

## CHAPTER III

### RESEARCH METHODOLOGY

#### Chemicals

All chemicals were analytical reagent grade and were used without further purification. Chemicals are listed as follows:

1. Potassium permanganate [ $\text{KMnO}_4$ ]: 99%, Ajax finechem, Australia
2. Potassium dichromate [ $\text{K}_2\text{Cr}_2\text{O}_7$ ]: 99.8%, Ajax finechem, Australia
3. Sulfuric acid [ $\text{H}_2\text{SO}_4$ ]: 96%, LAB-SCAN, Ireland
4. Ethanol [ $\text{C}_2\text{H}_5\text{O}$ ]: 99.8%, Merck, Germany
5. Acetaldehyde [ $\text{C}_2\text{H}_4\text{O}$ ]:  $\geq 99\%$ , Fluka, Switzerland
6. Isobutanol [ $\text{C}_4\text{H}_{10}\text{O}$ ]: 99.9%, BDH, England
7. Ethyl acetate [ $\text{C}_4\text{H}_8\text{O}_2$ ]: 99.8%, BDH, England
8. Acetic acid [ $\text{C}_2\text{H}_4\text{O}_2$ ]: 99.8%, Merck, Germany
9. Propanol [ $\text{C}_3\text{H}_8\text{O}$ ]: 99.5%, BDH, England
10. Glucose [ $\text{C}_6\text{H}_{12}\text{O}_6$ ]:  $>99\%$ , Ajax finechem, Australia
11. Fructose [ $\text{C}_6\text{H}_{12}\text{O}_6$ ]:  $>99\%$ , Fluka, Switzerland

#### Preparation of solutions

All solutions were prepared in distilled water obtained from water distillation system (Cyclon, Fistreem, England).

##### 1. Working standard solution of ethanol

Working standard solutions of ethanol were freshly prepared by dilution of 99.9 %v/v ethanol with water and kept at 4 °C when did not use.

##### 2. Acidic potassium permanganate solution (0.2 mmol/L $\text{KMnO}_4$ in 0.25 mol/L sulfuric acid)

A portion of 0.0079 g of  $\text{KMnO}_4$  was dissolved in 100 mL water. An aliquot of 12.50 mL of 5.0 mol/L  $\text{H}_2\text{SO}_4$  was added before making up to a volume of 250 mL in a volumetric flask with water.

### **3. Acidic potassium dichromate solution (0.15 mol/L $K_2Cr_2O_7$ in 4.0 mol/L sulfuric acid)**

A portion of 11.03 g of  $K_2Cr_2O_7$  was dissolved in 100 mL water. An aliquot of 83.30 mL of 12.0 mol/L  $H_2SO_4$  was added before making up to a volume of 250 mL in a volumetric flask with water.

#### **Preparation of sample solutions**

##### **1. Beer**

Beer sample was filled in a 250 mL beaker and poured ten times from one beaker into another, to decrease the amount of foam. Afterwards it was filtered through a filter paper (Whatman, No. 6). The resulting filtrate was bubble-free and could be analyzed directly. A portion of 8.0 mL of filtrate sample was diluted by making up to a volume of 10 mL in a volumetric flask with water.

##### **2. Wine**

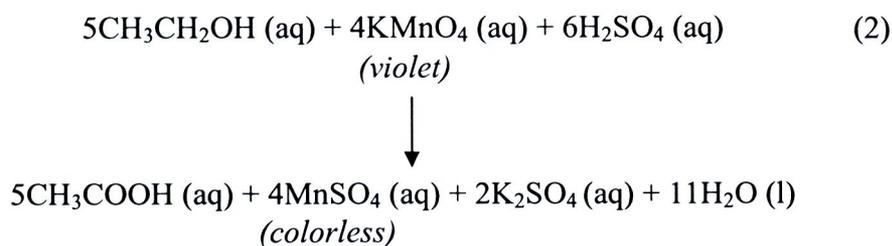
Wine sample was filtered through a filter paper (Whatman, No. 6). Finally, a portion of 4.0 mL of filtrate sample was diluted by making up to a volume of 10 mL in a volumetric flask with water.

