CHAPTER 1

INTRODUCTION

1.1. Flow Injection Analysis (FIA)

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 acceptable technique owing to its high sample throughput, cost effective performance and versatile technique that is now firmly established, with widespread applications in quantitative chemical analysis. This technique was first reported in 1975 (Ruzicka and Hansen, 1975). Since then, FIA methods have become widespread and of great interest due to its capability for rapid automated analysis of wet chemical samples using simpler and more flexible equipment.

1.1.1 Principles of the FIA

The three principles or cornerstones of FIA were identified by Ruzicka and Hansen (1981) 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. More recent developments of FIA showed that sample injection should be understood in a much broader sense. It follows that neither the timing of zone movement nor the control of dispersion should be restricted to those of the injected samples. Although dependent on the other two principles, the central issue of FIA is the control of dispersion, which is the very basis for under non equilibrium

conditions. A schematic diagram for a basic instrumental setup of a flow injection system is illustrated in Figure 1.1. The change will be proportional to the analyte concentration using controlled experiment conditions being kept equal both for samples and standards.

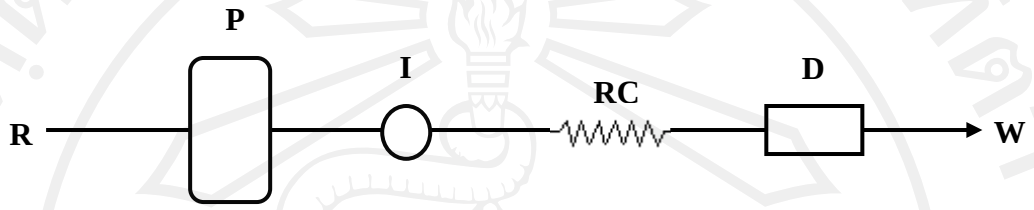


Figure 1.1 The Schematic diagram of the basic FIA system showing the various components. P=pump, R=Carrier stream, I=point of sample injection, RC=reaction coil, D=detector, W=waste

A simple FI system consists of a pump that provides a constant flow rate carrier stream, while a small, reproducible volume of a liquid sample is injected into the liquid carrier via an injector. A discrete sample zone is then transported through a narrow bore tube towards a detector.

1.1.2 Dispersion of Sample Zone

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 is illustrated in 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 form due to higher velocity at the center of tubing (about 2 times the average velocity).

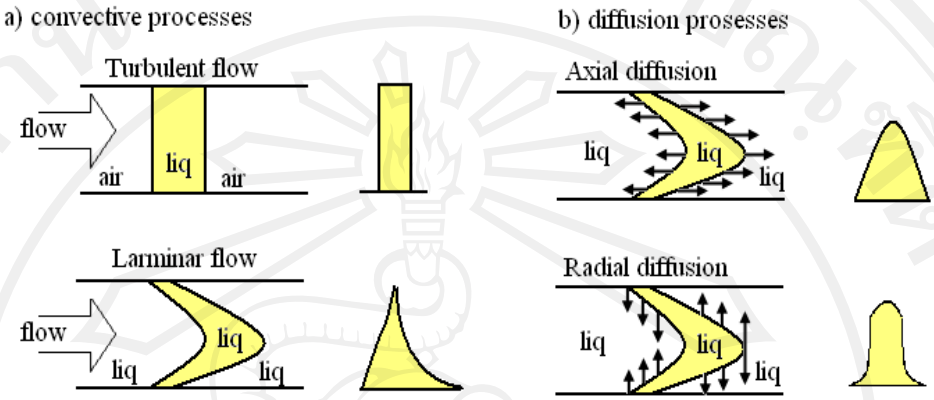


Figure 1.2 General types of transport in closed tubes and the recorded profiles at the detector (Valcarcel and Castro, 1987)

Diffusion transport is caused by concentration gradients. There are two types of diffusion processes: axial and radial, as shown in Figure 1.2b. Axial diffusion is insignificant compared to convective flow, but the radial diffusion contributes more significantly to sample dispersion. This process, termed "secondary flow", results in a washout effect accounting for the low mutual contamination of samples successively injected into the carrier stream and also serves to limit band spreading. At low flow rate it may even be the major mechanism for dispersion. In fact, flow injection analyses usually performed under conditions in which dispersion by both convective process and radical diffusion occurs as shown in Figure 1.3c.

