CHAPTER 1

Introduction

1.1. Flow injection analysis (FIA) [1-3]

Nowadays, low cost automatic and user-friendly analytical methods have become attractive for the determination of trace levels of metals in many kinds of samples. Among these, flow injection analysis is a well accepted technique owing to its high sample throughput, cost effective performance and versatile technique that is now firmly established, with widespread application in quantitative chemical analysis.

The designation of FIA was proposed in 1975 by Ruzicka and Hansen. FIA may be defined as the sequential insertion of discrete sample solution into an unsegmented continuously flowing stream with subsequent detection of the analyte. In the first edition, Ruzicka and Hansen defined FIA as "A method based on injection of a liquid sample into a moving unsegmented continuous stream of a suitable liquid. The injected sample forms a zone, which is then transported toward a detector that continuously records the absorbance, electrode potential, or any other physical parameter, as it continuously changes as a result of the passage of sample material through the flow cell". Seven years later, in the second edition, the definition has been revised to read: "information gathering from a concentration gradient formed from an injected, well-defined zone of a fluid, dispersed into a continuous unsegmented stream of a carrier" Furthermore, Fang defines FIA as "A flow analysis technique

performed by reproducibly manipulating sample and reagent zones in a flow stream under thermodynamically non-equilibrated conditions"

1.1.1 Principles of the FIA [2, 3]

The three principles or cornerstones of FIA were identified by Ruzicka and Hansen as sample injection, controlled dispersion of the injected sample zone, and reproducible timing of the movement of the injected zone from the injection point to the detector. This technique is based on a well-defined volume of a sample is injected into a non-segmental carrier stream which is continuously pushed down the narrow tube by the pump with constant flow rate. The reagent stream is continuously pumped down another tube and merged 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 transport 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). The change will be proportional to the analyte concentration using controlled experiment conditions being kept equal both for samples and standards.

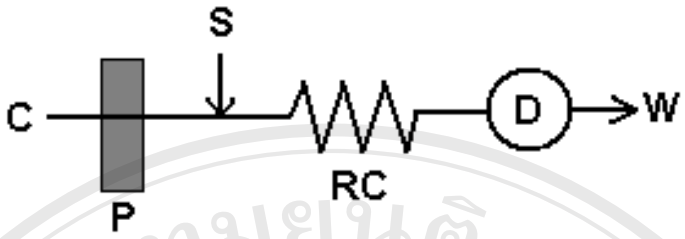

Figure 1.1 Schematic diagram of the basic FIA system showing the various components. P=pump, C=Carrier stream, S=point of sample injection, RC=reaction coil, D=detector, W=waste [2].

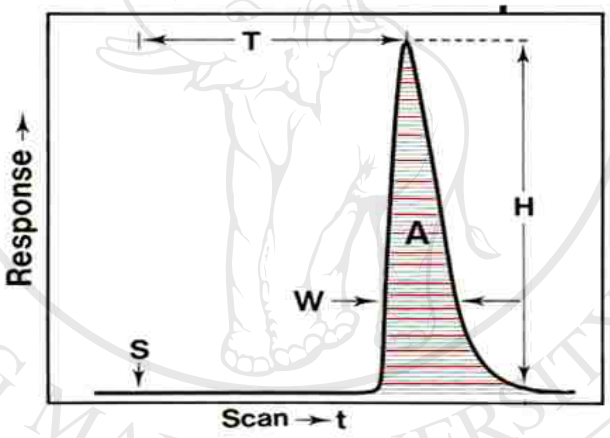

Figure 1.2 The analog output has the form of a FIA peak, the recording starting at s (time of injection t_0). H is the peak height, W is the peak width at a selected level, and A is a peak area. T is the residence time corresponding to the peak height measurement [3].

A typical recorder output has the form of a peak and subsequently recorded as a function of time (Figure 1.2), the height H of which is related to the concentration of the analyte. The time spend between the sample injection S and the peak maximum,

which yields the analytical readout, is the residence time T during which the chemical reaction takes place.

