CHAPTER II

REVIEW OF RELATED LITERATURE AND RESEARCH

Ethanol

Ethanol or "ethyl alcohol" is commonly called "alcohol". Ethanol in its pure state is a colorless, volatile, flammable liquid which is completely miscible with water. The chemical formula for ethanol is CH_3CH_2OH or C_2H_5OH (condensed structural formulas) and its molar mass is 46.07 g/mol. Ethanol has a boiling point of 78.5 ^{0}C, a low melting point of -114.5 ^{0}C and a density of 789 g/L [46].

Ethanol is produced by the fermentation of starch and sugar from plants, crops or biomass materials and also by the hydration of ethylene gas from petroleum industry. For the fermentation process, starches must first be broken down into simple sugars (maltose, glucose or fructose) by two main groups of amylolytical enzymes: liquefying (α-amylases) and saccharifying (glucoamylases, β-amylases and α-amylases) enzymes [47]. Then, enzymes in yeast (e.g. *Saccharomyces cerevisiae* or *S. elipsoideus* groups) changes simple sugars into ethanol, carbon dioxide and energy. The fermentation reaction is represented by simple equation (see equation (1)), that is actually very complex and impure cultures of yeast produce varying amounts of other substances, including glycerine, flavor and various organic acids [1, 2, 48].

$$\underset{\text{(starch)}}{(C_6H_{12}O_6)n} \xrightarrow[enzyme]{amylolytical} \underset{\text{(simple sugar)}}{nC_6H_{12}O_6} + \text{other substances} \qquad (1)$$

$$\downarrow \text{ Enzymes in yeast}$$

$$\underset{\text{(ethanol)}}{2CH_3CH_2OH} + 2CO_2 + \text{energy (which is stored in ATP)}$$

1. Importance of ethanol

Ethanol is widely used as a solvent in laboratory and industry (e.g. for esters, medicines, paints and perfumes), as a fuel and as a raw material for preparation

of other organic chemicals. Moreover, ethanol is found in alcoholic beverages and best known or commonly called "alcohol".

Alcoholic beverage production and consumption are an important part of social events in many countries. Thus, alcohol drinking plays a significant role in social interaction especially alcohols neurological effects. Ethanol is a psychoactive substance that has a depressant effect. High blood ethanol content is usually considered to be legal drunkenness because it reduces attention and slows reaction speed. Thus, ethanol in alcoholic beverages can be addictive, and the state of it addiction is known as alcoholism [7, 46, 47, 48].

2. Ethanol in alcoholic beverages

Generally, ethanol is the second main component in alcoholic beverages after water and its contents refer to control quality, conservation and tax collection. The concentration of ethanol in alcoholic beverage (i.e. beers, wines and distilled spirits) is usually stated as the percentage of alcohol by volume (ABV). Beer and wine have a lower %ABV while distilled spirits have a higher %ABV.

Beer is produced by fermentation of starches which are mainly derived from cereal grains such as malted barley, wheat, corn and rice. Beer is commonly divided into two main types including lager and ale. Ale is further classified into varieties such as pale ale, stout and brown ale. Most beer is flavored with hops, which add bitterness and act as a nature preservative. The ethanol percentage of beer is usually 4-6 %ABV, but it may be less than 2 %ABV or as much as 12 %ABV [1, 2, 3, 4, 5, 6, 7].

Wine is generally produced by fermentation from grapes and other fruits such as apples, cherries, or plum. Special types of wine include sparking wine and fortified wine. Sparking wine can be made by means of a secondary fermentation. Fortified wine is wine which a distilled spirit has been added. Wine involves a longer fermentation process than beer and also a long aging process (months or years), resulting in an ethanol content of 9-16 %ABV [1, 2, 3, 4, 5, 6, 7, 8].

