CHAPTER 2

EXPERIMENTAL

2.1 Chemicals

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

1. Ammonium ferrous sulfate hexahydrate: $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$, BDH

2. Ammonium ferric sulfate dodecahydrate: $NH_4Fe(SO_4)_2 \cdot 12H_2O$, BDH

3. Ferous sulfate heptahydrate: $FeSO_4 \cdot 7H_2O$, Aldrich

4. Ferric chloride hexahydrate: $FeCl_3 \cdot 6H_2O$, Aldrich

5. Salicylic acid: $HOC_6H_4CO_2H$, Merck

6. Sodium pyrophosphate: $Na_4P_2O_7$, Merck

7. Dipotassium hydrogen phosphate: K_2HPO_4, Merck

8. Ammonia solution: NH_4OH, BDH

9. Nitric acid: HNO_3, Merck

10. Perchloric acid: $HClO_4$, Merck

11. Hydrochloric acid: 37% (w/w) HCl, Merck

12. Sulfuric acid: 98% (w/w) H_2SO_4, BDH

13. Acetylsalicylic acid: $C_9H_8O_4$, Merck

14. Acetylsalicylic acid: 75% (w/w) $C_9H_8O_4$ available as aspirin, Osotsapa

15. Hydrogen peroxide: 30% (w/w) H_2O_2, Carlo Erba

16. Sodium molybdate dihydrate: $Na_2MoO_4 \cdot 2H_2O$, BDH

17. 2,4´-pyridylazoresorcinol (PAR): $C_{11}H_{14}O_2N_3$, Aldrich

18. Potassium dihydrogen phosphate: KH_2PO_4, BDH

19. Ammonium heptamolybdate tetrahydrate: $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, BDH

20. Tin(II) chloride: $SnCl_2$, BDH

21. Hydrazine sulfate: $H_2NNH_2 \cdot H_2SO_4$, Aldrich

22. L-Ascorbic acid: $C_6H_8O_6$, BDH

23. Sodium sulfide: Na_2S, Aldrich

24. Potassium permanganate: $KMnO_4$, BDH

25. Sodium hydroxide: NaOH, BDH

26. Phytic acid: $C_6H_{12}O_{26}P_4$, Sigma

27. Phenyl phosphate dihydrate: $C_6H_5OP(O)(ONa)_2 \cdot 2H_2O$, Sigma

28. Adenosin-5-monophosphate: Boenernge Mannheim GmbH

29. Glycerophosphate: $(HOCH_2)_2CHOP(O)(ONa)_2 \cdot xH_2O$, Sigma

30. 2-aminoethylphosphonic acid: $H_2NCH_2CH_2P(O)(OH)_2$, Sigma

31. Potassium chloride: KCl, BDH

32. Anion exchange resin: AG1-X8 (Chloride form), Bio-Rad

33. Sodium metasilicate: Na_2SiO_3, AJAX

34. Sodium chloride: NaCl, BDH

2.2 Preparation standard solutions and reagents

2.2.1 Stock standard solution of iron

Standard solutions of 1000 mg l^{-1} Fe(II) and Fe(III) were prepared by dissolving 0.7022 and 0.8640 g of ammonium ferrous sulfate ($(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$, M.W. = 392.14) and ammonium ferric sulfate ($NH_4Fe(SO_4)_2 \cdot 12H_2O$, M.W. = 482.19) in a portion of water. A 0.5 ml portion of concentrated sulfuric acid was added before making up to a volume of 100 ml with Milli-Q water.

Standard solutions of 0.01 M Fe(II) and Fe(III) were prepared by dissolving 0.1390 and 0.1352 g of ferrous sulfate $FeSO_4 \cdot 7H_2O$, M.W. = 277.91) and ferric chloride $FeCl_3$, M.W. = 270.32) in a portion of water. A 0.2 ml portion of concentrated sulfuric acid was added before making up to a volume of 50 ml with Milli-Q water.