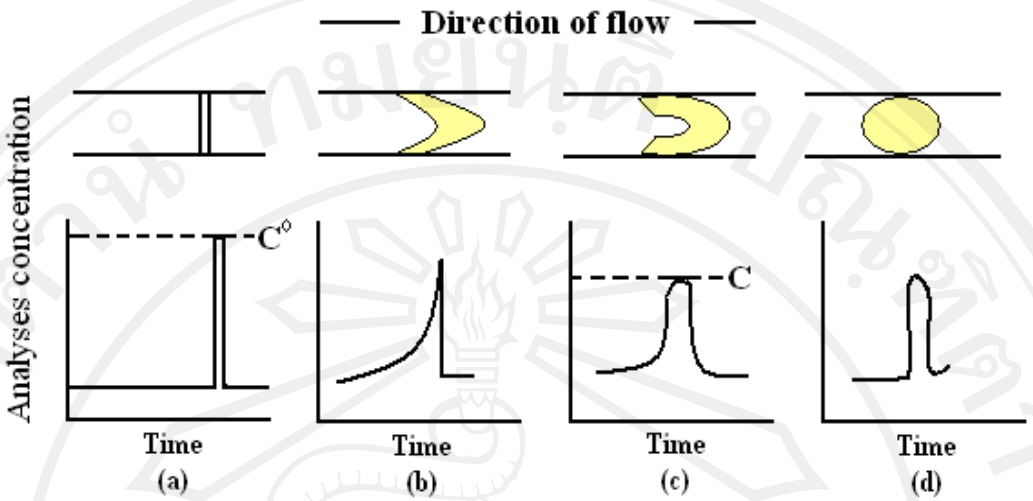


Figure 1.3 Effects of convection and diffusion on concentration profiles of analyses at the detector: (a) no dispersion; (b) dispersion by convective process; (c) dispersion by convective process and radial diffusion; (d) dispersion by diffusion (Skoog and Leary, 1992)

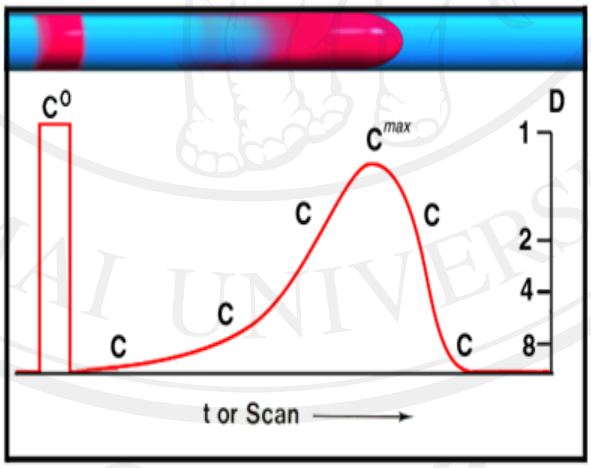


Figure 1.4 Dispersed sample zone in flow system; an original homogeneous sample zone (top left) disperses during its movement through a tubular reactor (top center), thus changing from an original square profile (bottom left) of original concentration C^0 to a continuous concentration gradient with maximum concentration C^{max} at the apex of the peak (Ruzicka and Hanson, 1988)

A simple dispersion experiment is used to describe the dispersion by means of the dispersion coefficient as shown in Figure 1.4. A sample solution, contained within the vale cavity prior to inject, is homogeneous and has the original concentration C^0 that, if it could be scanned by detector, would yield a square signal the height of square signal the height of which would be proportional to the sample concentration (Figure 1.4, left). When the sample zone is injected, it follows the movement of the carrier stream, 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 1.4, right), forming a concentration gradient, within which on single element of fluid has the same concentration of sample material as a neighboring one. It is useful, however, to view this continuum of concentration as being composed of individual elements of fluid, each of them having a certain concentration of material C, since each of these elements is potential source of readout.

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

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

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 .

Dispersion can be affected by various factors. Such factors include sample injection volume, physical dimensions of the FIA system (that is, the lengths and internal diameters of the tubes), residence time, and flow rate of the carrier stream (Kellner et al, 1998).

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

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 1.5)

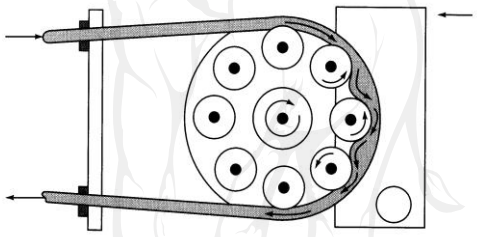


Figure 1.5 Relationship between the rollers of a peristaltic pump and the pump tubes (Skoog and Leary, 1992)

(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 and may be used as detectors for FIA. These include the spectrophotometer, nephelometer, fluorimeter, radiometric and various electrochemical detectors.

e) Data processing unit

In its simplest form, a FI system may be manually operated, with the detector connected to a chart recorder for data acquisition and display. More advanced and commercial systems may be fully or partially automated, utilizing an on-board microprocessor system for timing of analyzer operations (i.e., sampling and injection), signal display, calibration and calculation of the results. More commonly, however, this control is achieved using a portable computer connected to a suitable analogue or digital converter, and a control/ data acquisition software (McKelvie, 1999).