Distilled spirits are also called distilled liquors or spirits. Distilled spirits are produced by distillation of fermented base product. Distilling concentrates the ethanol and eliminates some of other volatile compounds. Distilled spirits may be classified into two types (Figure 1). First, it is represented by gin and vodka which

have no requirement for congeners. Second, it is the congeneric or self-flavored type including brandy, whisky and rum. Brandy is produced by distilling wine and aged in wooden cakes. Whisky is made by distilling from fermented grains (e.g. barley, malted barley, rye, wheat and corn) and aged in wooden cakes. Rum is made from sugarcane by-product such as molasses, or directly from sugarcane juice, by a process of fermentation and distillation. Gin is a spirit which derives its predominant flavor from juniper barriers. Vodka is made by distillation of fermented substance such as grains, potatoes or sometimes fruits. Vodka is composed primarily of water and ethanol with traces of impurities and flavoring. The ethanol content of distilled spirits is 30-60 %ABV [7, 8].

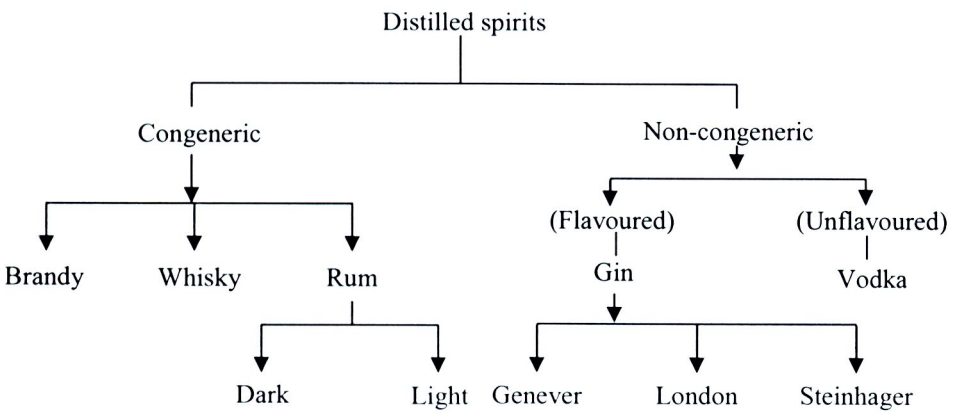


Figure 1 The main types of distilled spirits [2]

Methods for the determination of ethanol and literature reviews

Several analytical methods have been used for determination of ethanol in alcoholic beverage samples. These methods include titration, spectrophotometry, gas chromatography (GC), high performance liquid chromatography (HPLC), voltammetry, infrared spectroscopy (IR) and fourier transform infrared spectrometry (FT-IR).

The titration method was commonly used to determine ethanol in alcoholic beverage by titrating extracted ethanol with acidic potassium dichromate and utilizing ferrous ammonium sulfate and 1,10-phenanthroline ferrous sulfate as titrant and

indicator, especially it was adopted in the standard titrimetric method of AOAC [9]. The potassium iodide was also used to add and titrated the resulting iodine with sodium thiosulfate [10]. For the most spectrophotometric method, it is based on the reaction of ethanol with acidic potassium dichromate or ceric nitrate and then monitored Cr-(III) or a red colored product, respectively [11, 12, 13].

For GC method, alcoholic beverage samples were firstly separated ethanol by filtration, dilution or liquid-liquid extraction and further direct injected into the GC system using flame ionization detection [14, 15, 16, 17, 18]. For HPLC, those samples were also prepared by filtration, dilution, degasser and separation with column and separated ethanol was injected into HPLC system using UV-Vis spectrophotometric or conductometric detection [19, 20].

Other methods for ethanol determination in alcoholic beverage such as voltammetric method based on the oxidation of ethanol at Pt working electrode in electrolyte solution [21], IR method [22] and FT-IR method [23] were also reported.

In addition, many continuous-flow methods technique for ethanol separation using gas diffusion membrane have been applied to ethanol determination in alcoholic beverages. These methods are summarized in Table 1.