1.1.2 Dispersion of sample zone [2-4]

The most common physical phenomenon in manipulation of sample zone in the FIA system is the dispersion. The shape of the resulting zone is determined by two main processes: convective transport and diffusion transport. Convective transport results from mechanical flows driven by a propelling system. It consists two processes: turbulent and laminar flows (Figure 1.3a). 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 form due to higher velocity at the center of tubing (about 2 times the average velocity).

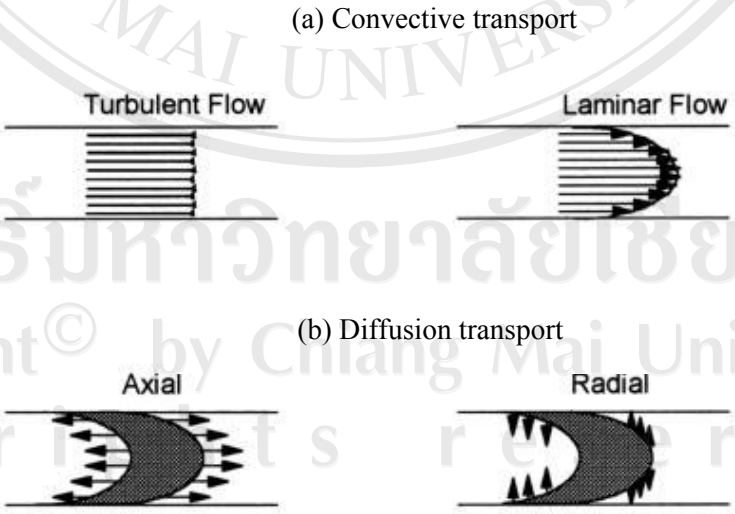

Figure 1.3 General types of transport in closed tubes [3].

Diffusion transport is caused by concentration gradients. There are two types of diffusion processes: axial and radial, as shown in Figure 1.3b. 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 radial diffusion occurs as shown in Figure 1.4c.

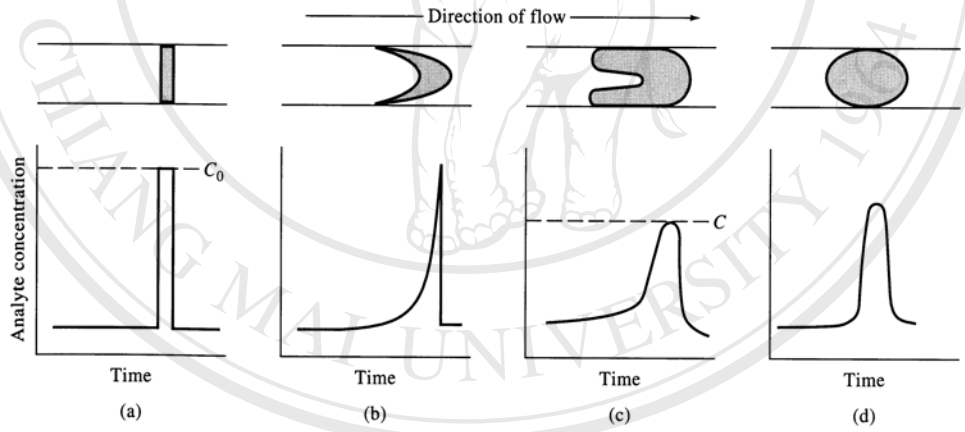

Figure 1.4 Effects of convection and diffusion on concentration profiles of analyses at the detector: (a) no dispersion; (b) dispersion by convective process; (c) dispersion by convective process and radial diffusion; (d) dispersion by diffusion [5].

A simple dispersion experiment is used to describe the dispersion by means of the dispersion coefficient as shown in Figure 1.5. 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 1.5, left). When sample zone is injected, forming 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 concentration (Figure 1.5, right), which composed of a certain concentration (C) of individual elements of fluid.

