CHAPTER 1

INTRODUCTION

1.1 Flow Injection Analysis (FIA)

Flow injection analysis (FIA) is a method as continuous flow analysis (CFA) requiring an injection valve, which was first used by Ruzica and Hansen [1] in 1975. Flow method provides the reproducible physical conditions, in contrast with batch method or segmented flow analysis (SFA).

In the first edition in 1981, Ruzicka and Hansen [2] 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 form a zone ,which is then transported toward a detector that continuously records the absorbance, electrode potential, or any other physical parameter, as it continuously changes as a result of the passage of sample material to through the flow cell". Seven years later, in the second edition, the definition has been revised to read "Information gathering from a concentration of gradient formed from an injected, well-defined zone of a fluid, dispersed into a continuous unsegmented steam of a carrier" Furthermore, Fang defined FIA as "A flow analysis technique performed by reproducibly manipulating sample and reagent zones in a flow stream under thermodynamically non-equilibrated conditions" [3].

1.1.1 Principle of FIA [1-10]

FIA is a method based on an injection of a well-defined volume of liquid sample into a continuously moving nonsegmented carrier stream of reagents or suitable solvents in a controlled way. The injected sample forms a zone that physically disperses small tubing, and reacts with components of carrier stream forming a detectable product as it passed through the mixing reactor, which is then transported towards a flow-through detector for measurement. The detector continuously records physical or chemical parameters such as absorbance, electrode potential, or other parameters as it continuously changes as a result of the physical or chemical process taking place during the passage of sample zone through the flow cell of detector. A typical recording is in a form of a peak (in high, width or area) that is proportional to the concentration of analyte and provides kinetic information on the chemical reactions taking place in the flowing stream. The degree of mixing or sample dispersion is controlled by factors such as flow rate, manifold geometry, etc. In short, the three basic principles of FIA are based on a combination of sample injection, controlled dispersion of injected fluid and reproducible time.

The simplest flow injection manifold (Figure 1.1a) typically consists of a propulsion unit (such as a peristaltic pump), a six-port rotary sample injection valve, and a flow-through detector (such as a spectrophotometer). Narrow-bore tubing is used for sample and reagent transport, and coiled reactor are often included to aid mixing. The manifold shown in Figure 1.1 is a single-line system in which the carrier stream transports the sample towards the detector. The corresponding output is shown in Figure 1.1b.

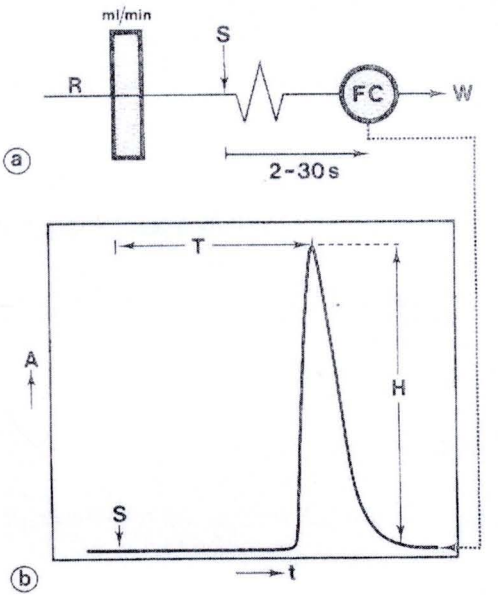

Figure 1.1 The simplest FIA system:

(a) The simplest single-line FIA manifold; S = injection port, D = flow cell and W = waste

(b) The analog output; S = Starting point of injection, W = peak width at a selected level, H = peak height, A = peak area, T = residence time corresponding to the peak height measurement and t_b = peak width at the base line [1]

This make FIA a sample, automated microchemical technique, capable of having a high sampling rate and a minimum sample and reagent consumption. FIA method is based on a combination of three principles.

(a) Sample injection

(b) Controlled dispersion of the injected sample zone

(c) Reproducible timing of its movement from the injection point toward a the detector

If the method requires more than reagent, addition streams can be merged with the carrier stream at suitable point in the manifold. Simultaneous FI system can be performed by designing spit-line manifolds in which the sample is injected into more than the flow channel and undergoes a different reaction in each channel. This can be achieved either by slitting the carrier stream after injection or by connecting two injection valves in series in two separate reaction systems. Other components that can easily incorporates into FI systems such as gas dialysis units, for the diffusion of a gaseous analyst from a carrier (donor) stream through a microporous membrane into a reagent (acceptor) stream, and solid-phase reaction columns, in which the injected sample reacts with, or selected components are retained by a column packed with solid material.

