CHAPTER I

INTRODUCTION

1.1 Sequential injection analysis (SIA)

1.1.1 Introduction

Flow injection analysis (FIA) has been widely accepted for many years in analytical laboratories for routine analysis. It has an important functional advantages from using a small volume of the sample as compared to conventional wet analytical procedures. However, it is not sufficiently advantageous as compared to numerous modern discrete analyzers used mainly in analytical analysis such as electrochemistry techniques. In order to compete with other techniques, the newest methodology of flow injection measurements, so-called sequential injection analysis (SIA), has been developed [1].

Sequential injection analysis is a new technique in automation analysis. It was reported for the first time by Ruzicka and Marshall in 1990 [2]. An analytical signal is measured in a flow-through detector from the flow of series of liquid segments of sample and reagents through a detector. Therefore, it is a typical flow measurement with essentially reduced consumption of sample and reagents as compared to other flow techniques [1].

1.1.2 Principle of SIA

In a typical SIA system, a syringe pump and a multiposition valve (instead of an injection valve) are used (Figure 1.1). Sample and reagent solutions are aspirated into a holding coil by a syringe pump. Then, the valve is switched to the detector position and the flow is reversed. The stacked zones are dispensed to the detector through the valve and a reaction coil. During this flow, segments of sample and reagents interdisperse and a detectable product is produced in the overlapped zone (Figure 1.2). The peak observed in continuous flow will correspond to the overlapped part of sample and reagent zones.

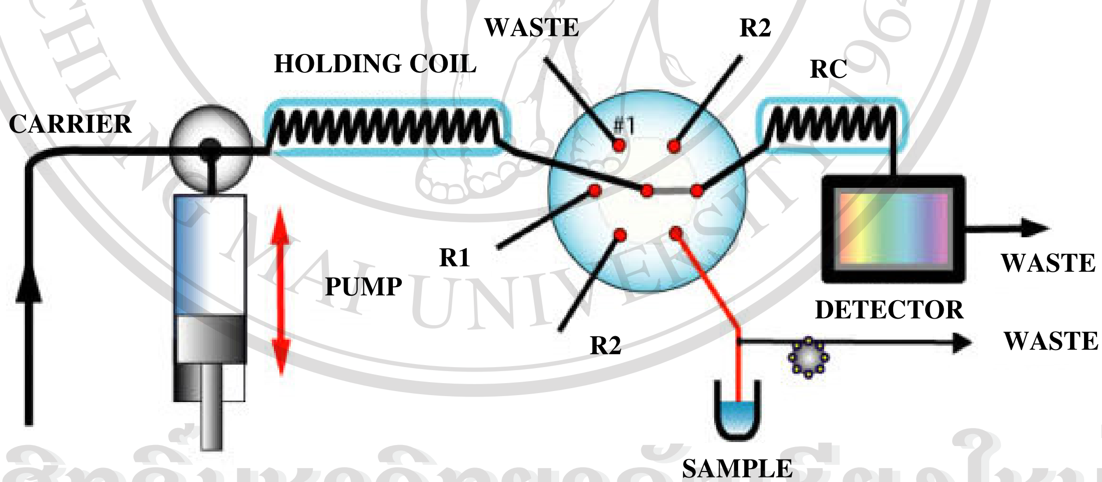

Figure 1.1 A conventional SIA setup is comprised of a bi-directional syringe pump, a holding coil, a multiposition valve, a reaction coil (RC) and a detector [3].

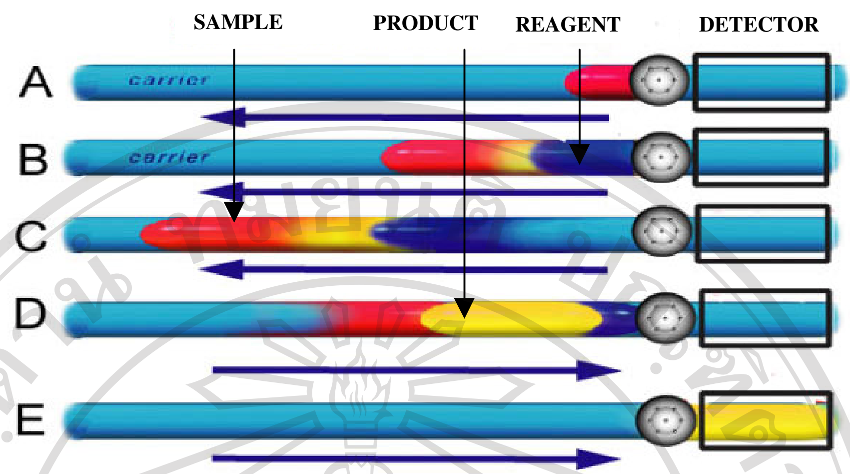

Figure 1.2 The principle of SIA [3]. Each step can be described as follows: A = The sample was loaded into the holding coil, B = The reagent was loaded into the holding coil, C = The stack zone was aspirated into the holding coil to improve mixing and dispersion, D = The product was produced and was propelled to the detector and E = The product was monitored by the detector and the signal was recorded.