##### **3. Thai white distilled liquor, brandy, whisky and rum**

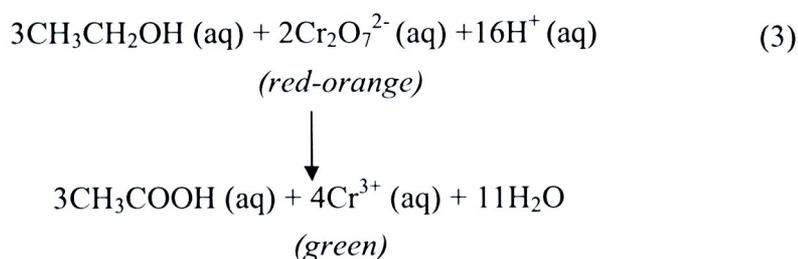
A portion of 1.0 mL of Thai white distilled liquor, brandy, whisky or rum sample was diluted by making up to a volume of 10 mL in a volumetric flask with water.

#### **Related reactions**

The determination of ethanol is based on two simple redox reactions. First reaction, ethanol reduces potassium permanganate in an acidic solution (e.g. sulfuric acid) and the decrease in color intensity of permanganate was monitored at a proper wavelength of colorimetric or UV-Visible spectrophotometric detection (see equation 2).



Second reaction, ethanol reduced potassium dichromate in an acidic solution (e.g. sulfuric acid) and the increase in color intensity of chromium-(III) was monitored at a proper wavelength of colorimetric or UV-Visible spectrophotometric detection (see equation 3).



In this work, above-mentioned reactions were firstly investigated by absorption spectra scanning using UV-Visible spectrophotometer for both methods of FI-colorimetric and HSI-spectrophotometric systems.

## Determination of ethanol in Thai white distilled liquor by FI-colorimetric system

### 1. Instrument setup

A FI-colorimetric system (Figure 8) was constructed by Jaroon Jakmune and groups (Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand). This system consisted of a 4-channel peristaltic pump (P; Masterflex, Cole-parmer, USA), a 6-port manual injection valve (I; Upchurch Scientific, USA), a flow through cell (FC; 10 mm path length, Perkin elmer, USA), a homemade colorimetric-LED detector (D; including visible red, green and blue LEDs). A personal computer with in-house built software (Recorder, version 5) and an eDAQ chart software were employed for collecting data and interpreting peak heights, respectively. All tubings for assembling the proposed system were Teflon tube of 0.89 mm i.d. except pump tubes.

## 2. Manifold of FI-colorimetric system

A FI-colorimetric system is shown in Figure 8 and Appendix A.1. A standard/sample (S) was manually injected into a carrier (C) stream of water, an acidic reagent stream (R), a reaction coil (RC) and a flow through cell (FC) of LED-colorimetric detector, respectively. A FI-peak was obtained. Calibration graph was a plot of peak height obtained versus ethanol concentration. Concentration of ethanol in sample was then evaluated using the calibration graph.

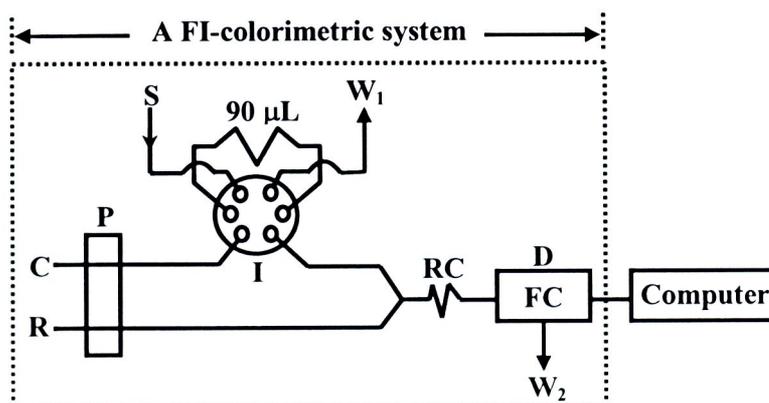
## 3. Optimization of FI-colorimetric system by using an acidic potassium permanganate reagent solution