2.2.2 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 500 ml deionized water.

2.2.3 Acetyl salicylate, 0.01 M

A 1.20 g portion of aspirin powder was dissolved in 20 ml of 2 M sodium hydroxide. The solution was boiled in water bath for 15 minutes before making to a volume of 500 ml with deionized water.

2.2.4 Ammonia ammonium chloride buffer, 0.1 M pH 9.0

A portion of water was used to dissolved 1.71 g of ammonium chloride before 2.3 ml of ammonia solution was added. Deionized water was added to a volume of 500 ml.

2.2.5 Sodium pyrophosphate, 0.1 M in ammonia ammonium chloride buffer pH 9.0

A solution was prepared by dissolving 22.30 g of sodium pyrophosphate in a portion of ammonia ammonium chloride buffer before making to a volume of 500 ml with the same solution.

2.2.6 Dipotassium hydrogen phosphate, 1.0 M

A 87.09 g portion of dipotassium hydrogen phosphate was dissolved and diluted to 500 ml with deionized water.

2.2.7 Borate buffer, 0.1 M pH 9.0

A 0.3091 g portion of boric acid was dissolved in 40 ml water. The pH was adjusted with 0.5 M sodium hydroxide before making up to a volume of 50 ml with deionized water.

2.2.8 2,4′-pyridylazo resorcinol (PAR), 0.005 M

PAR (0.0538 g) was dissolved in a portion of 0.5 M hydrochloric acid before making up to a volume of 50 ml with deionized water.

2.2.9 Sodium molybdate, 0.025 M in 1.3% (v/v) nitric acid

A 3.0244 g portion of sodium molybdate was dissolved in water. A 6.50 ml volume of nitric acid was added before making up to a volume of 500 ml with deionized water.

2.2.10 Ammonium molybdate, 1.0 and 0.5% (w/v)

Acidic molybdate solution was prepared by adding 17.5 ml of sulfuric acid to 300 ml water. To this mixture 5.0 g of ammonium molybdate tetrahydrate was added before making up to a volume of 500 ml with deionized water.

A 0.5% (w/v) acidic molybdate solution was prepared similarly to the 1.0% (w/v) solution changing the weight of ammonium molybdate tetrahydrate and sulfuric acid to 2.5 g and 12.5 ml, respectively.

2.2.11 Ascorbic acid, 0.5 and 2.0% (w/v)

Ascorbic acid solutions of 0.5% and 2.0% (w/v) were prepared by dissolving 2.50 and 10.0 g of ascorbic acid in 500 ml deionized water.

2.2.12 Tin(II) chloride reducing solution, 0.02% (w/v)

Tin(II) chloride reducing solution was prepared by adding 14 ml of sulfuric acid to 300 ml water. To this mixture 1 g of hydrazine sulfate and 0.1 g of tin(II) chloride were added before making up to a volume of 500 ml with deionized water.

2.2.13 Potassium permanganate, 0.01 M

A 0.40 g portion of potassium permanganate was dissolved and diluted to 250 ml with deionized water.

2.2.14 Sodium sulfide, 200 mg l^{-1} sulfide

A 200 mg l^{-1} sulfide solution was prepared by dissolving 0.1529 g of sodium sulfide nonahydrate in 100 ml water.

2.2.15 Potassium chloride, 0.1 M

A 7.45 g portion of dried potassium chloride was dissolved in water and diluted to 1000 ml with deionized water.

2.3 Instruments and apparatus

1. Peristaltic pump (Ismatec, Glattbrugg-Zurich, Switzerland)
2. Stopped flow injection analyser (Home made, Chiang Mai, Thailand)
3. Recorder (R100 A, Perkin-Elmer, USA)
4. Capillary electrophoresis (Capel 103 RE, USSR)
5. Voltammetric analyzers (Methrom 694VA Stand with 693 VA processor, Switzerland)
6. Spectrophotometer (CECIL CE 1010, England)
7. Spectrophotometer (Applied Biosystems 759A Absorbance detector, USA.)
8. Electrochemical detector (Princeton Applied Research model 400 EC, USA.)
9. FIA Workstation with FCS program (A-Chem Technologies, Australia.)