In contrast to a typical FIA, SIA system is carried out usually in a single channel setup, with the use of a single pump and a single valve. Precision and accuracy of the system depends on the accuracy of fixed volume of sample and reagent introduction into a holding coil. Therefore a computer software is used to control volume, time and direction of selection valve and a pump as well as data acquisition [1,4].

1.1.3 Components of SIA

The main components of SIA and their functions are discussed briefly as follows;

1.1.3.1 Propulsion system

Usually, a syringe pump is selected to be used in the SIA system because it offers pulse-free and highly precise flow. Besides, this pump can aspirate and dispense solution in microliters volume. However, it is relatively expensive, requires priming before use and has a limited reservoir volume [5].

1.1.3.2 Distribution system

A multiposition valve is commonly used in the SIA system. 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 [6].

1.1.3.3 Transportation system

This part consists of a holding coil and a reaction coil (typically made of 0.5-0.8 mm i.d. tubing). The dimension of this system must be designed according to volume dictated by the experimental protocol, i.e., the larger i.d. is used for dilution and mixing. Holding coil has a function to hold the reagents and the sample before propelling them to a detector. The position of the holding coil should be in between the syringe pump and the multiposition valve (see Figure 1.1) to prevent the aspirated solution from entering the pump [6]. The length of tubing depends on the volume of the syringe. Reaction coil provides longer reaction time so that the detector response can be increased.

1.2 Lab-on-valve (LOV)

1.2.1 Introduction

Lab-on-valve is the third generation of flow-injection analysis. The first report of lab-on-valve was described by Ruzicka in 2000 [7]. The purpose of this technique is to reduce the reagent consumption and waste generation down to micro and submicroliter level [8]. LOV system is robust which is an additional benefit offered by a conventional sized pump, valve and fiber optic spectrophotometer (Figure 1.3) that can be programmed to accommodate a wide variety of assays within the same microfluidic device. Sample metering, mixing, dilution, reagent addition, incubation, separation, and detection can be executed in any desired sequence in a system consisting of channels that are integrated with a multi-purpose flow cell (Figure 1.4). The channels are located on a monolithic object that is mounted atop a conventional multiposition valve [7]. Novel and unique applications can be conducted based on a variety of detection techniques. Moreover, LOV system offers high repeatability of microanalysis because the sample processing channel of this system is in the permanent rigid position. Besides, all of the advantages of the sequential injection system are also displaying in this novel LOV technique.

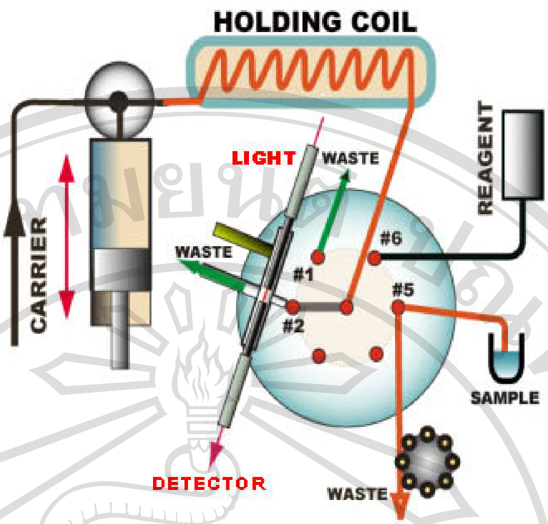

Figure 1.3 Lab-on-valve manifold. [3]

