CHAPTER 2

EXPERIMENTAL

2.1 Chemicals, Apparatus and Instruments

2.1.1 Chemicals

All chemicals were analytical reagent grade and were used without further purification. Deionized water (MilliRX, Millipore, USA) was used thoughout for preparing the solutions.

1. Tetramethylammonium bromide : $(CH_3)_4NBr$, BDH
2. Lithium chloride : LiCl, AJAX
3. Methanol : CH_3OH, Carlo Erba
4. Fumaric acid : $C_4H_4O_4$, Fluka
5. Citric acid : $C_6H_8O_7.H_2O$, Carlo Erba
6. Succinic acid : $(CH_2COOH)_2$, BDH
7. DL – Tartaric acid : $C_4H_6O_6$, Fluka
8. DL – Malic acid : $C_4H_6O_5$, MERCK
9. Oxygen free – nitrogen gas : N_2 99.999%, Lanna
10. Hydrochloric acid : HCl, 36.46% (W/V),
11. Sodium hydroxide : NaOH, UNIVAR
12. Acetic acid : CH_3COOH, Merck
13. Sodium acetate trihydrate : $CH_3COONa.3H_2O$,

14. L – Ascorbic acid : $C_6H_8O_6$, UNILAB
15. Disodium hydrogenorthophosphate dihydrate : $Na_2HPO_4 . 2H_2O$, BDH
16. Sodium dihydrogenphosphate dihydrate : $NaH_2PO_4 . 2H_2O$, Fluka
17. 2, 6 – Dichlorophenolindophenol sodium salt hydrate : $C_{12}H_6C_{l2}NaO_2$, Fluka
18. Metaphosphoric acid : HPO_3, Merck

2.1.2 Apparatus and instruments

The apparatus and instruments used are listed below:

1. Peristaltic pump, Alitea, Sweden
2. PTFE tube, Upchurch Scientific, USA
3. Solenoid valve, Cole Parmer, USA
4. Voltammograph model VA 693, Metrohm, Switzerland
5. Laboratory – made flow cell for HMDE
6. Peristaltic pump model MP-3, Eyela, Japan
7. Injection valve, Upchurch, USA
8. Amperometer (laboratory-made by Dr.Jaroon Jakmunee)
9. Flow through cell, BAS, USA
10. Dialysis unit (laboratory made by FBA group, Thailand)
11. Dialysis membrane, MWCO 12,000 Da, CelluSep, USA
12. Data acquisition system, eDAQ, Australia

2.2 Preparation of standard solutions and reagents

2.2.1 Preparation of solutions for the determination of fumaric acid by voltammetry

1. Standard solution of fumaric acid

Stock standard solution of fumaric acid (1000 mg/L) was prepared by dissolving 0.1000 g of fumaric acid in methanol and making up to 100 ml in a volumetric flask with methanol.

The 500 mg/L and 100 mg/L standard solutions of fumaric acid were prepared by diluting of 1000 mg/L fumaric acid stock standard solution in a 100 ml volumetric flask with methanol.

2. Supporting electrolyte solution: Tetramethylammonium bromide, 0.1 M + Lithium chloride, 0.01 M

The supporting electrolyte was prepared by dissolving 7.70 g of tetramethylammonium bromide and 0.210 g of lithium chloride in water, adjusting volume to 500 mL.

3. Acetate buffer, 0.1 M pH 4.5

A portion of water was used to dissolve 3.85 g of ammonium acetate before 4 ml glacial acetic acid was added, then adjusting volume to 1000 mL.

4. Hydrochloric acid, 0.1 M

Hydrochloric acid (35%) 8.30 ml was diluted in water and making the final volume to 1000 mL.

5. Sodium hydroxide, 0.1 M

A 2.0 g portion of sodium hydroxide was dissolved in water and diluted to 500 mL with deionized water.

2.2.2 Preparation of solutions for the determination of ascorbic acid by amperometric and titrimetric methods

1. Standard solution of ascorbic acid 1000 mg/L

A portion of 0.1000 g of ascorbic acid was dissolved and made up to volume of 100.00 mL with water.

