4. DISCUSSION AND CONCLUSIONS

Ion chromatography is regarded as a versatile analytical technique for separating and quantifying ions. Ion chromatography has been successfully applied to the analysis of ions in many extremely diverse types of sample. Determination of ions in difficult sample matrices such as toothpastes, brines and caustics and black liquors has become common ion chromatography practice. In this research, ion chromatography was employed to determine anions and cations (transition metals) in geological samples. The geological samples of interest were hot spring water, rain water, river water, waterfall, coal and reference materials but emphasis in this research was on geological water samples. For solid geological samples, the work attempted here could be regarded as preliminary development.

4.1 Discussion

Two ion chromatographic systems employed were the suppressed ion chromatography of anions with conductivity detection and the post-column derivatization system with a UV-vis detector. For ion-exchange processes in ion chromatography, they are based upon exchange equilibrium between ions in solution and ions of like charge on the surface of an essentially insoluble, high-molecular-weight solid. Natural ion exchangers, such as clays and zeolites, have been recognized and used for several decades. The most common active sites for cation exchange-resins are the sulphonic acid group $\_SO_3^-H^+$, a strong acid, and the carboxylic acid group $\_COO^-H^+$, a weak acid. Anionic exchangers contain quaternary amine groups $\_N(CH_3)_3^+OH^-$ or primary amine groups $\_NH_3^+OH^-$; the former is a strong base and the latter a weak one.

When a sulphonic acid ion exchanger is brought in contact with an aqueous solvent containing a cation M^{x+}, an exchange equilibrium is set up that can be described by :

$$\underset{\text{solid}}{xRSO_3^-H^+} + \underset{\text{solution}}{M^{x+}} \rightleftharpoons \underset{\text{solid}}{(RSO_3^-)_xM^{x+}} + \underset{\text{solution}}{xH^+}$$

where $RSO_3^-H^+$ represents one of many sulphonic acid groups attached to a large polymer molecule. Similarly a strong base exchanger interacts with the anion A^{x-} as shown by the reaction

$$\underset{\text{solid}}{xRN(CH_3)_3^+OH^-} + \underset{\text{solution}}{A^{x-}} \rightleftharpoons \underset{\text{solid}}{[RN(CH_3)_3^+]_xA^{x-}} + \underset{\text{solution}}{xOH^-}$$

The equilibrium constant, K_{ex} for the exchange reaction shown in **Equation 4.1 and 4.2** take the form

Cation exchange resins $$\frac{[(RSO_3^-)M^{x+}]_s[H^+]_{aq}}{[(RSO_3^-)H^+]_s[M^{x+}]_{aq}} = K_{ex} \quad \text{.....(4.1)}$$

Anion exchange resins $$\frac{[RN(CH_3)^{3+}A^{x-}]_s[OH^-]_{aq}}{[RN(CH_3)_3^+OH^-]_s[A^{x-}]_{aq}} = K_{ex} \quad \text{.....(4.2)}$$

where K_{ex} represents the affinity of the resins for the ion M^{x+} or A^{x-} relative to another ion. Where K_{ex} is large, a strong tendency exists for the solid phase to retain M^{x+} or A^{x-}; where $K_{ex}$is small, the reverse is obtained. By selecting a common reference ion such as H^+, distribution ratios for different ions on a given type of resin can be

experimentally compared. Such experiments reveal that polyvalent ions are much more strongly held than singly charged species. Within a given charge group, however, differences appear that are related to the size of the hydrated ion as well as to other properties. This sequence is somewhat dependent upon the type of resin and reaction conditions and should thus be considered only approximate [34].

For the suppressed ion chromatography of anions in this work, the column used was an IonPac AS4A column of 4 x 250 mm dimension. IonPac AS4A analytical column is composed of 16 micron polystyrene/ divinylbenzene substrate agglomerated with anion exchange latex that has been completely aminated. The 0.5 % crosslinked latex particles have a diameter of approximately 0.175 μm and carry the ion exchange sites. The ion exchange capacity of this column is 20 meq/ column and stable between pH 0 and 14. The latex particles are strongly held to the substrate surface by electrostatic and Van der Waals interactions. The column used with a Dionex Anion Self-Regenerating Suppressor (ASRS-I) requires a constant water feed through the regenerant chambers to achieve suppression. Water can be delivered to the suppressor regenerant chamber from recycled eluent in the Auto-suppression Recycle Mode of operation equipped with conductivity detector [8].

The factors which affect the separation efficiency and sensitivity were studied to obtain optimized analysis conditions. These factors are listed as follows.

- Type of eluent system
- Effect of eluent concentrations
- Effect of eluent flow rate

Type of eluent system

F^-, Cl^-, NO_2^-, Br^-, NO_3^-, PO_4^{3-} and SO_4^{2-} were anions of interest here, so sodium carbonate-sodium bicarbonate was used as the eluent. Because of its compatibility with a membrane-based suppressor system, which is essential for the sensitive conductometric detection of sample analytes [35], such eluent system can be used to elute standard inorganic anions rapidly and efficiently under isocratic conditions. Carbonate-bicarbonate also controls the selectivity of the analysis, by changing the CO_3^{2-}/ HCO_3^- ratio. The suppressor reaction product is carbonic acid, H_2CO_3, which results in a low background conductivity (15-17 μs off set in range).