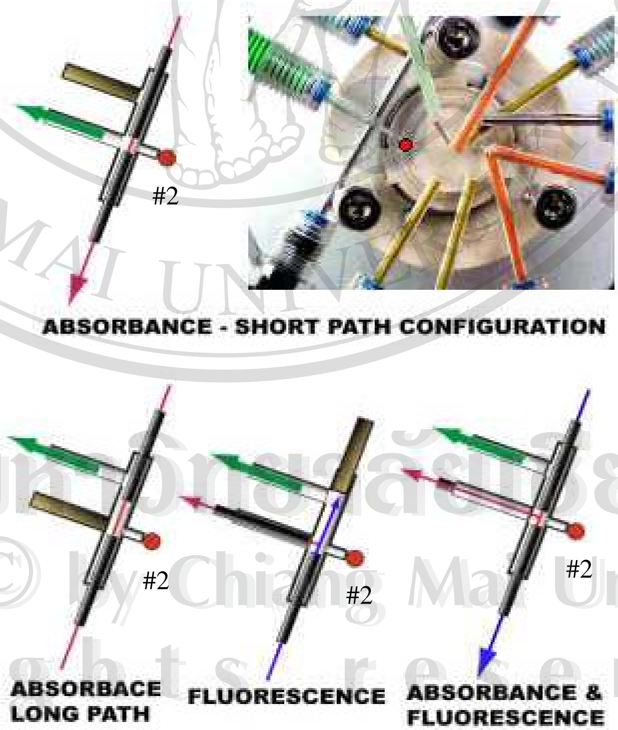

Figure 1.4 Multi-purpose flow cell. [3]

1.2.2 Principle of LOV

Sample and reagents are injected by flow reversal method into a holding coil via a multiposition valve. Mixing, dilution and incubation are taken place in the holding coil. The solution zones are dispensed into the flow cell (port #2) while the fiber optic spectrophotometer are monitoring the solution.

1.2.3 Components of LOV

Most components of lab-on-valve system are the same as those in the SIA system, except the detector is moved to attach onto the valve. For example, the fiber optic spectrometer is connected to the LOV as shown in Figure 1.3. Thus in the LOV system, the travel distance from the valve to the detector is shorten. In addition, the real time monitoring of reaction rate or its kinetic study is possible [7].

1.3 Acidity

1.3.1 General background

The meaning of acidity in modern food processing can be: (i) the quality of being sour, sourness, tartness and sharpness to the taste as the acidity of lemon juice [9], and (ii) the property of being acidic (pH values below 7) and the taste experience when something acidic is taken into the mouth [10].

Acidity of fruit juice is calculated as anhydrous citric acid which should not exceed 3.5 percent (w/v), unless otherwise provided for a specific fruit juice [11]. Various properties of acid allow it to be used as a flavoring agent, buffer, preservative, synergist, viscosity modifier, melting modifier and meat curing agent [12].

The acidity of juice depends on the content of several organic acids such as citric, malic, fumaric, acetic, ascorbic and galacturonic acid etc. Measuring of organic acid levels in foods and beverage is important in the fermentation process, checking product stability, validating the authenticity of juices, determination of the acid concentration and studying of the organoleptic properties of fermented products such as wine [13].

The most common acid in fruit juice is citric acid that can be obtained either in anhydrous or monohydrate form [14]. Citric acid or 2-hydroxy-1,2,3-propanetricarboxylic acid is different from other hydroxy acids in that it is a triacid as shown in Figure 1.5. It was first isolated from lemon juice by a Swedish chemist, Carl Wilhelm Scheele, in 1784 [15]. Its major advantage is the high solubility in water.

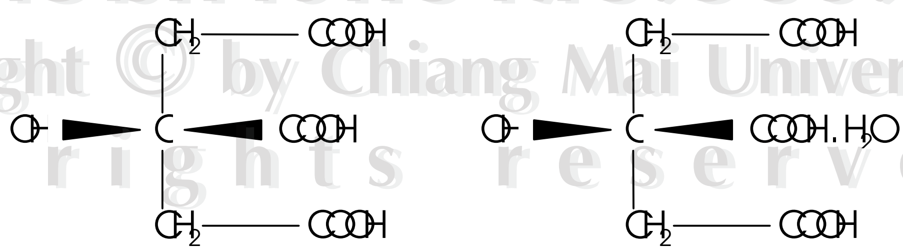

Figure 1.5 Structural formula of citric acid. [16]

1.3.2 Application of citric acid

Citric acid is often employed as a standard reference acid to evaluate the effects of other acidulants in various food products [12]. It takes part in the production of energy through the tricarboxylic acid cycle and plays an important role in the proper functioning of the prostate gland [17]. Because of its function and environmental acceptability, citric acid and its salts (primary sodium and potassium) are used in many industrial applications [18]. In pharmaceutical industry, they are used as an anticoagulants to preserve blood and in the preparation of pharmaceutical formulations in order to improve their tastes. In food industry, they are used as acidifiers for inhibiting the growth of micro-organisms in food, flavoring agents in carbonated beverages for imparting a tangy citrus flavor, sequestering agents of metallic ions, pH stabilizers, dispersing agents and synergists for antioxidants [17].