Table 1 Continuous-flow methods with gas diffusion membrane separation for determination of ethanol

Techniques	Details	Year [Ref.]
1. FA-TBS/ Immobilized alcohol oxidase and gas diffusion PTFE membrane/ Amperometry	• Analyte: ethanol • Sample: beer and wine • Condition: Ethanol was passed through the membrane into the acceptor stream, which was carried to the enzyme reactor (alcohol oxidase), where it was converted to acetaldehyde and hydrogen peroxide. The latter was detected hydrogen peroxide at 700 mV with amperometric detection. • Analytical characteristics : LR = 0-15 %v/v, DL = 0.0001 %v/v, SP = 30 h^{-1} , %RSD = 3.37	1995 [24]

Table 1 (Cont.)

Techniques	Details	Year [Ref.]
2. FIA/ Membrane permeation / UV-Vis spectrophotometry	• Analyte: ethanol • Sample: distilled spirits and wines • Condition: Ethanol was passed through the silicon tubular membrane into the acceptor stream, which reacted with Cr (VI) under acidic conditions, yielding Cr (III) which was monitored at 600 nm. • Analytical characteristics : LR = 5-20 %v/v, DL = 0.5 %v/v, SP = 20 h^{-1} , %RSD = 3.5	2003 [29]
3. FIA/ Immobilized enzyme and pervaporation (PTFE membrane) / Photometry and fluorimetry	• Analyte: ethanol and glycerol • Sample : wine • Condition: The ethanol was pervaporated by PTFE membrane into the acceptor solution which was containing potassium dichromate and sulfuric acid. The ethanol was oxidized. The Cr (III) was formed and monitored at 600 nm. For glycerol, it was introduced into channel, which containing a buffer solution and passed through the glycerol dehydrogenase immobilized reactor (GDR) to produce NADH formed, which is monitored fluorimetrically at λ_{ex} = 340 nm and λ_{em} = 460 nm. • Analytical characteristics : LR = 1-20 %v/v ethanol and 2-8 g/L glycerol , DL = 0.5 %v/v ethanol and 1.5 g /L glycerol , SP = 6 h^{-1} , %RSD = 3 for ethanol and 2 for glycerol	2005 [31]
4. FIA/ Pervaporation/ Density measurement	• Analyte: ethanol • Sample: beverage • Condition: The ethanol was pervaporated and collected into the acceptor stream (distilled water) and introduced to the density meter, where it is measured the density of water-alcohol mixture. • Analytical characteristics : LR = 0-40 %v/v, DL = 0.11 %v/v, SP = 15 h^{-1}, %RSD = 0.32	2006 [32]

Table 1 (Cont.)

Techniques	Details	Year [Ref.]
5. FIA/ On-line liquid-liquid extraction/ FTIR	• Analyte: ethanol • Sample: alcoholic beverage • Condition: The sample was introduced into the carrier stream and carried to mixed with the organic solvent in extraction coil, where ethanol was extracted into the organic phase. And then, the organic phase crossed the membrane and flowed continuously into the infrared flow cell, while the aqueous phase went to waste. Finally, the quantification was carried out measuring the ethanol wave number at 877 cm^{-1}. • Analytical characteristics: LR = 0.05-15 %v/v, SP = 25 h^{-1}, %RSD = 0.8-1.3	2005 [31]
6.FIA/ Membraneless gas diffusion (MGD) / Spectrophotometry	• Analyte: ethanol • Sample: liquor • Condition: A flow injection method was developed by employing the MGD unit to determine ethanol. This method was based on the reduction of dichromate to Cr (III) by ethanol vapor. Cr (III) was spectrophotometrically monitored and monitored at 600 nm. • Analytical characteristics: LR = 0.5-30 %v/v , DL = 0.27 %v/v, SP = 16 h^{-1}, %RSD = 0.5	2006 [32]
7. FIA/ Gas diffusion / Spectrophotometry	• Analyte: ethanol • Sample: alcoholic beverage • Condition: A tubular gas diffusion PTFE membrane was used in FI-spectrophotometric system. This probe was immersed into the sample. The ethanol was diffuse through the membrane into the acidic Cr-(VI) acceptor stream. Cr-(VI) was reduced by ethanol and the formed Cr-(III) was spectrophotometrically monitored at 600 nm. • Analytical characteristics: LR = 0-50 %v/v , SP = 30 h^{-1}, %RSD < 2	2006 [33]

Table 1 (Cont.)

