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 value with a 25 µl sample loop, Rheodyne

b. Guard columns, IonPac AG 4 A (5x50 mm), IonPac CG 5 (5x50 mm) and IonPac CG 12 (5x50 mm), Dionex Corp., U.S.A.

c. Separator columns, IonPac AS 4 A (5x250 mm), IonPac CS 5 (5x250 mm) and IonPac CG 12 (5x250 mm), Dionex Corp., U.S.A.

d. Anion self-regenerating suppressor - I (4 mm), Dionex Corp., U.S.A.

e. Cation self-regenerating suppressor - I (4 mm), Dionex Corp., U.S.A.

f. Post column, IonPac membrane reactor, Dionex Corp., U.S.A.

g. Conductivity detector, Dionex Corp., U.S.A.

h. uv/vis detector, Dionex Corp., U.S.A.

i. 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 µm, Sartorius, Germany.

6. Ultraviolet-Visible spectrophotometer, Shimadzu 265, Shimadzu Co., Japan.

7. Atomic absorption spectrophotometer, Shimadzu AAS-680, Shimadzu Co., Japan.

8. pH meter, Chemtrix Type 60 A, Chemtrix Inc., U.S.A.

9. pH meter 50 Fisher Scientific - EMD, U.S.A. with ion selective fluoride electrode.

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. Methanesulfonic acid (MSA), > 99%, Fluka, Switzerland.
14. Tetrabutylammonium hydroxide solution, assay 40%, puriss, Fluka, Switzerland.
15. Copper standard solution for atomic absorption spectrophometry, 1 g/l, Fluka, Switzerland.
16. Lead standard solution for atomic absorption spectrophotometry, 1 g/l, Fluka, Switzerland.

17. Manganese standard solution for atomic absorption spectrophotometry, Fluka, Switzerland.
18. Zinc standard solution for atomic absorption spectrophotometry, Fluka, Switzerland.
19. Nickel standard solution for atomic absorption spectrophotometry, Fluka, Switzerland.
20. Calcium chloride standard solution for atomic absorption spectrophotometry, BDH Chemical Ltd., England.
21. Strontium chloride standard solution for atomic absorption spectrophotometry, BDH Chemical Ltd., England.
22. Lead(II) nitrate, 99.999%, Aldrich, U.S.A.
23. Copper(II) sulphate pentahydrate, 99.999%, Aldrich, U.S.A.
24. Manganese(II) nitrate tetrahydrate, 98.5%, Fluka, Switzerland.
25. Zinc(II) nitrate hexahydrate, 99.999%, Aldrich, U.S.A.
26. Nickel(II) nitrate hexahydrate, 99.999%, Aldrich, U.S.A.
27. Magnesium ion chromatography standard solution, 1.000 g/l in nitric acid, Fluka, Switzerland.
28. Calcium ion chromatography standard solution, 1.000 g/l in nitric acid, Fluka, Switzerland.
29. Strontium chloride dihydrate, 99.5% puriss, Fluka, Switzerland.
30. Acetic acid, 100% AR grade, E Merck, Germany.
31. Ammonia solution, 30% NH_3 AR grade, BDH, Chemicals Ltd., England.

2.2 Preparation of Solutions

2.2.1 Preparation of eluent

In this work a solution of sodium carbonate/sodium bicarbonate ($Na_2CO_3/NaHCO_3$), oxalic acid and MSA were the three eluents investigated.

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_3/170mM $NaHCO_3$) 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 1.8 mM Na_2CO_3/1.7 mM $NaHCO_3$ by pipetting 10.0 ml of it into a one - litre volumetric flask and diluting it to the mark.

(b) Oxalic acid solution

An exact weight, to give needed concentration of oxalic acid, was dissolved in about 700 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.

(c) MSA solution

The MSA stock solution of 1.0 M was prepared by pipetting 6.54 ml of its standard solution into a 100 ml volumetric flask and adjusting the volume to the mark with deionized water. The MSA eluents were prepared by dilution of its 1.0 M stock solution to the desired concentration.