1.1.2 Modes of FIA [11-13]

FIA is classified as normal FIA (nFIA) mode and reverse FIA (rFIA) mode. The rFIA method was first discovered by Johnson and Petty which is based on an injection of a small amount of reagents into a flowing stream of sample and/or standard solution, in contrast with normal flow system which is based a flow of reagent throughout the system, increasing reagent use. The reverse FI method has proven suited in cases where sample material is abundant while reagent ought to be spared. This configuration is suitable for field applications in which the sample is in abundant supply (as is the in many environmental situations) and is particularly useful when expensive reagents are necessary because reagent consumption is low in the rFIA system. The reverse FI system also minimizes the quantity reagents discharged

to waste, which is advantageous if reagents which affect the environment are used in the system.

1.1.3 Dispersion [14-16]

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 1.2a). 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).

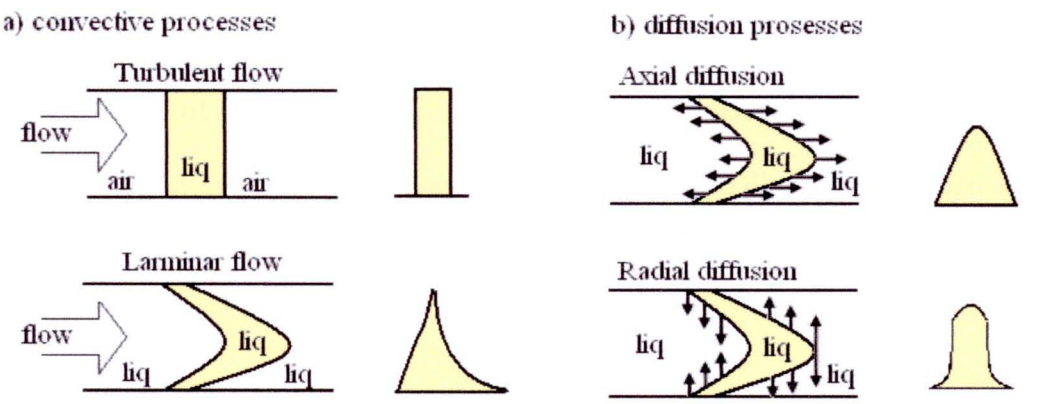

Figure 1.2 General types of transport in closed tubes and the recorded profiles at the detector [17]

A simple method for designing an FI system is based on the concept of dispersion. The most physical phenomenon in manipulation of sample zone in FI system is the dispersion. The shape of the resulting zone is determined by two phenomena, convection and diffusion (radial and longitudinal) as can be seen in Figure 1.3. In fact, the flow injection analyses usually perform under conditions in which dispersion by both convection and radial diffusion occurs as shown in Figure 1.3c.

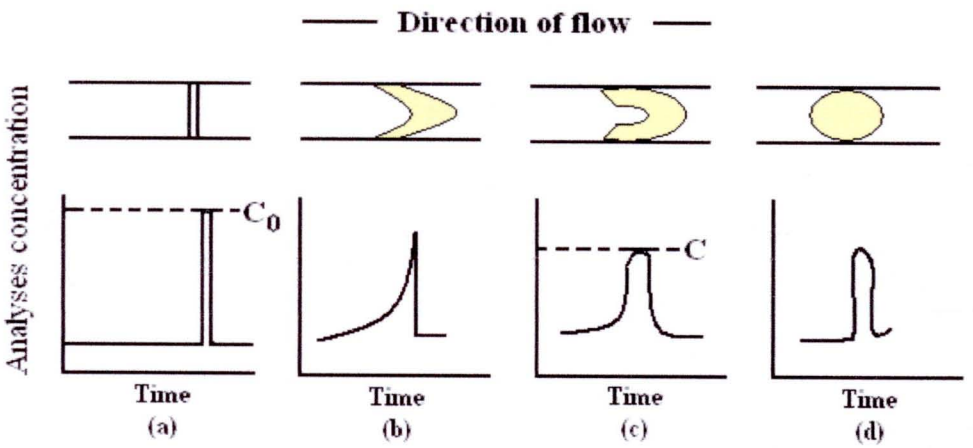

Figure 1.3 Effect of convection and diffusion on concentration profiles of analyses at the detector: (a) no dispersion; (b) dispersion convection; (c) dispersion by convection and radial diffusion; and (d) dispersion by diffusion [17]

A simple dispersion experiment is used to describe to dispersion by mean of the dispersion coefficient as shown in Figure 1.4. A sample solution is homogeneous and has the original concentration C° that would yield a square signal of which the height would be proportional to the sample concentration (Figure 1.4, left). When sample zone is injected, forming a dispersed zone of which form depends on

the geometry of the channel and flow velocity. Therefore, the response curve has the shape of peak reflecting a continuum of concentration (C) of individual elements of fluid.