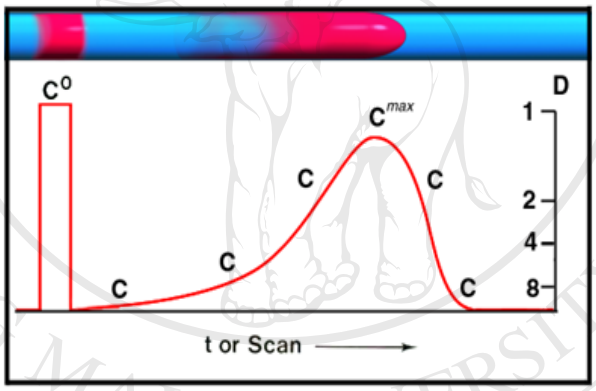

Figure 1.5 Dispersed sample zone in flow system; an original homogeneous sample zone (top left) disperses during its movement through a tubular reactor (top center), thus changing from an original square profile (bottom left) of original concentration C^0 to a continuous concentration gradient with maximum concentration C^{max} at the apex of the peak [4].

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

$$D = C^0/C_{max} \quad (1.1)$$

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 ($D<2$) 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 FIA experimental parameters or factors which may influence 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 value 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.

1.1.3 FIA Instrumentation [1-5]

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

(a) Propulsion system

The liquid propulsion system is a basic unit in all flow analysis system. FIA is a technique based on highly reproducible timing, a feature that demands pulseless 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. A 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 systems, 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 1.6)

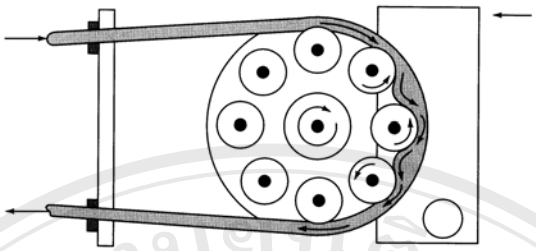

Figure 1.6 Relationship between the rollers of a peristaltic pump and the pump tubes [5].

(b) Injection or insertion system

The injectors employed in FIA are similar in kind to those used in HPLC, but it is necessary for FIA 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 1.0 mm I.D. The connectors used in an FIA system serve the proposed 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) or triple (T-, Y- or W-shaped).

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 sensing part of the FI manifold, 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 by used as detectors for FIA. These include the spectrophotometer, nephelometer, fluorimeter, radiometric and various electrochemical detectors.

1.2 Sequential Injection Analysis (SIA)

Sequential injection analysis (SIA) is automatic and user-friendly analytical methods. SIA is even a more convenient technique because it offers the robustness often required in process analysis. An analytical system based on SIA system is also simpler because it is totally computer controlled and has minimal needs for maintenance and recalibration. Reagent consumption and waste production are also minimal.

Sequential injection analysis (SIA) was first reported by Ruzicka and Marshall at the University of Washington in 1990 [6]. The principles upon which SIA is based are similar to those of FIA, namely controlled partial dispersion and reproducible sample

handling. In contrast to FIA, SIA employs a computer-controlled multiposition valve and pump (generally peristaltic) operated synchronously. In contrast to FIA, SIA employs a computer-controlled multiposition valve and pump (generally peristaltic). The SIA provides various advantages such as widespread recognition in the automation methods, easy to use, low sample and reagent consumption, low cost of analysis, versatility and tremendous flexibility in manifold design, detectors and high precision.

Sequential Injection (SI), the second generation of FIA techniques, is the most versatile one. In its simplest form (Fig 1.7), the sample zone (red) is injected along with a zone of reagent (blue) into a carrier stream (light blue). During flow reversals of the carrier stream, the sample and reagent zones disperse within each other, while on their interface the reaction product (yellow) is formed. A flow through detector records changes in a desired physical parameter when the reaction product reaches the flow cell. The underlying principle of SIA is flow programming. Sequential injection has been microminiaturized in the Lab-on-Valve format, and serves as a platform for Bead Injection.

Figure 1.7 Sequence zone of SIA systems [7].

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 multiposition valve, into a carrier stream using a single syringe pump placed upstream of the valve. Show 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. 1.7). 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.

