CHAPTER 1

INTRODUCTION

1.1 Chlorine-containing Anions

The presence of chlorine-containing anions in the water can be attributed to several pathways. One of many pathways is the use of inorganic salts of these species in the agro industry, paper industry, firework manufacture, matches manufacture and so on.[1] These species are circulated and remained in the environment. One pathway is the formation of chlorine-containing anions as disinfection by-product (DBP) in the water treatment process.[2] Chlorine gas (Cl_2) or hypochlorous acid (HOCl) solution as a disinfectant is added into the water in order to kill diseases via chlorination reaction. When the chlorine gas is dissolved in the water, it is rapidly converted to hypochlorous acid, as expressed in reaction 1.1.

$$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^- \quad .....(1.1)$$

$$HOCl \longleftrightarrow OCl^- + H^+ \quad .....(1.2)$$

At the equilibrium, hypochlorous acid dissociation depends upon pH, as in reaction 1.2. Organic acids, fulvic and humic acid, present in the water will react with hypochlorous acid to form a chlorinated organic disinfection by-products (DBPs), and hypochlorous acid also decomposes to form the chlorate species[3], as expressed in reaction 1.3.

$$3HOCl \rightarrow 3H^+ + 2Cl^- + ClO_3^- \quad .....(1.3)$$

In the treatment using chlorine dioxide (ClO_2) as a disinfectant, the organic materials remain in the water will be oxidized under the formation of the chlorite and chlorate species.[4] Ozonation of water is applied in the treatment process, the chlorine residual or chlorine dioxide residual is oxidized, which can then further react to result in the formation of the chlorate ion.

Chlorine-containing anions investigated in this research consisting of chloride, chlorite, chlorate and perchlorate ions. These species are stable under the ordinary condition except hypochlorite and chlorite ions.[5] Because both species are very reactive, chlorite can react with hypochlorite and disproportionate to chloride and chlorate ions, as in reaction 1.4.

$$ClO_2^- + ClO^- \rightarrow Cl^- + ClO_3^- \qquad \text{.....(1.4)}$$

The chlorite species can be degraded upon exposure to light. It has also been reported to react with metal[2] by forming an intermediate [$MClO_2^{2+}$] before yielding chlorine dioxide as in reaction 1.5.

$$ClO_2^- + M^{n+} \rightarrow [MClO_2^{2+}] \rightarrow ClO_2 + M^{(n-1)} \qquad \text{.....(1.5)}$$

The chlorine-containing anions are toxic to an organism, especially to human.[6] The species are irritating to the skin, eyes and respiratory tract. The chlorite and chlorate ions are irritating to the gastrointestinal tract and kidneys, corrosive to the mucous membranes and ingestion may cause abdominal pain, vomiting and swelling of the liver, and they can lead to the haemolysis of red blood cells and methaemoglobinaemia. For the perchlorate ion, it can interfere with the ability of the thyroid gland to utilize iodine to produce thyroid hormones. However, no information is available concerning the carcinogenicity and the mutagenicity of these species.

Due to the stability and presistence of by-product species in the environment, it is important to develop a rapid and sensitive analytical method for the quantification of chlorine-containing anions in a variety of samples.

1.2 Chromatographic Method

In any analytical process, the separation technique is important and necessary for simultaneous determination of chlorine-containing anions such as chlorite, chloride, chlorate and perchlorate. Chromatography is one of many techniques that has commonly been used and provides powerful separation of the individual species in a mixture. Beyond the separation, chromatographic technique can be applied for the qualitative and quantitative analysis.

In the late 1890s, Michael Tswett, a Russian botanist, invented the separation of plant pigments such as chlorophylls and xanthophylls, based on chalk column with petroleum ether solution. The pigments were separated as colored bands on the column. He called this separation process the chromatography (as liquid-solid chromatography). Then in 1931, Kuhn and his co-workers employed the same technique to separate lutein and xanthine and demonstrated about liquid chromatography, that it could be used for the preparative separation process. However, the development of this technique was still slow and desultory due to a lack of essential instrumentation. Until 1941, Martin and Synge developed the important separation process in liquid-liquid chromatography and proposed theory of elution chromatography, the plate theory.[7] After that they employed this theory to invent the chromatographic instrument, which detected the signal from the physical characteristics of the interested species.