1.2 Separation Methods in Flow Injection Analysis

Several methods of separation are often incorporated in flow injection analysis in order to obtain more efficient analytical results when working on food samples. Improved selectivity and sensitivity are the main targets for the incorporation of these methods to address hurdles arising from the analysis of food due to complex matrices. Separation methods are classified according to the type of interface involved during the mass transfer process and these include solid-liquid, liquid-liquid, and liquid-gas separation techniques (De Mattos et al, 1995). More specific examples are: gas diffusion (Baadenhuijsen and Seuren-Jacobs, 1979), hydride generation (Pacey et al, 2002), and gas diffusion (Amador-Hernandez and Luque de Castro, 2000) for gas-liquid separations; solvent extraction (Karlberg and Thelander, 1978 and Bergamin F^{o} et al, 1978), microdistillation (Lane et al, 1999), and dialysis (Hansen and Ruzicka, 1976 and Mayer et al, 1996) for liquid-liquid separations; and solid-phase extraction (Pereira and Arruda, 2003 and Pérez-Serradilla

and Luque de Castro, 2007), ion-exchange (Lopes et al, 1996 and Couto et al, 1998), precipitation (Yebra et al, 1995), and supercritical fluid extraction (Rahmanian and Ghaziaskar, 2008) for solid-liquid separations. However, these techniques are beyond the scope of this study and therefore will not to be discussed in deeper details. Nevertheless, one specific method has been essential to the study, namely, the gas diffusion method, and hence, shall be discussed more thoroughly in the succeeding paragraphs.

1.2.1 Gas diffusion method

Gas diffusion is a separation technique that is based on the combination of two principles – membrane permeation and evaporation. This technique, when applied in flow injection analysis, is effective for the determination of volatile and semi-volatile substances in complex, dirty samples requiring no further purification. The dehydration of organic solvents and the removal of organic compounds from aqueous solutions are some of the applications of a gas diffusion method (Lipski and Cote, 1990).

During gas diffusion in flow injection analysis, the volatile compounds that are liberated from the liquid donor stream evaporate into an air space, eventually permeate through a hydrophobic membrane, and subsequently absorbed by an acceptor flowing stream. The liquid sample is introduced into the donor chamber of the gas diffusion unit by injection or by continuous aspiration through the donor stream. Separation is achieved due to a temperature difference, which results in a vapor pressure difference across the membrane, hence providing a driving force for the separation (Luque de Castro and Papaefstathiou, 1998). The application of an auxiliary energy, such as heating, microwave or ultrasonication, usually aids in

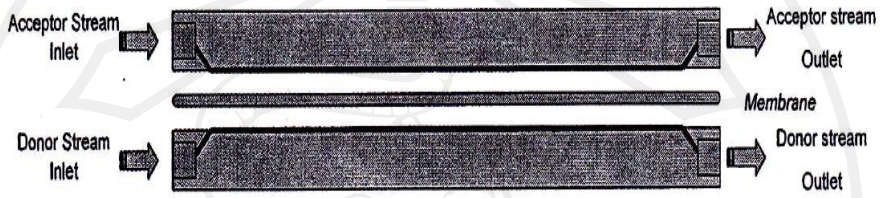
shortening the pretreatment step in driving an effective separation especially when solid samples are involved (Bryce et al, 1996). Other pretreatment steps that may aid in the effective separation of certain analytes in complex samples include: altering the pH of the carrier or donor stream in cases such as of those dealing with weak electrolyte samples; increasing the ionic strength of the medium by adding salt, and hence, causing a salting out effect; and, transforming the analyte into a more volatile form by subjecting it to a derivatization process prior to gas diffusion (Delgado-Reyes et al, 1998).

Gas diffusion unit is usually made of methacrylate or Perspex, which is a transparent material, in order to permit visual monitoring of the liquid sample/donor stream level in the donor chamber during analysis. The parts of the gas diffusion unit are usually clamped together using four metallic rods, screwed between two aluminum supports. An illustration of a gas diffusion unit incorporated in a flow injection system is shown in Figure 1.6 .

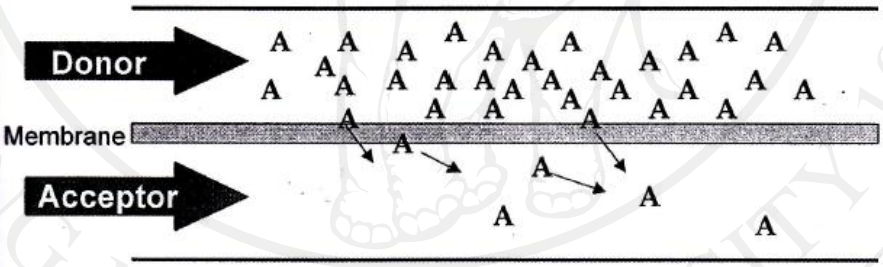
Gas diffusion unit consists of a lower donor chamber and an upper acceptor chamber separated by a hydrophobic membrane with an optional support. (Papaefstathiou and Luque de Castro, 1995: 2063, Wang et al, 2000: 177 and Mataix and Luque de Castro, 1999: 23). Moreover, an air-gap is provided within the donor chamber between the liquid sample and the lower surface of the hydrophobic membrane.

Gas Diffusion has a disadvantage of decreasing mass-transfer efficiency through the membrane due to the presence of an air-gap in the donor chamber of the gas diffusion unit, thus decreasing the sensitivity of the method. Nevertheless, this air-gap between the donor chamber and the hydrophobic membrane in the gas diffusion

unit provides a higher degree of selectivity during analysis by preventing the direct contact of the analytical sample that is injected through the donor stream and of the hydrophobic membrane, hence, preventing any contamination caused by other unwanted compounds that are also present in the complex dirty sample.