The key operational variables for the FI-colorimetric system by using an acidic potassium permanganate reagent solution were optimized to maximize the efficiency of LED-colorimetric detector and sensitivity (peak heights and slope) of the method. These variables were 1)  $\text{KMnO}_4$  concentrations (varied in the range of 0.1-0.4 mmol/L), 2)  $\text{H}_2\text{SO}_4$  concentrations (varied in the range of 0.05-0.5 mol/L), 3) flow rate of carrier and reagent streams (varied in the range of 0.9-1.8 mL/min), 4) reaction coil lengths (varied in the range of 100-210 cm), 5) sample volumes (varied in the range of 90-180  $\mu\text{L}$ ), 6) stability study of reagent solution (studied by using UV-Visible spectrophotometer, and 7) calibration graph and detection limit. Finally, the selected conditions were summarized and used to apply for ethanol determination in Thai white distilled liquor samples of different brands. The results obtained from this method were compared by other methods of FI-spectrophotometric, AOAC redox titration and micro-scale potentiometric redox titration.

## 4. Optimization of FI-colorimetric system by using an acidic potassium dichromate reagent solution

The key operational variables for the FI-colorimetric system by using an acidic potassium dichromate reagent solution were also optimized to maximize the efficiency of LED-colorimetric detector and sensitivity (peak heights and slopes) of the method. These variables were 1)  $\text{K}_2\text{Cr}_2\text{O}_7$  concentrations (varied in the range of 0.05-0.3 mol/L), 2)  $\text{H}_2\text{SO}_4$  concentrations (varied in the range of 1.0-4.0 mol/L), 3) reaction coil lengths (varied in the range of 100-200 cm), and 4) calibration graph and detection limit. Finally, the selected conditions were summarized and also used to apply for ethanol determination in Thai white distilled liquor samples of different

brands. The results obtained from this method were compared with other methods of FI-spectrophotometric, AOAC redox titration and micro-scale potentiometric redox titration.



**Figure 8** Manifold of FI-colorimetric system: C – carrier, R – reagent, P- peristaltic pump, S – sample/standard, I – 6-port manual injection valve, RC – reaction coil, D – homemade LED-colorimetric detector, FC - flow through cell, W<sub>1</sub> and W<sub>2</sub> – waste 1 and 2

## Determination of ethanol in alcoholic beverage by HSI-spectrophotometric system

### 1. Instrument setup

The HSI-spectrophotometric system (Figure 9) was constructed by Jaron Jakmune and groups (Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand). This system consisted of two peristaltic pumps (P<sub>1</sub> and P<sub>2</sub>; Masterflex, Cole-parmer, USA), a 6-port manual injection valve (I; Upchurch Scientific, USA), a homemade gas diffusion (GD) unit, a manual syringe (MS), two-way solenoid valves (SV<sub>1</sub>, SV<sub>2</sub>, SV<sub>3</sub> and SV<sub>4</sub>; Bio-chem valve Inc., USA), a three-way solenoid valve (SV<sub>5</sub>; Bio-chem valve Inc., USA), a flow through cell (FC; 10 mm path length, Perkin elmer, USA), UV-Visible spectrophotometric detector (D; Spectronic 21, Milton Roy Company, USA) and a homemade controller. A personal computer with in-house software (Recorder, version 5) and eDAQ chart software were employed

for collecting data and interpreting peak heights, respectively. All tubings for assembling the proposed system were Teflon tube of 0.89 mm i.d. except pump tubes.