The chromatographic method is a separation technique, which is achieved by distributing the analyte species between a stationary phase and a mobile phase. The stationary phase functions to retard the species based on various

interactions such as surface adsorption, relative solubility, charge etc, while the mobile phase functions to elute the species through the stationary phase. The separation process depends on the differences in physical and chemical characteristics of the species have the effect to the different interaction between the stationary phase and the mobile phase. Thus, the analyte species spend different time in the movement through column. After that, these species are detected using the suitable detector, giving the signal calls a chromatogram, as presented in Figure 1.1.

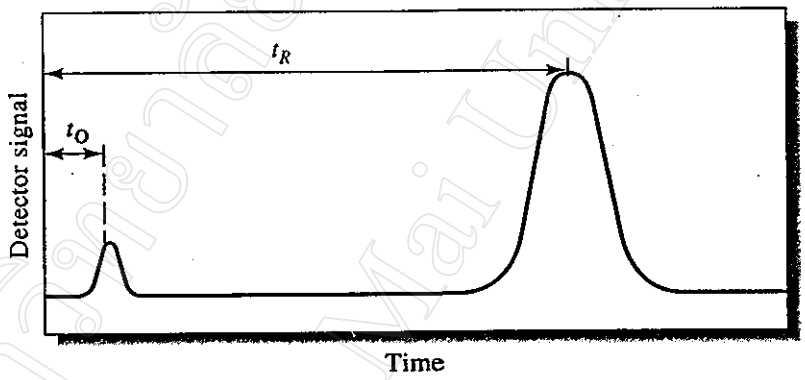

Figure 1.1 A chromatogram obtained from the chromatographic method.

The chromatographic method can be classified into three categories based on the physical nature of the phase, the separation process, or the physico-chemical phenomenon.[8] Figure 1.2 shows the classification of some chromatographic techniques as a function of the polarity of the stationary phase. Each chromatographic technique is a specific analytical method. Therefore, the application of these techniques can be suitably selected the most suitable one for a specific analytical problem. An ion chromatographic technique is basically a chromatographic method for solving analytical problems of ionic or ionizable compounds in aqueous solution. Ion chromatographic is a versatile, selective and sensitive method. It has been applied in various fields such as clinical, pharmaceutical, industrial, food and environmental

analysis.[9-11] Therefore, an ion chromatographic technique is applicable to separation and analysis of chlorine-containing anions, including chlorite, chloride, chlorate and perchlorate.

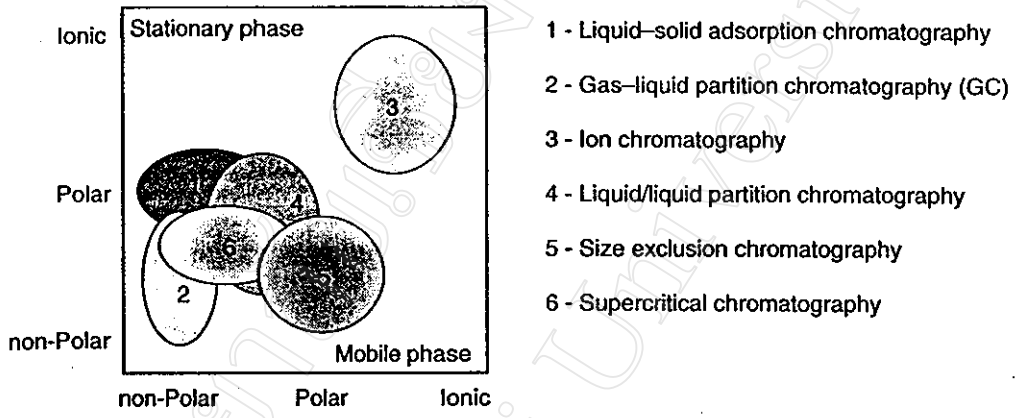

Figure 1.2 Classification of some chromatographic techniques.[8]