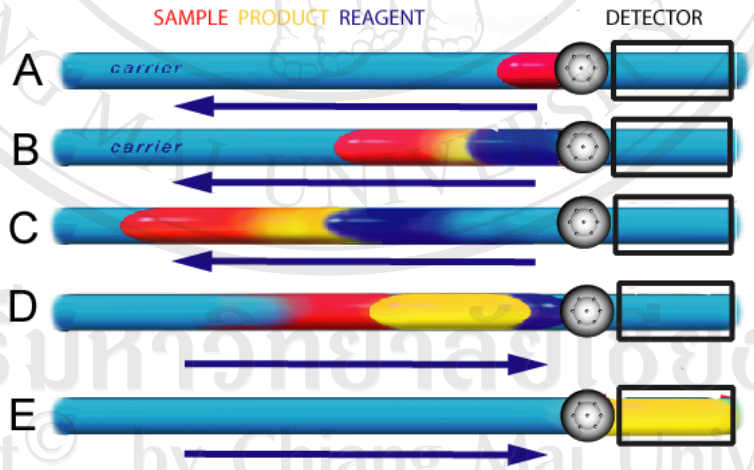

Figure 1.8 Structure of injected zones and concentration profiles as seen by the detector; R-reagent; P-sample; P-composite region where the analyte is transformed into a detector product [7].

1.2.2 Sequential Injection Analyzer

A general schematic flow diagram of a sequential injection analyzer is depicted in Fig. 1.8. The versatility of the technique is centered on a selection value (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 [8-11]. 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 [12-16]. 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 dispersions. Controlled dispersion and reproducible sample handing [17-25] are integral and indispensable prerequisite for the success of SIA. Computer control of the SIA system is, therefore, an essential prerequisite [26-32] 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 reagent should be very low.

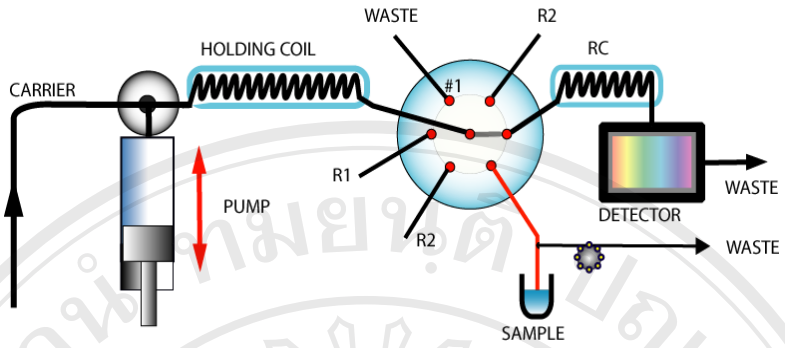

Figure 1.9 Structure flow diagram of a sequential injection analyzer [7].

The core elements of the SIA network were [23]:

(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.

The SIA assembly includes the following essential parts [33]:

(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.

1.2.3 SIA Dispersion Zones [1, 7, 34]

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 (C°) of analyte (red) and reagent (blue).

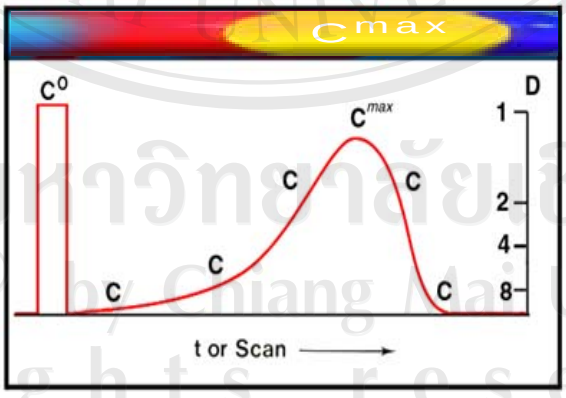

Figure 1.10 Dispersed sample zones of SIA system [7].

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:

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

Where C° is the original concentration of the constituents 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:

$$D = \frac{C^o}{C_{\max}} (0 \langle D \langle \alpha) \qquad (1.3)$$

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 ($D = C°/C_{max}$) 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 (T°) to the moment of peak maximum readout (T_{max}) or to the end of the stop flow period.

1.2.4 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.

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 in injected, zone overlap and mixing are further promoted.