Techniques	Details	Year [Ref.]
8. FIA/ Gas diffusion / Colorimetry	• Analyte: ethanol • Sample: beverage • Condition: Flow injection (FI) colorimetric method was developed for the determination of ethanol. This method was based on the reaction of ethanol with ceric ion in acidic medium to produce a red colored product which monitored at 415 nm. • Condition: LR = 0.1-10 %(v/v) , DL = 0.03 %v/v, SP = 20 h^{-1}, %RSD < 3	2011 [37]

Continuous-flow analysis

1. Flow injection analysis

1.1 General and Principle

Flow injection analysis (FIA) or unsegmented-flow analysis was reported for the first time by Ruzicka and Hansen in 1975 [49]. Its general procedure is based on the injection of sample solution into a continuous flowing stream of reagent or carrier. A zone of the injected sample solution is formed and moved constant downstream by pump into a reaction coil/mixing coil, in which the sample solution dispersed into the reagent. The product is formed and then transported toward a detector that continuously recorded the signal such as pH, conductivity, absorbance, fluorescence and electrochemical potential. The flowing stream in FIA system has two main purposes. One is to deliver the sample zone to the detector. The second is to merge the sample zone and carrier stream together. If the carrier contains reagent, this process brings the analyte and reagent together to promote chemistry that generates a detectable product. Although reaction is not necessary to reach equilibrium, the measure of reaction is the same for both standards and samples. The simplest FIA system and typical recorded output of FIA peak are shown in Figure 2 [49, 50, 51, 52].

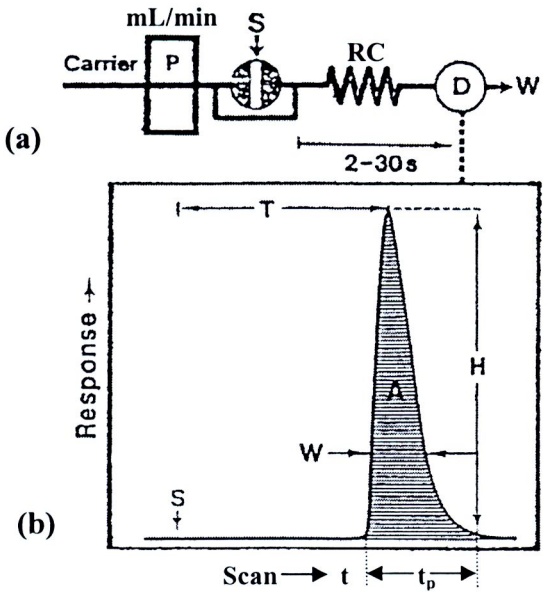


Figure 2 (a) The simplest single-line FIA manifold utilizing a carrier stream of reagent, P – pump, S – injection port, RC – reaction coil, D – flow cell and W – waste and (b) The analog output has the form of a peak, the recording stating at S (time of injection), H – peak height, W – peak width at a selected level, A – peak area, T – residence time corresponding to the peak height measurement, and t_b – peak width at the baseline [50]

For the basic principle, FIA is based on a combination of three principles; 1) sample injection, 2) controlled dispersion of the injected sample zone, and 3) reproducible timing of its movement from the injection point toward and into the detector. Controlled dispersion process relates with many parameters of the system such as length and size of tubing, tubing diameter, flow rate of the carrier/reagent stream and type of mixing/reaction part [49, 50, 51, 52]. The ***dispersion coefficiency (D)*** is defined by equation as follow (Figure 3):

$$D = C^0/C^{max} = H^0/H$$

Where C^0 is the original concentration of the injection sample. C^{max} is the maximum concentration of the sample zone after it has undergone all the dispersion processes and is passing through the detector. H is the peak height recorded for the sample zone

as it passes through the detector. And H^0 is the peak height corresponding to an undispersed sample zone [49, 50].

