CHAPTER 2

EXPERIMENTAL

2.1 Apparatus and Instruments

The apparatus and instruments used in this experiment were as follows:

1. Analytical balance, model BA210S, Sartorius Basic, Germany.
2. UV-VIS spectrophotometer, model U-2000, Hitachi, Japan.
3. ICP spectrophotometer, model JY238 Ultrace Ultima, JY Jobin Yvon, Horiba, France.
4. Spectronic 21, Milton Roy Company, USA.
5. Chart recorder, model 135A, National, Japan.
6. Flow-through cell for spectrophotometer, Hellma, Germany.
7. Peristaltic pump, EYELA MP-3, Tokyo Rikakikai, Japan.
8. Mercury vapour lamp (54 W), Cole-Parmer, USA.
9. Rotary injection valve, Upchurch Sciencetific Inc., Hungary.
10. Disposable syringe (1 ml), Nissho Nipro, Japan.
11. Tygon tube (i.d. 0.051, 0.076, 0.102, 0.127 cm), Cole-Parmer, USA.
12. PTFE tube (i.d. 0.051, 0.086, 0.107, 0.132 cm), Cole-Parmer, USA.
13. Silicone tube (i.d. 0.114 cm), Cole-Parmer, USA.
14. Test tube (o.d. 1.0, 1.5, 1.9, 2.4 cm).
15. Home made connectors, Y- and T-shaped.
16. Hot plate, Baird and Tatlock, England.
17. pH/mV meter, model 5986-2S, Cole-Parmer, USA.
18. Filter paper, 12.5 cm medium, Whatman No. 6.
19. Micropipet, model 5000, Nichiryo, Japan.
20. Polyethylene (PE) bottles.

2.2 Chemicals

All chemicals used in this study were of analytical-reagent grade or higher purity and used without further purification.

1. Cadmium chloride, $CdCl_2.2(1/2)H_2O$, M.W. 228.34, AnalaR, BDH Ltd., England.
2. Calcium chloride, $CaCl_2.2H_2O$, M.W. 147.02, GR, Merck, Germany.
3. Copper chloride, $CuCl_2.2H_2O$, M.W. 170.48, pure, Merck, Germany.
4. Ferric nitrate, $Fe(NO)_3.9H_2O$, M.W. 404.02, RPE, Carlo Erba, Italy.
5. Hydrochloric acid, HCl, 37.0% w/w, M.W. 36.46, Merck, Germany.
6. Magnesium chloride, $MgCl_2.6H_2O$, M.W. 203.31, purum, Fluka, Switzerland.
7. Nitric acid, HNO_3, 65.0% w/w, M.W. 63.01, Merck, Germany.
8. Poly(vinyl alcohol), M.W. 15,000, Fluka, Switzerland.
9. Potassium carbonate, K_2CO_3, M.W. 138.21, purum, Fluka, Switzerland.
10. Potassium chloride, KCl, M.W. 74.56, GR, Merck, Germany.
11. Potassium iodide, KI, M.W. 166.01, AnalaR, BDH Ltd., England.
12. Potassium nitrate, KNO_3, M.W. 101.11, purum, Fluka, Switzerland.
13. Potassium phosphate, $K_3PO_4.3H_2O$, M.W. 266.32, extra pure, Merck, Germany.
14. Potassium sulphate, K_2SO_4, M.W. 174.27, GR, Merck, Germany.
15. Rhodamine B, $C_{28}H_{31}ClN_2O_3$, M.W. 479.02, GR, Merck, Germany.
16. Sodium acetate, $CH_3COONa.3H_2O$, M.W. 136.08, extra pure, Merck, Germany.
17. Sodium chloride, $NaCl$, M.W. 58.44, RPE, Carlo Erba, Italy.
18. Sodium fluoride, NaF, M.W. 41.99, pure, RDH, Germany.
19. Sodium selenate, Na_2SeO_4, M.W. 188.94, Aldrich, USA.
20. Sodium selenite, Na_2SeO_3, M.W. 172.94, Aldrich, USA.
21. Zinc chloride, $ZnCl_2$, M.W. 136.29, GPR, BDH Ltd., England.