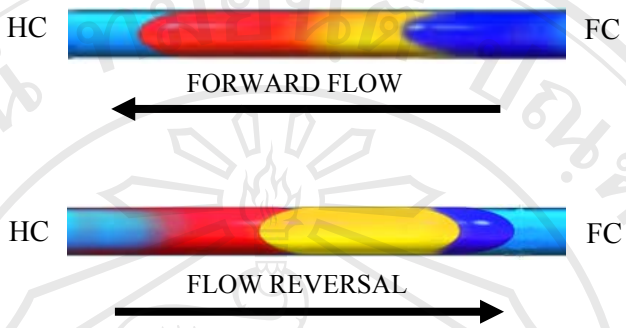

Figure 1.11 Forward and reversal flow of SIA system [7].

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 Iron [36, 37]

1.3.1 Physical and Chemical Properties

Iron (Fe, atomic number 26, atomic mass 55.8) is a shiny, white and malleable metal with a density of 7.9 g/cm^3 and a melting point of 1535 °C (its boiling point is almost 3000 °C). Naturally occurring iron is composed of the isotopes ^{56}Fe (92%), ^{54}Fe (6%) and ^{57}Fe (2%). Iron is a reactive metal, but it is stable in dry air and water free of carbon dioxide. This stability is the result of a coating of iron oxide which prevents further oxidation. Under these conditions, iron behaves similarly to aluminum and chromium. In biological environments iron is oxidized, even by atmospheric oxygen, first to the ferrous form (Fe^{2+}) and then to the ferric form (Fe^{3+}). The compounds FeO, Fe_3O_4, and δ-Fe_2O_3 are interconvertible. The ferric state of iron is very prone to undergo hydrolysis, i.e., to form insoluble ferric hydroxide polymers with hydroxyl ions generally referred to as “rust”. Because of this reaction iron is rarely found in nature in its elementary form, except in meteorites. The toxic intermediate iron pentacarbonyl is an orange liquid. Iron (IV) can be obtained by oxidizing iron (III) bound to certain strongly stabilizing ligands. Aside from the possibility of forming iron (IV) peroxidases, there is no evidence for involvement of iron in oxidation states other than II or III in biochemistry.

In aqueous solutions iron occurs as iron (II) or iron (III) or as organic ferrous and ferric complexes. Under aerobic conditions and when pH approaches neutrality, the ferric form of inorganic salts is by far the most prevalent one. Without the presence of iron chelators, the brown precipitate of ferric hydroxide, with its poorly organized crystal structure, will form. Its solubility is as low as 0.56 μg of iron per liter in a solution of neutral pH. Ferric hydroxide is only very poorly bioavailable.

1.3.2 Sources and Uses

Iron is the second most abundant metal after Al and the fourth most abundant element in the earth's crust. The earth's core is believed to consist mainly of iron and nickel, and the occurrence of iron meteorites suggests that it is abundant throughout the solar system. The major iron ores are hematite (Fe_2O_3), magnetite (Fe_3O_4), limonite [FeO (OH), and siderite ($FeCO_3$). Because of its high abundance, iron is often found as an impurity in other materials.

Iron and its alloy are used mainly in the construction, transportation, machine-manufacturing, and energy industries.

1.3.3 Toxicity and Limiting Concentrations

Iron is a moderately toxic element when compared with other transition metals. However, toxic doses of iron and its compounds can lead to serious problems. Excessive iron accumulation occurs in individuals with exceedingly high rates of erythropoiesis (e.g., thalassemia and sideroblastic anemia) and also in the genetic disorder idiopathic hemochromatosis. In these conditions, large amounts of iron are deposited in the hepatic parenchyma and other parenchymal tissues ultimately leading to tissue damage. In idiopathic hemochromatosis, the excessive body stores of iron can be depleted by phlebotomy. In anemic disorders associated with iron overload, treatment with an iron chelate such as desferrioxamine can reduce the iron accumulations and ameliorate tissue damage.