(a)



(b)

Figure 1.6 Gas diffusion unit: (a) Cross sectional diagram of a gas diffusion unit incorporated in a flowing system with PTFE membrane which help enhance reproducibility during analyte detection, (b)Transport of analyte from donor stream to acceptor stream

For this reason, the use of a gas diffusion unit becomes more importantly advantageous when incorporated in FIA since the presence of an air-gap in the gas diffusion unit prevents problems arising from membrane fouling due to the analysis of very contaminated complex matrices that may possibly cause blocking of the

membrane pores by large molecules with high molecular weights that are present in the dirty sample. Another problem that may be prevented is the possible effect of corrosive materials that may cause deterioration to the hydrophobic membrane, thereby increasing the lifetime of the membrane (Luque de Castro and Papaefstathiou, 1998).

Due to the lack of sensitivity of merely using flow injection analysis particularly when incorporated with gas diffusion technique, different sensitive detection methods have been applied in conjunction with flow injection methods.

1.3 Chemiluminescence Detection

1.3.1 Chemiluminescence Principle

Chemiluminescence (CL) is currently an attractive detection technique because of its high sensitivity, low detection limit, selective reactions and wide dynamic range achievable with relatively simple equipment, especially in conjunction with a flow injection system. As CL is defined as the production of light arising from a chemical reaction, the only important equipment required is a sensitive photomultiplier tube (Trojanowicz et al., 2003).

Chemiluminescence is defined as a process producing electromagnetic (ultraviolet, visible, or near-infrared) radiation as a result of a chemical reaction (usually an oxidation) in which one of the reaction products occurs in an excited state and emits light when returning to its ground state (Mervartová et al., 2007). Normally, the amount of energy released during a chemical reaction dissipates as heat. Therefore, CL is not a very common phenomenon. The CL phenomenon can be observed when a chemical reaction yields an electronically excited intermediate or

product that either luminesces or donates its energy to another molecule, which then luminesces. If radiation is emitted through energy-transfer; the process is usually called chemi-excitation. When the chemiluminogenic reaction is enzymatic and/or occurs within living organisms, such as fireflies, the phenomenon is called bioluminescence (BL). The process by which CL is generated is the same as that for photoluminescence (e.g., fluorescence and phosphorescence), except that the former does not require light excitation source.

The intensity of light (I_{CL}) is generally observed to increase initially and later decrease with time as the reactants are consumed (Grayeski, 1987). This can be described by the equation:

$$I_{CL}\,(\text{photon/s}) = \Phi_{CL}\,(\text{photons/molecules reacted}) \times dC/dt\,(\text{molecules reacted/s})$$

where Φ_{CL} is the CL efficiency and is equal to the efficiency of production of excited states (number of excited-state molecules per number of molecule reacted) multiplied by the emission efficiency (number of photons emitted per number of excited-state molecules), and dC/dt is the number of molecule reacting per unit of time. Because the emission intensity is determined by the rate of the chemical reaction, measurement of emission intensity can be used as the basis of quantitation for any species whose concentration is determined by the rate of the chemical reaction.

A CL process can be divided in two steps, which involve: (a) direct CL, where the analyte emits light upon oxidation; and (b) chemi-excitation or indirect CL, where the analyte plays a different role in the chemiluminescent reaction mechanism

(Townshend, 1990; Calokerinos et al., 1995). A general description for each CL reaction process is as follows:

(a) Direct CL

A direct chemiluminogenic reaction takes place in two steps, which can be simplified as follows:

Excitation reaction: $A + B \longrightarrow C^*$

De-excitation reaction: $C^* \longrightarrow C + hv$

The excited molecule C* can either be the final or the intermediate product of the reaction. The total Φ_{CL} of a chemiluminogenic reaction lies in the range 1-20% but very often can be much less than 1%, while bioluminogenic reactions may have efficiencies of up to 100%.

(b) Chemi-excitation

A great variety of applications is based on chemiluminogenic reactions, which take place through indirect or chemi-excitation. In this case, the excited molecule (C*) is an ineffective transmitter, but in the presence of an appropriate fluorophore CL can be obtained by sensitization of CL reaction. Sensitized CL involves the infection transmitter (C*) transfer its excitation energy to the effective fluorophore (F), which is added into the system as soon as collision each other resulting in an effective transmitter known as F*. After excited molecule (F*) retruns to ground state The CL emission take place. A considerable increase of luminescence intensity may be achieved by induct CL. The general reaction process can be represented as:

Energy transfer step: $C^* + F \longrightarrow C + F^*$

De-excitation reaction: $F^* \longrightarrow F + hv$

The molecule F is a fluorophore, which can also be excited by absorption of radiation (photoluminescence). If a molecule does not have the ability to participate in a chemiluminogenic reaction, it may still be converted into another molecule which does have this property. Alternatively, the molecule can be converted into a fluorophore by derivatization. The fluorophore can then be chemi-excited.

For a chemical reaction to be suitable for CL detection, it should meet three essential requirements:

1) The reaction molecule (C) should be capable of receiving the energy released from the reaction to form product (C*), and the efficiency of this process should be sufficiently high.
2) The product (C*) should be capable of luminescing under the condition of the reaction; the intensity of the radiation should be sufficiently high. Alternatively, a suitable acceptor molecule, F, capable of accepting energy should be available for chemi-excited and subsequently emission of radiation.
3) The energy required for excitation must be supplied by the reaction in one step, if possible. In a multi-step reaction, the necessary energy must be released in a single step since the excitation step should occur instantaneously.

