# 2. EXPERIMENTAL

## 2.1 Apparatus and Chemicals

### 2.1.1 Apparatus

(1) Ion chromatograph DX-300, manufactured by Dionex Corp., U.S.A. , consisting of :

- (a) Injection valve with a 25 $\mu$l sample loop, Rheodyne.
- (b) Separator columns, IonPac AS4A (4x250 mm.), IonPac CS 5 (4x250 mm.), Dionex Corp., U.S.A.
- (c) Guard columns, IonPac AG4A (4x50 mm.), IonPac CG 5 (4x50 mm.),Dionex Corp., U.S.A.
- (d) Anion self-regenerating suppressor-I (4 mm.), Dionex Corp., U.S.A.
- (e) Post column, IonPac membrane reactor, Dionex Corp., U.S.A.
- (f) Conductivity detector, Dionex Corp., U.S.A.
- (g) UV/vis detector, Dionex Corp., U.S.A.
- (h) BDS, Barspec Data System, Barspec System Inc., Israel.

(2) Vacuum pump, Water Associates, U.S.A.

(3) Ultrasonicator, model 8891, Cole-Parmer Instrument Co., U.S.A.

(4) Filter unit, Millipore, U.S.A.

(5) Membrane filter, 0.45 $\mu$m, Sartorius, Germany.

(6) Ultraviolet-Visible spectrophotometer, Hitachi U-2000, Hitachi Ltd., Japan.

(7) Atomic absorption spectrophotometer, Shimadzu AA-680, Shimadzu Co., Japan.

(8) Inductively coupled plasma spectrophotometer, Optima 3000, Perkin Elmer Co., U.S.A.

(9) pH meter, Chemtrix Type 60A, Chemtrix Inc., U.S.A.

(10) pH meter F-16, Horiba Ltd., Japan.

(11) Fluoride ion selective electrode, Model 9409, Orion, U.S.A.

(12) Reference electrode, Model 900001, Orion, U.S.A.

(13) Chloride electrode, Model 27502-13, Cole Parmer, U.S.A.

(14) Magnetic stirrer, Heidolph, Germany.

### 2.1.2 Chemicals

(1) Sodium carbonate anhydrous, purum, Fluka, Switzerland.

(2) Sodium bicarbonate, 99.8%, Carlo Erba, Italy.

(3) Sodium fluoride, 99.6%, Baker Analyzed, U.S.A.

(4) Sodium chloride, 99.5%, puriss, Fluka, Switzerland.

(5) Sodium nitrite, 99.0-100.5%, E.Merck, Germany.

(6) Sodium bromide, 99.5%, Baker Analyzed, U.S.A.

(7) Sodium nitrate, 99.5%, Carlo Erba, Italy.

(8) Sodium sulphate, 99.5%, Carlo Erba, Italy.

(9) Potassium phosphate trihydrate, 98.5%, Carlo Erba, Italy.

(10) Oxalic acid, AR grade, E.Merck, Germany.

(11) Lithium hydroxide monohydrate, >99%, Fluka, Switzerland.

(12) 4-(2-Pyridylazo)resorcinol (PAR), 99%, Fluka, Switzerland.

(13) Copper standard solution for atomic absorption spectrophotometry, 1g/l, Fluka, Switzerland.

(14) Lead standard solution for atomic absorption spectrophotometry, 1g/l, Fluka, Switzerland.

(15) Manganese standard solution for atomic absorption spectrophotometry, 1g/l, Fluka, Switzerland.

(16) Zinc standard solution for atomic absorption spectrophotometry, 1g/l, Fluka, Switzerland.

(17) Nickel standard solution for atomic absorption spectrophotometry, 1g/l, Fluka, Switzerland.

(18) Iron standard solution for atomic absorption spectrophotometry, 1g/l, Fluka, Switzerland.

(19) Cobalt Copper standard solution for atomic absorption spectrophotometry, 1g/l, Fluka, Switzerland.

(20) Cadmium standard solution for atomic absorption spectrophotometry, 1g/l, Fluka, Switzerland.

(21) Lead (II) nitrate, 99.999%, Aldrich, U.S.A.

(22) Copper (II) sulphate pentahydrate, 99.999%, Aldrich, U.S.A.

(23) Manganese (II) nitrate tetrahydrate, ≥ 97 %, Fluka, Switzerland.

(24) Zinc (II) nitrate hexahydrate, 99.999%,Aldrich, U.S.A.

(25) Nickel (II) nitrate hexahydrate, 99.999%, Aldrich, U.S.A.

(26) Cobalt (II) sulphate hydrate, 99.999%, Aldrich, U.S.A.

(27) Acetic acid, 100%, AR grade, E.Merck, Germany.

(28) Ammonia solution, 28-30% $NH_3$, AR grade, BDH, Chemicals Ltd., England.

(29) Sodium 2-(parasulphophenylazo)-1, 8-dihydroxy-3, 6-naphthalene disulphonate; SPADNS, for complexometry, Fluka, Switzerland.

(30) Zirconyl chloride octahydrate, ≥ 96 %, BDH Chemicals Ltd., England.

(31) Hydrochloric acid, 36.5-38 %, J.T Baker, USA.

(32) Sodium arsenic dioxide, ≥ 95 %, Carlo Erba, Italy.

(33) Potassium nitrate, > 99 %, May & Baker Ltd., Dagenham.

(34) Chloroform, Merck- Schuchardt, Germany.

(35) Barium chloride, 99 % Carlo Erba, Italy.

(36) Magnesium chloride, ≥ 98 %, Fluka, Switzerland.

(37) Sodium acetate, 99.5-100.5. %, E.Merck, Germany.