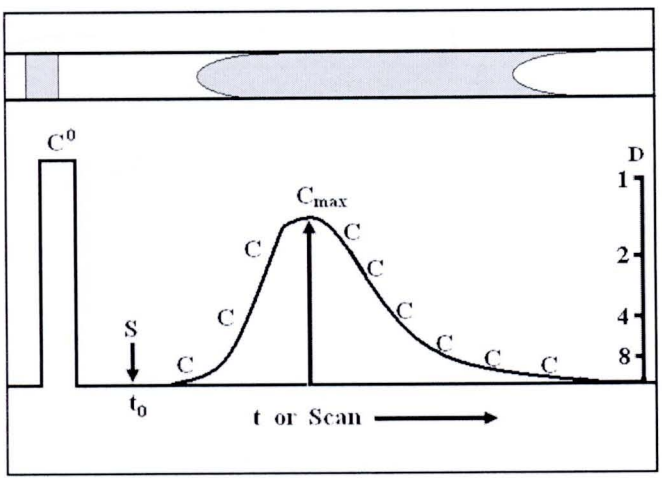

Figure 1.4 Dispersed sample zone in flow system; an original homogeneous sample zone (top left) disperses during its movement through a tubular reactor (top center), thus changing from an original square profile (bottom left) of original concentration C° to a continuous concentration gradient with maximum concentration C^{max} at the apex of peak [1]

The dispersion coefficient D is the ratio of the concentration of sample solution before and after the dispersion process has taken place.

$$D = C^{\circ}/C \qquad (1.1)$$

Where C° is the original concentration of the constituent of interest in to solution to be injected.

C is the concentration of that fluid element of the dispersed solution zone. When the fluid element with the highest concentration is concerned (i.e. readout at FI peak maximum), expressed as:

$$D = C^{\circ}/C^{max} \qquad (1.2)$$

Where C^{max} is the concentration of the constituent at peak maximum of the dispersed zone.

Dispersion is classified according to its magnitude as limited (D = 1-3), medium (D = 3-10), and large (D > 10) dispersion.

(a) Low dispersion concentration systems (D = 1-3) are used whenever one intends to prevent the original concentration of to analyte in the injected fluid zone being diluted by the carrier.

(b) Medium dispersion systems (D = 3-10) are used in signal-channel FI systems, where reagent are used as carrier streams, to attain adequate mixing of sample and reagent.

(c) Large dispersion systems (D > 10) 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 dispersion coefficient D including axial dispersion coefficient, sample volume, carrier flow rate ratio between sample carrier and merging reagent, geometrical dimensions of the tubular reactor, and configurations of manifold component. 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.

1.1.4 FIA instrumentation [18-24]

The FIA analyzer is comprised four basic components consisted of a propulsion system, an injection or insertion system, a transport and reaction system, and reaction system, and a detection system. The basic components of FIA system as shown in Figure 1.1 are:

(a) Propulsion system

The propulsion unit is a component to drive or propel the solution in FIA system. In FIA, the liquid propulsion system which propels the carrier stream needs pulseless feature and reproducible flow rate. The method of FIA can select the appropriate propulsion devices easily. This is the versatility of this technique. Various types of pumps have been used. They include peristaltic pump (set of rollers on a revolving that squeezes flexible tubing to produce a constant, pulsing flow) that is popularly used in FI system, syringe pump, gas-pressure reservoir and reciprocating piston pumps. One or several pump tubes are affixed so that they rest on a minimum of the rollers at all times (Figure 1.5)

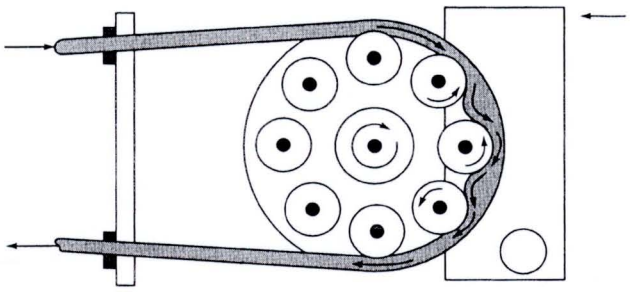

Figure 1.5 Relationship between the rollers of peristaltic pump and the pump tube [25]

(b) Injection system

An injection port by which a well-defined and accurate volume of sample solution is injected into a carrier stream. The injectors employed in FI system are similar to those used in HPLC, but it is necessary for FI valves to extremely high pressure as for HPLC. It is vital that the sample solution is the injected rapidly as a pulse or plug of liquid; in addition, the injections much 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, solenoid valve and multi-injection system.