The limiting factor for the occurrence of CL is that the energy required for luminescence in the visible region lies between 44 and 71 kcal mol^{-1}. Therefore, a minimum requirement for CL is that the reaction produces 44 kcal mol^{-1} of energy. A

variety of organic compounds meet this requirement and in some instances, their chemiluminogenic properties have been thoroughly studied during redox reactions. CL reactions can occur very rapidly (<1 s) or can be long lasting (>1 day), the duration being influenced by a wide range of the reaction condition. This presents a challenge to the development of an instrument for CL monitoring.

1.3.2 Measurement of CL

Traditional analytical uses rely on reactions that produce highly intense emission over periods as long as several minutes; this allows the reactions to be monitored with fairly simple analytical instrumentation and procedures. Figure 1.7 shows the variation of the CL intensity with the reaction time. The height or the peak area of the CL signal is proportional to the analyte concentration and is used for quantitation of the analyte (Mestre et al., 2001; Zamora et al., 2001).

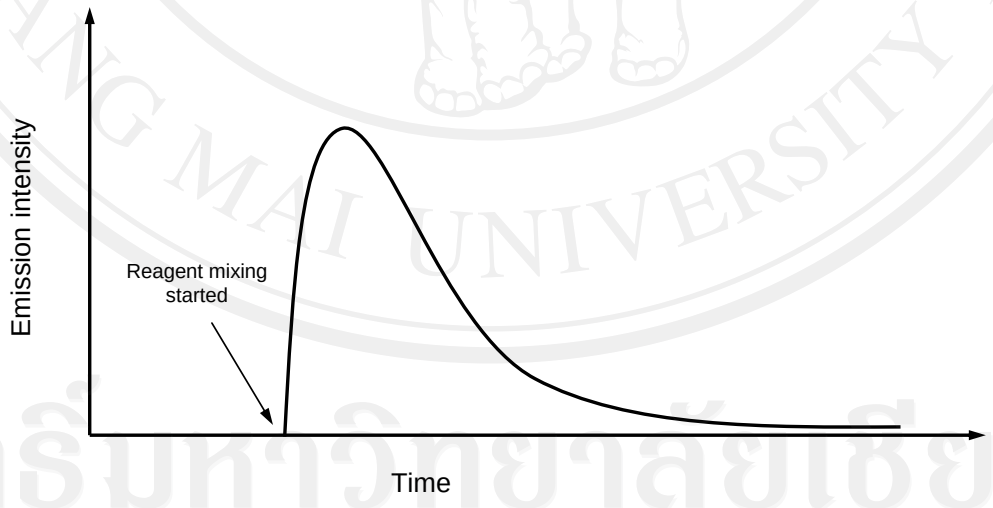


Figure 1.7 Temporal variation of the CL intensity

In the continuous flow method, the chemiluminescent reagent and the analyte are continuously circulated through separate channels prior to merging into a single stream. Figure 1.8 illustrates a basic FI-CL setup, where the chemiluminescent

reaction takes place upon merging of the reagent stream with the sample carrier stream. Satisfactory mixing is achieved effectively at a T-piece, but Y-junctions are equally efficient for this purpose.

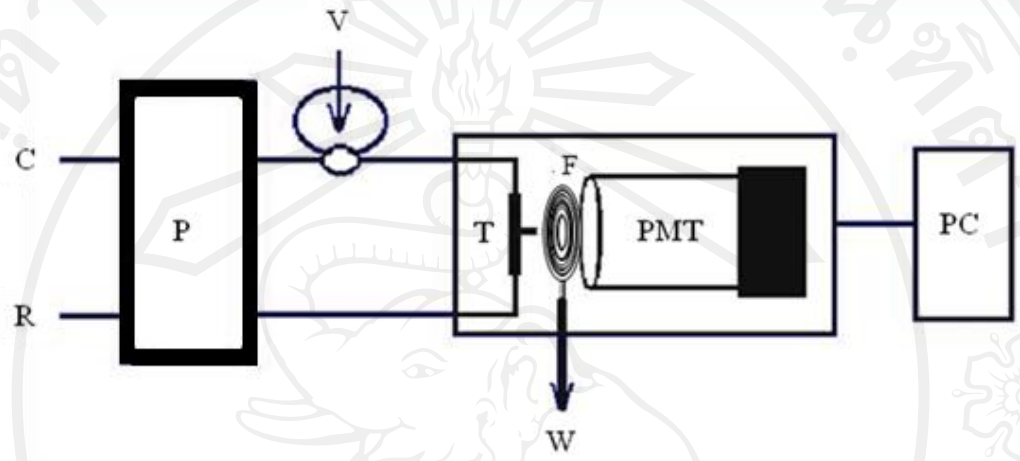


Figure 1.8 Schematic configuration of a basic flow injection chemiluminescence (FI-CL) manifold: C = carrier solution; R = reagent; P = peristaltic pump; V=injection valve; T=T-shaped connector; F=Flow cell; PMT=Photomultiplier tube; PC=Personal computer; W=Waste

During chemiluminescence detection in FIA, the peak height or the peak area of the CL signal detected is proportional to the concentration of the analyte injected into the flowing system. This data is used for the quantification of the analyte. Figure 1.9 shows a typical peak signal obtained during FI-CL analysis in which the CL signal starts to rise upon merging of the CL reagent with the sample carrier stream.