1.3 Ion Chromatographic Method

Ion chromatography is a separation technique, which was developed for solving several specific analytical problems in aqueous systems. The advance of ion chromatography was first introduced by Small *et al.* in 1975[12,13], the low-capacity packed column and suppressed conductivity were employed for the first prototype IC by this group. The original IC concept has developed into an independent analytical technique within the short time.[14,15] Presently, ion chromatographic technique has become a modern analytical technique. It has been continuously developed by modification of stationary phase[16], elution system[17] and detection technique.[18,19]

The separation of ion chromatographic technique is based on an exchange of analyte ions with the counter ions to the fixed ions of the ion exchanger. A copolymer, which is employed as the supporting material of ion exchanger, is usually synthesized with polystyrene and divinylbenzene, and then is chemically modified with

functional groups that have acidic or basic properties. Such exchangers are shown in Figure 1.3. Ion exchangers employed for the ion chromatographic system require low capacity which can be obtained by modifying with a low level of substitution and small particle size to improve the efficiency of column. These stationary phases can further be subdivided into strong and weak functional groups, as shown in Table 1.1. The various ionic species can be separated on the basis of the different affinities to functional group on stationary phase. The eluent or mobile phase is an aqueous solution of inorganic salt and often contains an organic solvent. The eluent performs a duty as the competing ion to the eluted analyte ion. The elution power depends on the type of competing ion which is influenced by its charge, size, molecular weight (MW) and concentration. However, the separation is the result of all conditions of the ion chromatographic system.

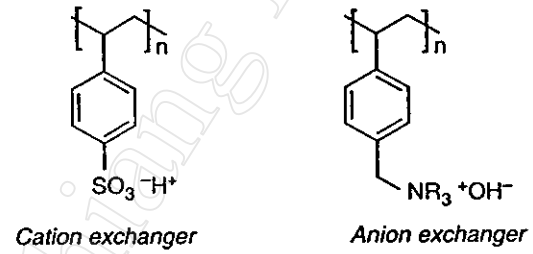

Figure 1.3 Two types of stationary phase in ion chromatography.[8]

Table 1.1 Functional groups on the synthetic ion exchange materials[20]

Type	Functional group	Classification
Cation exchangers		
Sulfonic acid	$-SO_3^-H^+$	Strong
Carboxylic acid	$-COO^-H^+$	Weak
Phosphonic acid	$-PO_3H^-H^+$	Weak
Phosphinic acid	$-PO_2H^-H^+$	Weak
Phenolic	$-O^-H^+$	Weak
Arsonic acid	$-AsO_3H^-H^+$	Weak
Selenonic acid	$-SeO_3^-H^+$	Weak
Anion exchangers		
Quaternary amine	$-N(CH_3)_3^+OH^-$	Strong
Quaternary amine	$-N(CH_3)_2(C_2H_5OH)^+OH^-$	Strong
Tertiary amine	$-NH(CH_3)_2^+OH^-$	Weak
Secondary amine	$-NH_2(CH_3)^+OH^-$	Weak
Primary amine	$-NH_3^+OH^-$	Weak

The separation mechanism is based on ion exchange on the stationary phase between the analyte ions and charged functional groups or counter ions by the electrostatic force and displacement, as shown in Figure 1.4.

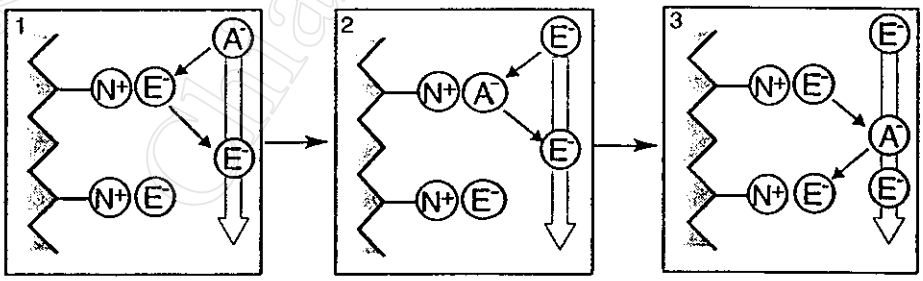

Figure 1.4 Separation mechanism based on ion exchange between analyte anion (A^-) and counter ion (E^-).[8]

