CHAPTER 1
INTRODUCTION

1.1 Flow Injection Analysis

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

1.1.1 Principle of FIA [1-8]

FIA is a method based on an injection of a well-defined volume of liquid sample into a continuously moving nonsegmented carrier stream of reagent(s) or suitable solvent(s) in a controlled way. The injected sample forms a zone that physically disperses in a small tubing, and reacts with component(s) 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 parameter 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 the form of a peak (in height, 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.

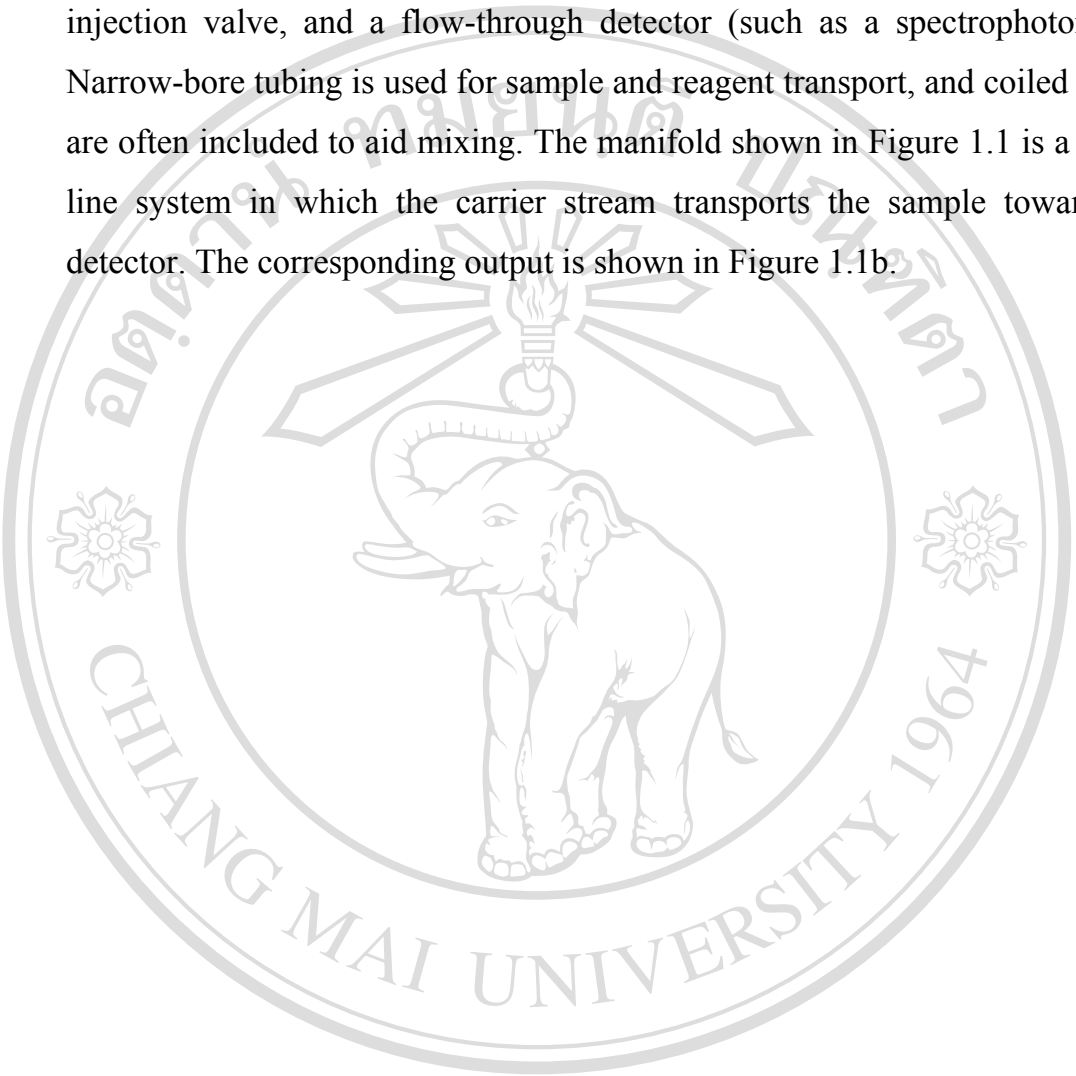

Figure 1.1 The simplest FIA system:

(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].