(c) Transport and reaction system

The transport system is an integral component of flow injection system. The function of transport system is to provide connection between the different components of the system. Normally, the transport system consists of narrow-bore tubes of inner diameter such as PTFE tubing which is chemically resistant, and

adsorbs the least solutes on its surface. Beside, polyethylene or polypropylene tubing is used because it is inexpensive and easy to flange. The connector 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 connector, but basically there are either dual (linear or V-shaped), triple (T-, Y- or W-shaped), or quadruple (usually in the shape of an arrowhead).

A reactor in which the sample zone disperses and reacts with the components of the carrier stream, forming a species (e.g. colored) to be sensed by a flow-through detector, is a major component of the transport system. The main function of reactor is to promote the reproducible radial mixing of two or PTFE tubing. There are many types of the reactor such as straight open tube, coiled tube, mixing chamber, single-bead string reaction (SBSR) and knitted or 3-dimention reactor as shown in Figure 1.6.

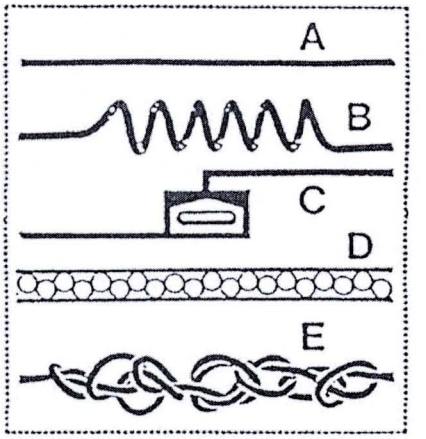

Figure 1.6 The microreactor geometries most frequently used in FIA: A = straight open tube; B = coiled tube; C = mixing chamber; D = single-bead string reactor (SBSR); and E = knitted reactor [1]

(d) Detection system

The detection system is sensing part of the FI manifold, which allows continuous monitoring of a given property of the sample or its reaction product and provide quantitative information of the analyte. In theory, any detection system, which could be adapted for flow through detection may be used as detector for FIA. Choice depends upon the method being used, the sensitivity and selectivity required. These include the spectrophotometer (UV and Visible), atomic absorption and inductively couple plasma spectrometer, chemiluminescence, nephelometer, fluorimeter, and various electrochemical detectors. The signal output from detector is displayed on a chart recorder, microprocessor or a computer as a peak. Recently, microcomputers have been incorporated to provide graphic or numeric displays measured peak heights, areas or widths of the results.

1.2 Sequential Injection Analysis (SIA)

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 [26].

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

2. In SIA, the multi-channel peristaltic pumps 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 handlings of sample and reagent zones necessitate computer control, so automation becomes essential

1.2.1 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 multi-position 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 (Figure 1.7). 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.

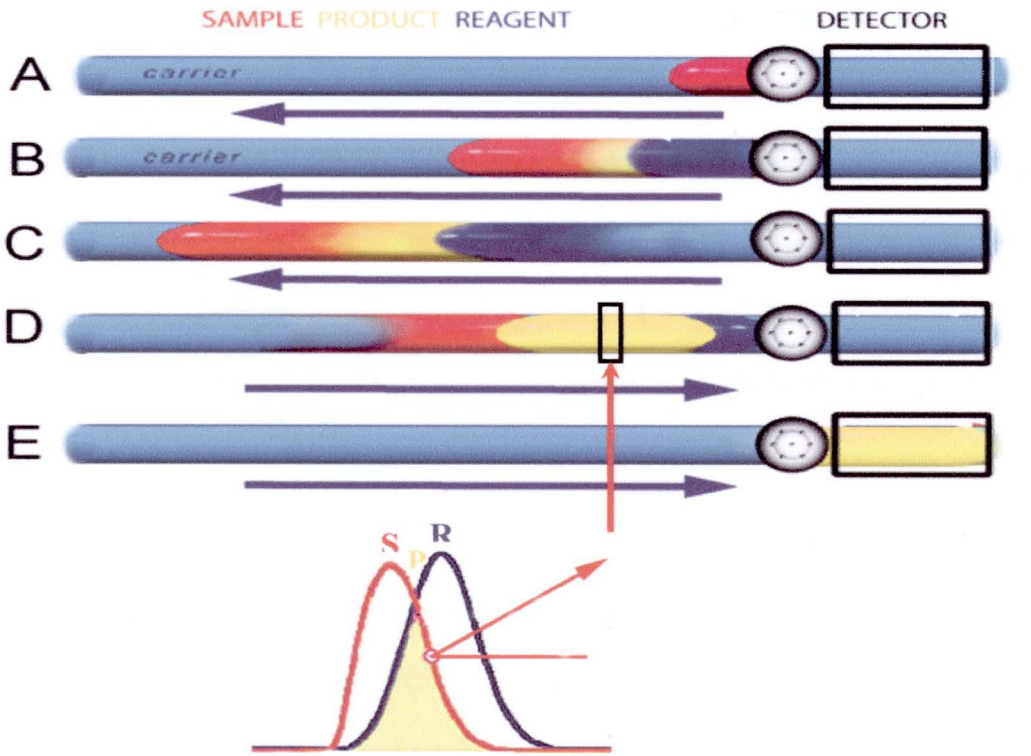

Figure1.7 Structure of injected zones and concentration profiles as seen by the detector; R-reagent; S-sample; P-composite region where the analyte is transformed into a detector product [27]