(38) Ammonium iron(III) sulphate, > 99 %, Fluka, Switzerland.

(39) Mercury(II) thiocyanate, > 99 %, Fluka, Switzerland.

(40) Ethanol, 95 %, Ayuttaya, Thailand.

(41) Sodium hydroxide, EKA Nobel, Sweden.

(42) Potassium hydroxide, 85.5 %, Carlo Erba, Italy.

(43) Potassium sulphate, 99 %, BDH Chemicals Ltd., England.

(44) Potassium bromate, 99.5 %, E.Merck, Germany.

(45) Formic acid, 98-100 %, E.Merck, Germany.

(46) Nitric acid, 64-70.5 %, BDH Chemicals Ltd., England.

(47) 2-Dimethylaminoethanol, 98 %, BDH Chemicals Ltd., England.

(48) Cyclohexylene diaminetetraacetic acid (CDTA), > 99.0 %, Fluka, Switzerland.

(49) Pyridine-2,6-dicarboxylic acid (PDCA), > 98 %, Fluka, Switzerland.

(50) Dimethyl sulphoxide, 98 %, E.Merck, Germany.

(51) Sodium peroxide, 99%, BDH Chemicals Ltd., England.

(52) Hydrogen peroxide, 30 %, E.Merck, Germany.

(53) Hydrofluoric acid, 29 %, Fluka, Switzerland.

(54) Sulphuric acid, 37 %, E.Merck, Germany.

## 2.2 Samples

Samples investigated were geological samples consisting of waters, coals and reference materials. Details of these samples are listed in **Table 2.1.**

**Table 2.1** Details of the samples investigated in this work.

| Code | Sample Type | Origin |
|---|---|---|
| Sample #1 | Hot spring water | Fhang, Chiang Mai |
| Sample #2 | Hot spring water | Sankampang, Chiang Mai |
| Sample #3 | Spring water | Mae Taeng, Chiang Mai |
| Sample #4 | Waterfall | Mae Taeng, Chiang Mai |
| Sample #5 | River water | Chiang Dow, Chiang Mai |
| Sample #6 | Water in cave | Chiang Dow, Chiang Mai |
| Sample #7 | Rain water | Kathmandu, Nepal |
| Sample #8 | Rain water | Kathmandu, Nepal |
| Sample #9 | Rain water | Jiri, Nepal |
| Sample #10 | Rain water | Pokhara, Nepal |
| Sample #11 | Rain water | Gorkha, Nepal |
| Sample #12 | Rain water | Kathmandu, Nepal |
| Sample #13 | Rain water | Jiri, Nepal |
| Sample #14 | Rain water | Pokhara, Nepal |
| Sample #15 | Rain water | Pokhara, Nepal |
| Sample #16 | Rain water | Pokhara, Nepal |
| Sample #17 | Rain water | Gorkha, Nepal |
| Sample #18 | Rain water | Kathmandu, Nepal |
| Sample #19 | Rain water | CMU, Chiang Mai |
| Sample #20 | Coal | Hod, Chiang Mai |
| Sample #21 | Coal | Ban Hong, Lampoon |
| Sample #22 | Soil 5 | Reference materials |
| Sample #23 | River sediment | Reference materials |

## 2.3 Preparation of Solutions

### 2.3.1 Preparation of eluent

In this work, mixed solutions of sodium carbonate/sodium bicarbonate ($Na_2CO_3$/$NaHCO_3$) and pyridine-2,6-dicarboxylic acid (PDCA) and oxalic acid were the three eluents investigated. The first eluent system was used for anion separations with the IonPac AS4A column. PDCA and oxalic acid eluent systems were used for transition metal separations with the IonPac CS5 column. The PDCA eluent was used for iron, copper, nickel, zinc, cobalt and manganese. The oxalic acid eluent was used for lead, copper, cobalt, zinc and nickel. For cadmium and manganese had been found to coelute in trial runs when oxalic acid was used as eluent.

The preparation of each eluent is as in the following:

a) Stock solution of 180 mM $Na_2CO_3$/ 170 mM $NaHCO_3$

Stock solution (180 mM $Na_2CO_3$/ 170 mM $NaHCO_3$) was prepared by dissolving 1.9078 g of sodium carbonate plus 1.4282 g of sodium bicarbonate in deionized water and diluting the resultant solution to 100 ml.

This stock solution was then used to prepare a solution of 1.80 mM $Na_2CO_3$/ 1.70 mM $NaHCO_3$ by pipetting 10.0 ml of it into a one-litre volumetric flask and diluting it to the mark.

b) Pyridine-2,6-dicarboxylic acid (PDCA) eluent system

PDCA eluent was prepared by methods :

1) Stock solution of PDCA eluent concentrate (5X)

Stock solution (PDCA eluent concentrate (5X)) was prepared by dissolving 18.5 g of potassium hydroxide in 200 ml deionized water and 5.8490 g of PDCA and 4.880 g of potassium sulphate stirred until all solids dissolved. Add 17.030 g of formic acid and the resultant solution was transferred to a one-litre volumetric flask and deionized water was added to the mark.

This stock solution was then used to prepare a solution of 7.5 mM PDCA/ 66 mM potassium hydroxide/ 5.6 mM potassium sulphate/ 74 mM formic acid by transfering 200.0 ml of it into one-litre volumetric flask and diluting it to the mark.

2) PDCA stock solution (6.0 M PDCA/ 40 mM Sodium hydroxide)

Stock solution (6.0 mM PDCA/ 40 mM solution hydroxide) was prepared by placing 200 ml of deionized water into a clean one-litre beaker and adding 43.7 ml of 9.2 M sodium hydroxide (50%), while stirring with a magnetic stirrer with addition of 10.0 g of PDCA. Stirring was allowed to continue for about 10 minutes or until all the PDCA had been dissolved. Dilution to one-litre was made from this solution with thorough stirring.