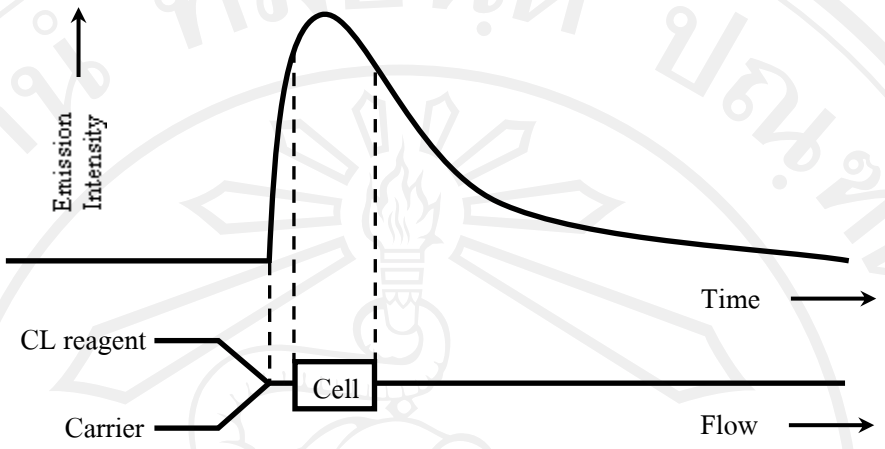
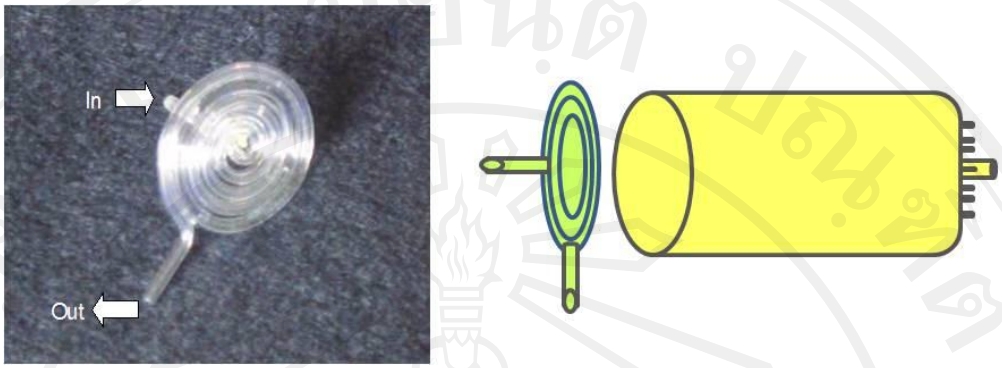


Figure 1.9 Typical CL signal showing CL intensity as a function of time. The observation window is positioned relative to the emission-time curve during flow-through CL measurements.

Chemiluminescence signals in flowing systems are maximized through manipulation of important factors such as the mixing of reagents and the detection cell. The preferred configuration for a CL flow cell is the spiral flat coil cell, which has been reported by Burguera et al. (1980). In this flow cell (Fig. 1.10), the light emission is directly observed in the spiral coil as the chemiluminescence mixture is formed.

The distance between the flow cell and the merging point of the reagents should be as short as possible, the length of the detection coil should be long enough to give a satisfactorily intense signal, and optimal reaction conditions should be set in order to obtain maximum CL intensity after mixing rather than to maximize the overall yield (Varcarcel and Luque de Castro, 1987)



(a) (b)

Figure 1.10 Flow cell: a) Spiral flat coil cell. (b) Spiral flat coil flow cell positioned against the window of a photomultiplier tube (PMT)

1.4 Photocatalysis

Photo-Catalysis is defined as "acceleration by the presence of as catalyst". A catalyst does not change in itself or being consumed in the chemical reaction. This definition includes photosensitization, a process by which a photochemical alteration occurs in one molecular entity as a result of initial absorption of radiation by another molecular entity called the photosensitized (Remin et al.,1995).

In chemistry, photocatalysis is the acceleration of a photoreaction in the presence of a catalyst. In catalysed photolysis, light is absorbed by an adsorbed substrate. In photogenerated catalysis, the photocatalytic activity (PCA) depends on the ability of the catalyst to create electron–hole pairs, which generate free radicals (hydroxyl radicals: ·OH) able to undergo secondary reactions. Its comprehension has been made possible ever since the discovery of water electrolysis by means of the titanium dioxide. Commercial application of the process is called advanced oxidation process (AOP). There are several methods of achieving AOP's, that can but do not

necessarily involve TiO_2 or even the use of UV light. Generally the defining factor is the production and use of the hydroxyl radical.