If the method requires more than one reagent, additional streams can be merged with the carrier stream at suitable points in the manifold. Simultaneous FI system can be performed by designing split-line manifolds in which the sample is injected into more than one flow channel and undergoes a different reaction in each channel. This can be achieved either by splitting the carrier stream after injection or by connecting two injection valves in series in two separate reaction systems. Other components that can easily incorporated into FI systems such as gas dialysis units, for the diffusion of a gaseous analyte 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 [9-11]

FIA is classified as normal FIA (nFIA) mode and reverse FIA (rFIA) mode. The rFIA method was first discovered by Johnson and Petty [9] which is based on an injection of a small amount of reagent(s) into a flowing stream of sample and/or standard solution, in contrast with normal flow system which is based on 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 reagents ought to be spared. This configuration is suitable for field applications in which the sample is in abundant supply (as is the case 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 of reagent(s) discharged to waste, which is advantageous if reagents which affect the environment are used in the system.

1.1.3 Dispersion [11-14]

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 1.2. In fact, the flow injection analyses usually perform under conditions in which dispersion by both convection and radial diffusion occurs as shown in Figure 1.2c.

Figure 1.2 Effects 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 [11].

A simple dispersion experiment is used to describe the dispersion by means of the dispersion coefficient as shown in Figure 1.3. 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 1.3, 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 (Figure 1.3, right), which composed of a certain concentration (C) of individual elements of fluid.

Figure 1.3 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 the peak [1].

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

$$D = C°/C$$

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:

$$D = C°/C^{max}$$

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

(b) Medium dispersion systems (D = 3-10) are used in single-channel FI systems, where reagents 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, flow rate ratio between sample carrier and merging reagent, geometrical dimensions of the tubular reactor, and configurations of manifold components. Varying the values of these parameters confers a significant degree of control over the dispersion characteristics and facilitates optimization of a flow injection system for many diverse applications.

1.1.4 FIA instrumentation [15-21]

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

Figure 1.4 Relationship between the rollers of a peristaltic pump and the pump tube [21].

(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 withstand extremely high pressures as for HPLC. 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, 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 connections 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. Besides, 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 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).

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 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 (SBSR) and knitted or 3-dimension reactor as shown in Figure 1.5.

Figure 1.5 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 qualitative and quantitative information of the analyte. In theory, any detection system, which could be adapted for flow through detection may be used as detectors for FIA. 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 out put 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.

1.1.5 Application of FIA

FIA has been used extensively in analytical applications because it is simple, sensitive, reproducible and inexpensive. This method provides 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 give 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 interested compounds 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.

Table 1.1 Examples of applications of FIA in various fields.

Field	Analyte	Method	Reference
Environment	Chromium	Electrothermal atomic absorption spectrometry	22
	Iron	Chemiluminescence	23
	Manganese	Spectrophotometry	24
	Arsenic	Hydride generation atomic absorption spectrometry	25
	Selenium	Flow injection analysis with inductively coupled plasma atomic emission spectrometry	26
Agriculture	Manganese	Potentiometry	27
	Ozone	Spectrophotometry	28
	Selenium	Flow injection analysis with hydride generation inductively coupled plasma mass spectrometry	29
Industry	Zinc	Solid phase spectrophotometry	30
	Silver	Flame atomic absorption spectrometry	31
	Ruthenium	Spectrophotometry	32
Food	L-glutamate	Potentiometry	33
	Oxalic acid	Spectrophotometry	34
	Copper and Cobalt	Fluorimetry	35
	Selenium	Flow injection analysis with hydride generation atomic absorption spectrometry	36
Biochemistry	Carbaryl	Chemilumenescence	37
	Calcium	Fluorimetry	38
	Selenium	Flow injection analysis with spectrofluorimetry	39
Pharmaceutical	Metronidazole	Amperometry	40
	Salicylamide	Chemiluminescence	41
	Neostigmine	Spectrophotometry	42

1.2 Selenium [43-46]

1.2.1 History

Selenium was discovered in 1817 by Swedish chemistry Jons Jacob Berzelius [43] by an examination of the red deposit which was found in the lead chambers used in the manufacture of sulphuric acid, when the sulphur dioxide was produced from pyrites.