This stock solution was then used to prepare a solution of 6.0 mM PDCA/ 40 mM sodium hydroxide/ 90 mM acetic acid by pipetting 100 ml of it into a one-litre volumetric flask and adding 100.00 ml of 0.90 M acetic acid. Dilution to one-litre was made with deionized water.

c) Oxalic acid solution

An exact weight, to give needed concentration of oxalic acid, was dissolved in about 800 ml deionized water, then adjusted to the desired pH with 1 M LiOH. Finally, the solution was diluted to 1000 ml in a volumetric flask with deionized water.

Because the system inlet line was so small that air or small particles in eluents could block in the system, causing baseline noise and shortening the life-time of the column, the eluents were filtered using a filter apparatus (0.45 μm membrane) and then degased, using vacuum and ultrasonication prior to analysis.

### 2.3.2 Post-column reagent

In this work, 4-(2-pyridylazo) resorcinol (PAR) is used for post column reagent. The preparation of PAR post column reagent can be prepared by two methods:

a) 0.5 mM PAR/ 1.0 M 2- dimethylaminoethanol (DMAE)/ 0.5 M ammonium hydroxide/ 0.3 M sodium bicarbonate

200 ml of deionized water was added with 30.5 ml of ammonium hydroxide and 0.0543 g of PAR with ultrasonication for five minutes. Stirring using a magnetic stirrer was allowed for several minutes to ensure that the PAR had been completely dissolved. 51.4 ml of DMAE and 12.63 g of sodium bicarbonate were added with thorough stirring. Dilution to 500 ml was made with deionized water.

b) 0.36 mM PAR/3.52 M ammonium hydroxide/1.0 M acetic acid

100 ml of ammonium hydroxide was added with 0.04 g of PAR and then 30 ml of acetic acid was added slowly as this reaction is highly exothermic. Next, ultrasonication was made for three-five minutes and stirring using a magnetic stirrer was made to ensure that

the PAR had been completely dissolved. The solution was transferred to a 500 ml volumetric flask and diluted it to the mark.

### 2.3.3 Standard anion stock solutions, 1000 ng/μl , 100 ml

These were prepared by dissolving each of the following standard anions in deionized water which was also used to make up the volume to 100.00 ml in a 100 ml volumetric flask:

Sodium fluoride, 0.2210 g
Sodium chloride, 0.1648 g
Sodium nitrite, 0.1500 g
Sodium bromide, 0.1288 g
Sodium nitrate, 0.1371 g
Potassium phosphate trihydrate, 0.2804 g
Sodium sulphate, 0.1479 g

### 2.3.4 Standard cation stock solutions, 1000 ng/μl, 100 ml

These were prepared by dissolving each of the following standard anions in deionized water which was also used to make up the volume to 100.00 ml in 100 ml of volumetric flask.

Lead (II) nitrate, 0.1594 g
Copper (II) sulphate pentahydrate, 0.3929 g
Manganese (II) nitrate tetrahydrate, 0.1430 g
Zinc (II) nitrate hydrate, 0.2897 g
Nickel (II) nitrate hexahydrate, 0.4954 g

Cobalt (II) sulphate hydrate, 0.2623 g

Cadmium (II) chloride, 0.1631 g

### 2.3.5 Metal ion standard solution for atomic absorption spectrophotometry

Each standard solution of metal ion with concentration of 1 g/l was used to prepare a range of working solutions from 1 ppm concentration downwards by pipetting an appropriate volume of the standard solution into a 100-ml volumetric flask and diluting it to the mark with deionized water.

### 2.3.6 Reagents for the determination of anions by spectroanalytical methods

Details of the preparation of reagents used in the determination of anions by spectroanalytical methods are given in the references listed in **Table 2.2**.

**Table 2.2** List of references for details of the preparation of reagents used in the determination of anions by spectroanalytical and ion selective electrode methods.

| Determination | Method | Reference |
|---|---|---|
| Chloride | Mercury(II) thiocyanate | 27 |
| | Potentiometric | 28 |
| Fluoride | SPADNS | 28 |
| | Ion selective electrode | 27, 28 |
| Nitrate | UV spectrophotometric screening | 28 |
| Sulphate | Turbidimetric | 27 |

## 2.4 Determination of Anions with IonPac AS4A column

The IonPac AS4A analytical column is designed specifically for the analysis of anions. Separation is accomplished by using a carbonate/ bicarbonate eluent system. To analyze mixtures of anions, the separator column is packed with a low-capacity surface-active anion-exchange resin, and the eluent is most usually a mixture of $Na_2CO_3$ / $NaHCO_3$. The eluent concentration must be low enough to match the low capacity of the column and allow sufficient retention of the ion to be analyzed, as well as to avoid overloading the suppressor column, which is in this case packed with a strong acid cation exchanger in the hydrogen form. This resin converts the excess eluent into a solution of carbonic acid, which has a very low conductivity, while the anions of sample emerge as their acids, which have a high conductivity. In this work, ion chromatographic conditions of IonPac AS4A column were optimized and applied for analysis of anions in water samples and geological samples. The column was run with Anion Self-Regenerating Suppressor-I (ASRS-I) equipped with a conductivity detector.

### 2.4.1 Investigation of retention times of ions of interest.

The retention times of some anions of interest were determined. Each of the studied anion standard solutions given in **Table 2.3** was injected onto the IonPac AS4A column using 1.80 mM $Na_2CO_3$/ 1.70 mM $NaHCO_3$ as eluent at a flow rate of 2.0 ml/min. The conductivity detector was used with a fixed temperature compensation at 1.7%/ °C and output range at 3 μS. The retention times obtained are shown in **Table 3.1**.