2.4 FIA set-ups

2.4.1 FI system for determination of iron using salicylic acid

The FI system for determination of iron using salicylic acid is depicted in Figure 2.1. Teflon tubing with 0.5-mm id was used throughout.

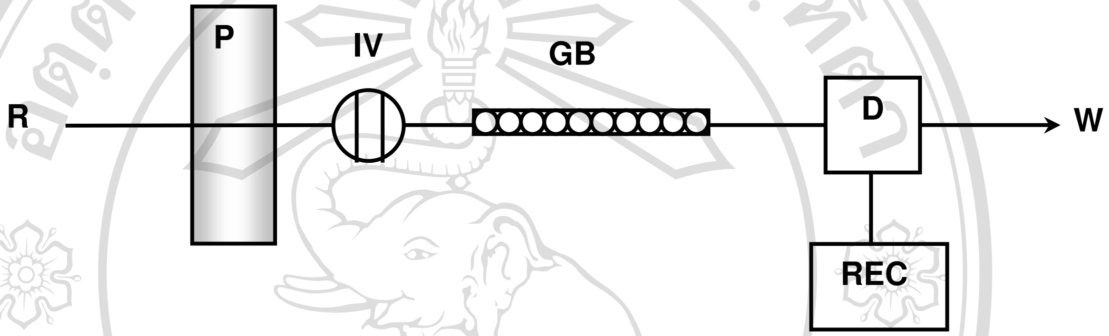
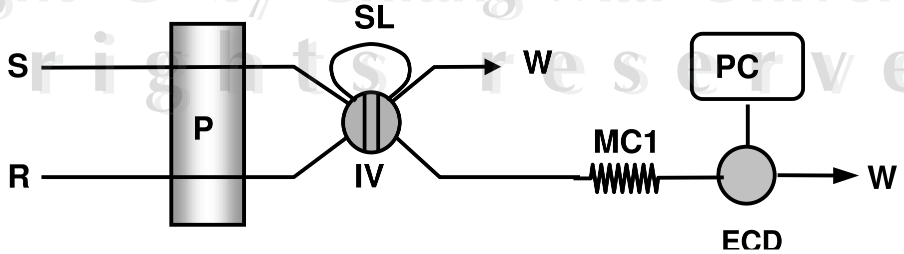


Figure 2.1 FI manifold for determination of iron using salicylic acid: R = 0.01 M salicylic acid solution, P = peristaltic pump, IV = 6-port injection valve, GB = 5 cm glass bead column, D = detector, REC = recorder, W = waste