1.2.2 Occurence

Selenium is rare element, almost never found in the native state. It is not abundant in the earth's crust (less than one part in ten million). However, some soils in the western regions of the United States contain selenium to the extent of several parts per million. This element occurs free in nature, is often associated with native sulfur. There are no ores from which selenium can be mined as a primary product. It is found principally in sulfide minerals. Occasionally, it is found in the form of selenites of other metals.

1.2.3 Preparation

Selenium is obtained as by-products of several industrial chemical processes. This element is obtained principally from the anode mud of the cell used in the electrolytic refining of copper, the flue dust from roasting sulphur and pyrites, and the lead chamber sulphuric acid process.

1.2.4 Physical properties

Selenium is an element belonging to group 16 (VIB) of the periodic table. This element has an atomic number of 34, an atomic weight of 78.96, a specific gravity of 4.79 (grey) at 20°C, a melting point of 217°C and a boiling point of about 685°C (grey). The electronegativity value is 2.48. There are several allotropic forms, including grey, red, and black selenium. There are six

natural isotopes. Selenium is nonmetal and it exists in the form of grey crystals which is the most stable form of semimetallic appearance at ordinary temperature.

1.2.5 Chemical properties

Selenium can exist in five oxidation states -2, 0, +2, +4 and +6. The common oxidation states are –2, +4, and +6 as selenide, selenite and selenate. Chemically, it resembles sulphur. Selenium burns in oxygen to form its most stable oxide, selenium(IV) dioxide. Selenium is active element combining easily with metals and nonmetals. Selenium is not dissolved by nonoxidizing acids.

1.2.6 Distribution to environment

Environmental selenium compounds originate from the glass and electronics industries, as well as from the combustion of fossil fuels and uses in agriculture. In the environment, Se levels generally are in the 0.1-400 ppb range in natural waters, 1 ppb in the atmosphere and 0-80 μg/g in soils. Se in plant is partly dependent on where it is grown. In recent years, interest in selenium as an essential element in biological systems has increased because not only it is essential trace nutrition but it can be toxic to living organism at higher concentrations as well. Biological role involves it is essential to humans about stimulatory; carcinogenic and teratogenic, and animals about growth. A safe and adequate daily intake of selenium for adults is between 50 and 200 μg per day. The biological effects of selenium are known depend on its chemical form, selenite is more toxic than selenate, it is important to measure the amount of each selenium species in the environment. The information about the availability and mobility of selenium in the environment and its biogeochemical cycle, however, requires the additional knowledge of the different chemical forms and oxidation states which this element can exist. The

inorganic Se species most frequently found in water and soils are selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}). Although selenium levels in natural waters are generally low, high concentrations of this element may occur in association with geological features or due to the corrosion of household plumbing by weakly acidic water. The selenium ions including selenite and selenate in water can more or less affect the health of living things. Therefore, it is necessary to investigate accurate and precise knowledge of the selenium species present in the environment.

1.2.7 Deficiency signs

In humans, low selenium levels in blood have, however, been observed in a few pathological conditions, including colonic, gastric and pancreatic carcinoma and cirrhosis. Muscle weakness, pain and tenderness are signs of deficiency. If deficiency is severe, Keshan disease (which adversely affects the heart) and Kashin-Beck disease (involving arthritis) can result. Both are very rare in industrialized countries. Keshan disease, which primarily affects children and women, is present in China, where selenium levels in soil are extremely low. The incidence of Keshan disease can be reduced by supplementation of the diet with sodium selenite. Chronically low selenium intake may result in an increase in the risk for cancer, heart disease and a weakened immune system. The signs in animals depend upon vitamin E status. For example, selenium- and vitamin E- deficient animals present numerous problems involving skeletal, heart, muscle, liver and pancrease. Low concentrations of Se in feed are common, therefore, Se is a feed additive. Selenium is added to feed as either sodium selenite or sodium selenate.

1.2.8 Effect of excess selenium

Low levels of selenium is an essential element in the diet whereas at higher concentrations it is toxic. Because of its toxicity and cumulative effect

in the body, selenium presents a health problem when it occurs in food or water even in minute amounts.

