CHAPTER 2

EXPERIMENT

2.1 Materials and Apparatus

1. Home-made semi-automatic stopped-FI analyzer consisting of
    - stopped flow controller
    - six-port injection valve
    - light emitting diode (LED)-colorimeter
2. Peristaltic pump model MP-3 (EYELA, Japan)
3. Tygon tubing (Saint-Gobain Performance Plastics, USA) i.d. 1.52 mm.
4. PTFE tubing (Cole-Palmer, USA) i.d. 1/32 inches
5. Syringe 1 ml (NIPRO, Japan)
6. Flow through cell 1 cm path length (HELLMA, Germany)
7. Spectronic 21 (Spectronic Instrument, USA)
8. Recorder model L6512 (LINSEIS, USA)

2.2 Chemicals

All chemicals were of analytical-reagent grade except when specified.

1. Sodium molybdate dihydrate: $Na_2MoO_4.2H_2O$, Fisher Sciencific Company
2. L-Ascorbic acid: $C_6H_8O_6$, APS
3. Nitric acid: HNO_3 35 %V/V, E. Merck
4. Potassium dihydrogen phosphate: KH_2PO_4, E. Merck
5. Potassium iodide: KI (iodate free), Carlo Erba

6. Hydrochloric acid: HCl 37%W/V, Carlo Erba
7. Potassium chlorate: $KClO_3$, E. Merck
8. Sodium acetate: CH_3COONa, E. Merck
9. Acetic acid: CH_3COOH, Merck

2.3 Preparation of standard solutions and reagents

Milli Q water was used through-out.

2.3.1 Stock standard solution of 1000 mg P l^{-1}

Standard solution of 1000 mg P l^{-1} was prepared by dissolving 0.2197 g of potassium dihydrogen phosphate (KH_2PO_4) in water and making up to volume of 500 ml in a volumetric flask.

2.3.2 Stock standard solution of chlorate

Standard solution of 1000 mg l^{-1} chlorate was prepared by dissolving 0.1474 g of potassium chlorate ($KClO_3$) in deionized water and making up to 100 ml in a volumetric flask.

2.3.3 Sodium molybdate, 0.02 M in 0.15 M nitric acid

A 2.4195 g portion of sodium molybdate was dissolved in water. A 5.4 ml volume of nitric acid was added before making up to a volume of 500 ml with water.

2.3.4 Ascorbic acid, 0.25% (w/v)

Ascorbic acid solution of 0.25% (w/v) was prepared by dissolving 1.25 g of ascorbic acid in 500 ml water (fresh preparation).

2.3.5 Potassium iodide, 0.1 M

A 4.1711 g potassium iodide was dissolved and diluted to 250 ml with water.

2.3.6 Hydrochloric acid, 7 M

Hydrochloric acid of 7 M was prepared by diluting 150 ml volume of conc. hydrochloric acid to 250 ml water.

2.3.7 Acetate buffer, 0.8 M pH 4.8

0.8 M acetate buffer pH 4.8 was prepared by mixing 1.5 M sodium acetate 532 ml with 1.5 M acetic acid 468 ml.

2.4 Preparation of Soil Samples

Sampling of soil samples was explained in Appendix C.

2.4.1 For determination of phosphate [40]

20 soil samples were collected from different areas in Chiang Mai city. 10 g of each soil sample was extracted with 25 ml of 0.8 M acetate buffer (pH 4.8) and shaked for 30 min, filtered through a filter paper and adjusted the volume to 50 ml with water prior to an analysis.

2.4.2 For determination of chlorate [41]

20 soil samples were provided by Department of Soil Resource and Environment, Faculty of Agricultural Production, Maejo University where all subjects were routinely checked up. A portion (20 g) of each soil sample was extracted with

20 ml water and shaked for 1 h, filtered through a filter paper and adjusted the volume to 25.00 ml prior to an analysis.

2.5 Manifold and operation steps

2.5.1 Stopped-FI system for determination of phosphate [5]

The diagram of stopped-FI system used is shown in Figure 2.1. All pump tubings were Tygon tube. The rest of flow lines were assembled from PTFE tubings. Reagent solutions were propeled by a peristaltic pump. Each sample was introduced by means of a six-port valve with a 55 μl sample loop. The system was controlled by a semi-automatic stopped-FI analyzer with a light emitting diode (LED)-colorimeter that monitered the color intensity of reaction product. The voltage signal from the colorimeter was recorded with a recorder.

