CHAPTER 2

EXPERIMENTAL

2.1 Materials and apparatus

1. Peristaltic pump (Ismatec, Switzerland).
2. Three-way valve (Cole-Parmer, USA)
3. Six-port injection valve (Upchurch Scientific, USA)
4. Spectronic 21 (Spectronic instrument, USA)
5. Flow through cell 1 cm path length, 8 µl (Starna Scientific Ltd., UK)
6. Stopwatch (Citizen, Thailand)
7. Syringe (Nipro Co. Ltd., Thailand)

2.2 Reagents

1. L-Ascorbic acid: $C_6H_8O_6$ MW 176.13, 99.5% (Fluka, Switzerland)
2. Potassium dihydrogen phosphate: KH_2PO_4, MW 136.39, 99.5% (Merck, Germany)
3. Sodium molybdate dihydrate: $Na_2MoO_4.2H_2O$, MW 241.95 (GR Merck, Germany)
4. Sulfuric acid: H_2SO_4, MW 98.08, 98% (Merck, Germany)
5. Acetic acid: CH_3COOH (Carlo Erba) , MW 60.05, 99.8% (Merck, Germany)
6. Disodium tartrate: $NaOCO(CHOH)_2COONa.2H_2O$, MW 230.08 (Fluka, Switzerland)
7. Sodium hydrogen carbonate : $NaHCO_3$, MW 84.01, (Merck, Germany)

8. Meta-phosphoric acid : $(HPO_3)_n$ (Merck, Germany)
9. 2,6-Dichlorophenol indophenol sodium salt hydrate : $C_{12}H_6Cl_2NNaO_2.H_2O$, MW 290.08, 97% (Fluka, Switzerland)

2.3 Preparation of standard solution and reagent

2.3.1 Stock standard solution of ascorbic acid 1000 mg/L

Ascorbic acid 0.1 g was dissolved in DI water and the final volume was adjusted to 100 ml (fresh preparation). The solution was store in a brown glass bottle. Standard solutions were freshly prepared before use by dilution of stock standard with DI water.

2.3.2 Reagent solution, molybdic acid

Sodium molybdate dihydrate 2.4679 g and potassium dihydrogen phosphate 0.7076 g were dissolved in 0.55 x 10^{-1} M sulfuric acid and the final volume was adjusted to 50 ml.

2.3.3 Sulfuric acid, 0.55×10^{-1} M

Concentrated sulfuric acid 3.0 ml was diluted in water and adjusted to 500 ml using DI water.

2.3.4 Metaphosphoric acid - acetic acid solution (extracting solution)

Metaphosphoric acid 15 g was dissolved in 40 ml of acetic acid. Then the solution was diluted to 500 ml with DI water and stored in refrigerator.

2.3.5 Standard dye solution (2,6-dichlorophenol indophenol)

2,6-dichlorophenol indophenol 0.1250 g and sodium hydrogen carbonate 0.1050 g were dissolved in DI water and the final volume was adjusted to 500 ml. After filtration through a filter paper (NO.42), the solution was kept in a brown glass bottle and stored in refrigerator. Prior to each use, the dye solution was standardized by titration with a standard solution of ascorbic acid.

2.3.6 Preparation of fruit samples

Fruit samples (mango, apple, chinese pear, pineapple, tangerine, star fruit, lemon, strawberry, jujube and guava) were collected from local markets in Chiang Mai city. Each fruit sample was blended with a blender. The suspended samples were filtered through a filter paper (NO.1) or centrifuged and the clear solutions of samples were determined by comparing results from stopped-flow method with titrimetric standard method.

2.4 Manifold and operation steps

2.4.1 Stopped-flow system for determination of vitamin C

The manifold of stopped-flow system used is shown in Figure 2.1. The reagent solution and carrier were propelled by a peristaltic pump. Each standard or sample solution was introduced by means of a six-port valve with a 100 μl sample loop. The detector was the Spectronic 21 with a flow cell of 1 cm path length 8 μl internal volume. The signal was converted to voltage and recorded with computer software (StampDAQ, Parallax, Inc., USA). The height and area of signal were calculated using

the eDAQ Chart 5 (Apache Software Foundation, USA) for determination of vitamin C.