The amounts in excess of 200 mg/day are considered toxic for man. A study group of the World Health Organization determined that the maximal iron content of drinking water should not exceed 100 μg/L. The United States Public Health Service

regards iron concentrations of up to 300 μg/L as acceptable. Elemental iron of small particle size is inert but may influence the functions of the respiratory organs. Therefore, a maximal concentration of 6 mg iron oxide/m^3 has been recommended. Certain organic iron compounds are very toxic, and maximal concentrations in air should not exceed 0.1 ppm of 0.8 mg/m^3.

1.3.4 Determination of Iron

There are many analytical techniques for the determination of iron including spectrophotometry, inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma atomic emission spectrometry (ICP-AES), absorption spectrometry (AAS), High performance liquid chromatography (HPLC) and ion chromatography (IC). Though all of these methods are highly sensitive, main disadvantages are the necessity of expensive and sophisticated instrumentation [38]. A brief review for iron determination is shown in Table 1.1.

Table 1.1 A brief review of the methods for the determination of iron.

Technique	Condition	Linear range, LOD (ug L^{-1}), %RSD	Reference
ICP-AES	1-(2-Thiazolylazo)-p-Cresol (TAC) as reagent, acetate buffer pH 4.75, λ = 259.93 nm, voltage = 650 W, integration = 3 s	0-2.0, 3.2%	39
ICP-AES	microwave digestion, temperature 0-200 °C, pressures 0-14.0 bar, voltage = 1200 W, λ=238.20 nm	0.05-5.0, 1	40

Table 1.1 (Continued).

Technique	Condition	Linear range, LOD (ug L^{-1}), %RSD	Reference
ICP-MS	Muromac® 1-X8 as resin, 50-100 mesh, 9 M HCL as eluent solution, voltage = 1000 W, integration = 20-60 s	2.5-10, 0.3, 2.5%	41
FAAS	digestion with conc.H_2SO_4 and 30%H_2O_2, flame = air-acetylene, slit width = 0.2, λ=248.8 nm	0-10000, 1%	42
FAAS	dry ashing at 600 °C, flame = air - acetylene, slit width = 0.2, λ = 248.3 nm	500-4000, 0.4%	43
FAAS	Diphenylcarbazide immobilized on an Amberite XAD-2000 resin, pH 9, 0.5 M HNO_3 as eluent, slit width = 0.2, λ = 248.3 nm,	0.32, < 2%	44
GF-AAS	λ = 248.3 nm, slit width = 1.0, pyrolysis temperatures = 1300 °C, atomization temperatures, = 2300 °C	610-1170, < 5%	45
ET-AAS	λ = 344.1 nm, pyrolysis temperatures = 1100 °C, atomization temperatures, = 2500 °C	0.07-150, 0.0002	46

Table 1.1 (Continued).

Technique	Condition	Linear range, LOD (ug L^{-1}), %RSD	Reference
HPLC	2-(5-bromo-2-pyridylazo)-5-diethylaminopheno as reagent, PEEK column with C18 as stationary phase, acetonitrile + water (90:10,v/v) containing sodium dodecyl sulfate as eluent, λ = 585 nm	20-500, 18	47
HPLC	sulfonylcalix[4]arenetetrasulfo-nate as pre-column chelating reagent, chromolith^{TM} performance RP-18e as analytical column, 50 wt.% methanol-water mixture containing TBABr (pH 5.6) as mobile phase, λ = 330 nm	0.00042	48

Nowadays, Flow injection analysis (FIA) and sequential injection analysis (SIA) systems equipped with a simple detector, such as a UV-VIS spectrophotometric detector for iron (III) determination, are the most effective and suitable approach for routine analysis, mainly owing to their simplicity, low instrumentation cost and high sample throughput. A brief review of FIA and SIA with spectrophotometric detection for determination of iron is shown in Table 1.2.

Table 1.2 A brief review of FIA and SIA with spectrophotometric detection for determination of iron.

