, is widely used as an activator and red phosphor materials in colour cathode-ray tubes for televisions, computer screen and solid state laser, and opto - magnetic materials in an optical isolator, etc [61].

1.3.3 Determination of Europium

Various methods have been developed for the determination of europium. They include spectrophotometry, spectrofluorimetry, polarography, voltammetry, inductively coupled plasma atomic emission spectroscopy (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS). In this account, only previous determination of europium will be discussed.

Taketatsu and Sato [62] described a fluorimetric method for the determination of europium(III) using thenoyltrifluoroacetone(TTA)-trioctylphosphine (TOPO) or phenanthroline(Phen)-Triton X-100 which is sensitive and reliable, and requires relatively simple equipment. They reported that the detection limit is 0.02 μg/kg Eu.

Yamada *et. al.* [63] described the laser fluorimetry combined with nitrogen laser and a pulse-gated photon counting method for determining europium(III) with 4,4,4-trifluoro-1-(2-thienyl)-1,3-butadione. The detection limit for europium(III) is 0.3 ng/l. The europium(III) complex can be determined with high selectivity and sensitivity in a large excess of the samarium(III) complex.

Lyle and Za'tar [64] presented the spectrophotometric method for the determination of 0.04 - 5 mg of europium in rare earth mixtures. In this method, europium(III) is reduced on a Jones reductor to europium(II) which in turn reduces molybdophosphoric acid to molybdenum blue. the absorbance is measured at 810 nm in aqueous solution or at 790 nm after extraction into n-amyl alcohol.

Fu *et. al.* [65] described the polarographic study of the Eu(III)-triethylenetetra-aminehexaacetic acid complex. They reported that this method convenient to determine trace Eu concentrations in the range 2.5×10^{-6} - 5.0×10^{-4} mol/l in 0.1 mol/l NH_4Cl + 0.1 mol/l NH_3 solution.

Mlakar and Branica [66] described a voltammetric method for the determination of europium(III) in the presence of 2-thenoyltrifluoroacetone (TTA). In this method, the Eu-TTA complex is strongly adsorbed at the surface of the mercury drop electrode and provides the possibility of measuring low concentration levels of europium. The detection limit in sodium chloride, with an accumulation time of 10 min at a potential of -0.2 V, was 5×10^{-9} mol/l Eu(III).

1.4 Research Aims

The aims of this research work may be summarised as follows:

(1) To design and construct a flow injection system for the determination of trace aluminium and europium in environmental samples

(2) To investigate the optimum conditions for the determination of trace aluminium and europium by flow injection spectrophotometric procedure

(3) To develop flow injection spectrophotometric procedures for aluminium and europium by using some tetracyclines as a complexing reagent

(4) To apply the proposed procedures to the determination of trace aluminium and europium in environmental samples