A homemade GD unit was consisted of two acrylic plates (15 cm length x 4.8 cm width x 1 cm height), engraving for donor and acceptor channels (with each of 350 mm length x 1.5 mm width x 0.75 mm depth). Two channels were separated by a PTFE membrane (0.15 mm thickness; Plainton, Australia)

## 2. Manifold of HSI-spectrophotometric system

A HSI-spectrophotometric system is shown in Figure 9 and Appendix A.2. The operation of the system was described as follows. Firstly, carrier<sub>1</sub> of water was propelled by P<sub>2</sub> through SV<sub>1</sub>, three T-connectors, RC, FC, SV<sub>5</sub> and W<sub>4</sub>, respectively, by electronic control of a homemade controller. And carrier solutions of water of donor (at carrier<sub>1</sub>) and acceptor (at carrier<sub>2</sub>) streams of the GD were flowed to fill all tubings and channels by P<sub>1</sub> to W<sub>2</sub> and by MS to W<sub>3</sub>, respectively.

Then, the operation cycle was followed. Firstly, a standard/sample solution (500  $\mu$ L) was loaded into sample loop (L<sub>S1</sub>) of a manual injection valve (I) for 5 s. Then, the solution was injected into the donor stream which was continuously flowed by P<sub>1</sub> to W<sub>2</sub>, while the acceptor stream was stopped during the diffusion period (73 s). For the first sequential zone, reagent solution (R<sub>1</sub>) of acidic potassium dichromate, diffuse zone containing ethanol and reagent solution (R<sub>2</sub>) of acidic potassium dichromate were flowed by P<sub>2</sub> to fill into L<sub>R1</sub> (at SV<sub>2</sub>) of 10 s, L<sub>S2</sub> (at SV<sub>3</sub>) of 16 s and L<sub>R2</sub> (at SV<sub>4</sub>) of 10 s, respectively, while excess of each solution was discarded by P<sub>2</sub> to WC and W<sub>4</sub>. Then, its sequential zone (R<sub>1</sub>+diffuse zone containing ethanol+R<sub>2</sub>) was further injected by P<sub>2</sub> into RC of 15 s, stopped at RC of 60 s, injected by P<sub>2</sub> from RC to FC and W<sub>4</sub> of 175 s and then measured the increase color intensity of chromium-(III) at 600 nm, respectively. During the first sequential zone was injected and stopped at RC, the donor (30 s) and acceptor (60 s) streams were cleaned and the second standard/sample solution was started to load into sample loop (L<sub>S1</sub>) at I. When a HSI peak of the first sequential zone was ended (with an analysis time of 175 s), the second sequential zone was started to flow and fill into L<sub>R1</sub>, L<sub>S2</sub> and L<sub>R2</sub>, respectively.

Under the selected condition was described above, the total analysis time for one injection was 286 s (4 min 46 s), resulting in sample throughput of

approximately 15 injections per hour. The operation is semi-automated and the order of solution sequence of [reagent]-[standard/sample]-[reagent] was obtained.

### 3. Optimization of HSI-spectrophotometric system

The key operational parameters for the HSI-spectrophotometric system by using an acidic potassium dichromate reagent solution were optimized to maximize the efficiency and sensitivity (peak heights and slopes) of the method. These variables were 1) diffusion times, sample volumes at  $L_{S1}$  and membrane thicknesses (varied in the ranges of 0.33-1.42 min, 200-500  $\mu\text{L}$ , 0.075-0.15 mm, respectively), 2) filling times of diffuse zone containing ethanol (varied in the range of 10-30 s), 3)  $\text{H}_2\text{SO}_4$  concentrations (varied in the range of 1.0-4.0 mol/L), 4)  $\text{K}_2\text{Cr}_2\text{O}_7$  concentrations (varied in the range of 0.05-0.20 mol/L), 5) reaction coil lengths (varied in the range of 20-50 cm), 6) sample/standard volumes at  $L_{S2}$  (varied in the range of 16-38  $\mu\text{L}$ ), 7) stopped times at reaction coil (varied in the range of 40-100 min), 8) interference study, and 9) calibration graph, precision and detection limit. Finally, the selected conditions were summarized and used to apply for ethanol determination in alcoholic beverage samples such as beer, wine, brandy, whisky, rum and Thai white distilled liquor. The results obtained from this method were compared by other methods of AOAC redox titration and micro-scale potentiometric redox titration.