2.3 Preparation of Standard and Reagent solutions

Deionized water was used to prepare all solutions. All labware used for handling solutions was cleaned with detergent solution, rinsed with tap water, soaked for at least 24 h in 1:2 (v/v) nitric acid/water, rinsed with deionized water and finally dried before use.

1) Standard solutions of selenium

1.1) Se(IV) standard solutions

Selenite stock standard solution of 1,000 ppm was prepared by dissolving 0.2235 g of sodium selenite in deionized water and adjusting to 100 ml with deionized water in volumetric flask, then transferred into PE bottle and kept in refrigerator for further use. Working standard solutions for calibration curve at concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5 ppm were prepared daily by transferring portions of 0.01, 0.02, 0.03, 0.04 and 0.05 ml of stock solution into 100-ml volumetric flasks and adjusting to the mark with deionized water. Other standard solutions of selenite were made by appropriate aqueous dilution of a stock solution with deionized water.

1.2) Se(VI) standard solutions

Selenate stock standard solution of 1,000 ppm was prepared by dissolving sodium selenate 0.2442 g in deionized water and diluting to 100 ml with deionized water in volumetric flask, then transferred into PE bottle and kept in refrigerator for further use. For lower concentrations, standard solutions in the range of 0.1-0.5 ppm, were prepared daily by transferring portions of 0.01-0.05 ml of stock standard solution into 100-ml volumetric flasks and diluting to the mark with deionized water. Other standard solutions of selenate were made by appropriate aqueous dilution of a stock solution with deionized water.

2) Standard solution of potassium

The stock standard solution of potassium (100 ppm) was prepared by dissolving an appropriate amount of 0.0191 g of potassium chloride in deionized water and diluting to 100 ml in a volumetric flask with deionized water, then transferred into PE bottle, and kept in refrigerator for further use.

3) Standard solutions of sodium

The stock standard solution of sodium (100 ppm) was prepared from NaCl, made by dissolving 0.0254 g in deionized water and adjusting to 100 ml in volumetric flask with deionized water, then transferred into PE bottle and kept in refrigerator for further use.

4) Standard solution of calcium

The stock standard solution of calcium (100 ppm) was prepared by dissolving 0.0254 g of calcium chloride in deionized water and adjusting to 100 ml in volumetric flask with deionized water, then transferred into PE bottle, and kept in refrigerator for further use.

5) Standard solution of magnesium

The stock standard solution of magnesium (100 ppm) was prepared by dissolving 0.0836 g of magnesium chloride hexahydrate in deionized water and adjusting to the mark in 100-ml volumetric flask with deionized water, then transferred into PE bottle and kept in refrigerator for further use.

6) Standard solution of zinc

The stock standard solution of zinc (100 ppm) was prepared by dissolving 0.0208 g of zinc chloride in deionized water and adjusting to 100 ml in volumetric flask with deionized water, then transferred into PE bottle, and kept in refrigerator for further use.

7) Standard solution of copper

The stock standard solution of copper (100 ppm) was prepared by dissolving 0.0268 g of copper chloride dihydrate in deionized water and adjusting to 100 ml in volumetric flask with deionized water, then transferred into PE bottle, and kept in refrigerator for further use.

8) Standard solution of cadmium

The stock standard solution of cadmium (100 ppm) was prepared by dissolving 0.0367 g of cadmium chloride in deionized water and diluting to 100 ml in volumetric flask with deionized water, then transferred into PE bottle, and kept in refrigerator for further use.

9) Standard solution of iron

The stock standard solution of iron (100 ppm) was prepared by dissolving 0.0723 g of ferric nitrate nonahydrate in deionized water and adjusting to 100 ml in volumetric flask with deionized water, then transferred into PE bottle, and kept in refrigerator for further use.

10) Standard solution of chloride

The stock standard solution of chloride (100 ppm) was prepared by dissolving 0.0210 g of potassium chloride in deionized water and adjusting to 100 ml in volumetric flask with deionized water, then transferred into PE bottle, and kept in refrigerator for further use.