2. Supporting electrolyte solution: 0.1 M phosphate buffer, pH 6

Supporting electrolyte solution was prepared by dissolving 14.71 g of sodium dihydrogenphosphate dihydrate and 1.00 g of disodium hydrogenphosphate dihydrate in water, adjusting volume to 500 mL.

3. 2, 6-Dichlorophenol indophenol reagent solution

A portion of 0.1250 g of 2, 6-dichlorophenol indophenol and 0.1050 g of sodium hydrogen carbonate were dissolved and made up to volume of 500.00 mL with water.

4. Metaphosphoric acid – acetic acid solution

A portion of 30.00 g of metaphosphoric acid and 80.00 mL of acetic acid were dissolved and made up to volume of 1000.00 mL with water.

5. Fruit juice samples

Samples were collected form supermarkets and local suppliers. The samples were opened and filtered with cotton wool before the analyses.

6. Pharmaceutical tablet samples

Pharmaceutical tablet samples were obtained form local pharmaceutical store. Twenty tablets of the sample were weighed and an average weight of a tablet was calculated before being ground into fine powder. Then, 0.6-0.4 g portions were accurately weighed, dissolved in water to get appropriate concentrations of ascorbic acid in solutions.

2.3 Systems for determination of fumaric acid by batch voltammetry and FI voltammetry

2.3.1 Batch differential pulse voltammetric system

Batch differential pulse voltammetric determination of fumaric acid is performed on a voltammograph as illustrated in Figure 2.1. The Metrohm VA 636 computrace voltammograph having Pt wire, hanging mercury drop, and Ag/AgCl electrodes as auxillary, working and reference electrodes, respectively was employed.

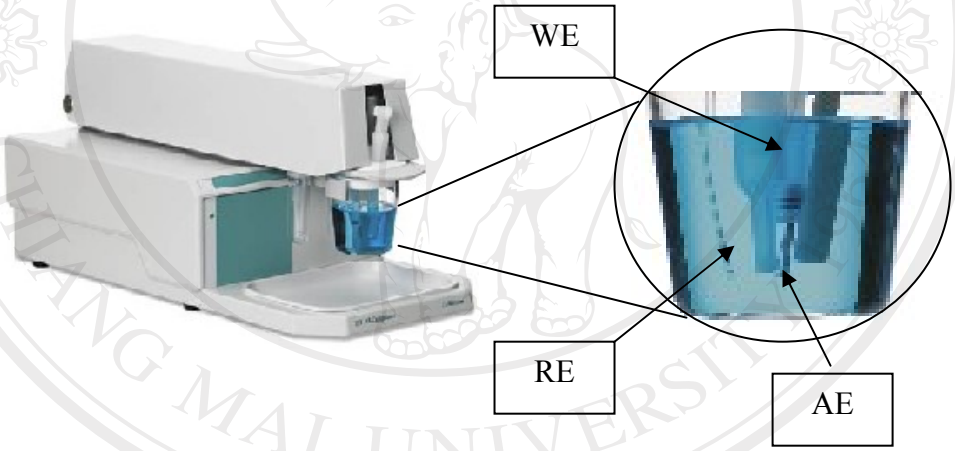

Figure 2.1 Metrohm 636 voltammograph. AE: auxiliary electrode, WE: working electrode, RE: reference electrode. Solution in system; 10 mL of $(CH_3)_4NBr$(0.1M) + LiCl (0.01M) as a supporting electrolyte.

2.3.2 Pumping devices for FI system

2.3.2.1 Nitrogen gas pressure driven pumping device

Nitrogen gas pressure pumping device was assembled as shown in Figure 2.2. Using a N_2 gas cylinder, a constant pressure gas (adjusting by restriction valve 1) was applied on top of a solution in a closed bottle and a restriction valve 2 on liquid side was adjusted to control flow rate of the outlet solution. Weight of water was continuously monitored and converted to volume per time unit or a flow rate (mL/min).

