CHAPTER 2

EXPERIMENTAL

2.1 Chemical and Reagent

All chemical were analytical-reagent grade except when specified.

(1) Acetic acid glacial, CH_3COOH, BDH
(2) Ammonium acetate, CH_3COONH_4, Ajax chemicals
(3) Ammonium heptamolybdate tetrahydrate, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, Carlo Erba
(4) Anion exchange resin, 1- X8, Dowex
(5) Hydrochloric acid, HCl, 37%, Merck
(6) Potassium chloride, KCl, BDH
(7) Potassium dihydrogen phosphate, KH_2PO_4, Merck
(8) Sodium hydroxide, NaOH, Carlo Erba
(9) Sulfuric acid, H_2SO_4, 98%, Merck

Preparation of Standard and Reagent Solutions

All solutions were prepared using milli-Q water

Preparation of the standard solutions

Standard Solution of Orthophosphate (PO_4-P) 1000 ppm

The stock standard solution of Orthophosphate (PO_4-P) was prepared by dissolving 0.2197 g of potassium dihydrogen phosphate in water making to 50 ml in volumetric flask with water.

Preparation of the Reagent solutions

Acetic Acid; 4.2, 4.0, 3.0, 2.0, 1.0 and 0.1 M

Acetic Acid solutions of 4.2, 4.0, 3.0, 2.0, 1.0 and 0.1M were prepared by dissolving 24.00, 22.80, 17.10, 11.40, 5.70 and 0.57 ml of glacial acetic acid in 100 ml in volumetric flask with water.

Acetate buffer, 0.1 M pH 4.5

A portion of water was used to dissolved 3.85 g of ammonium acetate before 4 ml glacial acetic acid was added. Water was added to a volume of 1000 ml.

Ammonium molybdate; 1.0, 0.4 and 0.2 % (w/v) in 2.5% H_2SO_4

Acidic molybdate solutions of 1.0 % (w/v) were prepared by adding 12.5 ml of sulfuric acid to 300 ml water. To this mixture, 10 g of Ammonium heptamolybdate tetrahydrate was added before making up to a volume of 1000 ml in volumetric flask with water.

The 0.4 and 0.2 % (w/v) acidic molybdate solutions were prepared similarly to the 1.0 % (w/v) solution changing the weight of ammonium molybdate to 4 and 2 g, respectively.

Potassium chloride, 0.1 M

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

Hydrochloric acid, 0.1 M

Hydrochloric acid 8.3 ml was dissolved in water and making the volume to the mark of a 1000 ml volumetric flask.

2.2 Equipment/Apparatus

1. 6-port injection valve (Upchurch Scientific, USA)
2. Peristaltic pump (Ismatec, Switzerland)
3. Peristaltic pump (Eppendorf, USA)
4. Electrochemical detector (CV 50W, BAS, USA)
5. Electrochemical detector (VA 757 computrace, Metrohm, Switzerland)

2.3 Experimental set up

2.3.1 Cyclic voltammetric system

Figure 2.1 illustrates batch cyclic voltammetric study for the phosphate analysis. The voltammeter was a Metrohm VA 757 computrace having Pt wire, glassy carbon and Ag/AgCl as auxiliary, working and reference electrodes, respectively.

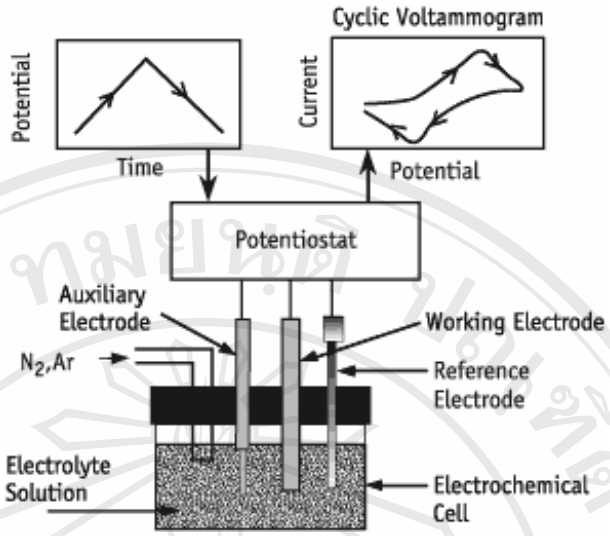

Figure 2.1 Schematic of cyclic voltammeter system. AE: auxiliary electrode, WE: working electrode, RE: reference electrode. Solution in system ; 5 ml KCl (0.1 M) and 5 ml 0.5% (w/v) ammonium molybdate in 2.5% (v/v) H_2SO_4.

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2.3.2 FI-Amperometric system

A FI-amperometric set-up is illustrated in Figure 2.2. A sample/standard solution is injected into the FI system via a sample loop (100µl) using a peristaltic pump. By switching the valve, it is then moved into a carrier stream (0.1 M KCl) before merging with 0.5% (w/v) ammonium molybdate in 2.5% (v/v) H_2SO_4 and flowing further through a mixing coil (50 cm.). The flowing reaction product (12-phosphomolybdate is continuously monitored at a cross flow cell (CV 50W, BAS) whereby Mo(VI) should be reduce to Mo(V) at fixed applied potential. The current change is recorded.