1.2.2 Sequential Injection Analysis

A general schematic flow diagram of a sequential injection analyzer is depicted in Figure 1.8. The versatility of the technique is centered on a selection valve (SV) where each port of the valve allows different operation to be performed.

An important advantage of SIA is the versatility that the multi-position valve provides [28-31]. Each port of the valve is dedicated to a specific purpose and the combinations of sample, standard, 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 [32-36]. 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 [37-45] are integral and indispensable prerequisite for the success of SIA. Computer control of the SIA system is, therefore, an essential prerequisite [46-52] 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.

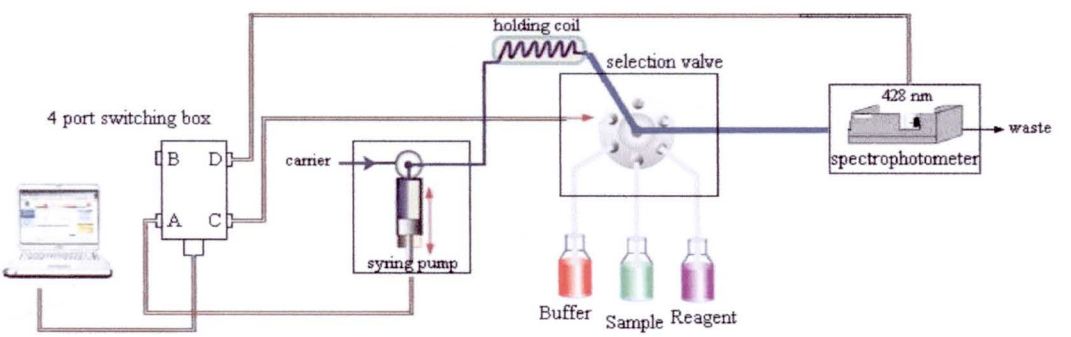

Figure 1.8 Schematic flow diagram of a sequential injection analyzer

The core elements of the SI network were [52]

(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

1.2.2.1 Essential Compartments of SIA [53]

The SIA assemble 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 I precise, reproducible, bi-directional, and able to measure mal volumes. Computer control 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 solution, 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. 0f 0.5 to 0.8 mm tubing is a typical how line for 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 advantage 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 even 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 is used for SIA such as AnalySIA, Flow TEK^{TM}, Lab VIEW, and FIAlab.

1.2.3 SIA Dispersion Zones [53]

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. Figure 1.9 is shown the sample and reagent injection provides the initial input, serving as a starting point for the initial concentration (C°) of analyte (red) and reagent (blue).

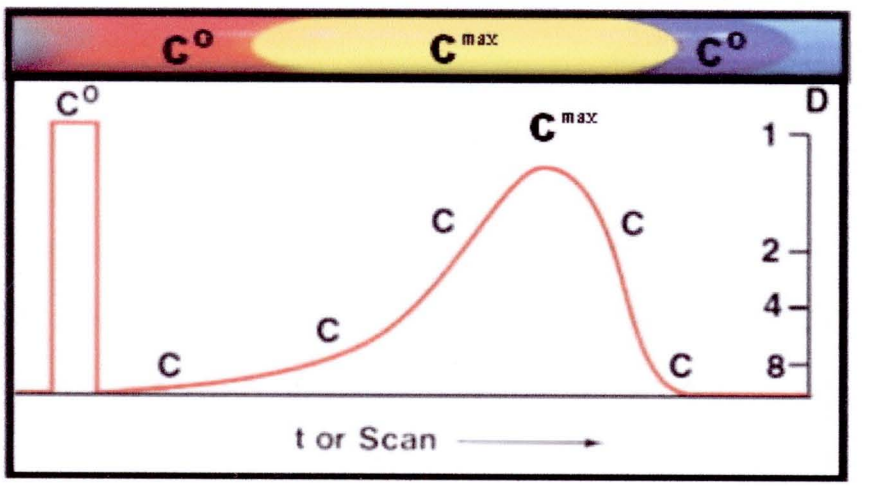

Figure 1.9 Dispersed sample zones of SIA system

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

$$D = \frac{C^0}{C} \qquad (1.3)$$

Where C° 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 under consideration. When the fluid element with the highest concentration id concerned (i.e. readout at SI peak maximum), equation 1.4 is expressed as:

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

$$D = \frac{C^0}{C^{max}} \quad (0<D<\alpha) \qquad (1.4)$$

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 concentration of analyte, product and reagent. To produce 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 ($D = C°/C^{max}$) 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 achieve 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 (T°) to the moment of peak maximum readout (T^{max}) or to the end of the stop flow period.

