CHAPTER 2

EXPERIMENTAL

2.1 Apparatus and instruments

The apparatus and instruments used are listed below:

1. Peristaltic pump, Lachat Instrument, USA
2. 6- Port Valve, Rheodyne, USA
3. Flow through cell, Hellma, Germany
4. Colorimeter: 5564-15, Cole Parmer, USA
5. Chart recorder PM 8251, Philips, Holland
6. UV/vis Spectrometer: Perkin Elmer Lambda 25, USA
7. Voltammograph: Metrohm VA 693, Switzerland
8. Laboratory -made flow cell for HMDE, Thailand
9. Laboratory -made gas diffusion unit, Thailand
10. PTFE membrane, Trace Biotech AG, Germany
11. Ag/AgCl reference electrode for HMDE, BAS, USA
12. HMDE working electrode, Metrohm, Switzerland
13. Pump tube (organic resistance), Fisher, USA

2.2 Chemicals

All chemicals were analytical reagent grade and were used without further purification.

1. Ammonia solution : 30% (w/v), BDH
2. Ammonium chloride : NH_4Cl, Merck
3. Ammonium ferric sulfate dodecahydrate : $Fe(NH_4)(SO_4)_2.12H_2O$, Fluka
4. Ammonium ferrous sulfate hexahydrate : $Fe(NH_4)_2(SO_4)_2.6H_2O$, Fluka
5. Calcium carbonate : $CaCO_3$, Fluka
6. Carbon disulfide : CS_2, Merck
7. Diphenylamine : $(C_6H_5)_2NH$, Fluka
8. Eriochrome Black T (EBT) : $C_{20}H_{12}N_3NaO_7S$, Aldrich
9. Ethanol : C_2H_5OH 99.8%(w/v), Merck
10. Ethylendiaminetetraacetic acid sodium salt : $Na_2C_{10}H_{14}N_2O_8.H_2O$, Merck
11. Hydrochloric acid : 36.46%(w/v), BDH
12. Hydroxy naphthol blue : $C_{20}H_{11}N_2Na_3O_{11}S_3$, Merck
13. Magnesium chloride hexahydrate : $MgCl_2.6H_2O$, Fluka
14. Mercuric chloride : $HgCl_2$, Carlo Erba
15. Mercury : Hg, 99.995%, Fluka
16. Oxygen free-nitrogen gas : N_2, 99.999% , Lanna
17. Phosphoric acid : 86.04 %(w/v), J. T. Baker
18. Potassium dihydrogen phosphate : KH_2PO_4, Carlo Erba

19. Potassiunm dicromate : $K_2Cr_2O_7$, Merck

20. Sodium hydroxide : NaOH, Eka Nobel

21. Sulfuric acid : 98.08%(w/v), BDH

22. Tetrabromofluorescein (Eosin) : $C_{20}H_8Br_4O_5$, BDH

23. Tetrabutyl ammonium hydrogen sulfate : $[CH_3(CH_2)_3]_4N(HSO_4)$, Fluka

24. Zinc powder : 90% assay, BDH

25. Zinc sulfate heptahydrate : $ZnSO_4.7H_2O$, Merck

2.3 Preparation of standard solutions and reagents

2.3.1 Standard solutions and reagents for the determination of calcium, magnesium, iron and zinc by flow injection colorimetry

1. Stock standard calcium solution, 0.10 M

A portion (1.0010 g) of $CaCO_3$ was dissolved in 1 mL of 1.0 M HCl. The solution was boiled to remove carbon dioxide and then made up to 100 mL with deionized water.

2. Stock standard magnesium solution, 0.10 M

A portion (2.0330 g) of $MgCl_2.6H_2O$ was dissolved in 1 mL of 1.0 M HCl and made up to 100 mL with deionized water.

3. Stock standard iron(II) solution, 0.10 M

A portion (3.9214g) of $Fe(NH_4)(SO_4)_2.6H_2O$ was dissolved in 1 mL of 1.0 M HCl and made up to 100 mL with deionized water.

4. Stock standard zinc solution, 0.10 M

A portion (2.8750g) of $ZnSO_4.7H_2O$ was dissolved in 1 mL of 1.0 M HCl and made up to 100 mL with deionized water.

5. Ammonia/ammonium chloride buffer, 0.1 M pH 9.5

A portion (5.34 g) of NH_4Cl was dissolved with water, 6.8 mL of ammonia solution was added. The solution was checked for pH before making up to 1000 mL with deionized water.

6. Eriochrome Black T, 0.01% w/v

A portion (0.01 g) of EBT was dissolved in 100 mL of 0.1 M ammonia/ammonium chloride buffer pH 9.5.