2.4.2 FI system for on-line preconcentration and electrochemical detection of phosphate.

a) FI system A



b) FI system B

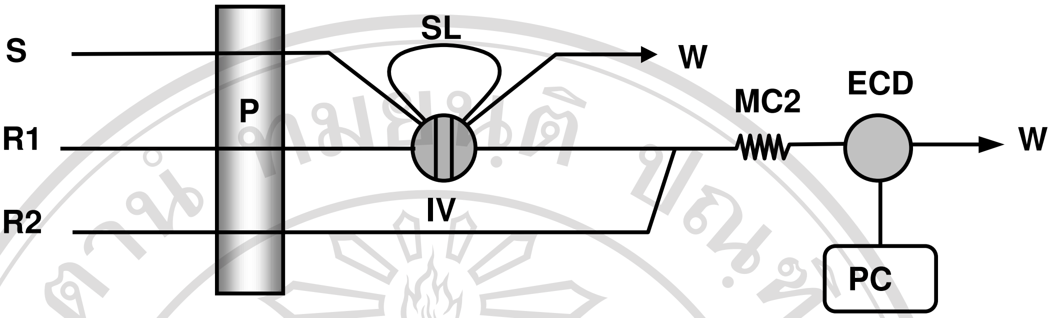


Figure 2.2 FIA manifolds for the direct determination of orthophosphate: (A) one reagent manifold: 30 cm mixing coil (MC1), 50 μL sample loop, R = 0.4% ammonium molybdate in 2% (v/v) sulfuric acid (B) two reagent manifold: 60 cm mixing coil (MC2), 50 μL sample loop, R_1 = 0.1 M potassium chloride, R_2 = 0.5% ammonium molybdate in 2.5% (v/v) sulfuric acid (C) Replace the sample loop in FI system B with an ion-exchange mini-column (5 mm long x1.5 mm i.d.) packed with Bio-Rad AG1-X8 of 200-400 mesh, chloride form

2.4.3 FI system for on-line sulfide removal for phosphate determination.

The manifold was modified from the one previously reported [42].

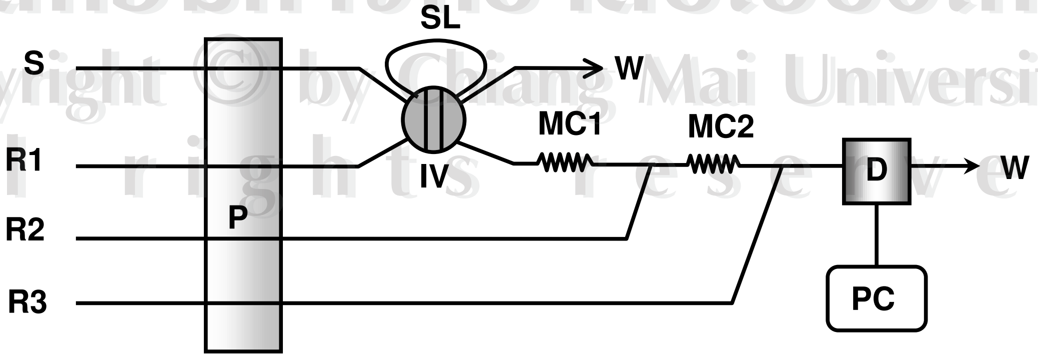


Figure 2.3 FIA manifold for on-line sulfide removal for phosphate determination: S = sample, R1 = water, R2 = 1.0% (w/v) ammonium molybdate in 3.5% (v/v) sulfuric acid, R3 = reducing solution, P = peristaltic pump, IV = 6-port injection valve, MC1 = mixing coil 1 (30 cm), MC2 = mixing coil 2 (120 cm), D = spectrophotometer, PC = computer, W = waste