1.2.3.1 Factors Influencing Dispersion [53]

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.

1.2.4. Mixing and Zone Overlap of SIA

Since the reaction product (yellow) (Figure 1.10) 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.

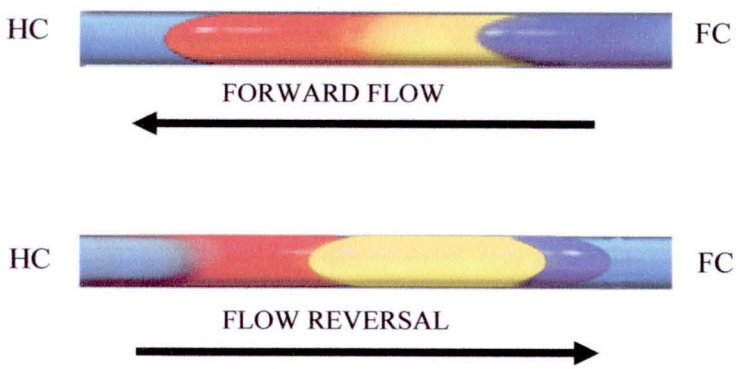

Figure 1.10 Forward reversal flow of SIA system

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.

1.3 Cadmium [54-59]

1.3.1 Physical and chemical properties

Cadmium is a heavy metal, a chemical element with the symbol Cd, atomic number 48 and molecular weight 112.41 g. The soft, bluish-white metal is chemically similar to the two other metals in group 12, zinc and mercury. Similar to zinc it prefers oxidation state +2 in most of its compounds and similar to mercury it shows a low melting point compared to transition metals.

1.3.2 Sources and Uses

Cadmium has a wide variety of sources in the environment and from industry. One source is from ingestion of grown foodstuffs, especially grain and leafy vegetables, which readily absorb cadmium from the soil. The cadmium may occur naturally or as a contaminant and the contaminant include sewage sludge, fertilisers, polluted ground water and mining effluents. Cadmium may also contaminate fish.

Cadmium is also a constituent of alloys, pigments, batteries, metal coatings for example protective coatings on steel, plastics and fertilisers. Occupational exposure may occur from the manufacture of these products and from welding, and smelting of lead, zinc and copper as these occur in mixed ores with cadmium. Cadmium is also found in cigarette fumes (0.007 to 0.35 μg per cigarette) and fumes from vehicles. Residential sites may be contaminated by municipal waste or leaks from hazardous waste sites.

1.3.3 Toxicology [60]

Humans have a daily intake of cadmium form ingestion and inhalation which is around 20 to 40 μg per day, but only to 10% of this absorbed. After absorption, cadmium is transported in the blood bound to albumin. It is taken up by the liver, and, due to its similarity to zinc, causes this organ to induce the synthesis of the protein metallotheionin to which it binds. The cadmium-metallotheionin complex then becomes transported to the kidneys, and it is filtered at the glomerulus, but is reabsorbed at the proximal tubule. Within the renal tubular cells, the cadmium-MT complex becomes degraded by digestive enzymes, which releases the cadmium. Renal tubular cells deal with the release of this toxic substance by synthesizing MT to neutralise it, but eventually the kidneys lose their synthetic capacity for MT. At this point the cadmium has accumulated to a high level in the renal tubular cells, and irreversible cell damage occurs. As can be seen above, the renal cells do not have an effective elimination pathway for the cadmium complex, which means that the half-life in the kidney is between 15 and 30 years. Some members of the local agricultural communities consuming the contaminated rice developed itai-itai disease and renal abnormalities, including proteinuria and glycosuria.

1.3.3.1 Environmental effects of cadmium

Cadmium waste streams from the industries mainly end up in soils. The causes of these waste streams are for instance zinc production, phosphate ore implication and bio industrial manure. Cadmium waste streams may also enter the air through (household) waste combustion and burning of fossil fuels. Because of regulations only little cadmium now enters the water through disposal of wastewater from households

or industries. Another important source of cadmium emission is the production of artificial phosphate fertilizers. Part of the cadmium ends up in the soil after the fertilizer is applied on farmland and the rest of the cadmium ends up in surface waters when waste from fertilizer productions is dumped by production companies. In aquatic ecosystems cadmium can bio accumulate in mussels, oysters, shrimps, lobsters and fish. The susceptibility to cadmium can vary greatly between aquatic organisms. Salt-water organisms are known to be more resistant to cadmium poisoning than freshwater organisms. Animals eating or drinking cadmium sometimes get high blood-pressures, liver disease and never or brain damage.