11) Standard solution of nitrate

The stock standard solution of nitrate (100 ppm) was prepared by dissolving 0.0163 g of potassium nitrate in deionized water and adjusting to 100 ml in volumetric flask with deionized water, then transferred into PE bottle, and kept in refrigerator for further use.

12) Standard solution of carbonate

The stock standard solution of carbonate (100 ppm) was prepared by dissolving 0.0230 g of potassium carbonate in deionized water and adjusting to 100 ml in volumetric flask with deionized water, then transferred into PE bottle, and kept in refrigerator for further use.

13) Standard solution of sulphate

The stock standard solution of sulphate (100 ppm) was prepared by dissolving 0.0181 g of potassium sulphate in deionized water and adjusting to 100 ml in volumetric flask with deionized water, then transferred into PE bottle, and kept in refrigerator for further use.

14) Standard stolution of phosphate

The stock standard solution of phosphate (100 ppm) was prepared by dissolving 0.0280 g of potassium phosphate trihydrate in deionized water and adjusting to 100 ml in volumetric flask with deionized water, then transferred into PE bottle, and kept in refrigerator for further use.

15) Standard solution of fluoride

The stock standard solution of fluoride (100 ppm) was prepared by dissolving 0.0221 g of sodium fluoride in deionized water and adjusting to 100 ml in volumetric flask with deionized water, then transferred into PE bottle, and kept in refrigerator for further use.

16) Rhodamine B solutions

Stock solution of 0.0020 M was prepared by dissolving the amount of rhodamine B (0.0958 g) in deionized water and diluting to the mark in a 100-ml volumetric flask. The working solutions at concentrations of 1.6, 1.8, 2.0, 2.2 and 2.4×10^{-4} M were prepared by transferring portions of 4.0, 4.5, 5.0, 5.5

and 6.0 ml of stock solution into 50-ml volumetric flasks, and then diluting to 50 ml with deionized water.

17) Hydrochloric acid solutions

Stock solution of 6.0 M was prepared by adding 500 ml of concentrated HCl solution in deionized water and adjusting to 1000 ml with deionized water in volumetric flask. The working solutions of 0.20, 0.25, 0.30, 0.35 and 0.40 M were prepared by transferring portions of 3.4, 4.2, 5.0, 5.8 and 6.6 ml of stock solution into 100-ml volumetric flasks and diluting to the mark with deionized water.

18) Potassium iodide solutions

Stock solution of 1.0 M was prepared by dissolving potassium iodide 16.6009 g in deionized water and adjusting to 100 ml in volumetric flask with deionized water. The working solutions of KI at concentrations of 0.19, 0.21, 0.23, 0.25 and 0.27 M were prepared by transferring portions of 0.19, 0.21, 0.23, 0.25 and 0.27 ml of stock solution into 100-ml volumetric flasks and adjusting to the mark with deionized water.

19) Sodium acetate solutions

Stock solution of 1.0 M was prepared by dissolving sodium acetate 13.6080 g in deionized water and adjusting to 100 ml in volumetric flask with deionized water. The working solutions of NaOAc at concentrations of 0.05, 0.10, 0.15, 0.20 and 0.25 M were prepared by transferring portions of 5, 10, 15, 20 and 25 ml of stock solution into 100-ml volumetric flasks and diluting to the mark with deionized water.

20) Poly(vinyl alcohol) solutions

Stock solution of 5.0% w/v was prepared by weighing PVA 5 g in beaker, then a small volume of deionized water was added and the solution was heated to a gentle boil with occasional stirring for 5 min on the hot plate to completely dissolved the solution. Beaker sides were washed with water. After cooling, the solution was brought to 100 ml in volumetric flask with deionized water. The working solutions of PVA at concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5% w/v were prepared by transferring portions of 2.5, 5.0, 7.5, 10.0 and 12.5 ml of stock solution into 25-ml volumetric flasks and adjusting to the mark with deionized water.