**Table 2.3** Standard solutions of studied anions used to determine retention times.

| Anion | Concentration (ng/μl) |
|---|---|
| $F^-$ | 0.5 |
| $Cl^-$ | 1.0 |
| $NO_2^-$ | 1.0 |
| $Br^-$ | 1.0 |
| $NO_3^-$ | 1.0 |
| $PO_4^{3-}$ | 2.0 |
| $SO_4^{2-}$ | 1.0 |

### 2.4.2 Optimization of IC conditions.

Some parameters affecting the sensitivity and resolution of analysis were investigated. Experimental details are given below.

#### 2.4.2.1 Effects of eluent concentration

A standard mixture of $F^-$, $Cl^-$, $NO_2^-$, $Br^-$, $NO_3^-$, $PO_4^{3-}$ and $SO_4^{2-}$ from Section 2.4.1 was injected onto the IonPac AS4A column with the following $Na_2CO_3$/ $NaHCO_3$ concentration: 1.44/1.36, 1.62/1.53, 1.80/1.70, 1.98/1.89, 2.14/2.06 mM. The results are given in **Figure 3.1,Tables 3.2 and 3.3**.

#### 2.4.2.2 Effects of the eluent flow rate

Optimum eluent flow rate should provide good separation and short analysis time. After optimal eluent concentration was selected, optimization of the flow rate was carried out by injection of the standard mixture in Section 2.4.1 onto the IonPac AS4A column at varying flow rate from 1.80-2.20 ml/min. The data of retention times, peak areas, resolution of each ion pair and the number of theoretical plates are shown in **Figures 3.2 and 3.3** and **Tables 3.4 and 3.5**.

### 2.4.3 Repeatability and reproducibility of results

Precision of analysis may be defined as the concordance of a series of measurements of the same quantity. The number of measurements required will depend on the accuracy required and on the known reproducibility of the method. The precision can be expressed as the standard deviation (SD) and the relative standard deviation (RSD). The smaller the value of the relative standard deviation, the greater precision of an analysis. The standard deviation and the relative standard deviation are defined by **equations 2.1 and 2.2**, respectively [30, 31].

$$\mathrm{SD} = \sqrt{(x_i-\bar{x})^2/(n-1)} \qquad \text{....................(2.1)}$$

$$\%\,\mathrm{RSD} = (\mathrm{SD}/\bar{x})100 \qquad \text{....................(2.2)}$$

where $x_i$ = The individual value in data

$\bar{x}$ = The mean of data

n = The number of measurement

In this work, the mixture of standard anions was replicately injected onto the IonPac AS4A column under the obtained condition to observe reproducibility of the peak areas and retention time of each anion. The calculated relative standard deviations and other relevant results are presented in **Tables 3.7 and 3.8.**

### 2.4.4 Determination of linearity

The mixed solutions of studied anions from 0.20-300 ng/µl were injected via the 25 µl injected loop onto the IC column under the appropriate conditions except $F^-$ from 0.20-100 ng/µl and $PO_4^{3-}$ from 1.20-600 ng/µl. The results are shown in **Table 3.9**. The calibration curve is illustrated in **Figure 3.4** and the chromatogram is shown in **Figure 3.5**.

### 2.4.5 Detection limit and minimum detectable quantity (MDQ)

To determine the detection limit under the established condition, the 25 µl volume of the mixture at the lowest concentration which gave an appropriate peak height was injected onto the column at a suitable output range (3 µS). From the value of the peak signal response (R) and noise level (n), the detection limit can be calculated as follows [29].

$$L = 2\,nm_x/R \qquad \text{(weight)} \qquad \textbf{.....................(2.3)}$$

where L = detection limit, defined as the weight of substance giving a signal twice the standard deviation of the noise level

n = noise level

$m_x$ = amount of compound injected

R = peak signal response

In practice, the noise level is obtainable by means of integrating the zoomed baseline of the chromatogram of each anion. For example, noise level of $SO_4^{2-}$ is shown in **Figure 3.6.**

The minimum detectable quantity (MDQ) is related to the detection limit. This is the amount of sample that produces a peak signal two times the noise and can be expressed via the following relationship.

$$MDQ = LW_{1/2} \qquad \text{....................}\mathbf{(2.4)}$$

where $W_{1/2}$ = width at half height (sec)

The calculated results are given in **Table 3.10.**

### 2.4.6 Preparation of geological samples for anions analysis

#### 2.4.6.1 Geological water samples

Sampling was carried out in the northern part of Thailand. The sample size was 1000 ml and water samples were collected in polyethylene bottles. After collecting, the samples were filtered through filter cellulose acetate with the pore size of 0.45 micron.

### 2.4.6.2 Coal samples

This study was aimed at investigating the determination of common anions content in coal samples for analysis by IC technique. A variety of existing reports [14-19, 23] provide procedures of geological sample preparation. The coal sample preparations used in this work are as follows.

**1) Extraction method with dimethyl sulphoxide (DMSO)**[15]

1.1) The extraction procedure was performed by weighing 1.0 g of 90 mesh coal into a beaker and adding about 20 ml of DMSO containing 5 ml of 0.1 M $KNO_3$.
The solution was well mixed with the aid of magnetic stirring for :

a) 1 hour at room temperature, and
b) 15 mins at 70-90 °C

1.2) The extraction procedure for 1.1b was followed but the ratio of extracting solvent was changed to the following.

a) 10 DMSO : 10 $KNO_3$
b) 12 DMSO : 8 $KNO_3$
c) 15 DMSO : 5 $KNO_3$
d) 18 DMSO : 2 $KNO_3$

After extraction, the leachates were filtered through a Whatman No. 42 paper and diluted with deionized water to 50 ml. Because DMSO is incompatible with the analytical column, the filtrate was diluted 100-fold with deionized water prior to the chromatographic step.