1.3.4 Determination of Cadmium

The determination of cadmium, there are several analytical techniques for metal analysis including atomic absorption spectrometry (AAS) [61-63], inductively couple plasma mass spectrometry (ICP-MS) [64-65], inductively couple plasma atomic emission spectrometry (ICP-AES) [66-67], spectrometry [68], fluorescence, [69], electrochemical techniques [70] and chromatography [71]. A brief review for cadmium determination is shown in Table 1.1 Although these analytical methods provides sensitive, selective and high sample throughput, but they generated large amount of toxic chemical waste and used expensive analytical instrumentation; some of them needed more analysis time and also complicated system operation and maintenance.

Table 1.1 A brief review of the methods for the determination of cadmium(II)

Method	Condition	Sample	LOD (μgL^{-1}), %RSD	Reference
FAAS	Ultrasound-assisted extraction, flow rate 3.5 ml min−1, A minicolumn : resin (Chelite P)	Mussel	0.011, 2.0%	61
GF-AAS	Liquid phase microextraction (LPME), reagent pH 7, extraction time 10 min	Water	0.0032, 0.1 and 0.4%,	62
ET-AAS	SS-ET AAS, solid sampling platforms, heat sampling graphite tube λ=283.3 nm	Fresh meat	0.13, 14%	63
ICP-MS	Argon, radio frequency (RF), RF generator 1250 kW, plasma flow rate = 15 l min^{-1}, sample flow rate 1.0 mL min^{-1}, temperature of spray chamber = 10 °C	Offal (lung, liver, kidney)	0.35, 2.25%	64

Table 1.1 (Continued)

Method	Condition/Reagents	Sample	LOD ($\mu g\ L^{-1}$), %RSD	Reference
ICP-MS	Electrothermal, Isotope dilution, gas flow 15.0 rate L min^{-1} Dwell time 10 ms, 1 Points per spectral peak	Seawater	0.002, 20%	65
ICP-AES	Preconcentration (DPTH-gel), pH 9.0, DPTH-gel method column flow rates 6.5 mL min^{-1}	Water	1.1, 2.5%	66
ICP-AES Electrothermal vaporization system (ETV)	Ar carrier gas flow rate 0.8 L min^{-1}, drying sample 100 °C, heating 1000°C, tungsten boat furnace, λ = 214.4 nm	zinc-base materials	0.85%	67
Spectrometry	Form complex with (TCPP), diode-array detector, 10 mm quartz cells, Spectra range 400-700 nm	Natural waters	0.9, 2.7%	68

Table 1.1 (Continued)

Method	Condition/Reagents	Sample	LOD ($\mu g\ L^{-1}$), %RSD	Reference
Fluorescence	Self-ordered ring (SOR) technique and α,β,γ,δ - tetra(5-sulfophenyl) porphine, pH 9.1, λ = 510–550 nm	Water	0.5, 3.2-3.8%	69
Anodic stripping voltammetry (ASV)	Mercury microelectrode 1M KNO3 pH = 2, high overpotential −0.1V	Rain water	5.3%	70
Chromatography (HPLC-ICP-MS)	reversed-phase C_{18} column mobile phase A (20mM ammonium acetate buffer), mobile phase B (80% methanol	Plant	0.492,	71

1.3.5 Application of FIA and SIA for Cadmium(II) Determination

FIA and SIA have been used extensively in analytical applications because it is simple, reproducible and inexpensive. The both methods provide automated sample processing and waste reduction due to low reagent consumption. It is also equipped with various detection systems easily for enhancement of efficiency of technique. In addition, the method gives rapid analysis that can apply to use in routine analysis which need to analyze a large number of samples. Therefore, FI method is applied to determination of cadmium (II) in various fields, such as agriculture, food, industry, biochemistry and especially in environment, due to increasing pollution from many factories and increasing population in the world at the present time. Applications of the FIA and SIA for the determination of cadmium (II) shown in Table 1.2 [72-80].