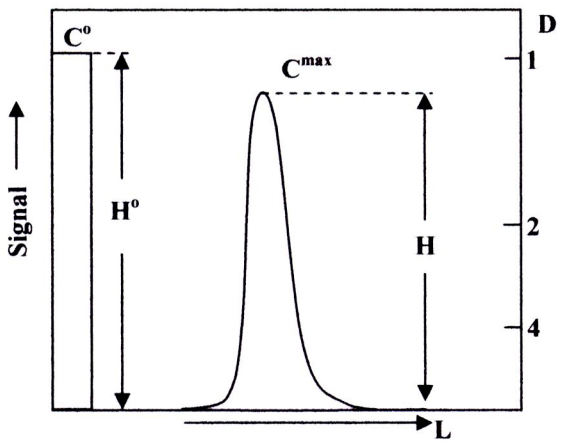


Figure 3 Dispersion (D) in the system defined as the ratio between the original concentration (C^0) and the concentration of the dispersed specie (C^{max}) [49]

Dispersion is classified into three types. There are as followed:

1. Limited dispersion (D = 1-3): An FIA system designed for using this range of dispersion is for a sample being presented to the detector without reaction e.g. in FIA-AAS, FIA-ICP-AES measurement.

2. Medium dispersion (D = 3-10): Popular for used in most FIA systems which chemical reaction is required. Peak height decreases as dispersion increases. The system is useful for FIA colorimetry.

3. Large dispersion (D ≥ 10): When using long tubing of mixing coil/reaction part between the points of injection and detection, resulting peaks will be very broad. Such a system is useful for FIA-titration [52, 53].

In general when increased dispersion, the chemistry effect causes predominant effect between analyte and reagent by enhance sensitivity, but the dilution effect leads to a lowering sensitivity, increasing peak broadening and reducing sample throughput. Therefore, in developing a new methodology, the analyst must find a set of conditions that gives the best balance between enhancement of chemistry and dilution for the application of interest [51, 52, 53].

1.2 Basic Components of FIA System

An ordinary FIA system usually consists of a least five essential parts (Figure 4):

1.2.1 A *propelling system*, which drives the carrier/reagent stream to the different elementary unit of the system with perfectly reproducible, pulse-free, and constant flow. A propelling system can be propelled by various types of pump. These system is such as peristaltic pump and syringe pump [52, 53].

1.2.2 An *injection system* for introduction of variable sample volume into the carrier stream in a highly accurate and reproducible manner. Different of injection valves are used such as a rotary injection valve, hydrodynamic valve and chromatographic injection valve [52, 53].

1.2.3 A *transport system* of reaction zone to interconnect the different elements and accomplish the desired extent of mixing or dispersion of the sample in the carrier/reagent stream as they flow along the system. There are straight tube, coiled tube, mixing chamber, glass bead column and knitted reactor [52, 53].

1.2.4 A *detection system* allowing continuous monitoring of a given property of the sample or its reaction product and providing qualitative and quantitative information about the former. In general, detectors are designed for used in colorimetric, spectrophotometric, electrochemical and atomic absorption techniques. A good detector should have high stability and fast response [52, 53].

1.2.5 A *recorder* is for the output from the detector as a peak by means of a chart recorder, a microprocessor, a computer or a microcomputer [52].