Effect of eluent concentrations

Results on the effect of eluent concentrations on retention time, peak area and resolution are shown in **Figure 3.1, Tables 3.2 and 3.3**. It was found that the retention times of all ions decreased with increasing Na_2CO_3/ $NaHCO_3$ concentration as eluent. Peak areas of monovalent ions also tended to decrease with increasing Na_2CO_3/ $NaHCO_3$ concentration while those of divalent and trivalent ions tended to increase with increasing Na_2CO_3/ $NaHCO_3$ concentration, as shown in **Table 3.2. Table 3.3** shows that separation efficiency decreases with increasing Na_2CO_3/ $NaHCO_3$ concentration. It should be noted that the resolution for each anion pair of the same charge did not change much whereas the resolution for each anion pair of unlike charge changed downwards with increasing Na_2CO_3/ $NaHCO_3$ concentration, especially that for the anion pair of NO_3^- and PO_4^{3-}. The optimum Na_2CO_3/ $NaHCO_3$ concentration was considered to be 1.80 mM Na_2CO_3/ 1.70 mM

$NaHCO_3$ with the analysis time less than 8 minutes. Peak areas of anions were sufficiently high and the resolution for each anion pair was clearly abundant as the resolution value was greater than 1.5 for every pair of peaks. With 1.80 mM Na_2CO_3/ 1.70 mM $NaHCO_3$ as eluent, the fluoride peak was not affected by either positive or negative signal, as shown in **Figure 3.5**. A change in the chemical composition of the eluent (sodium carbonate/ sodium bicarbonate ratio) is proportional to the capacity factor, k′[36]. The capacity factor can be calculated from **Equation 4.3**:

$$k' = \frac{t_s - t_m}{t_m} \qquad \text{..... (4.3)}$$

where t_s is the solute retention time and t_m the column dead time. The capacity factor, k' is, according to **Equation 4.4**, proportional to the column capacity C and inversely proportional to the concentration of the eluent E.

$$\log k' = \log C - \log E + \text{constant} \qquad \text{..... (4.4)}$$

If it is assumed that the capacity of the column remains constant, then a significant change in chemical composition of the eluent should occur when a sample containing an increased concentration of fluoride is injected. The change in the chemical composition of the eluent may be explained by column back-conversion from the fluoride to the carbonate/ bicarbonate form. Owing to its stronger affinity, the carbonate/ bicarbonate eluent replaced completely retained fluoride from the stationary phase. The sample plug in the separation column is therefore followed by a modified eluent which is very poor in carbonate/ bicarbonate but enriched with fluoride.

Effect of the eluent folw rate

The effects of the eluent flow rate on retention time, peak area, the number of theoretical plates and resolution are presented in **Figures 3.2 and 3.3** and **Tables 3.4 and 3.5**. It was found that all retention times, peak areas, the number of theoretical plates and resolutions decreased with increasing eluent flow rate. The best result in terms of total analysis time, sensitivity, column efficiency and separation factor was obtained at the eluent flow rate of 2.0 ml/ min. Optimized IC conditions giving high sensitivity and good resolution of analysis are summarized in **Table 3.6**.

From **Table 3.1** which showed the retention times of the investigated ions, it was found that the elution sequence was F^-, Cl^-, NO_2^-, Br^-, NO_3^-, PO_4^{3-} and SO_4^{2-}. The order of separation can be simply explained via the affinity of the resin for various ions. In general, the affinity of an ion exchanger for an ion increases with the charge on the ion and with increasing atomic weight of the ion. High solvated size of the solution ion is another factor which makes ions retained for a short time [44].

Repeatability and reproducibility

The repeatability and reproducibility of the retention time and peak area were investigated by performing five and six replicate injections, respectively. The results are given in **Tables 3.7 and 3.8**. The results were found to be satisfactory with %RSD value for the retention times of anions between 0.23–1.11 (for repeatability) and 0.93–2.48 (for reproducibility). As

for the values of %RSD for the peak areas, they were found to be between 0.81–5.14 (for repeatability) and 0.93–2.62 (for reproducibility).

Linearity

The linearity range for each anion was determined in the range 0.2-300 ng/µl except for fluoride and phosphate ions it was determined in the ranges 0.2-100 ng/µl and 1.2-600 ng/µl, respectively. The results are presented in **Table 3.9**, with the linearity curve shown in **Figure 3.4** and the relevant chromatogram shown in **Figure 3.5**. The linearity ranges of all anions investigated together with the corresponding correlation coefficients are listed in **Table 4.1**.

Table 4.1 Analytical characteristics of anions obtained with IonPac AS4A column under the established conditions.