Table 1.2 Applications of the FIA and SIA methods for the determination of cadmium

Method	**Condition/Reagents**	**Sample**	**LOD ($\mu g\ L^{-1}$), %RSD**	**Reference**
FIA Spectrophotometry	Preconcentration : resin Reagents : borate buffer pH 10, H_2O Sample volume : 70 µl λ : 500-650 nm	Surface water	0.12, 4.0%	72

Table 1.2 (Continued)

Method	Condition/Reagents	Sample	LOD ($\mu g\ L^{-1}$), %RSD	Reference
FIA Spectrophotometry	Preconcentration : SRC Reagents : dithizone, acetate buffer (pH 4.9), 0.2 M HCl, 0.5 M, NaOH, Triton X-100, λ : 544 nm	Natural water	5.4, 4.5%	73
FIA Spectrophotometry	Preconcentration : solid reagent (SRC), Reagents : dithizone, $1x10^{-3}$ M NaOH, 1 M HCl, Triton X-100 λ : 544 nm	Surface, well, drinking water	5.4, 3.7%	74
FIA Spectrophotometry	Ion-exchange separation : AG1-X8 resin, Reagent : MG, KI , 0.2M NaOH, λ : 690 nm	Foodstuffs and plant materials	0.11, 2.26%	75

Table 1.2 (Continued)

Method	Condition/Reagents	Sample	LOD ($\mu g\ L^{-1}$), %RSD	Reference
FIA Spectrofluorimetry	Reagents : $3x10^{-4}$ M o-vanillin furoylhydrazone (OVFH), acetate buffer pH 10.00, λ : 494 nm	Sludge	7.6, 0.5%	76
FIA Fluorescence spectrometry	Preconcentration : SPE column : resin Reagents : 200 µgL−1 Co and 1% (v/v) H_2SO_4, NaOH (carrier)	Tea	1.08, 0.97%	77
FIA-ICP-MS	Microwave digestion, SPM sampling Reagents : buffer solution pH 5.0, 0.8 M HNO_3, 0.5 M HCl	Seawater	0.078%	78

Table 1.2 (Continued)

Method	Condition/Reagents	Sample	LOD ($\mu g\ L^{-1}$), %RSD	Reference
FIA-FAAS	Preconcentration : minicolumn PVC tubing , resin (Chelite P) Minicolumn Reagents : 3 M HCl (elution), ultrapure water, Sample pH 8 Sample flow rate: 2.0 mL min^{-1}	Seawater	0.27, 0.5%	79
FIA and SIA stripping voltammetry	Electrode : a bismuth-film electrode (BiFE) Reagents : 0.1 M acetate buffer pH 4.5, 0.1 M HNO_3, 2 mg L^{-1} Bi(III) Preconcentration potential ; -1.2 V, Pulse height : 20 mV	Water	0.001, 5.5%	80

1.4 Rhodamine B [81]

The complexing agent, namely Rhodamine B, N-[9-(20carboxyphenyl)-6-(diethylamino)-3H-xanthene-3-ylident]-N-ethyllethanaminium chloride, is an organic reagent in chemical family of xanthene. The molecular formula is $C_{28}H_{31}N_2O_3Cl$. The molecular weight is 479.02 g/mol. Its appearance is red or brown or green crystal its melting point is 165 °C. It can dissolve in water and alcohol. It is bluish red colored solution when it dissolves in water. It is stable but incompatible with strong oxidizing agent. The maximum absorption of this reagent is at 452.75 nm. The chemical structure of rhodamine B is shown in Figure 1.11.

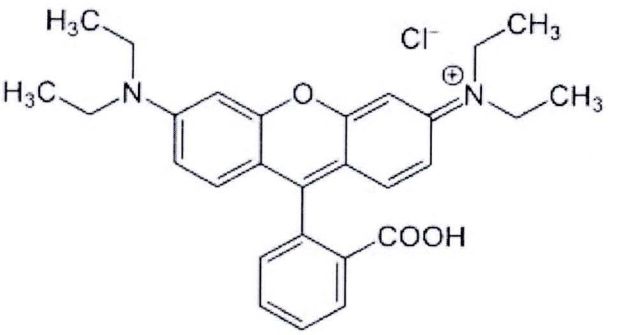

Figure 1.11 Chemical structure of rhodamine B [81]

In this study, FI system for determination of cadmium was developed from conventional spectrophotometric method based on cadmium(II) oxidation of iodide to triiodide in a weak-acid medium., then formation of an ion-association complex of thiiodide with rhodamine B which has the maximum absorption coefficient (ε) of 1.97x105 at 612 nm, in the presence of Hydroxylamine HCl,

which was then accomplished by measurement of the absorbance due to the complex according to the following chemical reaction.

1.5 Research Aims

The aims of this research can be summarized as follows:

1. To design and fabricate flow injection and sequential injection system with spectrophotometric detection for the determination of cadmium(II)

2. To investigate the optimum conditions for the determination of cadmium(II) by flow injection and sequential injection with spectrophotometric method

3. To apply of the proposed flow injection and sequential injection system for the determination of cadmium(II) in wastewater samples