2.4 Sample Preparation

In this study, investigated natural spring water samples were collected from different locations in Chiang Mai Province in 1000-ml PE bottles. Subsequently the water samples were filtered through wet quantitative filter paper, then the filtrated samples were acidified with 3 ml of concentrated nitric acid, and then kept in the cold room at 4 °C for preservation.

2.5 Instrumentation

In this study, the reverse FIA system was designed and constructed as can be seen arrangement in Figure 2.1. The FIA manifold for the determination of Se(IV) and Se(VI) was of tygon and teflon tubing. The FI system consisted of a six-port injection valve with an external loop for reagent injection; the tubing connecting the ports was made PTFE (0.107 cm i.d.), two individually two-channel peristaltic pumps used to sample and propel the sample and/or standard and reagent solutions; the flow rates being achieved using 0.152 cm i.d. tygon tubing, mixing reactors made of tygon tubing and PTFE tubing, spectrophotometer equipped with a flow-through cell for measurement and chart recorder.

The photoreactor was designed and constructed. It consisted of a PTFE tubing (0.086 cm i.d.) surrounding a mercury vapour lamp (23 cm long×1.3 cm diameter). The source emits short wavelength light at λ_{max} = 254 nm. The unit was covered with thick paper to prevent eye exposure to ultraviolet radiation.

Figure 2.1 Three-line FI manifold used for determination of Se(IV) and Se (VI): S = sample solution; R1 = HCl solution; R2 = KI solution; R3 = rhodamine B solution/NaOAc solution/PVA solution; P1, P2 = peristaltic pumps, PR = photoreactor; M1 = knitted reactor; M2 and M3 = mixing coils, I = injection valve; D = detector; REC = recorder; W = waste.

For operating method the standard and/or sample and hydrochloric acid solutions were introduced (via pump P1) and allowed to pre-mix in the first reactor (M1). Subsequently, the mixed stream was then mixed with potassium iodide solution and dilivered (via pump P2) in the second reactor (M2) where the oxidation of iodide with selenite was taken place producing triiodide. The complexing agent solution was injected into the continuous mixed solution through a rotary valve (I) and then triiodide was complexed with rhodamine B producing a purple complexes after passing through the third reactor (M3). The concentrated zone was then passed directly into the measuring cell and detected with spectrophotometer. The peak-height signal was measured and recorded. When absorbance due to selenite was measured at 590 nm, three-way stopcock was turned to another position, so that the solution passed through a photoreactor in order to reduce selenate to selenite and the solution was moved in the same way. Selenate was determined from the difference in absorbance values.

2.6 Procedure

2.6.1 Preliminary study of absorption spectra

In order to investigate the absorption spectrum of the ion-association complex, to start the reaction, 0.1 ml of stock standard solution of selenite was transferred into a 25-ml volumetric flask, then 0.1 ml of 6 M hydrochloric acid were added and followed after 2 min by 0.5 ml of 1 M potassium iodide, 0.4 ml of 1 M sodium acetate, 1.0 ml of 1% w/v PVA and 0.2 ml 0.002 M rhodamine B solution, then the solution was diluted to the mark with deionized water and a portion was transferred into a glass cell. The change in the color of rhodamine B is followed spectrophotometrically, by recording the absorbance versus wavelength plot on UV-VIS spectrophotometer. Record the change in absorbance against a reagent blank prepared similarly over the range from 500-800 nm, and the absorption spectrum of reagent blank against water reference was measured.

2.6.2 Optimization of analytical parameters in FI system

The various analytical parameters in the flow system were investigated (Table 2.1) by univariate optimization method to achieve the best sensitivity obtained by slope of the calibration graph between peak height and selenium concentrations (0.1-0.5 ppm), low reagent consumption, and high sample throughput obtained by measuring the t_{base} value.

Table 2.1 The studied ranges of analytical parameters.