Technique	Condition	Linear range, LOD (ug L^{-1}), %RSD	Reference
FIA	2-(5-nitro-2-pyridylazo)-5-(*N*-propyl-*N*-sulfopropylamino) phenol (Nitro-PAPS) as reagent, pH = 4.9, λ_{max} = 582 nm	0-100, 1, 1.3%	49
Stopped-FIA	methylene blue as reagent, pH = 2.8, λ_{max} = 663 nm	3-28, 1.80%	50
reverse-FIA	norfloxacin as reagent, pH 3.5 λ_{max} = 435 nm	200-1400, 10, 1.77%	51
FIA	*N*,*N*-dimethyl-*p*-phenylenediammonium dichloride (DmPD), λ_{max} = 554 nm	0-60.0, 5, < 1.5%	52
reverse-FIA	chlortetracycline as reagent, pH = 8.0, λ_{max} = 435 nm	500-20000, 100, 0.43%	53
SIA	tiron as reagent, pH = 1, λ_{max} = 635 nm	112000-145000 0.8%	54

1.4 Eriochrome cyanine R (ECR) [55]

Eriochrome cyanine R (ECR), 5-[α-(3-carboxy-5-methyl-4-oxo-2,5-cyclohexadien-1-ylidene)-2-sulfobenzyl]-3methylsalicylic acid was for the first time introduced in analytical practice by Eegriewe in the year 1937 and has been widely used since then. The molecular formula of ECR is $C_{23}H_{15}Na_3O_9S$ and the molecular weight is 536.4. The structure of ECR is shown in Figure 1.12.

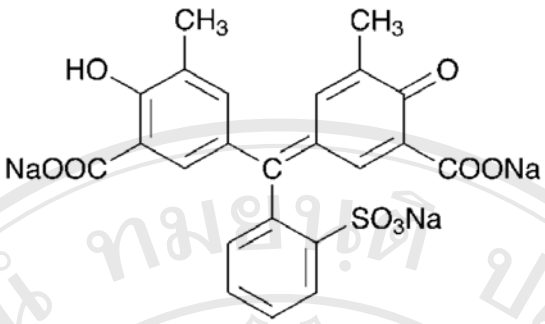

Figure 1.12 The structure of Eriochrome cyanine R [56].

Eriochrome cyanine R belongs to triphenylmethane dyes which are useful in analytical practice as sensitive spectrophotometric reagent for the determination of a large number of metal ions. Eriochrome cyanine R is a good chromogenic reagent for the determination of various metal, and it ternary complexes with cetyltrimethyl ammonium bromide (CTMAB) as cationic surfactant have been described and used for spectrophotometric analysis in solution [57]. The surfactant sensitized systems are based on the ability of certain surfactants to sensitize the binary complexes of the metal ion with chromogenic ligands. The ligands, most often, are metallochromic indicators and for sensitization, cationic surfactants are used [58]. The review of ECR reagent for determining some metals were concluded in Table 1.3.

Table 1.3 A brief review of ECR reagent for the determination of some metals.

Element	Surfactant	pH/acidity	Molar absorptive, ε (L mol^{-1} cm^{-1}) / λ_{max}, (nm)	Beer's law range (mg L^{-1})	Samples	Reference
Mg	Cetyltrimethylammonium bromide	11.5	-	-	Silicates low aluminium	59
Ga	Cetyltrimethylammonium bromide	5.4	1.20×10^5 ,(588)	0-0.6	Aluminium metal	60
Fe	Cetyltrimethylammonium bromide	4.0	1.28×10^4 ,(635)	0-0.3	Analytical-grade sodium hydroxide	61
Cr	Cetyltrimethylammonium bromide	-	7.8×10^4 ,(590)	0-14	Ores	62

Table 1.3 (Continued).

Element	Surfactant	pH/acidity	Molar absorptive, ε (L mol^{-1} cm^{-1}), / λ_{max}, (nm)	Beer's law range (mg L^{-1})	Samples	Reference
Al	Cetyltrimethylammonium bromide	7.5	-	0.8-4	Natural water	63
Cu	Cetyltrimethylammonium bromide	-	9.64×10^4,(590)	0.05-0.6	-	64
V	-	4.7-5.0	- ,(563)	0.6-25.0	Natural water	65
Sc	Cetyltrimethylammonium bromide	6.5	5.6×10^4,(610)	-	Monazite	66

1.5 Research Aims

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

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

3. To apply the proposed procedures to the determination of iron in water samples.