7. Magnesium- EDTA solution, 0.10 M

A portion (20.3300 g) of $Na_2C_{10}H_{14}N_2O_8.H_2O$ and a 37.2240 g of $MgCl_2.6H_2O$ were dissolved and made up to 1000 mL with 0.1 M ammonia/ammonium chloride buffer pH 9.5.

2.3.2 Standard solution and reagents for on-line derivatization of ethanol prior to voltammetric detection

1. Standard aqueous ethanol solutions

A series of standard aqueous ethanol solutions was prepared by appropriate dilution of absolute ethanol 99.8% (w/v) with deionized water.

2. Supporting electrolyte solution

A mixture of 8.02 g NH_4Cl, 8.76 g KH_2PO_4, 1.20 g NaOH and 0.20 g eosin were dissolved. The solution was made up to 1000 mL with deionized water.

2.4 Studies on FI-colorimetric determination

2.4.1 Absorption spectra

An aliquot of 12.0 mL of 0.01% w/v EBT and 50 mL of 0.10 M Mg-EDTA were delivered to a volumetric flask and made up to 100 mL volume with 0.1 M NH_3/NH_4Cl buffer (solution A). The absorption spectrum of solution A was recorded over the range of 400-800 nm using deionized water as reference. Solution B was prepared similar to solution A, but adding 2.25 mL of 0.10 M zinc before making up to volume 100 mL with 0.1 M NH_3/NH_4Cl buffer. The absorption spectrum of solution B was recorded using deionized water as reference.

2.4.2 Manifold for flow injection colorimetry for the determination of calcium, magnesium, iron and zinc

The FI system used for the determination of calcium(II), magnesium(II), iron(II) and zinc(II) is schematically represented in Figure 2.1. Sample or standard solution (50 μL) was injected into a Mg-EDTA stream where the displacement between the analyte cation and magnesium ion from Mg-EDTA complex occurred. After that the sample zone was propelled to Y-connection and merged with EBT stream then to mixing coil and detector, respectively. An increase of wine red color complex of Mg-EBT was detected by a colorimeter using a green filter (530 nm). A calibration graph of individual cation was a plot of peak height versus each ion concentration.

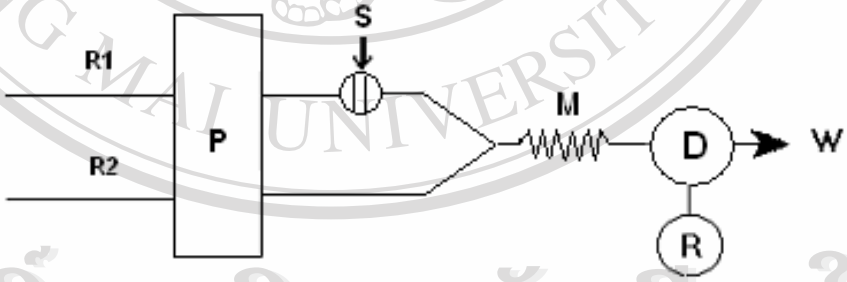

Figure 2.1 Flow injection system for the determination of calcium(II), magnesium(II), iron(II) and zinc(II); R1= Mg-EDTA reagent in NH_3/NH_4Cl buffer solution, R2 = EBT reagent in NH_3/NH_4Cl buffer solution, P = peristaltic pump, S = injection valve, M = mixing coil, D = colorimeter, R = recorder, and W = waste

2.4.3 Optimization

Effect of Mg-EDTA concentrations on peak height was studied. A series of standard Ca(II), Mg(II), Fe(II) and Zn(II) solutions in the range of 4.0- 10.0 mM were injected. From the FIAgram obtained, linear calibration equations were calculated. The slope and correlation coefficient (r^2) were considered for choosing a suitable Mg-EDTA concentration that can be utilized for all of the analytes.

Effect of EBT concentration on FIAgram was observed by injecting standard Ca(II) solution as a model for this study. The higher peak height and lower baseline noise was desired.

Effect of pH of standard solution was checked. Standard solutions of 5.0 mM of Ca(II), Mg(II), Fe(II) and 4.0 mM Zn(II) were prepared in various pH values (2, 3 and 5). The solutions were injected into the system. The obtained peak heights were measured.

2.5 FI-voltammetric system with on-line derivatization for determination of ethanol

The FIA manifold for on-line derivatization of ethanol and voltammetric detection is shown Figure 2.2. A standard/sample containing ethanol was injected into a stream of 0.1 M NaOH (R2), then pass through a gas diffusion unit where CS_2 was introduced through the PTFE membrane. The reaction product, ethyl dithiocarbonate or xanthate was merged with supporting electrolyte (R1) before entering a laboratory -

made flow cell for voltammetric detection by OSWV technique, using a 693 VA processor (Metrohm, Herisau, Switzerland).