Ion	Linearity range ng/µl	Correlation Coefficient	Sensitivity (peak area)(1x 10^5ng/µl)
F^-	0.2-100	0.9998	381
Cl^-	0.2-300	0.9995	934
NO_2^-	0.2-300	0.9991	410
Br^-	0.2-300	0.9990	356
NO_3^-	0.2-300	0.9993	430
PO_4^{3-}	1.2-600	0.9992	293
SO_4^{2-}	0.2-300	0.9984	534

All of the linearity ranges of anions given in **Table 4.1** are seen to cover wide concentration ranges. The regression constants (r^2) were > 0.9980

for all seven solutes. This reflects the fact that ion chromatography is one of the most widely used techniques for inorganic anion analyses in natural water and waste water samples.

Detection limit and minimum detectable quantity

The results of the investigation on the detection limit and minimum detectable quantity were determined by injection of the chosen test solution onto the IonPac AS4A column to produce a chromatogram with a peak height at least twice the noise level. The obtained chromatogram is shown in **Figure 3.6**. The detection limit and minimum detectable quantity were calculated via **Equations 2.1 and 2.2**, as described under Section 2.3.5. The calculated results presented in Table 3.9 indicate that the detection limits of F^-, Cl^-, NO_2^-, Br^-, NO_3^-, PO_4^{3-} and SO_4^{2-} under the investigated conditions were 0.03, 0.05, 0.62, 0.21, 0.16, 0.20 and 0.06 ng, respectively, whereas the minimum detectable quantities were 0.002, 0.003, 0.025, 0.016, 0.016, 0.046 and 0.007 ng.sec, respectively.

Determination of anions in geological samples

The obtained conditions were applied to the determination of anions in geological water samples and coal samples. Anions found in both river water samples and rain water samples were F^-, Cl^-, NO_3^- and SO_4^{2-}. But in hot spring water samples, only F^-, Cl^- and SO_4^{2-} were found. For coal samples, the anions found were seen to be dependent on the method of sample preparation.

For quantitative purposes, the calibration curve of each anion was constructed by plotting the peak area against the concentration injected. The calibration data are presented in **Tables 3.11-3.14**. The calibration curves with correlation coefficient, r^2 = 0.99818-0.99992, are presented in **Figures 3.7-3.10**. The results for the application of the method to the analysis of anions in geological samples are summarized in **Tables 3.15 and 3.16** and chromatograms of some geological samples are shown in **Figures 3.11-3.16**.

As for the preparation of coal samples, 4 methods were used.

Method #1 Extraction with Dimethyl sulphoxide (DMSO) [15]

This method was used in extracting F^-, Cl^-, NO_3^- and SO_4^{2-} from coal samples. Dimethyl sulphoxide which is well suited to the dissolution of inorganic salts, has been demonstrated to be a good solvent for bituminous and sub-bituminous coals [15]. The efficiency of the extraction can be improved by increasing the temperature. The results are given in **Table 3.17** and **Figure 3.17**. Extraction efficiency appeared to be increased with an increase in the DMSO/ KNO_3 ratio. But using pure DMSO in the solvent extraction, the efficiency of the extraction could be decreased by 12 percent. When magnetic stirring is used, the deletion of 0.1 M KNO_3 from the DMSO caused the extraction efficiency to decrease because the appearance of chemically and/or physically released different non-chloride salts from the coal to cause a displacement of the fraction of Cl^- bound by ion exchange which ion-exchanged chloride that is displaced by NO_3^- [15]. The difference between the amount of anions (especially Cl^-) leached into KNO_3 containing DMSO and into the DMSO alone provided an estimate of the fraction of anions that are held to the coal matrix by an

ion-exchange mechanism. The results are presented in **Table 3.18** and **Figure 3.18.**

Methods #2 and 3 involved fusion with $Na_2O_2$and fusion with Na_2CO_3.

Both methods are based on fusion with alkali salts. In general, the effectiveness of a flux in attacking geological materials increases in the order $Na_2CO_3 < NaOH < Na_2O_2$ [33]. In fusion with Na_2O_2, various forms of sulphur in the samples are converted into sulphate with less than a 10 percent loss [16]. The dilution effect caused the oxidation to change total sulphate and a sample to flux ratio, 0.15g/1.0 g was determined to be optimum for this analysis. Using flux greater than 1.0 g in 50 ml caused the ion peak shapes to be asymmetrical (probably caused by overloading the column)[16]. The slope of the calibration curve of peak area versus concentration remained constant throughout the analyses with a linear correlation coefficient of 0.9999. This method was used to extract NO_3^- and SO_4^{2-} in samples.