1.3.3 Determination of citric acid and acidity as citric acid content

There are a large number of methods for determination of acidity. The acidity is usually reported as the citric acid contents. Some methods are briefly reviewed.

1.3.3.1 Volumetric method

This classical method is based on acid-base titration of citric acid with sodium hydroxide using phenolphthalein [19] or ammonium hydroxide and phenol red or thymol blue as an indicator [20]. However, titration method was the draw back of tediousness and long time and large amount of reagents consumption.

1.3.3.2 Spectrophotometry

This method is based on the chromophore formed between pyridine, acetic anhydride and citrate. It can be used to determine citric acid in milk powders. The linear range and recovery were found to be 0-300 mg l^{-1} and 95.2%, respectively [21].

1.3.3.3 Ion chromatography (IC)

A gradient ion chromatographic method was developed to separate and determine main organic acids in fruit juices [22]. The method allowed separation of organic anions by Dionex OmniPac PAX-500 column using NaOH gradient elution and a conductometric detection. The detection limit was 10 mg l^{-1}. Similarly, citric acid in grape juice could be determined using Dionex As11 and Dionex AG11 as a pre-column. The method gave the detection limit of 164 µg l^{-1} [23]. A high-performance anion-exchange chromatographic method for the simultaneous separation and determination of citric acid and four artificial sweeteners in a single injection was developed [24]. This method was performed using an anion-exchange gradient program and a conductometric detection. The detection limit of citric acid was 0.22 µg ml^{-1}.

1.3.3.4 High performance liquid chromatography (HPLC)

A direct injection high performance liquid chromatographic procedure was developed for the separation and determination of the major carboxylic acids in wine [25]. The acids were separated on a reversed-phase column C-18, eluted with dilute acetic acid and monitored by UV spectrophotometry at 254 nm. The limit of detection of citric acid was 0.05 g l^{-1}.

1.3.3.5 Flow injection analysis (FIA)

Several FIA methods have been reported for determination of citric acid in foods, beverages and pharmaceutical formulations. For example, a photochemical method based on a flow-injection system was developed to determine citric acid in beverage [17]. The basis for the determination was the reaction that took place between citric acid and Fe^{3+} upon irradiation with visible or UV light. Fe^{3+} was photochemically reduced by citric acid to Fe^{2+}, which reacted with 1,10-phenanthroline, forming the reddish orange $Fe(phen)_3^{2+}$ complex. This complex was monitored by spectrophotometry at 512 nm. The method showed a linear range between 1 and 120 $\mu g\ ml^{-1}$ and detection limit was 0.5 $\mu g\ ml^{-1}$. Similarly, a method based on the inhibitory effect of citric acid upon the Fe^{3+} catalytic oxidation of 2,4-diaminophenol (DAP) by H_2O_2 was also reported [26]. A linear calibration graph was obtained from 0 to 1000 mg l^{-1} citric acid and the detection limit was 0.96 mg l^{-1}.

Two flow injection procedures for the determination of acidity (expressed as citric acid content) in fruit juices were developed [27]: conductimetric involving injection of sample into ammonia followed by gaseous diffusion into acetic acid; and spectrophotometric FI titration in which the sample was mixed with sodium hydroxide in the presence of phenolphthalein. The latter method gave a limit of detection of 0.1% w/v. Color of sample and the other matrices in the samples did not interfere the analysis.

1.3.3.6 Sequential injection analysis (SIA)

This technique was applied to determine citric acid in soft drink with potentiometric detection [28]. A linear calibration range was obtained from 0.1-0.6 % (w/v). Similar technique was developed for an assay of acidity (expressed as citric acid content) in fruit juice [29]. The method was based on an on-line titration of the acid contents with sodium hydroxide using phenolphthalein as an indicator. Absorbance was spectrophotometrically monitored at 552 nm. A linear calibration graph was obtained in the range of 0.2-1.0 and 0.5-2.5 % (w/v), with a relative standard deviation of 1%.

1.4 Research aims

1. To develop the sequential injection analysis with lab-on-valve for micro-titration of acidity.
2. To apply the proposed method to determine acidity in fruit juice samples.