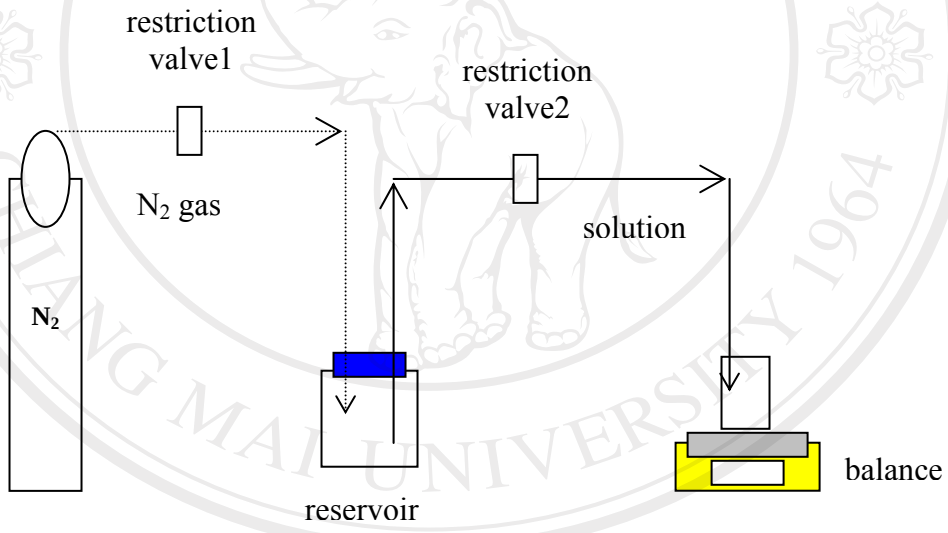

Figure 2.2 Nitrogen gas pressure driven pumping system.

2.3.2.2 Gravity driven pumping device

A gravity driven pumping system based on a mariotte bottle [64] was set up to be used as a solution propelling device as shown in Figure 2.3. Flow rate can be controlled by adjusting a restriction valve. A delivered volume per unit of time was investigated by measurement weight of water versus time.

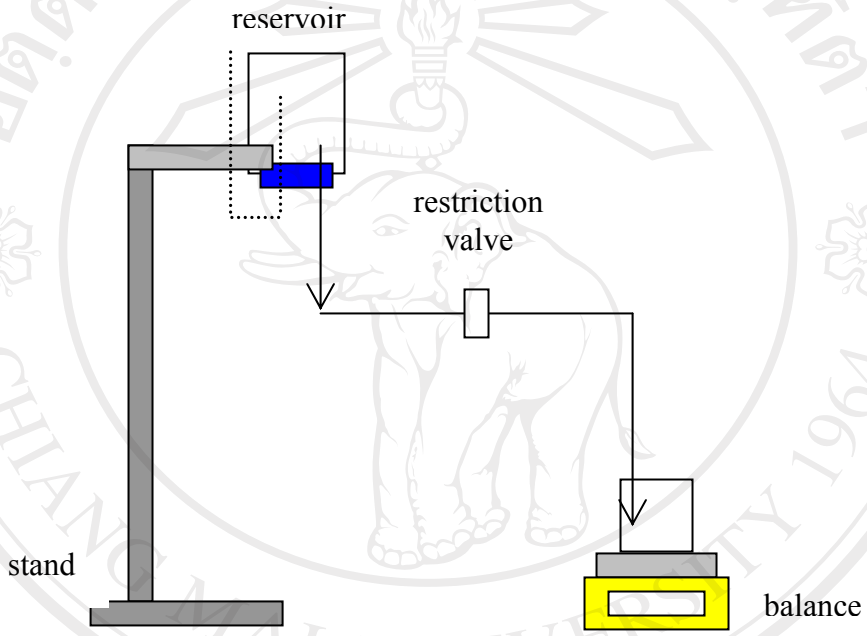

Figure 2.3 Gravity driven pumping device.

2.3.3 FI differential pulse voltammetric system

A FI – differential pulse voltammetric set up is illustrated in Figure 2.4. A gravity driven pumping device was used to propel an electrolyte solution. A sample/standard solution is injected into the FI system via 2 of three way solenoid valves connecting to provide a sample loop of 100µL. By switching the valves, the solution in the loop was then inserted into a carrier stream and moved along to a detector. The fumarate should be reduced to succinate at reduction potential of –1050 mV vs. Ag/AgCl reference electrode. The current change is recorded by the voltammograph.