For carbonate fusion, only SO_4^{2-} can be extracted. This is due to the fact that carbonate fusion induces carbonate level in the eluent (carbonate-bicarbonate), by assuming that it caused another anion before any elution because the output range was set at 30 μS. Combustion by furnace and that by Bunsen were not significantly different in the determination of sulfate in samples. The results are presented in **Table 3.19** and **Figure 3.19.**

Method #4 is simply a leaching method by water [15]. The efficiency of the leaching can be improved by increasing the temperature. The results are presented in **Tables 3.20-3.23.** As for the leaching time,

a period of 4 hours was found to be optimal. The leaching time longer than this would gave low yield due to the non- homogeneity of the samples. The total variance measured from the sum of the variances resulting from the analytical method used, the non-homogeneity of the samples and other random errors are related as shown in the following relationship [38]. The results are presented in **Tables 3.24-3.26**.

$$(S.D._{tot})^2 = (S.D._{method})^2 + (S.D._{heterog.})^2 + (S.D._{random})^2$$

The results from the analysis of the samples were obtained from replicate analyses by the spike method in terms of % recovery. The results of % recovery of anions found in geological samples are shown in **Tables 3.27–3.30**. The values of % recovery of anions are as follows : 97.7 for F^-, 99.7 for Cl^-, 98.7 for NO_3^- and 98.3 for SO_4^{2-}. Confirmation of peaks of anions of interest in geological samples was made by comparison of chromatograms of samples with those samples spiked with each anion. It was found that peaks obtained from samples spiked with anions were taller than that those from the unspiked samples, as shown in **Figures 3.20-3.22**. It can be seen that all results comfirm that the peaks in the samples were F^-, Cl^-, NO_3^- and SO_4^{2-}, respectively.

In order to verify quantitative accuracy, the amounts of F^-, Cl^-, NO_3^- and SO_4^{2-} were also determined by the spectroanalytical method. In order to decide whether there was a significant difference between the results obtained by the IC technique and the spectroanalytical technique, **the t-test** was conducted for comparison.

The tabulated t value (t_{table}) for three degrees of freedom at 95% confidence level is 3.18. **Tables 3.31–3.34** illustrate the results from the comparison of F^-, Cl^-, NO_3^- and SO_4^{2-} determinations obtained by the two techniques. For F^-, Cl^- and NO_3^-, it was found that the calculated t values were smaller than the tabulated ones. But in the case of SO_4^{2-}, the calculated t values were higher than the tabulated t values. The results of F^-, Cl^-, NO_3^- and SO_4^{2-} analysis by the two techniques suggest that the IC technique would be superior as it possesses higher sensitivity than the spectroanalytical techniques.

The amounts of F^- and Cl^- were also determined by ISE method as well as by IC. The results are shown in **Tables 3.35–3.36.** But the amounts of F^- in this analysis by ISE were found to be lower than those obtained by the IC technique, because analyses were performed at different time. Adsorption effects of F^- on the surface of plastic bottles might have been attributed to the lost of F^-, as a result of the prolonged storage of water samples [39].

Under ion chromatographic conditions, fluoride always elutes in the range of the water dip, making determination at concentration levels of less than 0.1 ng/μl difficult, if not impossible, owing to interference from the negative water dip [40]. The water dip occurs when the injected water passes the conductivity cell, decreasing the background conductivity of carbonic acid formed in the suppressor by exchanging the eluent cations with hydronium ions. There have been numerous attempts to circumvent this problem by sample pretreatment, by changing the eluent conditions or by tailoring the stationary phase design. An easy way to compensate for the negative dip is to add carbonate to the sample, matching the carbonate concentration in the mobile phase, thus making the negative dip invisible. However, this approach does not work with real samples such as rain water samples. If the carbonate

concentration in the sample is higher than the total carbonate concentration in the mobile phase, a positive signal that is almost indistinguishable from the fluoride peak is obtained within the void volume of the separator column. Diluting the sample with deionized water does not solve this problem because even small amounts of carbonate in the sample lead to a significant decrease in the fluoride peak height [41]. Another problem for the verification of fluoride, especially in environmental samples, is monocarboxylic acids. As many of these acids co-elute with or are only partly resolved from fluoride, interpretation of the signals near the void volume is extremely difficult. From results, the peak at 1.0 min which appears to be F^-, is actually F^- plus some organic anions (probably formate plus acetate, propionate, butyrate and lactate, but possibly also some others) which co-elute using the carbonate-bicarbonate eluent [20].

The exact determination of fluoride is possible if the advantage of the simultaneous determination of other mineral acids is eliminated and if the chromatographic conditions are changed so that fluoride is separated from the carbonate-bicarbonate travelling with the mobile phase. An increase in fluoride retention can be achieved by using an eluent of lower eluting power such as sodium tetraborate. This method has only limited applicability because sulfate has a much longer retention time under these conditions, interfering with subsequent analyses [40]. The chemical complexity of these waters can be illustrated using a weaker (borate) eluent. For example, using a gradient elution ranging from 7 to 21 mM $Na_2B_4O_7$, as many as 30 anionic species were found in a chromatogram of well water [20]. Approximately 10 peaks are seen before Cl^- eluents and another 20 peaks before SO_4^{2-}. Except for the

inorganic anions plus glycolate and formate, most of these peaks have not yet been identified.