According to Figure 1.4, the counter ion (E^-) fixed on the stationary phase is initially exchanged with the analyte ion (A^-) present in the mobile phase. Then

the elution inverses the phenomenon and regenerates the stationary phase with the eluent ion. The ion exchange separation relies on the different affinities of analyte ions to the counter ions. The exchange process is dependent on the ion exchange equilibrium constant, which is the distribution coefficient between the stationary phase and mobile phase. With an anion mode, the process can be expressed as in equations 1.6 and 1.7.

$$\underset{\text{ion exchange}}{RN(CH_3)_3^+E^-} + \underset{\text{solution}}{A^-} \longleftrightarrow \underset{\text{ion exchange}}{RN(CH_3)_3^+A^-} + \underset{\text{solution}}{E^-} \quad .....(1.6)$$

$$K = \frac{\left[RN(CH_3)_3^+A^-\right]\left[E^-\right]}{\left[RN(CH_3)_3^+E^-\right]\left[A^-\right]} \quad .....(1.7)$$

Where $RN(CH_3)_3^+E^-$ is the strong anion exchange with quaternary ammonium groups, A^- is the analyte anion and K is the ion exchange equilibrium constant for the exchange reaction. Generally, when the K value for analyte ion is large, the analyte ion has a tendency to retain on the stationary phase, whereas when the K value is small, the opposite is obtained.

1.4 Chromatographic Characteristics

1.4.1 Retention time and peak width

The elution curve is a function of detector response to time following a chromatographic separation, called a chromatogram, as shown in the Figure 1.5

For a simple chromatogram, the small peak on the left is for a species that is not retained by the stationary phase. The dead time provides a measure of the average rate of migration of the mobile phase and is an important parameter in

identifying analyte peaks. The time parameter t_o, t_R and t'_R can be converted into the dead volume (V_o), retention volume (V_R) and net retention volume (V'_R) using the constant flow rate.

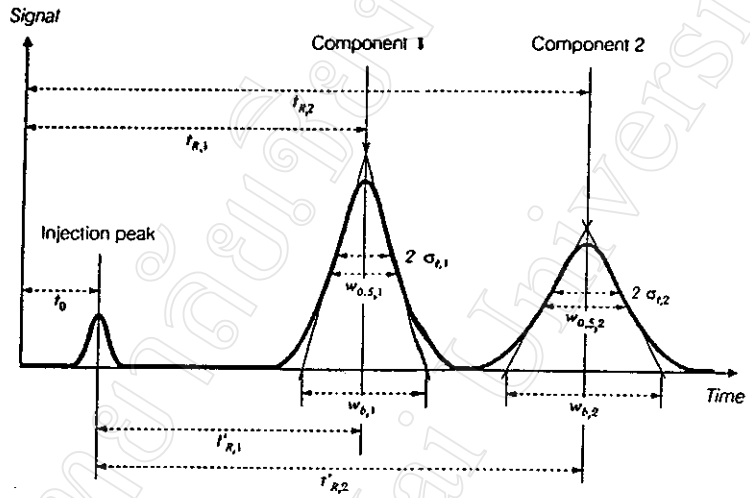

Figure 1.5 A typical chromatogram for two components.

According to Figure 1.5, t_o is the dead time, the time of eluent required to flow through the separation system, t_R is the retention time, the time between the injection point and the peak maximum of the component injected appears at the end of the separation system, t'_R is the net retention time, the time between retention time (t_R) and dead time (t_o), $w_{0.5}$ is the peak width at half height, the distance between each side of a peak measured at the half height of peak, w is the peak base width, the distance between each side of a peak measured at the base peak.

1.4.2 Capacity factor

The retention time t_R is the qualitative information of a chromatogram. It is constant for a given component provided the chromatographic conditions remain unchanged (column, mobile phase, temperature, etc.). For the characterization of a substance, it is more convenient to quote the capacity factor k′ since, in contrast to the

retention times, this is dependent neither on the flow of the eluent nor on the column length:

$$k' = \frac{t'_R}{t_o} = \frac{t_R - t_o}{t_o} \quad \text{.....(1.8)}$$

$$k' = \frac{V_s C_s}{V_m C_m} = \frac{K V_s}{V_m} \quad \text{.....(1.9)}$$

Where V_s is the volume of the stationary phase and V_m is the volume of the mobile phase. Low values of k′ signify that the corresponding ions are eluted in the vicinity of the injection peak and the separation is consequently very poor. Capacity factors between 1 and 5 are optimum in practice; larger k′ values lead only to peak broadening, lower detection sensitivity and long analysis times.