1.5 Nanoparticles

Nanoparticles are particles that have one dimension that is 100 nanometers or less in size. The properties of many conventional materials change when formed from nanoparticles. This is typically because nanoparticles have a greater surface area per weight than larger particles; this causes them to be more reactive to certain other molecules (Xu and Cui, 2007 and Safavi et al., 2008)

There is no strict dividing line between nanoparticles and non-nanoparticles. The size at which materials display different properties to the bulk material is material dependant and can certainly be claimed for many materials much larger in size than 100nm. Definitions certainly become more difficult for materials that are a very long way from being a sphere, such as carbon nanotubes for example. One of the aims for these materials is to grow them into long tubes, certainly not 'nano' in length, but as they have a diameter in the order of 3nm for a single walled tube, they have properties that distinguish them from other allotropes of carbon, and hence can be described as 'nanomaterials'. This sort of nanomaterial has led to the extension of the idea of nanomaterials being considered as such if any one of their structural features are on a scale of less than 100nm, that cause their properties to be different from that of the bulk material.

Many of these nanomaterials are made directly as dry powders, and it is a common myth that these powders will stay in the same state when stored. In fact, they will rapidly aggregate through a solid bridging mechanism in as little as a few

seconds. Whether these aggregates are detrimental will depend entirely on the application of the nanomaterial.

Zinc oxide nanoparticles, the novel classes of semiconductors have recently received considerable interest because of their increasingly intensively explored materials that bridge bulk material and molecular behavior and offer novel chemical properties (Li et al., 2009). Thus, zinc oxide nanoparticles were chosen as catalyst for the chemiluminescence reaction based on the luminol-hydrogen peroxide system which provided new approaches to enhance the inherent sensitivity and expand new applications.

In recent years, nanoparticles have been a rapidly growing in analytical field which involves the use as catalysts for a variety of organic and inorganic reaction. For example, gold nanoparticles as a novel nanocatalyst on luminol-hydrazine chemiluminescence flow injection system (Safavi et al., 2008), carbon nanoparticles as catalytic support for chemiluminescence of sulfur compounds in a molecular emission cavity analysis system (Safavi et al., 2009) and platinum nanoparticles on the luminol–hydrogen peroxide–platinum colloids chemiluminescence flow injection system for the determination of phenollic compounds (Xu and Cui, 2007). The results indicated that low detection limit, increasing the rate and selectivity of the chemical reaction were observed. Thus, zinc oxide nanoparticles seem promissing to be used as catalyst on the chemiluminescence reaction of lumimol-hydrogen peroxide system after on-line photochemical reduction of nitrate to nitrite in order to improve the sensitivity.

1.6 Nitrate and Nitrite

1.6.1 Physical and Chemical Properties

Nitrate and nitrite are compounds that contain a nitrogen atom joined to oxygen atoms, with nitrate containing three oxygen atoms and nitrite containing two. In nature, nitrates are readily converted to nitrites and vice versa. Both are anions, or ions with a negative charge. They tend to associate with cations, or ions with a positive charge, to achieve a neutral charge balance (Siu and Henshall, 1998).

Nitrate is not normally dangerous for the health unless it is reduced to nitrite (NO_2). Nitrite is the univalent radical NO_2 or a compound containing it, such as a salt or an ester of nitrous acid.

1.6.2 Source and Uses

Nitrates are naturally present in soil, water, and food. In the natural nitrogen cycle, bacteria convert nitrogen to nitrate, which is taken up by plants and incorporated into tissues. Animals that eat plants use the nitrate to produce proteins. Nitrate is returned to the environment in animal feces, as well as through microbial degradation of plants and animals after they die. Microorganisms can convert nitrate or the ammonium ion (which is a nitrogen atom combined with four hydrogen atoms) to nitrite; this reaction occurs in the environment as well as within the digestive tract of humans and other animals. After bacteria convert (reduce) nitrate to nitrite in the environment, the nitrogen cycle is completed when they then convert the nitrite to nitrogen. Normally, this natural cycling process does not allow excessive amounts of nitrates or nitrites to accumulate in the environment. However, human activities have increased environmental nitrate concentrations, with agriculture being the major source. This includes increased use of nitrogen-containing fertilizers as well as

concentrated livestock and poultry farming; the latter two produce millions of tons of nitrate-containing manure each year. Nitrate and nitrite compounds are very soluble in water and quite mobile in the environment. They have a high potential for entering surface water when it rains, as nitrates in applied fertilizers can dissolve in runoff that flows into streams or lakes; they also have a high potential for entering groundwater through leaching. The concentration associated with soil particles has been estimated to be about half the concentration in interstitial water (the water in the pore spaces between the soil particles).

1.6.3 Toxicity and Limiting Contamination

Naturally occurring nitrate levels in surface and ground water are generally a few miligrams per litre. In many ground waters, an increase of nitrate levels has been observed due to the intensification of farming practice. Concentrations can reach several hundred miligrams per litre. In some countries, up to 10% of the popullation may be exposed to nitrate levels in drinking water of above 50 mg L^{-1} (Kazemzadeh and Ensafi, 2001).