For changing retention time during the analyses of geological samples, the retention times of the anions typically decreased slightly with time, compared with these of the standards. This may be due to the presence of dissolved organic material in the geological samples adsorbing on the resin of the separation column and slightly changing its anion retention properties. In order to confirm the retention times of the anions, a blind standard (SO_4^{2-} and PO_4^{3-} standard) was injected in triplicate after every tenth sample injection.

In sample preservation, the measured concentrations of NO_2^- are not directly affected by sample oxidation. But NO_2^- is sensitive to oxidation, however, the water sample device would have had to be collected and subsampled under an inert atmosphere such as nitrogen and filtered under nitrogen. These capabilities were not available at the time of sample collection and preparation. Thus, by the time the samples had been filtered there may have been considerable oxidation of nitrite.

Although the analytical method described here is suitable for the quantitative measurement of these species, improvements are required in sample collection, preparation and preservation against oxidation.

The second system employed in this work was the post-column derivatization system. The column used was an IonPac CS5 column, with the following characteristics: 13 μm of particle diameter, 2% substrate cross-linking, 110 nm of latex diameter, 2% latex cross-linking, 150 μeq of sulphonic acid, 70 μeq alkanol quanternary ammonium functionalities and low hydrophobicity [57]. The most common factors of on-line chromatographic detection of transition metals is post-column derivatization using a metallochromic indicator with subsequent absorbance detection. The post-column reagent in the operation of this system was forced to diffuse under helium gas pressure into the column effluent through a permeable fiber membrane to yield complexes which could be detected by a UV/vis detector. Metals of interest were chosen to cover those of the main components of geological water samples, i.e., Fe^{3+}, Cu^{2+}, Zn^{2+}, Co^{2+} and Mn^{2+}. Since the post-column derivatization detection system was used, it was necessary to find a suitable color-forming reagent for complexation with the studied metals. The non-specific metallochromic indicator 4-(2-pyridylazo)resorcinol (PAR) was used in this research because the main requirements were complex stability and a high molar absorptivity for a range of metal complexes of interest. The latter consideration reflects the popularity of spectrophometric detection. In this role, kinetic stability or charge on the complex are not important. But these are based on thermodynamic stabilities and optical properties are not important. Furthermore, the stability of the complexes must be such that $K_{s\ (complexes)} << K_{s\ (Post\text{-}column\ derivatized\ complexes)}$ in order to achieve post-column derivatization and spectrophotometric detection [42]. The PAR-metal complexes show relatively high molar absorptivities (>20000) in the 500-540 nm range while the reagent itself shows relatively low background absorbance at the monitoring wavelengths[25]. Preparation of PAR reagent used could be done by 2 methods, both giving the same results. So in this work, method #2 was selected, because it is

a convenient method and it does not require an addition of dimethylaminoethanol (DMAE) which has a strong smell.

The choice of the most suitable eluent in this work was based on an investigation of two eluents, namely oxalic acid and pyridine 2-(6-dicarboxylic acid), PDCA solutions. Although both of them were compatible with the IonPac CS5 column, it was found that oxalic acid was a better choice. As shown in **Table 3.38**, retention times of the metal ions obtained with oxalic acid eluent spread over a wider range than those obtained with the PDCA, suggesting better separation. But with the oxalic acid eluent system, Fe^{3+} could not be readily eluted. Thus PDCA eluent was used in this work.

For preparation of PDCA eluent, 2 methods were compatible with determination of metal ions of interest. It was found that method #1 was a better choice. As shown in **Table 3.39**, retention times of the metal ions obtained with the method #1 were shorter than those obtained with the method #2.

The factors which affect the separation efficiency and sensitivity were studied of obtain optimized analysis conditions. These factors are listed as follows :

- Effect of eluent concentration
- Effect of eluent pH
- Effect of eluent flow rate
- Optimum detection wavelength
- Effect of PAR concentration
- Effect of PAR pH
- Effect of PAR flow rate

Effects of eluent concentraion

The optimum eluent concentration was found to be 7.5 mM PDCA. The dependence of retention times, peak areas and resolution on the concentration of eluent could be seen in **Table 3.40** and **Figures 3.24 and 3.25**. It can be seen that when 7.5 mM PDCA was used as eluent, the highest analysis sensitivity was obtained and resolution of each metal ion pair was well within an acceptable value (1.5). Also, the analysis time of 6.73 minutes for 5 metal ions can be regarded as satisfactory.

Effects of eluent pH

Often the pH will determine the predominant change on the elution. A di-ions in the eluent will be taken up more strongly by the resin than a mono-ion and will therefore have a greater eluting power [44]. Also, the eluent pH will determine the charge of sample ion. Different ion charges of the sample will move at different rates through the column for the same eluent concentration. Post-column reagent conditions were selected such that the resultant pH of the combined column eluent PDCA solution was greater than pH 4.0. An increase in the eluent pH was found to have decreased the retention time of the analytes. In general, the concentration or pH of PDCA would shorten the retention time of the transition metal ions. When pH of PDCA was lower than 4.0, it was found that manganese was not determined. However, when the pH was above 6, the shape for Fe^{3+} became asymmetric because the Fe^{3+} may produce insoluble hydroxide in the column and the elution order for Fe^{3+} and Cu^{2+} was reversed [26]. Part of Fe^{3+} may produce insoluble hydroxide in the column. Assuming this to be and in considering the

behaviour of other cations, optimum concentration and pH of PDCA chosen were described under analytical procedure.