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.

2.2.2 Post-column reagent

The post column reagent was a solution of $4x10^{-4}$ M PAR in 3 M NH_4OH/1 M CH_3COOH. At first 0.0435 g of PAR was dissolved in a mixture of 112 ml of NH_4OH and 28 ml of CH_3COOH. Then, the solution was diluted with deionized water to the mark in a 500 ml volumetric flask.

2.2.3 Regenerant in the chemical suppression mode

The regenerant was a solution of 25 mM tetrabutyl ammonium hydroxide 16.54 ml of tetrabutyl ammonium hydroxide, assay 40% w/v, was pipetted into a one-litre volumtric flask and deionized water was added to a final volume of 1000 ml.

2.2.4 Standard anion stock solutions, 1000 ng/μl, 100 ml

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

2.2.5 Standard cation stock solutions, 1000 ng/µl, 100 ml

These were prepared by dissolving 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) sulfate 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
Strontium chloride hexahydrate, 0.3043 g
Magnesium ion standard solution 1.000 g/l
Calcium ion standard solution 1.000 g/l

2.2.6 Metal ion standard solutions for atomic absorption spectrophotometer

Each standard solution of metal ion with the 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.

2.2.7 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 2.1**.

Table 2.1 List of references for details of the preparation of reagents used in the determination of anions by spectroanalytical methods.

Determination	Method	Reference
Chloride	Mercury(II) thiocyanate	20
Fluoride	Ion selective electrode	21
Nitrate	UV spectrophotometric screening	21
Phosphate	Ascorbic acid	21
Sulphate	Turbidimetric	21

2.3 Determination of Anions with IonPac AS 4 A Column

The IonPac AS 4 A analytical column is designed specifically for the analysis of anions. Separations are accomplished by using 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 and $NaHCO_3$. 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 acid, which have a high conductivity [12].

2.3.1 Investigation of retention times of anions of interest

The retention times of some anions of interest were determined. Each of the anion standard solutions given in **Table 2.1** was injected onto the IonPac AS 4 A column using 1.8 mM Na_2CO_3/1.7 mM $NaHCO_3$ as eluent at flow rate 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 2.2 Standard solutions of studied anions used to determine retention times

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

2 .3.2 Optimization of IC conditions

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

2.3.2.1 Effects of eluent concentration

A standard mixture of F^-, Cl^-, NO_2^-, Br^-, NO_3^-, PO_4^{3-} and SO_4^{2-} from Section 2.3.1 was injected onto the IonPac AS 4 A column with the following $Na_2CO_3/NaHCO_3$ concentrations : 1.44/1.36, 1.62/1.53, 1.80/1.70, 1.98/1.89 and 2.16/2.07 mM. The results are given in **Table 3.2**.

2.3.2.2 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 injecting the standard mixture in section 2.3.1 onto the column at varying flow rates from 1.8-2.2 ml/min. The data of retention times, peak areas, resolution of each ion pair and the number of theoretical plates are shown in **Table 3.3**.

2.3.3 Determination of linearity

The mixture solutions of studied anions from 0.20-400.00 μg/ml were injected via the 25 μl injection loop onto the IC column under the appropiate conditions. The results are shown in **Table 3.5**. The calibration curve is illustrated in **Figure 3.1** and the chromatogram is shown in **Figure 3.2**.

2.3.4 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 [24].

$$L = \frac{2\,nm_x}{R} \quad \text{(weight)} \qquad \text{.....................(2.1)}$$

where L = detection limit, defined as the weight of substance to give 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 3.3**.

The mininum 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.

$$MDQ = LW_{1/2} \qquad \text{......................(2.2)}$$

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

The calculated results are given in **Table 3.6**

2.3.5 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 the precision of an analysis. The standard deviation and the relative standard deviation are defined by equations 2.3 and 2.4, respectively [25].

$$SD = \sqrt{(x_i - x)^2/(n - 1)} \qquad \text{.....................(2.3)}$$

$$\%RSD = (SD / \bar{x})\, 100 \qquad \text{.....................(2.4)}$$

where x_i = the individual value in data
x = the mean of data
n = the number of measurement

In this work, the mixture of 1 ng/µl of standard anions was replicately injected onto the IonPac AS 4 A 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 **Table 3.7**.