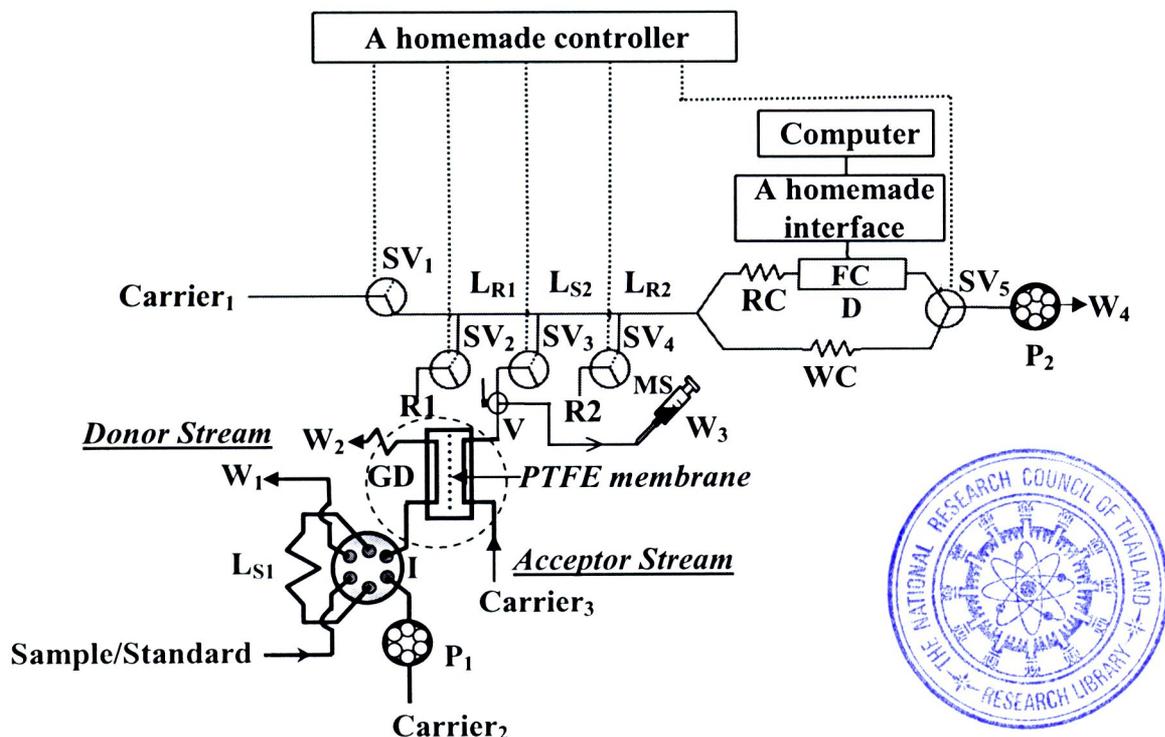


Figure 9 Manifold of HSI-spectrophotometric system:  $R_1$  and  $R_2$  – reagents at position 1 and 2,  $I$  – 6-port manual injection valve,  $GD$  – gas diffusion unit,  $L_{S1}$  and  $L_{S2}$  – sample loops 1 and 2,  $L_{R1}$  and  $L_{R2}$  – reagent loops 1 and 2,  $SV_1$ ,  $SV_2$ ,  $SV_3$  and  $SV_4$  – two-way solenoid valves 1, 2, 3 and 4,  $SV_5$  – three-way solenoid valve,  $V$  – manual two-way valve,  $RC$  – reaction coil,  $FC$  – Flow through cell,  $D$  – UV-Visible spectrophotometric detector,  $WC$  – waste coil,  $P_1$  and  $P_2$  - peristaltic pump 1 and 2,  $MS$  – manual syringe,  $W_1$ ,  $W_2$ ,  $W_3$  and  $W_4$  – waste 1, 2, 3 and 4