### 2) $Na_2O_2$ fusion method [16]

$Na_2O_2$ fusion procedure was performed by fusion of 0.15 g of dry samples with 1.0 g of $Na_2O_2$ at 800 °C in a furnace for 5 mins. The fusion melt was reacted with 15 ml of deionized water and the resulting slurry was transferred into a 50 ml volumetric flask and diluted to the volume with deionized water prior to the chromatographic step.

### 3) Carbonate fusion method [18]

Carbonate fusion was performed by mixing 0.1 g of dry samples with 0.5 g of $Na_2CO_3$ in a crucible. The mixture was fused for 30 mins, employing

a) a furnace at 860 °C and
b) a Bunsen burner

After the fusion, the fusion melts were dissolved and diluted to 100 ml with deionized water prior to the chromatographic step.

**4) Leaching with water** [15]

4.1) Optimization of temperature of leaching

The procedure was carried out by mixing 0.5 g of dry samples with 50 ml deionized water in a beaker.

The solutions were leached by magnetic stirring at

a) room temperature (~ 30 ± 2 °C)
b) 50 °C (50 ± 2 °C)
c) 100 °C (100 ± 2 °C)

For these temperatures, three following steps were taken, respectively:

Step 1. Solution was leached for 30 mins and diluted with deionized water into 100 ml.
Step 2. Solution was leached for 30 mins and diluted with deionized water into 50 ml.
Step 3. Solution was leached for 60 mins and diluted with deionized water into 100 ml.

4.2) Optimization of time of leaching

4.2.1) The procedure is the same as in (4.1) but the solution was leached at room temperature and the time of leaching was changed to

a) 1 hour
b) 2 hours
c) 4 hours
d) 8 hours
e) 16 hours
f) 24 hours

The coal sample in 4.2.1 was obtained from Mae Lai, Hod District, Chiang Mai Province (sample #20).

4.2.2) The procedure is the same as in (4.2.1) but the coal sample was obtained from Ban Hong District, Lamphun Province instead of Mae Lai District (sample #21) and the time of leaching was changed to the following :

a) 1 hour
b) 2 hours
c) 4 hours
d) 6 hours
e) 8 hours
f) 16 hours
g) 25 hours

### 2.4.7 Determination of anions in geological samples by IC

#### 2.4.7.1 Construction of calibration curves

Quantitative analysis of anions, namely $F^-$, $Cl^-$, $NO_3^-$ and $SO_4^{2-}$ in geological water samples was carried out by the external standard method. Before construction of a calibration curve, various concentrations of each anion in geological water samples were prepared. The peak areas obtained for the anion peaks are shown in **Tables 3.11-3.14** and the corresponding calibration curves in **Figures 3.7-3.10**.

### 2.4.7.2 Analysis of anions in geological water samples

Results on the analysis of anions in geological water samples are shown in **Tables 3.15 and 3.16** and typical chromatograms of some geological water samples are shown in **Figures 3.11-3.16**.

### 2.4.7.3 Analysis of anions in coal samples

Results on the analysis of anions in coal samples are shown in **Tables 3.17 -3.26** and typical chromatograms of some coal samples are shown in **Figures 3.17-3.19.**

### 2.4.8 %Recovery of anions in geological water samples

%Recovery of each anion in geological water samples was quantitatively confirmed by using the "spike" method, with the addition of known amount of standard solution of each anion to geological water samples at various concentrations. Calculation for the % recovery was made via the following relationship.

$$\%\text{Recovery} = (A-B)100/C \qquad \textbf{.....................(2.5)}$$

where A = the amount of anion in geological water sample obtained with standard anion solution spiked at various concentrations

B = the amount of anion in geological water sample obtained without spiking anion solution

C = concentration of standard anion solution spiked to
geological water sample

The results of % recovery of the anions investigated are shown in **Tables 3.27 -3.30** and corresponding chromatograms in **Figures 3.20-3.22**.

### 2.4.9 Analysis of anions in geological water samples by spectroanalytical method

The amounts of fluoride in geological water samples were determined by the SPADNS colorimetric method [28]. This method is based on the reaction between fluoride and a zirconium-dye lake. Fluoride reacts with the dye lake, dissociating a portion of it into a colorless complex anion ($ZrF_6^{2-}$), and the dye. As the amount of fluoride increases, the color produced becomes progressively lighter. The results are shown in **Table 3.31**.

The amounts of chloride in geological water samples were determined by the mercury(II) thiocyanate method [27]. This method is based on the displacement of thiocyanate ion from mercury (II) thiocyanate by chloride ion. In the presence of iron(III) ion, a highly colored iron(III) thiocyanate complex is formed, and the intensity of its color is proportional to the original chloride-ion concentration.The results are shown in **Table 3.32**.

The amounts of nitrate in geological water samples were determined by the ultraviolet spectrophotometric screening method [28]. This technique was used only for screening samples with low organic matter contents. The nitrate calibration curve usually follows Beer's law up to 11 mg $NO_3^-$/litre. The results are shown in **Table 3.33**.