2.3.6 Water samples

Sampling was carried out from ten sources of commercially bottled drinking water and ten sampled well water in the vicinity of Muang District of Chiang Mai province of Thailand. The sample size was 1000 ml and water samples were collected in polyethylene bottles. After collecting, the samples were filtered throught filter cellulose acetate with the pore size 0.45 micron. The ten bottled drinking water and ten well water samples were labelled b.w.$_1$ to b.w.$_{10}$ and w.w$_{.1}$ to w.w.$_{10}$, respectively.

2.3.7 Determination of anions in drinking water samples by IC

2.3.7.1 Construction of calibration curves

Quantitative analysis of anions, namely F^-, Cl^-, NO_2^-, Br^-, NO_3^-, PO_4^{3-} and SO_4^{2-} in drinking water samples was carried out by the external standard method. Before construction of a calibration curve, various concentrations of each anion in drinking water samples were prepared. The peak areas obtained for the anion peaks are shown in **Tables 3.8-3.12** and the corresponding calibration curves in **Figures 3.4-3.8**.

2.3.7.2 Analysis of anions in drinking water

Results on the analysis of anions in drinking water samples are shown in **Tables 3.13-3.14** and typical chromatogram of some drinking water samples in **Figures 3.9-3.10**.

2.3.8 % Recovery of anions in drinking water samples

% Recovery of each anion in drinking water samples was qualitatively confirmed using the "spike" method, with the addition of known amount of standard solution of each anion to drinking water samples at various concentrations.

Calculation for the % recovery was made via the following relationship.

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

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

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

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

The results % recovery of the anions investigated are shown in **Tables 3.15 - 3.19** and corresponding chromatograms in **Figures 3.11 - 3.13**.

2.3.9 Analysis of anions in drinking water samples by ion-selective electrode method and spectroanalytical method

The amounts of fluoride in drinking water samples were determined by the ion-selective electrode method [21] using the fluoride electrode in measuring the ion activity of fluoride in solution. Fluoride ion activity depends on the solution total ionic strength and pH, and on fluoride complexing species. The results are shown in **Table 3.20**.

The amounts of chloride in drinking water samples were determined by the mercury(II) thiocyanate method [20]. This method is based on the displacement of thiocyanate ion from mercury(II) thiocyanate by chloride ion. The results are shown in **Table 3.21**.

The amounts of nitrate in drinking water samples were determined by the ultraviolet spectrophotometric screening method [21]. This technique was used only for screening samples with low orgainc matter contents. The nitrate calibration curve usually follows Beer's law up to 11 mg N/L. The results are shown in **Table 3.22**.

The amounts of phosphate in drinking water samples were determined by the ascorbic acid method [21]. This method involves the reaction of ammonium molybdate and potassium antimonyl tartrate in acid medium with orthophosphate to form a heteropoly acid-phosphomolybdic acid which is reduced to intensely colored molybdenum blue by ascorbic acid. Minimum detectable concentration with this method is approximately 10 μg P/L. The results are shown in **Table 3.23**.

The amounts of sulphate in drinking water samples were determined by the turbidimetric method [21]. Sulphate ion is precipitated in an acetic acid medium with barium chloride ($BaCl_2$) so as to form barium sulphate ($BaSO_4$) crystals of uniform size. Light absorbance of the $BaSO_4$ suspension is measured by a photometer and the SO_4^{2-} concentration is determined by comparison of the reading with a standard curve. The results are shown in **Table 3.24**.