Table 3.41 showed the effect of eluent pH on resolution of each metal ion pair at various eluent pH values between 3.7-4.6 and the retention times of the metal ions at various eluent pH are given in **Figure 3.26** . It was found that eluent pH at 4.1 yielded the shortest analysis time and the resolution for any of the metal ion pair was more than 1.5, indicating that separation between components was adequate [11], and there would be no use to employ other eluent pH values with higher resolution.

Effect of eluent flow rate

The dependences of the resolution, peak area and the number of the theoretical plates on the eluent flow rate were considered. From **Figures 3.28 and 3.29**, these can be seen that each metal ion peak area and retention time decreased regularly with increasing eluent flow rate. The numbers of the theoretical plates also tended to decrease when the eluent flow rate was increased, as shown in **Table 3.42** and **Table 3.43** which show that all resolution values are acceptable over the range of the employed flow rates. In order to obtain high sensitivity and high column efficiency with reasonable analysis time, the eluent flow rate at 1.0 ml/ min was chosen.

Optimum detection wavelength

Spectrophotometers are responsive only to those materials that absorb in the UV or visible range of the spectrum. Therefore, the wavelength of the UV/vis detector used could affect the peak area of each metal ion. Peak areas

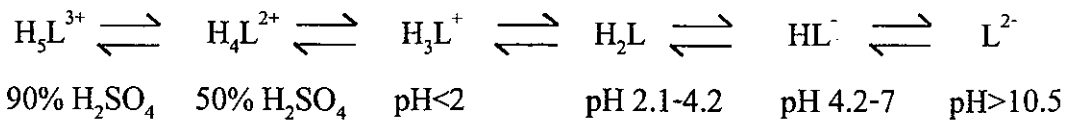
of the metal ions were plotted against the wavelengths, as shown in **Figure 3.30**. It could be seen from this figure that the optimum detection wavelength was 510 nm. But other experimental factors that affect the signal-to-noise ratio should also be optimized for maximum sensitivity, accuracy, and reproducibility. Controlling these factors was important because the magnitude of the analyte signal approaches the magnitude of the noise when analyzing at trace levels. For **Figure 3.31**, the top chromatogram (a) yielded a signal-to-noise ratio (S/N) for Fe^{3+}, Cu^{2+}, Zn^{2+}, Co^{2+} and Mn^{2+} as 66.2, 67.0, 22.4, 116.3, and 21.9, respectively. For the bottom chromatogram (b) the S/N values were 125.2, 66.9, 21.4, 155.8 and 21.7, respectively. Signals become more difficult to quantify as S/N decreases [34]. Thus, the optimum detection wavelength chosen in this work was 530 nm.

Effect of PAR concentration

The determination of transition metal ions was based on PAR concentration. **Figure 3.32** shows the peak areas of each metal ion at various PAR concentrations. It was found that PAR concentration at 0.36 mM was optimal PAR concentration, as this concentration yielded the highest peak area.

Effect of PAR pH

Depending on the acidity, PAR can exist as six species in solution[45].



The absorption maxima of the six forms, H_5L^{3+} to L^{2-}, lie at 433, 390, 395, 385, 413 and 490 nm. In weakly acidic or basic solutions the reagent has an orange color. Absorptiometric determinations of most metals are carried out in weakly acidic or weakly basic solution (**Table C-1** in **Appendix C**). **Figure 3.33** shows the peak area of each metal ion at various PAR pH values between 9.0-9.9. It was found that PAR at pH 9.70 yielded the highest peak area of each metal ion, indicating that pH 9.7 was optimal PAR pH.

Effect of PAR flow rate

The resolution, retention time and the number of theoretical plates in this work were based on an analytical column. So, after sample components had been eluted from the analytical column, PAR flow rate affected only the peak area of each metal ion. **Figure 3.34** shows the peak area of each metal ion against the PAR flow rate which indicated that each metal ion peak area decreased regularly with increasing PAR flow rate. Thus, the PAR flow rate at 0.5 ml/min was chosen.

For the purpose of convenience, the optimum conditions obtained with the IonPac CS5 column for the analysis of metal ions in this work are summarized in **Table 3.45**.