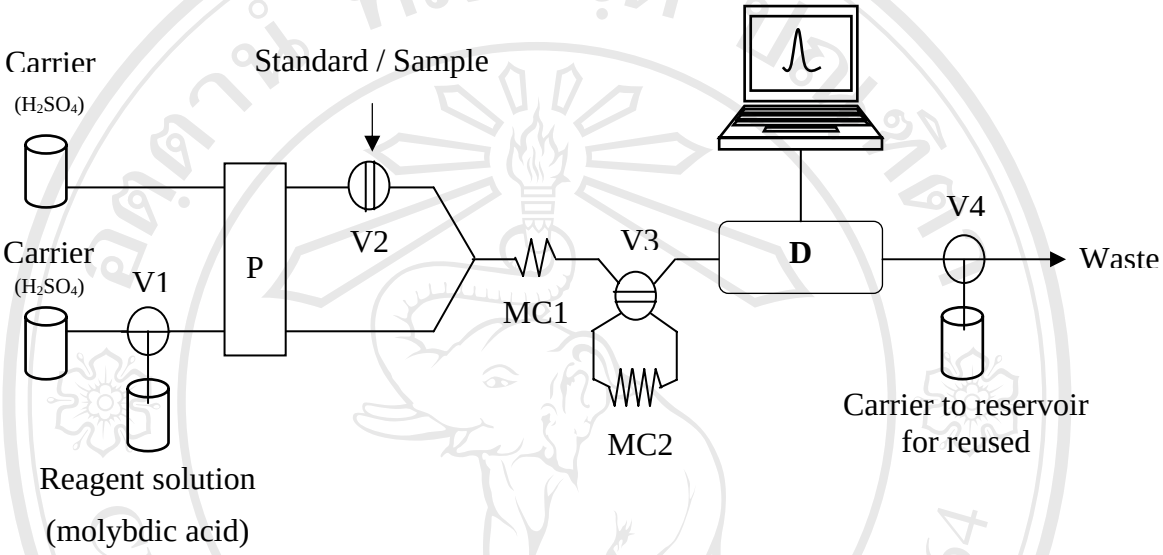

Figure 2.1 Stopped-FIA manifold for determination of vitamin C : P = Peristaltic pump; V2 and V3 = Six-port valves; V1 and V4 = Three-way valve; MC1 and MC2 = Premixing coil and stopping coil (30 and 200 cm); D = Detector (Spectonic 21)

The operation steps of the stopped-FIA system (Figure 2.1) are as follows, the reagent solution (molybdic acid) was introduced into the system through V1 using a peristaltic pump. Standard (ascorbic acid) or sample solution was injected into the carrier stream by the six-port value (V2). After the period of 12 sec, the reagent stream was stopped and changed to carrier stream by switching V1. Then, standard and reagent were premixed in the premixing coil (MC1) and stopped in the

stopping coil (MC2) (which is a loop of six-port value (V3)). After a desired stopped-time, V3 was switched for the carrier stream to flush the mixture into the detector. During the stopped-period, the carrier was flowed to reservoir for reuse by switching V4, while V4 was switched to waste before the product was injected into the detector by switching V3. The signal was recorded at 680 nm. The peak height or peak area of the stopped-FIA gram was used for vitamin C determination.

2.4.2 Optimization

The parameters such as operation time, reagent concentrations were optimized. Accuracy and precision of the developed method were evaluated. Results obtained from the stopped-flow method were compared with those obtained from the conventional 2,6-dichlorophenol indophenol titrimetric method.

2.4.3 Titrimetric determination of vitamin C, 1000 mg/L

The standard titrimetric method suggested by AOAC. Metaphosphoric acid-acetic acid solution (prepared as described in section 2.3.4) was added to the sample solution, which was then titrated with 2,6-dichlorophenol indophenol solution (prepared as described in section 2.3.5).