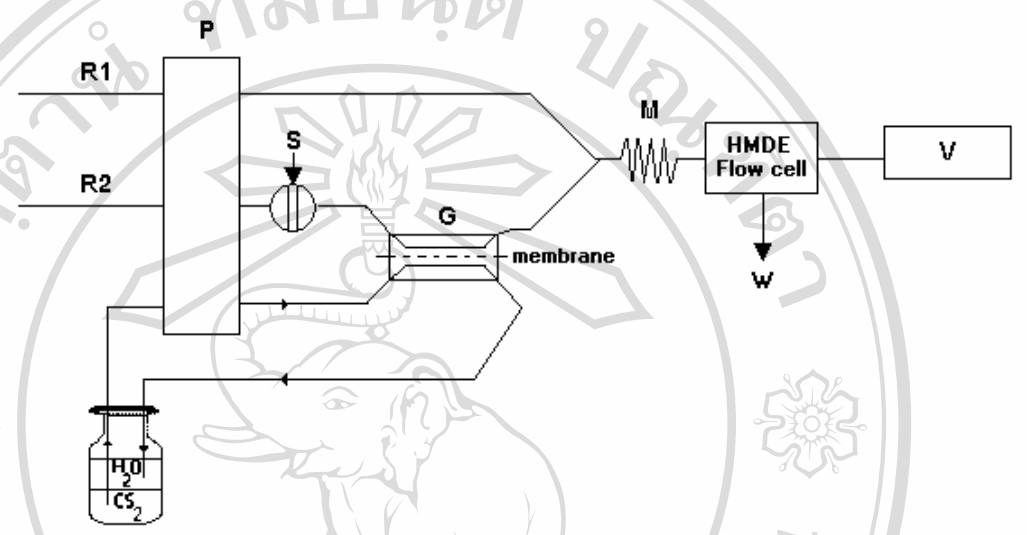

Figure 2.2 Schematic diagram of the FIA manifold for on-line derivatization and detection of ethanol; R1 = supporting electrolyte (0.15 M NH_4Cl, 0.03 M NaOH, 0.05 M KH_2PO_4 and 3 x 10^{-4} M eosin), R2 = 0.1 M NaOH, M = mixing coil, G = gas diffusion unit, V= voltammograph and W = waste

The schematic of flow cell used is shown in Figure 2.3. A body of flow cell was made of acrylic. An auxiliary (A.E.) electrode was made from stainless steel, fabricating as a nut to be fit on the block. The hole was drilled through the AE nut for removing of a mercury waste. The HMDE was inserted into a flow chamber (i.d. 0.7 cm, height 1.0 cm). A normal solution outlet was next to a R.E. (outlet 1). An overflow outlet (outlet 2) was placed at the flow chamber.

The flow cell was tested for flow injection voltammetric determination of zinc using acetate buffer as a supporting electrolyte. Peak current was measured by OSWV

using silver/silver chloride as a reference electrode. The effects of NaOH and CS_2 flow rates were studied.

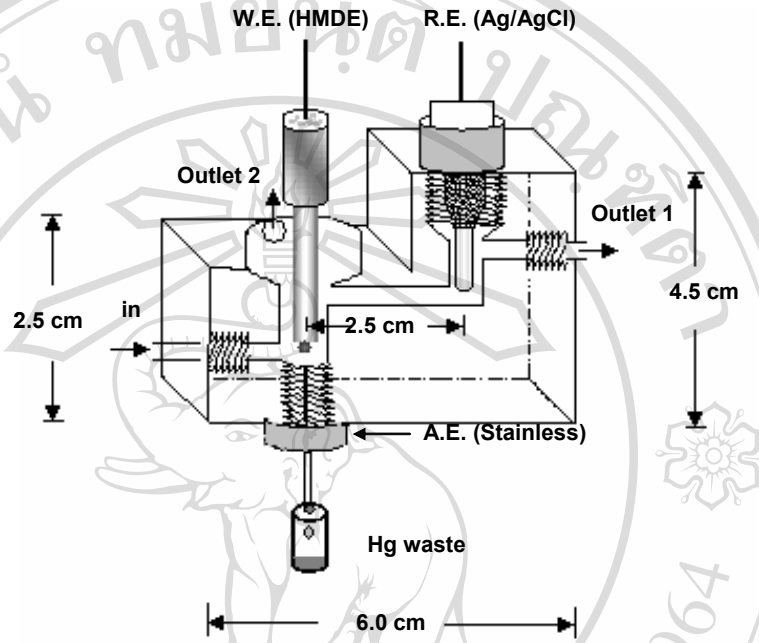

Figure 2.3 A side view of laboratory-made hanging mercury dropping electrode flow cell.