In order to compare the results obtained from the IC technique with those from the spectroanalytical 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 [25]. 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 $\bar{D}$ are used to compute a standard deviation, S_d . The t value is calculated from [25] :

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

and

$$S_d = \sqrt{\frac{\sum (D_i - \bar{D})^2}{N - 1}} \qquad \text{.......................(2.7)}$$

where D_i = The individual differences 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 cancluded 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 A.

Amounts of F^- , Cl^- , NO_3^- , PO_4^{3-} and SO_4^{2-} in drinking water samples by the IC technique and the spectroanalytical technique were compared using the t-test (Equation 2.6) and the results are given in **Tables 3.20-3.24**.

2.4 Determination of Alkaline Earth Metal Ions Using IonPac CS 12 Column

The IonPac CS 12 analytical column is designed specifically for the analysis of alkali metals, alkaline earth metals and ammonium ion. Its stationary phase consists of a 8.0 micron poly(ethylvinylbenzene/divinylbenzene) macroporous substrate resin that is functionalized with a relatively weak carboxylic acid. It has both cation exchange and reverse phase properties. It differs from other cation exchange columns which have a sulfonic acid functionality, making them strong cation exchangers, while the IonPac CS 12 column has a carboxylate functionality, making it a relatively weak cation exchanger with a high selectivity for hydronium ion. This difference makes it possible to use a low ionic strength eluents to elute isocratically both monovalent and divalent cations in a relatively short period of time [7]. In this research, ion chromatographic conditions of IonPac CS 12 column were optimized and applied for the analysis of alkali earth metal ions in selected drinking water samples. The column was run with cation self-regenerating suppressor - I (CSRS - I) which was used in the chemical suppression mode. The chemical suppression mode makes use of tetrabutylammoinum hydroxide (TBAOH) as a chemical regenerant instead of using an applied current and deionized water. The chemical suppression mode must be used for eluents containing chloride ions such as those containing hydrochloric acid [26]. In this work, methanesulfonic acid eluents was used in place of hydrochloric acid eluents. The cation self-regenerating suppressor-I (CSRS-I) was equipped with a conductivity detector.

2.4.1 Investigation of retention times of ions of interest

The retention times of some alkaline earth metal ions of interest were detemined, namely Mg^{2+} , Ca^{2+} and Sr^{2+}. Each studied ion standard solution given in Table 2.3 was injected onto the IonPac CS 12 column using 20 mM MSA at flow rate 1.0 ml/min as eluent and 25 mM TBAOH at flow rate 6.0 ml/min as regenerant solution. The conductivity detector was used with a fixed temperature compensation at 1.7%/ °C and output range 10 μS. The retention times obtained are shown in **Table 3.25**.

Table 2.3 Standard solutions of studied cations used to determine their retention times.

Cation	Concentration (ng/μl)
Mg^{2+}	1.00
Ca^{2+}	1.00
Sr^{2+}	1.00

2.4.2 Optimization of IC conditions

To obtain the highest separation efficiency and sensitivity of analysis, the related parameters were studied.

2.4.2.1 Effect of eluent concentration

The mixture of standard alkaline earth metal ions content of 1.0 ng/µl Mg^{2+} ,1.0 ng/µl Ca^{2+} and 1.0 ng/µl Sr^{2+} was triplicately injected onto the IonPac CS 12 column using MSA solution concentration between 10-25 mM MSA as eluent and 25 mM TBAOH as regenerant solution. The flow rates of the eluent and regenerant were constant at 1.0 and 6.0 ml/min, respectively. The retention time, peak area and resolution values at various eluent concentrations are given in **Table 3.26**.

2.4.2.2 Effect of eluent flow rate

From varying eluent flow rate, the optimum eluent flow rate obtained should provide good separation and short analysis time. The mixture standard solution from Section 2.4.2.1 was injected onto the column at various eluent flow rates between 0.80-1.10 ml/min and optimal eluent concentration was selected. The data of retention times, peak areas, resolution of each ion pair and the number of theoretical plates are shown in **Table 3.27**.