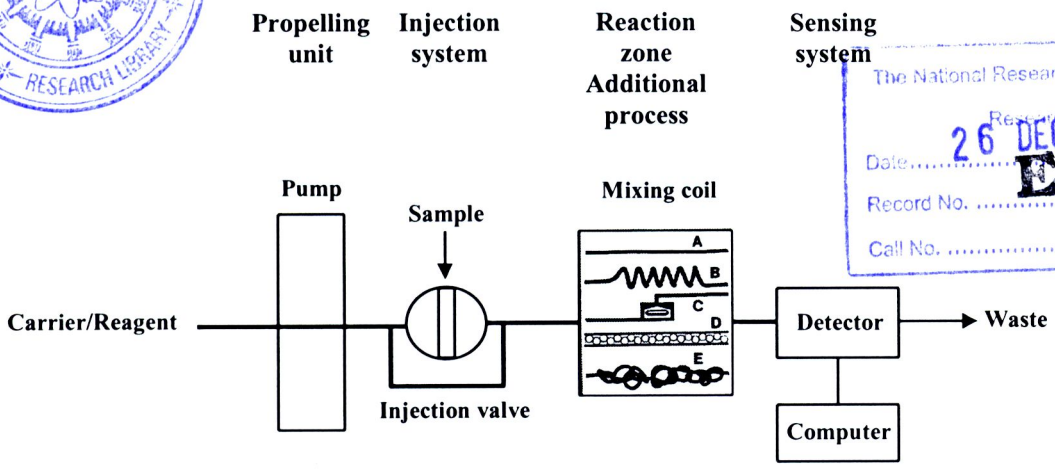


Figure 4 General FIA system, showing its essential components of propelling unit (peristaltic pump), injection system, reaction zone (A – straight open tube, B – coiled tube, C – mixing chamber, D – single-bead string reactor and E – knitted reactor). Adopted from Keit A. Smith and Malcolm S. Cresser [56] **and Ruzicka and Hansen** [50]

2. Hydrodynamic sequential injection analysis

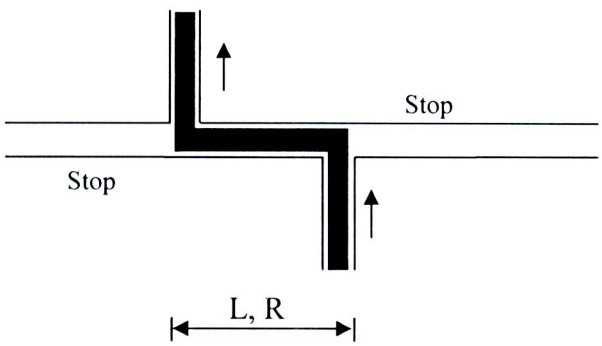
2.1 Hydrodynamic injection

Hydrodynamic (HD) injection is the alternative way for introducing sample into the FIA system with good reproducibility. The principle of HD injection technique is best explained by referring to Figure 5. By this approach a column of liquid can be exactly metered into a geometrically well-defined conduit (of length L and internal radius R) which is at all time open to other channels, provided that these channels are filled by a stagnant liquid (Sampling, Figure 5 (a)). These columns of liquid exert a hydrodynamic force, which serves as a lock while the sample volume ($Sv = \P R^2 L$) is being filled. Thus by an alternate and intermittent pumping of carrier and sample solutions, a well-defined sample zone can be formed and then inserted into the carrier stream (Injection, Figure 5 (b)). The manifold serving for this purpose (Figure 6 (a)) uses two peristaltic pumps to control the movement of sample and carrier solutions. It is obvious that if x=z and if the pumps follow the sequence indicated in Figure 6 (b), then the sample conduit L will be filled by sample solution from sample reservoir S when pump 1 is in motion, and during the next cycle, when

pump 1 is stopped and pump 2 operates, the carrier stream will empty this exactly metered volume of sample from conduit L and will carry it further toward the detector (D) [50].