2.4.4 FI system for simultaneous determination of phosphate and silicate using stopped FIA

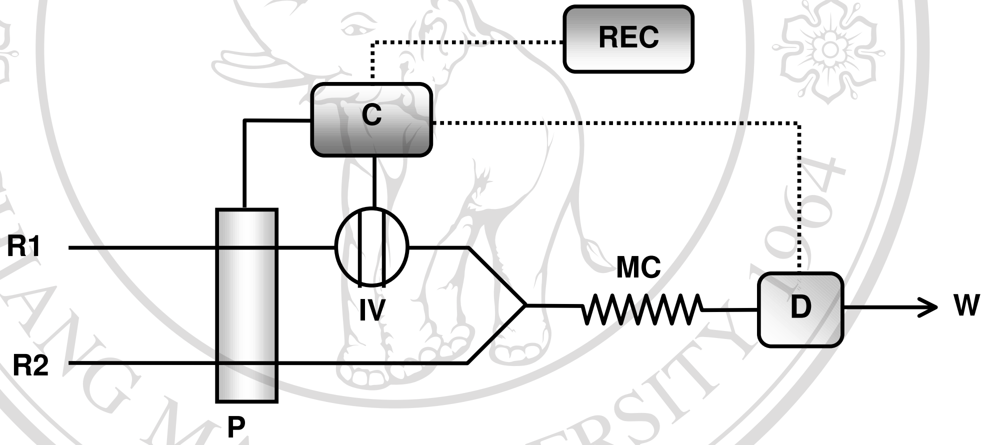


Figure 2.4 Stopped-FIA manifold for simultaneous determination of phosphate and silicate: R1 = 0.025 M sodium molybdate in 1.3% (v/v) nitric acid, R2 = 0.5% ascorbic acid, P = peristaltic pump, C = controller, IV = 6-port injection valve, MC = mixing coil, D = detector, REC = recorder, W = waste

2.5 Other set-ups

2.5.1 Capillary electrophoresis

The set-up was used for speciation of Fe(II) and Fe(III).

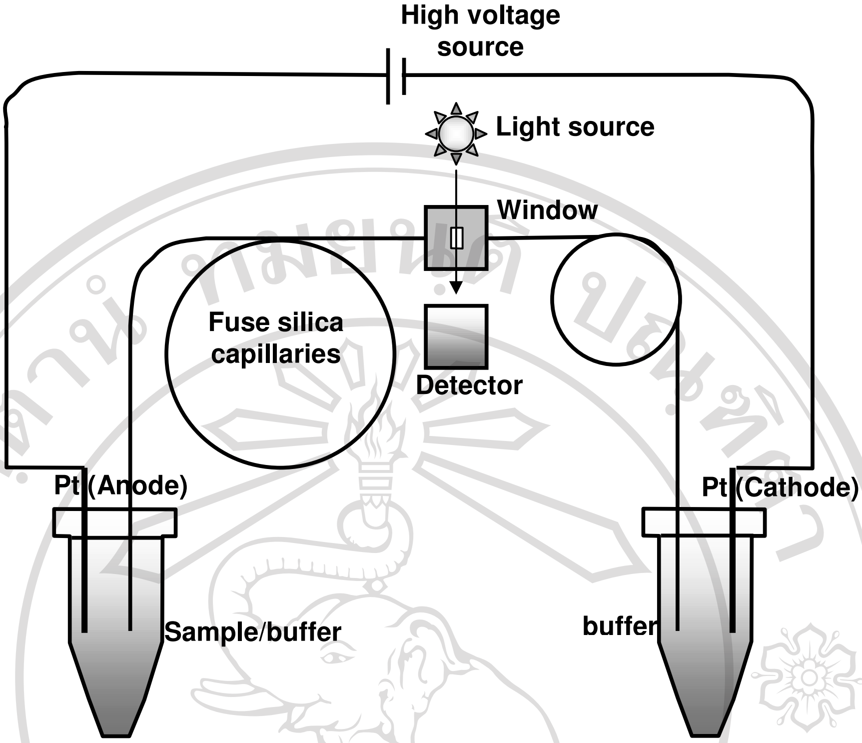


Figure 2.5 Capillary electrophoresis diagram

2.5.2 Voltammetry

The voltammetric cell used was for Metrohm 694VA stand as depicted in Fig. 2.6.

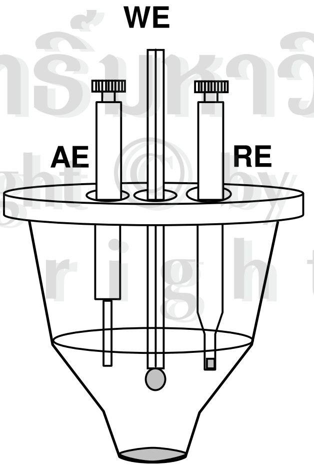


Figure 2.6 The voltammetric cell and electrode system: AE = Auxiliary electrode (Pt electrode), WE = Working electrode (Static mercury drop electrode), RE = Reference electrode (Silver/silver chloride electrode)