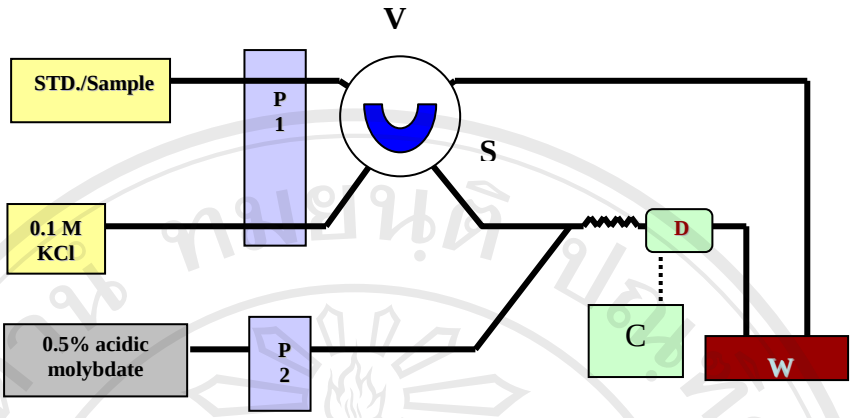

Figure 2.2 Manifold of FI-amperometric system for determination of orthophosphate (V=6 port injection valve, S=sample loop D=detector, C=computer, P1 and P2=Peristaltic pump, W=waste)

2.3.3 FI-Amperometric with preconcentration column system

A FI-amperometric set-up with preconcentration column is depicted in Figure 2.3. Operation sequence tried is summarized in Table 2.1. Using 3-way valves, the solutions were sequentially selected to pass through a column. For example in sequence B, a 4.2 M HOAc solution is passed through the preconcentration column packed with resins (Dowex 1-X8, Cl^- form) to convert the resin into acetate form. By switching valve B, water is passed to reduce the acidity and to wash the column. Via the valve C, PO_4-P standard solution is flowed to the column for a desired period. Switching the valve A to allow the 0.1 M HOAc flow to column for 1 min. This is to prevent baseline shift due to concentrated acid. The sorbed phosphate onto the resin in the column is eluted by passing the eluent (0.1 M HCl). The eluate flows to merge with the molybdate and flows further to mixing coil and the cross flow cell for detection. It should be noted that for sequence A, another 3-way valve was used to introduce 1 M NaOH.

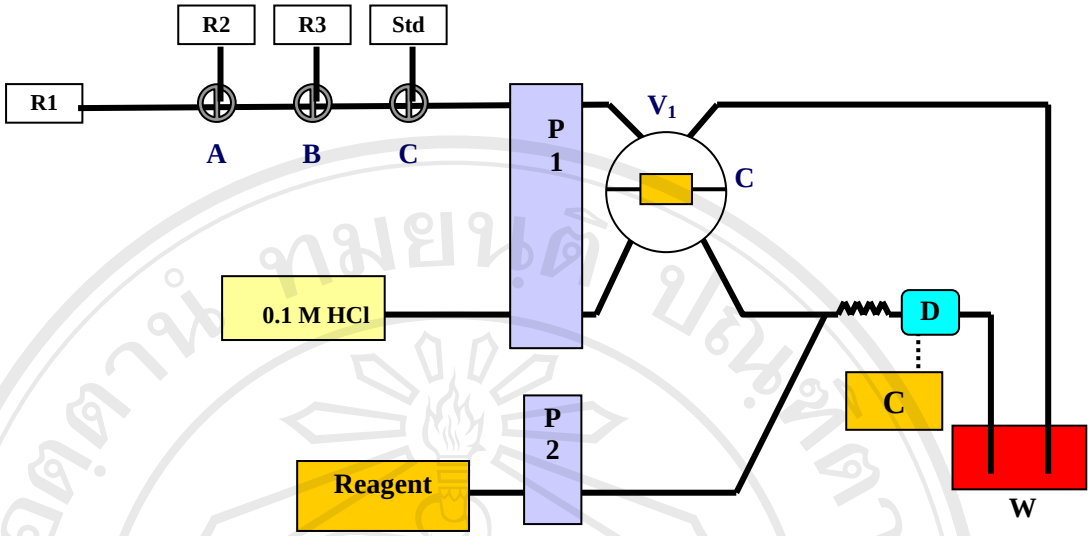

Figure 2.3 Manifold of FI-amperometric system with preconcentration column for determination of orthophosphate (R1= Milli-Q water, R2=0.1 M Acetic acid, R3=4 M Acetic acid, Std= Standard/sample solution, Reagent=0.5% (w/v) ammonium molybdate in 2.5% (v/v) H_2SO_4 (V_1=6 port injection valve, V_A-V_C=3 way switching valve, C= preconcentration column, D =detector, C=computer, P1 and P2 = Peristaltic pump, W= waste)

Table 2.1 Sequence tried for regenerating the column

Sequence A		Sequence B	
Sequence	Time (min)	Sequence	Time (min)
1 M NaOH	3	4.2 M HOAc	3
Milli-Q water	3	Milli-Q water	1
1 M HOAc	8	Std/sample	5
Acetete Buffer pH 4.5	3	0.1 M HOAc	1
Std/sample	5	0.1 M HCl	2
Milli-Q water	3		
0.1 M HCl	2		
Milli-Q water	3		
Total time	30		12