2.4.3 Determination of linearity

The mixture solutions of studied ions from 0.20-300.00 µg/µl were injected in the 25 µl injection loop onto the IC column under the appropriate conditions. The results are shown in **Table 3.29**. The calibration curve is illustrated in **Figure 3.14** and the chromatogram is shown in **Figure 3.15**.

2.4.4 Detection limit and minimum detectable quantity (MDQ)

Determination of the detection limit under the established conditions was carried out with the injection of 25 μl volume of the mixture at the lowest concentration which gave an appropriate peak height at a suitable output range (10 μS). The detection limit and the minimum detectable quantity were calculated as described under Section 2.3.4 using the peak height and the height of the noise level, as shown in **Figure 3.16** instead of peak area. The calculated results are presented in **Table 3.30**.

2.4.5 Reproducibility of results

The mixture standard solution from Section 2.4.2.1 was replicately injected onto the IonPac CS 12 column under optimized conditions to observe reproducibility of the retention time and peak area of each ion. The results of the calculated relative standard deviation are shown in **Table 3.31**.

2.4.6 Determination of alkaline earth metal ions in drinking water samples by IC

IonPac CS 12 column was used to determine Mg^{2+} , Ca^{2+} and Sr^{2+} in bottled drinking water samples and well water samples. The water samples were filtered through membrane filter cellulose acetate with pore size 0.45 micron. The drinking water samples were investigated for cations from Section 2.3.6.

2.4.6.1 Construction of calibration curves

After concentrations of Mg^{2+} , Ca^{2+} and Sr^{2+} in drinking water samples were estimated, quantitative analysis of cations in drinking water samples were performed by the external standard method. Calibration curve of each cation was constructed to cover a range of various concentrations. The corresponding peak areas are shown in **Tables 3.32-3.34** and calibration curves in **Figures 3.17-3.19**.

2.4.6.2 Analysis of cations in drinking water samples

The results of the analysis for cations in both drinking water and well water samples are shown in **Table 3.35** and typical chromatogram of some drinking water samples in **Figures 3.20 and 3.21**.

2.4.7 % Recovery of each cation in drinking water samples

Each cation in drinking water samples was qualitatively confirmed using the spike method. The standard solution of each cation at various concentrations was spiked into drinking water samples. The results and the chromatogram of % recovery of each cation are shown in **Tables 3.36-3.38** and **Figure 3.22**.

2.4.8 Analysis of alkaline earth metal ions in drinking water samples by atomic absorption spectrophotometric method

The amounts of Mg^{2+} , Ca^{2+} and Sr^{2+} in drinking water samples were determined by AAS technique using the external standard method.

2.4.8.1 Atomic absorption experiment [20]

The determination of Mg^{2+} , Ca^{2+} and Sr^{2+} in potable water is very straightforward; very few interferences are encountered when using an acetylene-air flame. Many chemical interferences are countered in the acetylene air flame and the use of releasing agent such as strontium chloride, lanthanum chloride or EDTA is necessary. In this work, strontium chloride was used as releasing agent. The amounts of cations determined from a calibration curve of each cation for use in atomic absorption measurement were plotted between the concentrations versus absorbance of each cation. The results are shown in **Tables 3.39-3.40.**

2.5 Determination of Metal Ions with IonPac CS 5 Column

The IonPac CS 5 analytical column is designed specifically for rapid and sensitive determination of transition metals. Seperations are accomplished using complexing eluents with pellicular cation and/or anion exchange materials. The mode of detection is post-column derivatization with visible absorbance detection [27]. In this work, ion chromatographic conditions of IonPac CS 5 column were optimized and applied for the analysis of trace transition metals in drinking water samples.