1.2.8.1 Selenium overexposure in humans

The public health aspects of excess selenium exposure first became of concern after the discovery that selenium caused alkali disease in animals, since it was quickly realized that selenium from grains or vegetables grown on seleniferous soils could also enter the human food chain. Daily doses of 2-3 mg over a period of time can cause toxicity. Selenium toxicity is characterized by skin rashes, a garlicky breath, nausea, vomiting, liver disease, hair and fingernails problems, depression and muscle weakness. In certain rural Chinese communities, chronic intakes of very high amounts (several milligrams per day) of selenium were linked to skin, hair and nail abnormalities which disappeared upon consuming regular selenium intakes. In rural farming and ranching families living in the Great Plains area of the United States known to have a history of alkali disease in animals. Uncertain symptoms of anorexia, general pallor, and malnutrition were reported, and more pronounced disease states such as bad teeth, yellowish discoloration of the skin, skin eruptions, chronic arthritis, diseased nails, and subcutaneous edema were seen.

1.2.8.2 Selenium toxicity in animals

Three distinct forms of selenium poisoning in animals are as follows:

(a) selenosis: acute selenium poisoning is caused by the consuming of a large quantity of highly seleniferous accumulator plants in a short period of time. Signs of severe distress include labored breathing, abnormal movement and posture, and prostration and diarrhea, and are followed by death in a few hours.

(b) Chronic of the blind-staggers type: blind staggers occurs in animals that consume a limited amount of selenium accumulator plants over a period of

weeks or months, but this disease has not been produced in animals by the administration of pure selenium compounds. The affected animals have impaired vision, stumble, and finally they succumb to respiratory failure.

(c) Chronic of the alkali-disease type: selenium is the active principle in forages and grains. Animals that consume grains containing 5 to 40 mg selenium/kg over a period of several weeks or months suffer from chronic selenious, known as alkali disease. Signs include liver cirrhosis, lameness, hoof malformations, loss of hair, and emaciation. The disease is associated with the consumption of seleniferous grains or grasses.

1.2.9 Therapeutic uses

Selenium is an essential micronutrient in the human diet. It functions as a component of enzymes involving in antioxidant protection and it involves metabolism of thyroid hormone. Selenium may enhance the immune system in its role as a cofactor of the enzyme glutathione peroxidase, which also fights toxic substances in the body. Selenium works with vitamin E in preventing free-radical damage to cells by inhibiting the production of free radicals. Because it prevents free-radical production, selenium appears to protect against heart disease, strokes and some kinds of cancer. Recent studies suggest that selenium supplements may prevent some forms of cancer and may boost immune status. Studies with selenium for treatment of psoriasis, arthritis and macular degeneration have also been conducted. Relevant Studies are as follows:

(a) Protecting against cancer-causing compounds

Beta-carotene and selenium, separately and in combination, appear to inhibit pancreatic carcinogenesis.

(b) Depression and selenium deficiency

Low selenium levels were found to depress mood, thus the belief that selenium could somehow influence brain function.

(c) Heart attack and selenium

According to Havard researchers, no evidence has been found demonstrating a link between increased plasma selenium and a reduced risk of myocardial in farction at the current levels of selenium intake in the U.S.

(d) HIV, selenium and beta-carotene

Selenium supplementation (and beta-carotene to a lesser degree) could have some influence in protecting cells against oxidative stress in subjects with human immunodeficiency virus (HIV) who are known to be deficient in selenium and vitamin A.

1.2.10 Uses

Selenium is widely used in many different ways. The applications of selenium are as follows:

(a) Electricity: Grey allotrope of selenium is light-sensitive. The most important uses of selenium are in the manufacture of photoelectric cells and rectifiers due to remarkable increasing in electric conductivity upon exposure to light. The photoelectric cells are utilized for the operation of alarm apparatus and various automatic devices such as automatic doors, escalators, and especially for sound films and telegraphic transmission of pictures. It is also used as semiconductors. Rare earth selenides, such as Gd_2Se_3, have been proved recently as semiconductor with valuable properties.

(b) Chemistry: Se is used in organic chemistry. Considerable quantities of selenium dioxide are used as oxidizing agent in organic chemical synthesis or a catalyst in a number of reactions.

(c) Glass: Se is used in glass industry. It is used as decoloriser of glass because it neutralized the green colour due to ferrous ion. Elemental selenium is used sometimes as an additive to glass, for the purpose of adding a pink, red or reddish yellow color.

(d) Rubber: Se is used as rubber additive in rubber industry for making rubber more resistant to abrasion, selenized rubber is used as a fire-proof material for cables.

(e) Drug: Minor amounts of compounds of selenium such as selenide have been used for the treatment of dandruff.

(f) Agriculture: Se is used as insecticides and fertilizers.