HD injection is the most widely used method of sample introduction because it has the advantage that sample composition is unaltered by the injection. A small volume of sample can be introduced into the tubing by hydrostatic pressure, the volume is constant. The sample volume introduced by hydrodynamic injection can be manipulated by varying the injection time. A major limitation of the hydrodynamic is that it is not suitable for the injection of highly viscous samples [42, 50-61].

(a) Sampling



(b) Injection

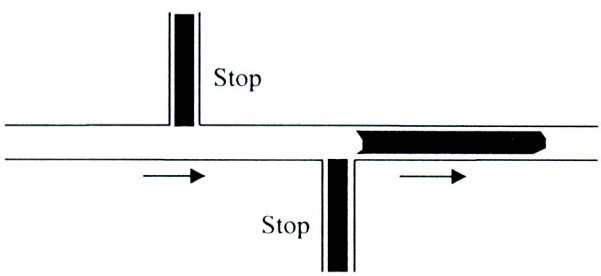


Figure 5 The principle of hydrodynamic injection. A fixed volume of sample solution is metered into a conduit, of length L and internal radius R (a - Sampling), and this volume is subsequently propelled downstream by the carrier stream (b - Injection). During the sample cycle, the carrier stream circuit is stopped, and vice versa. When aspirating the next sample, the column of carrier stream solution contained within the common conduit L is emptied to waste along with surplus of sample solution [50]

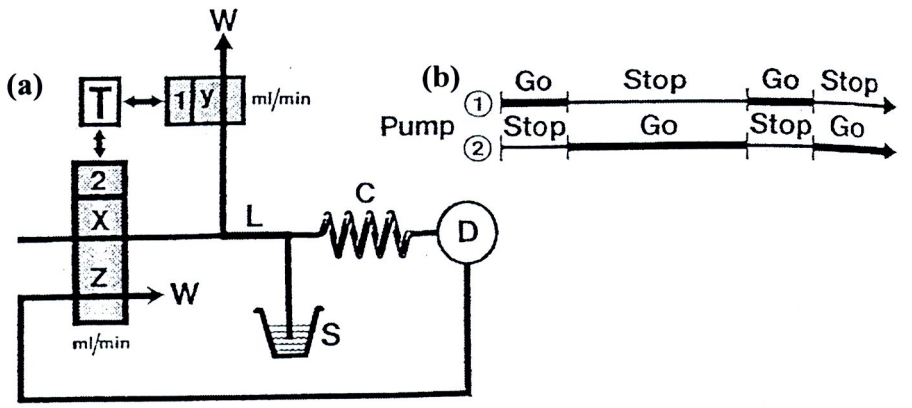
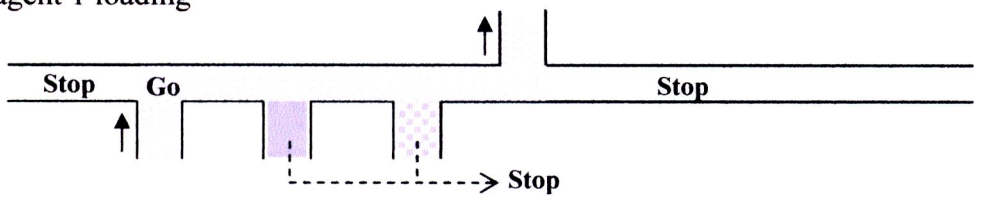


Figure 6 (a) Manifold for hydrodynamic injection. The sample volume is aspirated by pump 1, operating rate of y mL/min, and a fixed volume of sample from reservoir S passes into conduit L. Subsequently pump 2 is activated, pumping at rates x = z mL/min, and the sample is flushed through reactor C to the detector D. The operations of the two pumps is controlled by the time T, the time sequence of events being as depicted in (b) [50]