Parameter	Range
Wavelength	575-630 nm
HCl concentration	0.20-0.40 M
KI concentration	0.19-0.27 M
NaOAc concentration	0.05-0.25 M
PVA concentration	0.5-2.5% w/v
RhB concentration	$1.6\text{-}2.4 \times 10^{-4}$ M
carrier flow rate	0.7-1.9 ml/min
Size of mixing tubing (tygon)	0.051-0.127 cm i.d.
Size of mixing tubing (PTFE)	0.051-0.132 cm i.d.
Size of test tube for mixing coil	1.0-2.4 cm o.d.
Length of mixing tubing	50-250 cm
Type of mixing part	straight, coiled, knitted, zig zag
Injection volume	150-350 μl
Irradiation time	0-210 s

For studied ranges of injection volume and irradiation time these were varied by varying the lengths of tubing (statistical formulae for calculations of tube lengths are shown in Appendix B).

2.6.3 Analytical characteristics of the method

1) Linearity

In order to study linear ranges, selenium (as selenite and selenate) standard solutions at concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1.0, 1.5, 2.0, 3.0 and 4.0 ppm were investigated.

2) Precision

The precision of the method and instrument were studied by analyzing ten determinations of 0.2 ppm selenium standard solutions and analyzing 0.2 ppm selenium standard solutions ten times. The relative standard deviation was calculated.

3) Detection limit

The detection limit of the method for determination of selenium, as selenite and selenate, was investigated by analyzing blank ten times and selenium standard solutions at low concentrations (0.0050, 0.010, 0.030, 0.050, 0.070 ppm).

4) Calibration curve

The calibration curve for determination of selenium was constructed by analyzing various selenium standard solutions over the range of 0.1-0.5 ppm and the curve was plotted between absorbance and selenium concentrations.

5) Accuracy

The method was validated to study matrix effect on the determination of selenium by analyzing the spiked water sample which known concentration of selenium standard solutions (0.1 to 0.5 ppm). The remainder of the procedure (to construct the calibration curve) was the same as described for obtaining the calibration graph. The method of standard addition was carried out and recoveries of added selenium, as selenite and selenate, in sample solution were calculated.

6) Interferences

The effect of interfering ions including cations and anions was studied by transferring portions of each standard of the ions to aliquots of 0.1 ppm selenium standard solutions to obtain concentration ratio between analyte and interfering ion over the range from 1:2 to 1:50. The percentage error of the absorbance was calculated by comparing the peak height to that obtained after the introduction of analyte solution containing no interfering ion, as reference.

2.6.4 Application of FI system to water samples

Application of FI method for speciation of selenite and selenate in water samples was carried out. After the optimum experimental conditions were obtained, the proposed method was applied to seven natural water samples collected from differrent areas in Chiang Mai Province to determine selenite and selenate concentrations. Speciation of selenium was carried out in two steps.

Determination of selenite: The sample was pumped into the continuous-flow manifold.

Determination of selenate: Total selenium was determined after on-line reduction of selenate to selenite. The selenate concentration was calculated as the difference between total Se and selenite.

When the analysis was completed, the concentrations of the selenite and selenate were evaluated from the peak-height signal by using standard addition method.

2.6.5 Comparison with ICP method

In order to determine selenium by, two set of samples were examined because Se(IV) is the oxidation state that provides the most favorable kinetics in generation of hydride. Consequently, prereduction was performed before detection. First set, the water samples was examined without treatment prior to analysis. The second set was determined Se(IV) contents in all samples. The later, the water samples was reduced to convert Se(VI) to Se(IV) in order to determine Se(VI) by HCl digestion: 10 ml aliquot of each sample was pipetted and transferred into 50-ml beaker, followed by 10 ml of concentrated hydrochloric acid, stirred and covered the beaker with a watch-glass. The mixed solution was warmed in water bath at 60-70°C for 20 min. After it was cooled to room temperature, the sample was transferred into a 25-ml volumetric flask and made up to 25 ml with deionized water and mixed well. The selenium contents in each sample were then determined by.

2.6.6 Application of the proposed method to spiked water samples

Spiked water samples containing 0.010, 0.050, 0.100, 0.300, 0.500, 0.700 and 1.000 ppm selenium were prepared and analysed three times using the recommended FI method under the optimum conditions. The average recoveries of added selenium for sample solutions were calculated.