In general, for humans vegetables will be the main source of nitrate intake when levels in drinking water are below 10 mg L^{-1}. When nitrate levels in drinking water exceed 50 mg L^{-1}, drinking water will be the major source of total nitrate intake. Extensive epidemiological data support the current guideline value proposed by the World Health Organization (WHO) for nitrate-nitrogen of 10 mg L^{-1}. However, this value should not be expressed on the basis of nitrate nitrogen but on the basis of nitrate itself, which is the chemical entity of concern to health, and the guideline value for nitrate is therefore 50 mg L^{-1}.

The U.S. Environmental Protection Agency (EPA) has developed toxicity values to estimate the risk of non-cancer health effects from ingesting nitrates and nitrites. The toxicity value used to estimate a non-cancer effect following ingestion is called a reference dose (RfD). An RfD is an estimate of the highest dose that can be taken in every day without causing an adverse effect. The RfD for nitrate was developed considering the concentration at which methemoglobinemia was indicated at levels above 10% for 0- to 3-month-old infants. This was based on a daily intake of formula made with water containing 10 mg per liter (mg L^{-1}) of nitrate as nitrogen.

Table 1.1 Chemical toxicity values for non-cancer effect of nitrate and nitrite

Chemical Toxicity Values	
Oral RfD: NO_3	Oral RfD: NO_2
1.6 mg kg^{-1}-day	0.1 mg kg^{-1} -day

In the European standards for drinking water, 2nd edition, published by the WHO after the meeting in Geneva 1970, we find the following: Constituents in water which, if present in excessive amounts, may give rise to trouble:

Table 1.2 Nature of trouble and approximate level of nitrate

Substance	**Nature of trouble which may arise**	**Approximate level above which trouble may arise**
Nitrate (as NO_3)	Danger of infantile methaemoglobinaemia if the water is consumed by infants.	- Reccommended: less than 50 mg L^{-1} - Acceptable: 50 to 100 mg L^{-1} - Not reccommended: more than 100 mg L^{-1}

1.6.4 Determination of Nitrate and Nitrite

Several methods have been reported regarding the flow injection chemiluminescence detection for determination of nitrate and nitrite in various sample matrices.

The determination of nitrate and nitrite in gas phase chemiluminescence can offer greater scope for the elimination of matrix effects in that it is based upon monitoring of the chemiluminescence emission intensity arising from the reaction between gaseous nitric oxide (NO) and ozone. The chemiluminescence reaction require further reduction of nitrite to nitric oxide prior to reaction with ozone. A number of reducing agent have been reported to achieve this conversion including ferrous/molybdate (VI) (Cox,1980), vanadium(III) (Braman and Hendrix,1989 ; Hendrix and Braman,1995) and titanium(III) chloride (Aoki and Wakabayashi, 1995; Aoki et al., 1997). Some of the method described are not simple and require expensive reducing agent and/or chemiluminescent reagent.

The determination of nitrate by reducing nitrate to nitrite using selective reducing agent such as zinc, amalgamated cadmium, hydrazine-copper, and

copperised cadmium (Moorcroft and Compton, 2001). Copperised cadmium column is the most common arrangements with efficiencies for nitrate to nitrite conversion approaching 100%. However, cadmium is toxic for human and environment. This problem can be eliminated by using photochemical redactor.

Photochemical reductions have been applied to the on-line reduction of nitrate in flow injection chemiluminescence methods based on the formation of peroxynitrite from the reaction of nitrite with hydrogen peroxide. Nitrate is on-line reduced to nitrite due to absorption of ultraviolet light at quartz capillaries (Mikuška and Večeřa, 2002; Mikuška and Večeřa, 2003; Takeda and Fujiwara, 1993) or polytetrafluoroethylene tubing (Renmin et al., 1995; Motomizu and Sanada, 1995). The developed methods offer simplicity, sensitivity, rapidity and non-toxic.

In recent years, nanoparticles have been a rapidly growing in analytical field which involves the use as catalysts for a variety of organic and inorganic reaction. For example, gold nanoparticles as a novel nanocatalyst on luminol-hydrazine chemiluminescence flow injection system (Safavi et al., 2008), carbon nanoparticles as catalytic support for chemiluminescence of sulfur compounds in a molecular emission cavity analysis system (Safavi et al., 2009) and platinum nanoparticles on the luminol–hydrogen peroxide–platinum colloids chemiluminescence flow injection system for the determination of phenollic compounds (Xu and Cui, 2007). The results indicated that low detection limit, increasing the rate and selectivity of the chemical reaction were observed. Thus, zinc oxide nanoparticles seem promissing to be used as catalyst on the chemiluminescence reaction of lumimol-hydrogen peroxide system after on-line photochemical reduction of nitrate to nitrite in order to improve the sensitivity.

Accordingly, the purpose of this research is to design and construct the FI-CL system with photo-reactor for on-line reduction of nitrate to nitrite prior to chemiluminescence reaction using zinc oxide nanoparticles (as catalyst) for enhancement in chemiluminecence emission intensity for determination of nitrate and nitrite in food samples.

1.7 Objectives of the Study

The purpose of this research is to design and construct the GDFI-CL system with photo-reactor for on-line reduction of nitrate to nitrite prior to chemiluminescence reaction using zinc oxide nanoparticles, as catalyst, for enhancement in chemiluminecence emission intensity for determination of nitrate and nitrite in food samples.