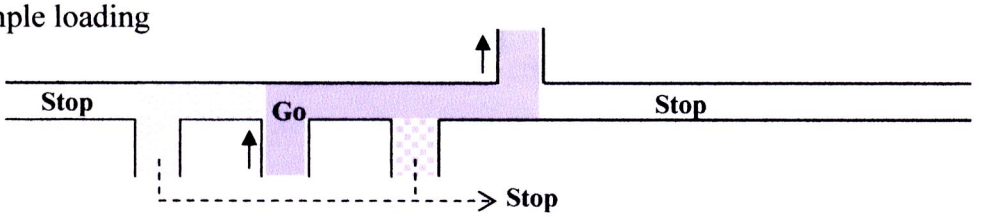
2.2 Hydrodynamic sequential injection

Hydrodynamic sequential injection (HSI) analysis, the introduction of sample and reagent solutions into tubing can be achieved by hydrodynamic flow concept with used of cost-effective devices, solenoid valves and 3-way connectors, leading to the name HSI analysis. With the similar principle to HD injection in FIA, a new HSI analysis was proposed. In this HSI, reagent and sample are sequentially introduced into the system while the carrier stream is stopped. The exact volume of solutions is introduced without the use of injection device but employing hydrodynamic injection principle. Figure 7 illustrates the principle of HSI for injection of two reagents and one sample into the system [42, 57].

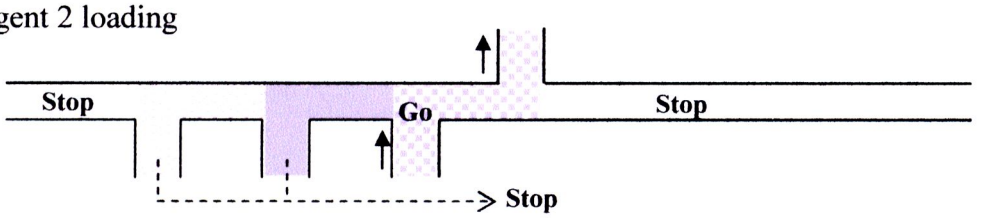
a) Reagent 1 loading



b) Sample loading



c) Reagent 2 loading



d) Injection

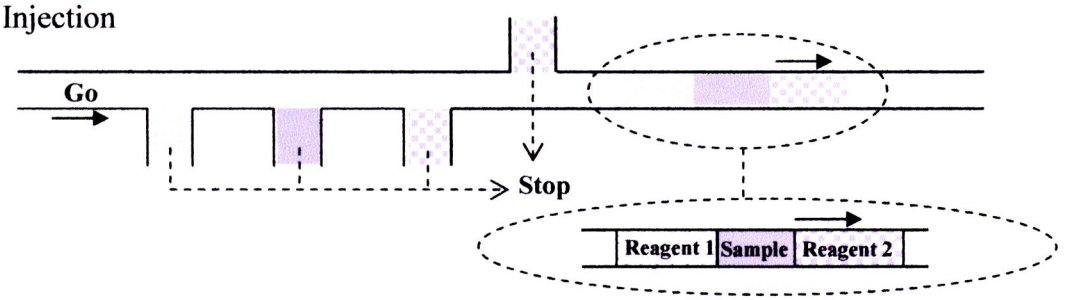


Figure 7 The general principle of hydrodynamic sequential injection (HSI) analysis; reagent 1, sample and reagent 2 are loaded sequentially based on hydrodynamic injection principle into the fixed-volume conduit (a-c) following by propelling the mixture zone by the carrier stream (d) [57]

The HSI analysis and sequential injection (SI) analysis are similar in concept and advantage for example in precise volume of loading solution, operational in automatic made and low chemical consumption. However, HSI system is better than SI system in term of instrumental cost because SI needs more expensive and higher quality of pump, selection valve and computer controller. In addition, HSI method overcomes some drawbacks of the general FIA which is used high amounts of reagent or chemical even though it is simple and low cost devices.

In this work, the semi-automatic, simple and cost-effective instruments of HSI system was assembled and applied to determine ethanol in alcoholic beverage.