(g) Pigments: Se is used in the manufacture of pigments and paints. Some metal selenides is used in production of various enamels.

(h) Metallurgy: Se is used in production of alloys. The machinability of various alloys of iron and of copper is improved by its presence.

(i) Food: Se in the food supplements (singly or in combinations with vitamins and minerals) may be organically bound in the form of selenomethionine as in brewer's yeast, or it may be present in an inorganic form as selenite or selenate.

1.2.11 Determination of selenium [47-59]

There has been an increasing demand for sensitive methods for the determination of selenium content in different matrices such as environmental, agricultural, and biological materials. There are various methods have been used to determine the element such as spectrophotometry, fluorimetry, etc. The method chosen depends on several factors such as instruments, researchers, and so on.

(a) Gravimetric method

Gravimetrically, selenium is commonly determine by reduction of selenium (IV) to the elemental state. The milligram amounts of selenium can be determined gravimetrically by using many reagents such as ascorbic acid, sulphur dioxide, hydroxylammonium chloride that selenium(IV) is successfully converted to elemental selenium. Selenium(IV) is gravimetrically precipitated with many precipitants such as hydroxylamine, lanthanum hydroxide.

(b) Titrimetric method

Selenium can be determined by iodometric method. In this method, iodide is oxidized to iodine with selenium(IV) and then obtained iodine is titrated by sodium thiosulphate using starch solution as indicator. The method can be determined selenium at part per million levels. This method has disadvantages in terms of sensitivity and sample throughput.

(c) Spectrophotometric method

The spectrophotometric method can determine selenium contents at microgram levels. This method is specific to determine selenium(IV) in aqueous solution. The common reagent used are 3,3-diaminobenzidine providing low sensitivity at maximum absorption of 340 nm and using toxic reagent, and 2,3-diaminonaphthalene (DAN) giving a brightly colored piazselenol compound, which is extract in cyclohexane and measured colorimetrically at 490 nm. The serious interference such as ferrous ion can reduce the concentration of DAN by oxidizing it. The method is time consuming and the reagents used are toxic and relatively unstable.

(d) Fluorimetric method

The 2,3-diaminonaphthalene is the common reagent used in this method. Selenium(IV) reacts with the reagent to produce a strongly fluorescent piazselenol compound extracted in cyclohexane and then is measured fluorometrically. High concentrations of nitrite may interfere with the formation of the fluorescent piazselenol complex. This method can examined selenium at submicrogram levels. The method is sensitive, but organic reagent are toxic.

(e) Hydride generation-atomic absorption spectrometry (HG-AAS)

Atomic absorption spectrometry with hydride generation is the technique most often used to determine selenium in environmental samples because of its sensitivity, speed and minimization of interferences. This method consists of the conversion of selenium in its selenium(IV) to form gaseous selenium

hydride (hydrogen selenide), and subsequently analyze by flame atomic absorption spectrometry at 196.0 nm. Mercury and arsenic at part per million levels may inhibit the formation of selenium hydride.

(f) Graphite furnace atomic absorption spectrometry (GFAAS)

The sample containing selenium is determined by placing the sample in the furnace where it are dried, charred (ashed or pyrolyzed), and then the sample is atomized, the absorbance being determined during atomization. The effective interferences such as sulfate and phosphate suppress the response during atomization.

(g) Other methods

Several analytical techniques have been reported for determining selenium including atomic spectrometric methods such as inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS); the sensitivity of ICP-MS for selenium is limited because the isotopes amenable to ICP-MS are present in low natural abundance (<10%), neuclear methods such as instrumental neutron activation analysis (INAA), radiochemical neutron activation analysis (RNAA), instrumental photon activation analysis (IPAA); although, nuclear techniques are very sensitive, but its use is limited by the high cost, time involved and the requirement for trained staff, electrochemical techinques, such as cathodic stripping voltammetry, also offer a highly sensitive approach to the determination of selenium in most matrices; however, there are associated matrix effects, liquid chromatography, gas chromatography, X-ray spectrometric analysis and so on. Although these techniques offer the required precision and sensitivity for the determination of selenium in various matrices, many of them have to be dismantled after a single analysis and therefore require considerable operator manipulation.

FIA is a modern method used in determination of selenium compounds due to high sample throughput, high precision and inexpensive apparatus and instruments. Some previous researches on FIA method for determination of selenium will be discussed.