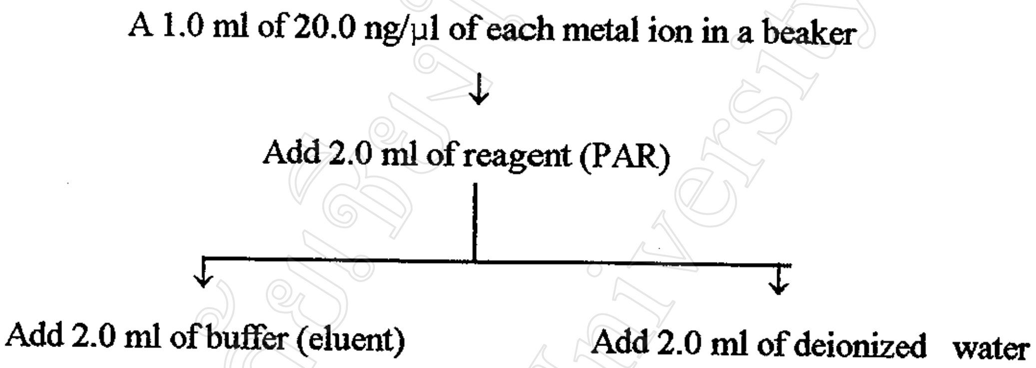
2.5.1 Investigation of the post-column reagent

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 [17]. 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 for the most versatile and convenient of the photometric reagent to use.

2.5.2 Investigation of color-forming reagent

Absorption maximum wavelength (λ max) of metal-PAR complexes was investigated using a uv-vis spectrophotometer. Each cation standard was prepared in both water and buffer as media in the presence of eluent, solution of oxalic acid pH 5.3, in order to make the condition nearly within the ion chromatographic system.

The preparation procedure of metal ion-complexes is shown in the diagram as in the following



Absorbance measurements of the final volume of 5.0 ml of metal ion-complexes was made on the spectrophotometer to obtain spectra from 450 to 550 nm.

The blank solution used was 1.0 ml of deionized water. The results are shown in **Tables 3.41 and 3.42**.

2.5.3 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 drinking water, namely, Pb^{2+} , Cu^{2+} , Mn^{2+} , Zn^{2+} and Ni^{2+}. Each studied cation standard solution given in **Table 2.4** was injected onto the IonPac CS 5 column using 50 mM oxalic acid pH 5.3 as eluent, at the flow rate 1.0 ml/min and 4.0×10^{-4} M 4-(2-pyridylazo) resorcinol (PAR) in 3.0 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 510 nm. The retention times obtained are shown in **Table 3.43**.

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

Ion	Concentration (ng/µl)
Pb^{2+}	4.0
Cu^{2}+	0.5
Mn^{2+}	2.0
Zn^{2+}	2.0
Ni^{2+}	4.0

2.5.4 Optimization of ion chromatographic conditions

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

2.5.4.1 Effect of eluent pH

The mixture standard cation solution from Section 2.5.3 was triplicately injected onto the IonPac CS 5 column using 50 mM oxalic acid pH 3.8-6.3 as eluent as shown in **Figure 3.23**. The results of the resolution of each metal ion at various values of eluent pH are shown in **Table 3.44.**

2.5.4.2 Optimum detection wavelength

The optimum wavelength for the metal ion analysis was determined by triplicately injecting 25 μl of the mixture solution from Section 2.5.3 at wavelengths between 480 to 540 nm at constant flow rates of eluent and the post-column reagent (1.0 and 0.5 ml/min, respectively). The results for each metal ion are shown in **Figure 3.24**.

2.5.4.3 Effect of eluent flow rate

The mixture standard cation solution from Section 2.5.3 was injected onto the IonPac CS 5 column at various eluent flow rates from 0.9 to 1.3 ml/min and at the constant flow rate 0.5 ml/min of the post-column reagent using 50 mM oxalic acid pH 5.3 as eluent and $4x10^{-4}$ M PAR in 3.0 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 the reservoir with helium when using a reagent via the pressurized helium gas at about 60 psi at the flow rate 0.5 ml/min. The detector wavelength was fixed at 510 nm with the absorbance unit (AU.) set at 0.05. The resolution, peak area of each metal ion at various eluent flow rates are given in **Table 3.45** and **Figure 3.25**, respectivety. The numbers of theoretical plates which indicate the column efficiency are shown in **Table 3.46**.