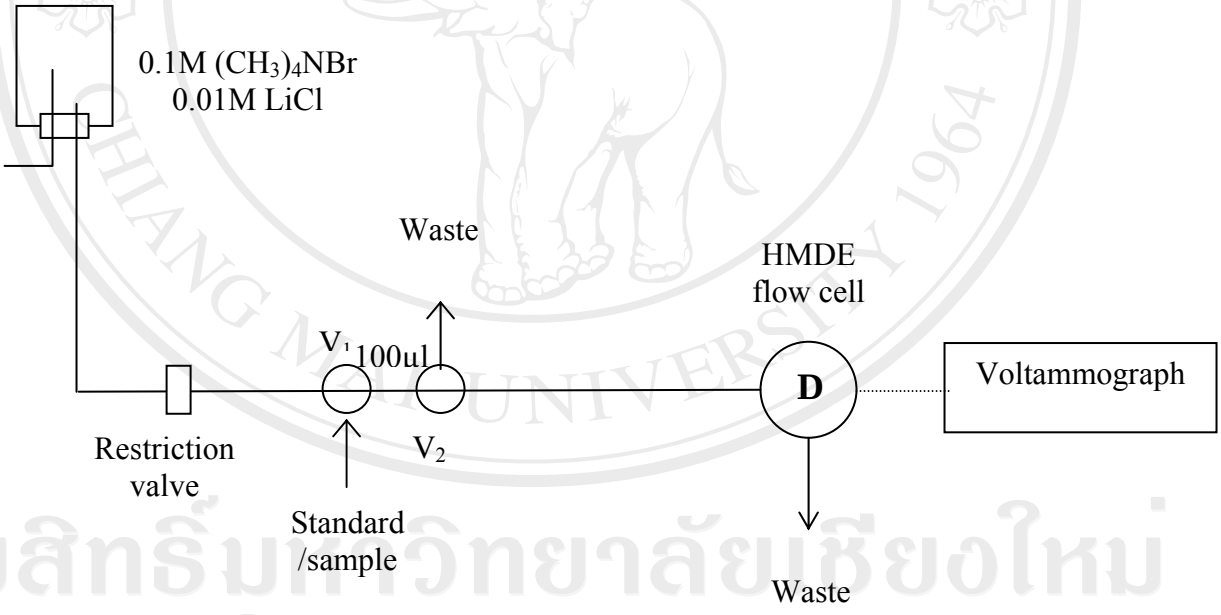

Figure 2.4 Manifold of FI – differential pulse voltammetric system for fumaric acid determination.

2.4 Systems for determination of ascorbic acid by FI amperometry

2.4.1 Flow injection - amperometric system

Using FI amperometric system as shown in Figure 2.5, ascorbic acid standard or sample solution (50 µL) was introduced via a six-port valve into phosphate buffer carrier stream. The injected solution moves through a flow cell whereas ascorbic acid should be oxidized to dehydroascorbic acid at a working electrode applying with constant potential (+800 mV versus Ag/AgCl). Three electrodes system was used, planar glassy carbon electrode as a working electrode, silver/silver chloride (Ag/AgCl) as a reference electrode and stainless steel as an auxiliary electrode. The current change is continuously recorded as FI peak.