Ahmed *et al.* [39] described the determination of selenite and selenate contents using FIA method with spectrofluorimeter in natural water samples based on oxidation of non-fluorescent reagent 2-(α-pyridyl)thioquinaldinamide (PTQA) in sulfuric acid solution with selenite as a result of fluorescent product at excitation wavelength of 350 nm and emission wavelength of 500 nm. Selenate was reduced to selenite in reduction coil with photoreactor. The sample throughput of 25 samples per hour was obtained. The relative standard deviation was 0.1-2.0%. This method was also applied to food and biological samples.

Anderson and Isaacs [26] presented the determination of selenium contents in environmental samples by FIA incorporated with hydride generation inductively coupled plasma atomic emission spectrometer (HG-ICP-AES) for determination of water samples. Samples were digested with the mixture of nitric, perchloric, and sulfuric acid. After that hydrochloric acid was added to reduce selenate to selenite. The digested sample was introduced to HG-ICP-AES by FIA method. The detection limit of the technique is 1.0 ppb and the percentage recoveries of 87 to 108% were obtained. This method is applied to environmental samples.

Oernemark and Olin [36] presented the determination of selenium contents in drinking water sample by FIA equipped with HG-AAS. Water sample was treated by wet digestion with permanganate and digested sample was passed through column containing Dowex 1×8 that in conjuction with HG-AAS. The results obtained from the proposed method agree well with those obtained by HG-AAS. The advantages of the proposed method are continuous and rapid analysis.

Olivas and Donard [60] presented the speciation of selenite and selenate by FIA in conjunction with HG-ICP-MS using microwave to reduce selenate to selenite within 5 min. The detection limits of the method were 6 and 8 pg/L for selenite and selenate, respectively. This method was applied to aqueous solution.

Tao and Hansen [61] described the determination of trace amounts of selenite in water samples by FIA equipped with hydride generation atomic absorption spectrophotometry (HG-AAS). Sample was concentrated in FI system based on reaction between selenite and 20 ppb lanthanum hydroxide in ammonium buffer (pH 9.1) producing precipitate collected in microline reactor and eluted the precipitate with hydrochloric acid and then directed to HG-AAS. The sample throughput of 33 samples per hour and detection limit (3SD) of the method of 0.001 ppm were obtained with precision (%RSD) of 0.7 (n = 11) for 0.5 ppm selenite. This method was applied to determination of selenium in tap water and ground water.

Quijano *et al.* [29] presented the determination of selenium contents in water samples by FIA equipped with HG-ICP-AAS. Sample was done by adding hydrochloric acid and then boiling the mixture at 100°C to reduce selenate to selenite. After that the solution was passed into HG-ICP-MS. Detection limit of the method was 35 ng/l and the precision of less than 5 was obtained with recovery of 92 to 104%. The serious interferences were some transition metals.

1.3 Rhodamine B [62]

The complexing agent, namely Rhodamine B, N-[9-(2-carboxyphenyl)-6-(diethylamino)-3H-xanthene-3-ylidene]-N-ethylethanaminium chloride, is an organic reagent in chemical family of xanthene. The molecular formular 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 blulish red colored solution when it dissolves in water. It is stable but incompatible with strong oxidizing agents. The maximum absorption of this reagent is at 542.75 nm. The chemical structure of rhodamine B is shown below.

Figure 1.6 Chemical structure of rhodamine B [62].

In this study, FI system for determination of selenium was developed from conventional spectrophotometric method based on selenium (IV) oxidation of iodide to triiodide in a weak-acid medium, then formation of an ion-association complex of triiodide with rhodamine B which has the maximum absorption coefficient (ε) of 1.97×10^5 at 580 nm, in the presence of poly(vinyl alcohol) (PVA), which was then accomplished by measurement of the absorbance due to the complex according to the following chemical reactions.

$$SeO_3^{2-} + 6H^+ + 6I^- \longrightarrow 2I_3^- + Se + 3H_2O$$

$$I_3^- + RhB^+ \longrightarrow [RhB^+][I_3^-]$$

1.4 Aims

The main purposes of this research are as follows:

1. To develop a flow injection system with spectrophotometric detection for speciation of Se(IV) and Se(VI).
2. To evaluate the proposed flow injection system by analysis of Se(IV) and Se(VI) standard solutions.
3. To apply the recommended method to the determination of Se(IV) and Se(VI) in real water samples.