2.5.4.4 Effect of eluent concentration

The last parameter of optimization condition of metal ions was the eluent concentration. This was carried out by triplicately injecting the mixture standard from Section 2.5.3 using oxalic acid concentrations between 35-65 mM as eluent and 4×10^{-4} M PAR in 3.0 M NH_4OH/1.0 M CH_3COOH as 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 510 nm with the absorbance unit at 0.05. The retention times, resolution values and peak areas at various eluent concentrations are given in **Table 3.47**.

2.5.5 Determination of linearity

In this work, determination of linearity was studied by injecting exact amount of the mixture of metal standard solution onto the IonPac CS 5 column operated at previously obtained conditions. The concentrations of the mixture standard solution were in the range from 0.2-400.0 ng/µl. The results are shown in **Table 3.49** and the linearity curve is shown in **Figure 3.26**. One example of the obtained chromatograms is shown in **Figure 3.27**.

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 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 (0.002) and the detection sensitivity (1000) 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 2.1. The calculated results are given in **Table 3.50**. For example, noise level of Mn is shown in **Figure 3.28**. From the value of the detection limit (L) obtained, the minimum detectable quantity was calculated via Equation 2.2.

2.5.7 Reproducibility of Results

The standard cation mixture solution from Section 2.5.3 was replicately injected onto the IonPac CS 5 column with appropriate optimized conditions to observe reproducibity of the retention and peak area of each cation. The results of calculated relative standard deviation and % relative standard deviation are shown in **Table 3.51**.

2.5.8 Determination of heavy metal ions in drinking water samples by IC

The amounts of Pb^{2+} , Cu^{2+} , Mn^{2+} , Zn^{2+} and Ni^{2+} in drinking water were determined by the IC technique . 25 µl of drinking water sample solution from section 2.3.6 was duplicately injected onto the IonPac CS 5 column under the established conditions.

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 Pb^{2+} , Mn^{2+} , Zn^{2+} and Ni^{2+} are shown in **Tables 3.52-3.55** and calibration curves in **Figures 3.29-3.32**.

2.5.8.2 Analysis of metal ions in drinking water samples

Pb^{2+}, Mn^{2+} , Zn^{2+} and Ni^{2+} were found in both types of drinking water but Cu^{2+} was not found in any of the water samples analysed. The results of the analysis are shown in **Tables 3.56 and 3.57** and typical chromatogram of some drinking water sample in shown in **Figure 3.33**.

2.5.9 % Recovery of each metal ion in drinking water samples

The chromatographic peaks produced from drinking water samples were qualitatively confirmed using the spike method. The results and the chromatogram of % recovery of each metal ion are shown in **Tables 3.58-3.61** and **Figure 3.34**.

2.5.10 Analysis of metal ions in drinking water samples by atomic absorption spectrophotometric method

The amounts of Pb^{2+} , Mn^{2+} , Zn^{2+} and Ni^{2+} in drinking water samples were determined by the AAS technique using the so-called heating method and external standard method.

2.5.10.1 Atomic absorption experiment [22]

When performing a hot acid digestion, regardless of the matrix, there are several methods of heating the sample. Each one has its own advantages and can be used in different situations.

For drinking water samples, one typically starts with 100 ml of aqueous solution in a beaker and adds 3 ml of nitric acid. This solution is put on the hot plate and heated to just below its boiling point (about 85 to 95 °C) with gentle refluxing along the sides of the beaker. The sample volume is reduced by a specified amount, the beaker is cooled slightly and 2 ml of concentrated hydrochloric acid is added. The addition of the HCl will dissolve most of any precipitate that has formed with the exception of silicates. The digestate is taken up to the original volume 100 ml and analyzed directly. The amounts of metal ions determined from a calibration curve of each standard cation, the results of each cation in drinking water sample by the IC technique and the AAS technique were compared using the t-test (Equation 2.6) and the resultant results are shown in **Tables 3.62-3.65**.