The elution behavior of the ions of interest, i.e., Fe^{3+}, Cu^{2+}, Zn^{2+}, Co^{2+} and Mn^{2+} was studied using 7.5 mM PDCA pH 4.1 as eluent at the flow rate 1.0 ml/min and 0.36 mM PAR pH 9.7 at the flow rate 0.5 ml/min as post-column reagent and λ_{max} at 530 nm. These ions were chosen by a criterion that they were likely to be contaminants in geological samples and some are known

to be the main components of geological reference materials. **Table 3.37** shows the retention times of the investigated ions. It was found that the elution sequence was Fe^{3+}, Cu^{2+}, Zn^{2+}, Co^{2+} and Mn^{2+}, respectively. The order of separation can be simply explained via the affinity of the resin for various ions as in the case of anions analysis. In general, ion exchangers favor the binding of ions of higher charge, decreased hydrated radius and increased polarizability [46] because interaction in ion-exchange systems is mainly ionic. In the chelates formed by PAR, the H of the ortho OH group is replaced by an equivalent of metal, with bonding of the metal to pyridine N and azo N (two five-membered rings). Charged (+ or -) as well as uncharged complex species can be formed, and the composition can vary with the concentrations of HL^- and L^{2-}, that is, with the pH. The reaction ratios of metal and PAR may be known, but the composition of the product is not always clear.

Evidence has been presented to show that Ni combines with PAR in the ratio 1:2 in both acidic and basic solutions, but different species are present. In slightly acidic solutions (pH 3.3), $Ni(HL)_2$ is present, having a red color ($\in_{520}$=37200). When the solution is made basic, the color changes to orange ($\in_{496}$=79400, pH 8). The color change is attributed to the formation of NiL_2^{2-}. Cobalt forms $Co(HL)_2$ in acidic solution, and the monoprotonated species $Co(HL)L^-$ in basic solution. Some metals form complexes whose M:PAR ratio depends on the pH (see **Appendix C**) [45].

Repeatability and reproducibility

The results of repeatability and reproducibility indicating the precision of analysis are presented in **Tables 3.46 and 3.47**. It was found that the

relative standard deviations (% RSD) of the metal ion retention times were in the range 0-0.34 (for repeatability) and 0.21-0.43 (for reproducibility). Those of peak area measurements were in the range 0.60-2.56 and 0.29-4.76, respectively.

Linearity

The linearity ranges for the metal ions were examined and the results are shown in **Table 3.48** and the linearity curve is shown in the **Figure 3.35**. The chromatogram of Fe^{3+}, Cu^{2+}, Zn^{2+}, Co^{2+} and Mn^{2+} obtained with IonPac CS5 column is shown in **Figure 3.37.** Analytical characteristics of the metal ions studied which are based on the data derived from **Figure 3.35** are presented in **Table 4.2.**

Table 4.2 Analytical characteristics of metal ions studied obtained with IonPac CS5 column.

Metal ion	Linearity range (ng/μl)	Correlation coefficient	Sensitivity (peak area)(x 10^5 ng/μl)
Fe^{3+}	0.08-8.0	0.998591	138.72
Cu^{2+}	0.08-8.0	0.999765	85.44
Zn^{2+}	0.08-8.0	0.999513	22.40
Co^{2+}	0.08-8.0	0.999769	212.08
Mn^{2+}	0.08-8.0	0.999447	29.36

The linearity ranges of metal ion given in **Table 4.2** are seen to cover the narrow concentration range 0.08-8.0 ng/μl, because the analytical column used in this work could not tolerate a higher loading as it has been used for

The linearity ranges of metal ion given in **Table 4.2** are seen to cover the narrow concentration range 0.08-8.0 ng/μl, because the analytical column used in this work could not tolerate a higher loading as it has been used for quite some time. Generally speaking, a new column gives a better separation of metal ions than an aged column [47]. Evidence for this problem can be seen in **Figure 3.23**.

Another problem could be attributed to a guard column. In this work, a guard column which would provide an effective and vital function in protecting the separator column was not employed. Soluble organic compounds or other soluble components of the sample, such as trace metals, present in samples collected for environmental studies gradually accumulate on the guard column [48]. When there was no guard column, this deterioration was in the separator column instead, producing loss in separation efficiency in the system. Both afore-mentioned problems appeared to have decreased the retention times of metal ions. It should be noted here that, due to some technical difficulties, linearity cut-off values were not obtained in this work.

Detection limit and minimum detectable quantity.

The results of the investigation on the detection limit, defined as the weight of substance giving a signal twice the standard deviation of the noise level, and the minimum detectable quantity, defined as the amount of sample producing a peak signal two times the noise of metal ions. The obtained chromatogram is shown in **Figure 3.49**. The calculated results are presented in **Table 3.36**. It was found that the detection limits of Fe^{3+}, Cu^{2+}, Zn^{2+}, Co^{2+} and Mn^{2+} under the investigated condition were 0.008, 0.010, 0.025, 0.004, and

After the analytical characteristics such as linearity range, detection limit, minimum detectable quantity, repeatability and reproducibility had been investigated and found that their values were in an acceptable level, the obtained conditions were then employed to determine metals in geological water samples and geological reference materials.

Determination of metal ions in geological samples.