The amounts of sulphate in geological water samples were determined by the turbidimetric method [28]. Sulphate ion was precipitated in an acetic acid medium barium chloride ($BaCl_2$) so as to form barium sulphate ($BaSO_4$) crystals of uniform size. Light scaftering of the $BaSO_4$ suspension was measured by a photometer and the $SO_4^{2-}$ concentration was determined by comparison of the reading with a standard curve. The results are shown in **Table 3.34.**

### 2.4.10 Analysis of anions in geological water sample by ion selective electrode method (ISE)

The amounts of fluoride and chloride in geological water samples were determined by the ion selective electrode method [27, 28] using the fluoride electrode and the chloride electrode measuring the ion activity of fluoride and chloride in the solution of interest, respectively. Fluoride ion activity and chloride ion activity depend on the solution total ionic strength and pH, and on complexing species. The results are shown in **Tables 3.35 and 3.36**.

In order to compare the results obtained from the IC technique with those from the spectroanalytical technique, the ISE technique or the AAS technique, whether there is a significant difference between them, an application of statistics was used. For this work, comparison was determined by means of **the student *t* test** whereby comparison is made between two sets of replicate measurements obtained from two different methods. A statistical t value is calculated and compared with a tabulated value for the given number of tests at the desired confidence level. If the calculated t value exceeds the tabulated t value, then there is a significant difference between the results by the two methods at that confidence level. If it does not exceed the tabulated value, it means that there is no significant difference between the methods.

In this work, the t-test with multiple samples was adopted as it is applicable to several different samples of slightly varying composition[31]. In this case, the difference between each of the paired measurements on each sample is computed. An average, $\bar{D}$ is calculated and the individual deviations of each from $D_i$ are used to compute a standard deviation, SD. The t value is calculated from [30, 31]:

$$t = \bar{D}\sqrt{N \ / SD} \qquad \text{....................(2.6)}$$

and

$$SD = \sqrt{\Sigma(D_i-\bar{D})^2 / (N-1)} \qquad \text{....................(2.7)}$$

where $D_i$ = the individual difference between the two methods for each sample, with regard to sign.

$\bar{D}$ = the mean of all the individual differences.

Usually, a test at the 95% confidence level is considered significant, while one at the 99% level is highly significant. That is, the smaller the calculated t value, the more confident one can be that there is no significant difference between the two methods. If too low a confidence level (e.g., 80%) is employed, it can be concluded erroneously that there is a significant difference between the two methods. On the other hand, too high a confidence level will require too large a difference to detect.

Unless otherwise stated, the t-test with multiple samples in this work was used at the 95% confidence level. Values of t for various degrees of freedom for various confidence levels are given in **Appendix D**.

Amounts of $F^-$, $Cl^-$, $NO_3^-$ and $SO_4^{2-}$ in geological water samples by the IC technique, the spectroanalytical technique and the ISE technique were compared using the t-test (**Equation 2.6**) and the results are given in **Tables 3.31-3.36**.

## 2.5 Determination of Metal Ions with IonPac CS5 Column

The IonPac CS5 analytical column is designed specifically for rapid and sensitive determination of transition metals and lanthanide metals in a wide variety of matrices. Separations are accomplished by using complexing eluents with pellicular cation and/or anion exchange materials. The mode of detection is post-column derivatization with visible absorbance detection. In this work, ion chromatographic conditions of IonPac CS5 column were optimized and applied for the analysis of trace transition metals in geological water samples.

### 2.5.1 Investigation of the post-column reagent

The post column reagents such as arsenazo I, arsenazo III and PAR should react quickly and their formation constants are usually high. They should form a complex of high molar absorptivity and the free indicator should have a low absorptivity at the wavelength of the maximum absorbance of the complex. The complexing reaction should take place in solutions in which close pH control is not necessary to eliminate the need for the presence of large amounts of buffer or close control of reagent flow rate [3]. In this work, the colorimetric reagent chosen was 4-(2-pyridylazo) resorcinol (PAR) which has been found to give a colored species with transition metals. PAR was by far the most versatile and convenient of the photometric reagents to use.

### 2.5.2 Investigation of retention times of metal ions of interest

Retention times of metal ions of interest employing post-column derivatization were obtained in the simultaneous determinations of some heavy metals in geological water samples, namely $Fe^{3+}$, $Cu^{2+}$, $Zn^{2+}$, $Co^{2+}$ and $Mn^{2+}$. Each studied cation standard solution given in **Table 2.4** was injected onto the IonPac CS5 column using 7.5 mM PDCA pH 4.1 as eluent, at the flow rate 1.0 ml/min and 0.36 mM 4-(2-pyridylazo) resorcinol (PAR) in 3.52 M $NH_4OH$ / 1.0 M $CH_3COOH$ as the post-column reagent at the flow rate 0.5 ml/min. The wavelength of the UV/vis spectrophotometer was set in the visible region at 530 nm. The retention times obtained are shown in **Table 3.37**.

**Table 2.4** Standard solution of studied metal ions used to determine their retention times.

| Metal Ion | Concentration (ng/µl) |
|---|---|
| $Fe^{3+}$ | 1.0 |
| $Cu^{2+}$ | 1.0 |
| $Zn^{2+}$ | 1.0 |
| $Co^{2+}$ | 1.0 |
| $Mn^{2+}$ | 1.0 |

### 2.5.3 Optimization of IC conditions.

To obtain the high test separation efficiency and good sensitivity of analysis, some parameters affecting were studied as described below.