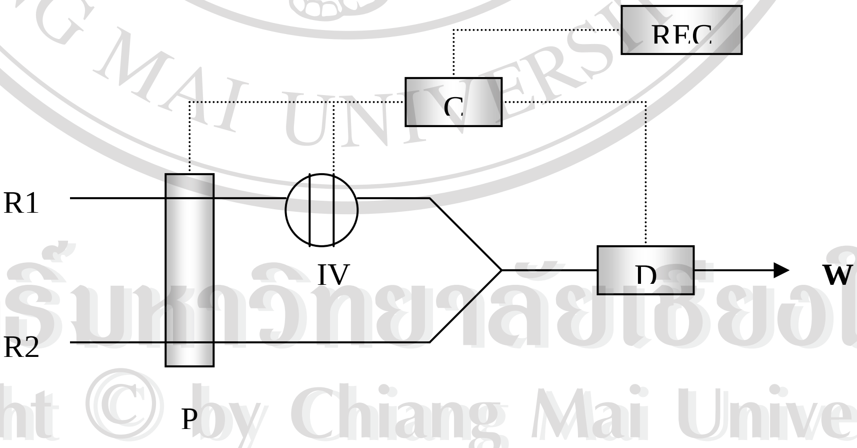

Figure 2.1 Stopped-FIA manifold for determination of phosphate: R1 = 0.02M sodium molybdate in 0.15M nitric acid, R2 = 0.25% (w/v) ascorbic acid, P = Peristaltic pump, C = controller, IV = six-port injection valve, D = detector, REC = recorder, W = waste

Before an analysis, the analyzer was set for the values of timing of operation cycle; travelling time, stopping time and washing time. During the mixture zone of reagent and sample was halted in a flow cell, the intensity of color of the product from the reaction increased continuously, gaving a higher signal. The slope of stopped-FIgram was used for phosphate determination via a calibration graph which is a plot of the slope and phosphate concentration.

2.5.2 Stopped-FI system for determination of chlorate

The system described in 2.5.1 was used, but the detector was changed to be the Spectronic 21 with a flow cell of 1 cm path length and the stopped-FI analyzer controlled only a peristaltic pump and an injection valve. A similar operation cycle as in 2.5.1 was used, but the flow was stopped while a sample zone was being in a mixing coil. Then the flow was restarted again to push the injected zone into a flow cell for continuous recording of signal. The peak height of the FIgram was measured for determination of chlorate.

The diagram of stopped-FI system for chlorate determination is shown in Figure 2.2.

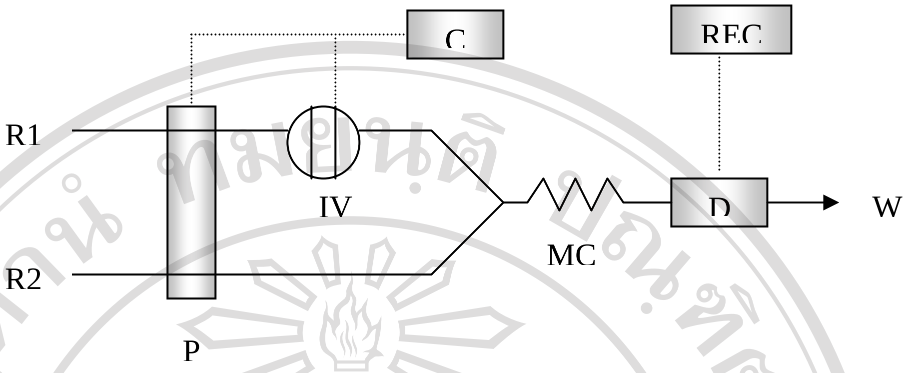

Figure 2.2 Stopped-FIA manifold for determination of chlorate: R1 = 0.1 M potassium iodide, R2 = 7 M hydochloric acid, P = Peristaltic pump, C = controller, IV = six-port injection valve, MC = mixing coil, D = detector, REC = recorder, W = waste

2.6 Optimization

2.6.1 For determination of phosphate

Effects of concentrations of reagents: sodium molybdate, ascorbic acid and nitric acid, on the slope of the FIAgram were studied. A standard phosphate solution of 0.3-6 mg P l^{-1} was injected into a system. For each concentration of the reagent, a calibration graph was plotted of slopes of the stopped-FIAgrams obtained versus phosphate concentration. Slopes of calibration graphs were considered for selection of reagent concentrations.

2.6.2 For determination of chlorate

In this study, effect of stopping time on the peak height of the FIAgrams was studied by variation of the time for stopping pump. A series of chlorate solutions (5-

50 mg ClO_3^- l^{-1}) was injected into the system. Peak heights from each of stopping time were plotted versus chlorate concentrations to make a calibration graph. Slopes of calibration graph were considered for selection of suitable stopping time. In addition, effect of concentration reagents: potassium iodide and hydrochloric acid were also studied. For each concentration of the reagent, a calibration graph similar to the above was plotted. Slopes of calibration graphs were considered for selection of reagent concentrations.