In geological water samples in this work, only small amounts of metal ions were found. For geological reference materials with all methods of preparation, each of the samples gave the same result; the main metal ion found was only Fe^{3+}. Concentrations of analyte ions were determined from the calibration curves in **Tables 3.50-3.54**. Calibration curves of these metal ions are shown in **Figures 3.38-3.42**. It can be seen that the concentrations of the metal ions in geological water samples lie between the following ranges : 0.001-0.005 ng/μl for Fe^{3+}, 0.0004-0.001 ng/μl for Cu^{2+}, 0.001-0.002 ng/μl for Zn^{2+}, 0.008 ng/μl for Co^{2+} and 0.003 ng/μl for Mn^{2+}, respectively. Chromatograms of some geological water samples and geological reference material are shown in **Figures 3.43-3.50**.

Quantitative confirmation of metal ions in geological water samples was done by the AAS technique and ICP technique using the external standard method. In order to decide whether there was a significant difference between the results obtained by the IC technique and the AAS or the IC technique and the ICP technique, **the t-test** was conducted for comparison. A t-test for a situation where two similar treatments on a sample need to be compared can be accomplished if the treatments on a sample need to be compared can be accomplished if the treatments are accomplished on the same

sample. This statistical test is primarily used for situations such as analysis of the same sample by two methods.

The tabulated t value (t_{table}) for four degrees of freedom at 95% confidence level is 2.776. **Table 3.64** illustrate the results of comparison of Zn^{2+} obtained by the two techniques. It was found that the calculated t values were smaller than the tabulated t values. So there was no significant difference in the results of Zn^{2+} analysis by the two techniques. For the case of the other metal ions, the calculated t values could not be calculated because they were found in a few samples and there were differences in the sample composition at different time intervals.

During the metal ion analysis, tailing of the Fe^{3+} peak was quite obvious. In general, the oxidation of Fe^{2+} ion which is in the reference materials causes the column contaminated with Fe^{3+} for the next injections. The poisoned column would show tailing of the Fe^{3+} peak and a discrete Fe^{2+} peak. The Fe^{2+} peak reflects reduction of Fe^{3+} at the beginning of the column. Tailings show deterioration of the retention behavior for Fe^{3+} [37]. This causes an error in the determination of other metal ions in geological reference materials or in the sample containing a large amount of Fe. Regarding this problem, ion exchanger resin (e.g. Dowex 1) should be used in the preparation of samples [49] for separating Fe from other metal ions in the samples.

4.2 Conclusions

In this research, anions and metal ions in geological samples, especially geological water samples, were determined using two systems of ion chromatographic technique, namely, suppressor anion chromatography and the post-column derivatization. The chemical composition of geological water is generally derived

from many different sources of solutes, including gases and aerosols from the atmosphere, weathering and erosion of soil and rocks, solution or precipitation reactions occurring below the land surface and cultural effect resulting from activities of man. Chemical analyses may be grouped and statistically evaluated by averaging frequency distributions, or ion correlations to summarize large volume of data. The investigation of optimum conditions for separation and analysis of anions was accomplished with the IonPac AS4A column with 1.80 mM Na_2CO_3/ 1.70 mM $NaHCO_3$ as eluent at the flow rate 2.0 ml/ min, employing a conductivity detector at output range 3 μS . The linearity ranges of F^-, Cl^-, NO_2^-, Br^-, NO_3^-, PO_4^{3-} and SO_4^{2-} were found to be 0.2-100, 0.2-300, 0.2-300, 0.2-300, 1.2-600 and 0.2-300 ng/μl, respectively. The detection limits of these anions were found to be 0.03,0.05, 0.62, 0.21, 0.16, 0.20 and 0.06 ng, respectively. The relative standard deviations of peak areas were found to be between 0.81-5.14 %. The results of anions in geological water samples were compared with those from the spectroanalytical technique, based on t-test. There was no significant difference in the results obtained from the two techniques except in the results for the sulfate ion.

In the post-column derivatization, an investigaton of optimum conditions for separation and analysis of transition metal ions as impurities in geological water samples and as the main constituents of metal ions in geological reference materials was accomplished with an IonPac CS5 column with 7.5 mM PDCA at 4.1 as eluent at flow rate 1.0 ml/ min and 0.36 mM PAR in 3.52 M NH_4OH/1.0 M CH_3COOH at pH 9.7, at flow rate 0.5 ml/min as the post-column reagent with a UV/vis detector at 530 nm. The linearity ranges for Fe^{3+}, Cu^{2+}, Zn^{2+}, Co^{2+} and Mn^{2+} were found to be 0.08-8.0, 0.08-8.0, 0.08-8.0, 0.08-8.0 and 0.08-8.0 ng/μl, respectively. The detection limits of these metals were found to be 0.008, 0.010, 0.025, 0.004 and 0.037 ng, respectively. The relative standard deviations of peak area were found to be between

0.58-4.76%. The results of metal ion analysis by IC technique were compared with those by the AAS and ICP techniques. Based on the t-test, there was not a statistical difference in the results of the metal ions by the two techniques. As for the post column reaction detection, it can be regarded as an alternate means of achieving enhanced sensitivity and selectivity, and in many ways the preferred means.

From the results obtained it can be concluded that ion chromatography is applicable to the determination of anions and cations in geological samples as it is a very sensitive and efficient analytical technique.