#### 2.5.3.1 Effect of eluent concentration

The standard solution of mixed cations from Section 2.5.2 was triplicately injected onto the IonPac CS5 column using PDCA concentrations between 6.0-8.0 mM as eluent and 0.36 mM PAR in 3.52 M $NH_4OH$/ 1.0 M $CH_3COOH$ as post-column reagent. The flow rate of the eluent was 1.0 ml/min and the post-column reagent 0.5 ml/min. The detector wavelength was set at 530 nm with the absorbance unit (AU.) at 0.2. The retention times, resolution values and peak area at various eluent concentrations are given in **Table 3.40, Figures 3.24 and 3.25.**

#### 2.5.3.2 Effect of eluent pH

The mixture standard cation solution from Section 2.5.2 was triplicately injected onto the IonPac CS5 column using 7.5 mM PDCA pH 3.7-4.6 as eluent and 0.36 mM PAR in 3.52 M $NH_4OH$/ 1.0 M $CH_3COOH$ as post-column reagent. The flow rate of the eluent was 1.0 ml/min and the post-column reagent 0.5 ml/min. The detector wavelength was set at 530 nm with the absorbance unit (AU.) at 0.2. The results of the retention time and the peak area as shown in **Figures**

**3.26 and 3.27**. The results of the resolution of each metal ion at various values of eluent pH are shown in **Table 3.41**.

### 2.5.3.3 Effect of eluent flow rate

The standard cation solution from Section 2.5.2 was injected onto the IonPac CS5 column at various eluent flow rates from 0.9-1.3 ml/min and at the constant flow rate 0.5 ml/min of the post-column reagent using 7.5 mM PDCA pH 4.1 as eluent and 0.36 mM PAR in 3.52 M $NH_4OH$/ 1.0 M $CH_3COOH$ as the post-column reagent. PAR is readily oxidized by oxygen. Therefore, it was necessary to pressurize helium gas about 70 psi at the flow rate 0.5 ml/min. The detector wavelength was fixed at 530 nm with the absorbance unit (AU.) set at 0.2. The resolution, retention time of each metal ion and peak area of each metal ion at various eluent flow rates are given in **Table 3.42**, **Figures 3.28 and 3.29,** respectively. The numbers of theoretical plates which indicate the column efficiency are shown in **Table 3.43**.

### 2.5.3.4 Optimum detection wavelength

The optimum wavelength for the metal ion analysis was determined by triplicately injecting 25 µl of the mixture solution from Section 2.5.2 at wavelength between 480-540 nm at constant flow rate of eluent and the post-column reagent (1.0 and 0.5 ml/min, respectively) using 7.5 mM PDCA pH 4.1 as eluent and 0.36 mM PAR in 3.52 M $NH_4OH$/ 1.0 M $CH_3COOH$ as the post-column reagent. The detector wavelength was set at 530 nm with absorbance unit (AU.) at

0.2. The results for each metal ion are shown in **Figure 3.30**. The results for signal to noise ratio at each wavelength of interest are shown in **Table 3.44** and the chromatograms are shown in **Figure 3.31**.

### 2.5.3.5 Effect of PAR concentration

The mixture standard cation solution from Section 2.5.2 was injected onto the IonPac CS5 column at eluent flow rate 1.0 ml/min and at the 0.5 ml/min of the post-column reagent using 7.5 mM PDCA pH 4.1 as eluent and using PAR concentration between 0.28-0.55 mM as post-column reagent in 3.52 M $NH_4OH$/ 1.0 $CH_3COOH$. The detector wavelength was set at 530 nm with absorbance unit (AU.) at 0.2. The peak areas of each metal ion at various PAR concentrations are shown in **Figure 3.32**.

### 2.5.3.6 Effect of PAR pH

The mixture standard cation solution from Section 2.5.2 was triplicately injected onto the IonPac CS5 column using 7.5 mM PDCA pH 4.1 at flow rate 1.0 ml/min as eluent and 0.36 mM PAR in 3.52 M $NH_4OH$/ 1.0 M $CH_3COOH$ pH 9.0-9.9 at flow rate 0.5 ml/min as reagent. The detector wavelength was fixed at 530 nm with the absorbance (AU.) set at 0.2. The results of the peak area of each metal at various values of PAR pH are shown in **Figure 3.33**.

#### 2.5.3.7 Effect of PAR flow rate

The last parameter of optimization condition of metal ions was the PAR flow rate. The mixture standard cation solution from Section 2.5.2 was triplicately injected onto the IonPac CS5 column using 7.5 mM PDCA pH 4.1 at flow rate 1.0 ml/min as eluent and 0.36 mM PAR in 3.52 M $NH_4OH$/ 1.0 M $CH_3COOH$ pH 9.7 at various PAR flow rates from 0.3-0.7 ml/min as reagent. The detector wavelength was fixed at 530 nm with the absorbance (AU.) set at 0.2. The results of the peak area of each metal at various values of PAR pH are shown in **Figure 3.34**.

### 2.5.4 Repeatability and reproducibility of results

The standard cation mixture solution from Section 2.5.2 was replicately injected onto the IonPac CS5 column with appropriate optimized condition to observe repeatability and reproducibility of the retention and peak area of each cation. The results of calculated relative standard deviation and % relative standard deviation are shown in **Tables 3.46 and 3.47**.

### 2.5.5 Determination of linearity

In this work, determination of linearity was studied by injecting exact amounts of the mixture of metal standard solutions onto the IonPac CS5 column operated at previously obtained conditions. The concentrations of the mixture standard solution were in the range from 0.08-8.0 ng/μl. The results are shown in **Table 3.48** and **Figure 3.35**. One example of the obtained chromatograms is shown in **Figure 3.36**.

### 2.5.6 Determination of detection limit (L) and minimum detectable quantity (MDQ)

Since the ratio of the signal to the noise determines sensitivity, it is logical to use the signal to noise ratio to evaluate the limit of detectability of the system. This, in turn, is the convention to express the limit of the detectability as that level (concentration or mass) of solute that produces a signal of which the average amplitude is twice the average amplitude of the noise. Under the established condition, the 25 μl volume of suitable concentration of each metal ion standard solution which gave a suitable peak at the lowest detector absorbance unit (AU.) range (0.2) was injected on to the column. From the value of the peak signal response (R) and the noise level (n), the detection limit can be calculated from **Equation 2.3**. The calculation results are given in **Table 3.49**. For example, noise level of mixed standards are shown in **Figure 3.37**. From the value of the detection limit (L) obtained, the minimum detectable quantity was calculated via **Equation 2.4**.