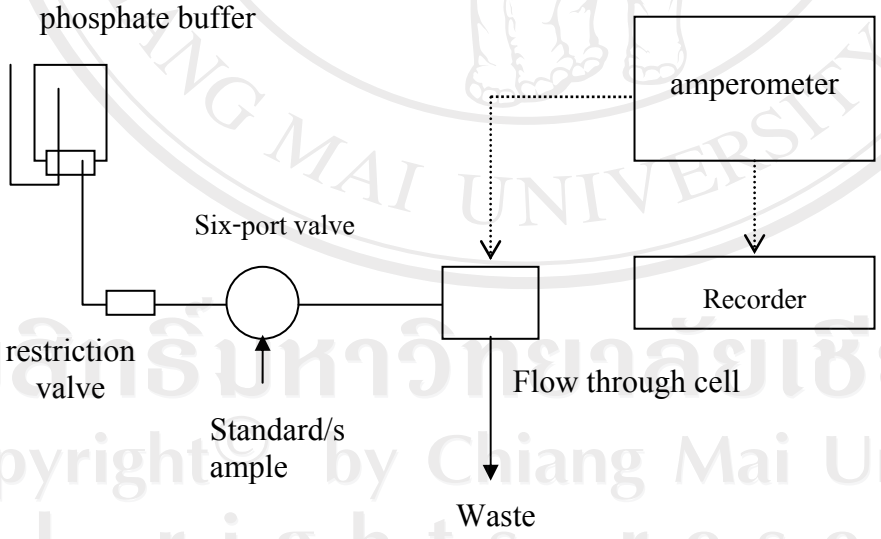

Figure 2.5 Manifold of FI amperometric system for ascorbic acid determination.

2.4.2 FI amperometric system with dialysis unit for sample pretreatment

FI amperometric system with dialysis sample pretreatment unit is illustrated in Figure 2.6. Ascorbic acid standard or sample solution (50 µL) was introduced via a six-port valve into the donor stream of deionized water flowing at 1.5 mL/min. An acceptor stream of 0.1 M phosphate buffer pH 5.6 was flowed at 1.5 ml/min as well. Sample was diluted and ascorbic acid was separated from other matrices in real samples by dialyzing through a dialysis membrane into the acceptor stream. The dialyzed zone containing ascorbic acid entered a flow through detector where a constant potential was applied on a glassy carbon working electrode. The peak of current change due to electrooxidation of ascorbic acid was recorded.

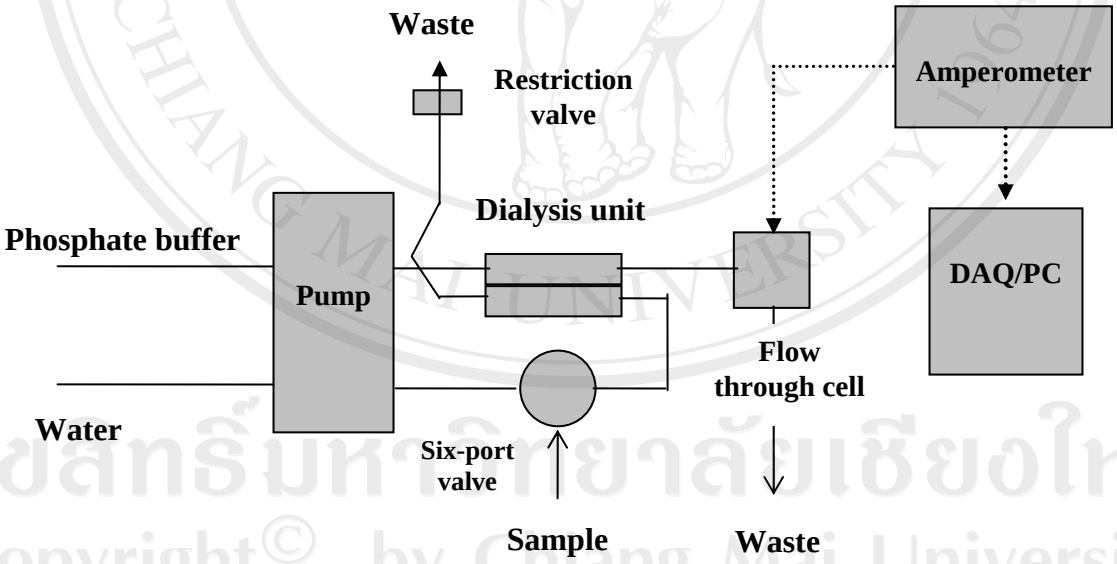

Figure 2.6 Manifold of FI amperometric system with dialysis pretreatment for ascorbic acid determination.