### 2.5.7 Preparation of geological samples for heavy metal ions analysis

#### 2.5.7.1 Geological water samples

The amounts of $Fe^{3+}$, $Cu^{2+}$, $Zn^{2+}$, $Co^{2+}$ and $Mn^{2+}$ in geological water samples were determined by the IC technique, using the nonspecific metallochromic indicator 4-(2-pyridylazo)resorcinol (PAR). 25 μl of each of the geological water samples from Section 2.2 was duplicately injected onto the IonPac CS5 column under the established conditions. The preparation is the same as in Section 2.4.6.1.

### 2.5.7.2 Geological reference materials

Soil 5 and river sediment 320 were used in the this work. First, the samples were cleaned with 30% $H_2O_2$ for 3 hours. This cleaning was done to remove tissues and organic substances. Finally, the samples were leached in deionized water, dried at 110°C for 3 hours and stored in cleaned plastic bottles, which was kept in a desiccator to prevent any hygroscopic activity. The sample preparations used in this work are as follow.

**Method #1** Digestion with conc. $HNO_3$ [32]

0.1 g of samples was dissolved in 1 ml of conc. $HNO_3$. The solutions were buffered to pH 4.4±0.1, using 2.0 M ammonium acetate, and a final volume of sample solution of 50 ml was made with deionized water prior to the chromatographic step.

**Method #2** Digestion with 1+1 $HNO_3$ [33]

0.1 g of samples was weighed into a beaker and 10 ml of 1+1 $HNO_3$ was added. Next, the solution was heated until the volume of the final solution became the lowest volume possible. Heating was allowed to continue and 1+1 $HNO_3$ was added as necessary until digestion was complete as shown by a light-colored, clear solution. The sample was not allow to dry during digestion. After digestion, the solution were diluted to 50 ml with 1% $HNO_3$ prior to the chromatographic step.

**Method #3** Digestion with 1+1 HCl [33]

The procedure is the same as in method #2 but change from 1+1 $HNO_3$ and 1% $HNO_3$ were used instead of 1+1 HCl and 1% HCl, respectively.

**Method #4** Digestion with HF [26]

0.1 g of sample was weighed into a Teflon beaker and 5 ml of 1+1 $H_2SO_4$ and 1 ml of 26 M HF were added. Heating was performed on a water bath until the volume of final solution became the lowest volume possible. The solution was transferred into a plastic bottle, containing a masking solution (10 ml of saturated boric acid and 84 ml of deionized water) The final volume of 100 ml with deionized water prior to the chromatographic step.

### 2.5.8 Determination of heavy metal ions in geological samples by IC

The amounts of metal ions in geological samples, namely $Fe^{3+}$, $Cu^{2+}$, $Zn^{2+}$, $Co^{2+}$and $Mn^{2+}$ were determined by the IC technique. The nonspecific metallochromic indicator 4-(2-pyridylazo) resorcinol (PAR) has been widely used for chromatographic detection of metals. 25 µl of geological water samples solution from section 2.5.6 was duplicately injected onto the IonPac CS5 column under the established condition.

### 2.5.8.1 Construction of calibration curves

A calibration curve for use in the IC technique was plotted using the peak areas (arbitrary unit) of standard solution containing known concentrations of the ion to be determined. Calibration curve of each metal ion was constructed from peak area data at various concentrations. The peak areas of $Fe^{3+}$, $Cu^{2+}$, $Zn^{2+}$, $Co^{2+}$and $Mn^{2+}$ are shown in **Tables 3.50-3.54** and calibration curve in **Figures 3.38-3.42**.

### 2.5.8.2 Analysis of metal ions in geological water samples

$Fe^{3+}$, $Cu^{2+}$, $Zn^{2+}$, $Co^{2+}$and $Mn^{2+}$ were found in geological water samples. The results of the analysis are shown in **Tables 3.55 and 3.56** and typical chromatograms of some geological samples in shown in **Figures 3.43-3.46**.

### 2.5.8.3 Analysis of metal ions in geological reference materials

$Fe^{3+}$, $Cu^{2+}$, $Zn^{2+}$, $Co^{2+}$and $Mn^{2+}$ were found in geological reference materials. The results of the analysis are shown in **Tables 3.57 and 3.58** and typical chromatograms of some geological samples in shown in **Figures 3.47-3.50.**

### 2.5.9 % Recovery of each metal ion in IC technique

The chromatographic peaks produced from standard solutions were qualitatively confirmed using the spike method. The results and the chromatogram of % recovery of each metal ion are shown in **Tables 3.59-3.63** and **Figures 3.51 and 3.52.**

### 2.5.10 Analysis of metal ions in geological water samples by atomic absorption spectrophotometry (AAS) and inductively coupled plasma spectrophotometry (ICP)

The amounts of $Fe^{3+}$, $Cu^{2+}$, $Zn^{2+}$, $Co^{2+}$and $Mn^{2+}$ in geological water samples were determined again by the AAS technique and ICP technique using external standard method. To indicate whether there is a significant difference of results between the IC technique and the AAS technique or the IC technique and the ICP technique, t test was used for the comparison. In this case, the value is calculated in a slightly different form from Section 2.4.10, because it is considered to be a t test with multiple samples. The t value calculated via **Equation 2.6** [30, 31]. The result is shown **Table 3.64**.