1.4.3 Selectivity

The two components are separated only if they have different k′ values. A measure of the separation efficiency of a chromatographic system is the selectivity α, which is also known as relative retention.

$$\alpha = \frac{k'_2}{k'_1} = \frac{t_{R,2} - t_o}{t_{R,1} - t_o} \quad ; \; k'_2 > k'_1 \quad \text{.....(1.10)}$$

1.4.4 Resolution

The separation of components in ion chromatography is based on ion exchange and an equivalent exchange of eluent ions on the fixed site of stationary phase. The extent of separation of the peaks is measured by resolution (R)[8], which is proportional to the ratio of the distance between the peak maxima, Δt_R, to the peak width at half height of the two neighboring peaks.

$$R = \frac{1.18(t_{R,2} - t_{R,1})}{w_{0.5,1} + w_{0.5,2}} \qquad .....(1.11)$$

It is evident from Figure 1.6 that a resolution of 1.5 gives an essentially complete separation of component A and B, whereas a resolution of 0.75 does not. At a resolution of 1.0, zone A contains about 4% of zone B and zone B contains about 4% of zone A, resulting in their separation being 98% complete. At a resolution of 1.5, the overlap is about 0.3%, which corresponds to 99.7% separation.

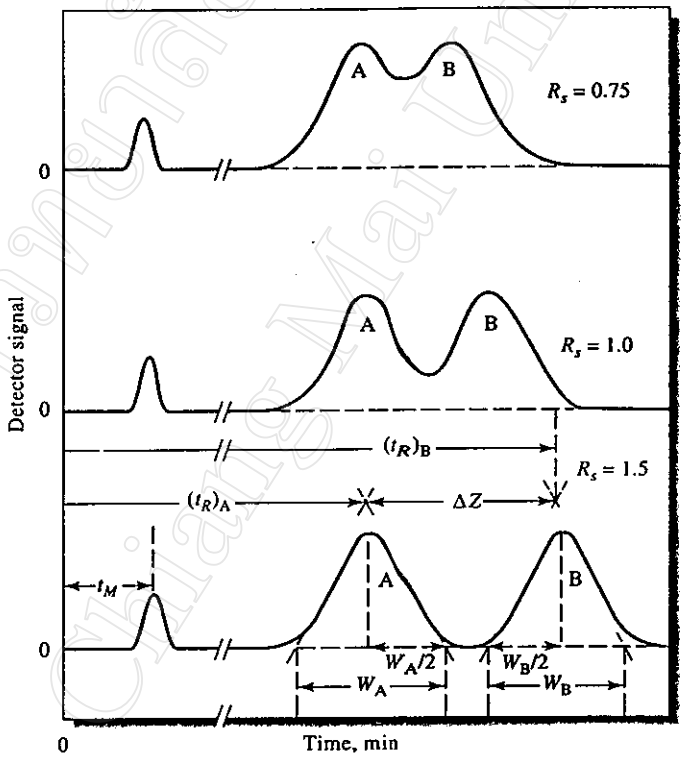

Figure 1.6 Separation at three resolutions between the two components.[21]

1.4.5 Asymmetry factor

The elution of chromatographic signals as Gaussian peaks is often not achieved in practice. An asymmetric peak shape, which is known as tailing, is often found.

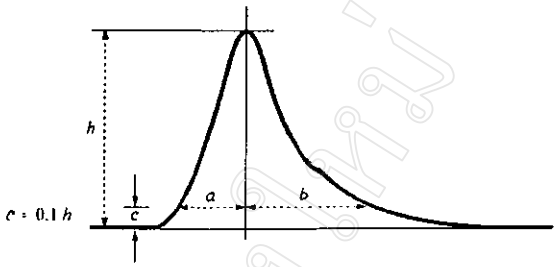

Figure 1.7 An asymmetric peak shape of chromatographic signals.

The peak asymmetry is quantified by the asymmetry factor (T) with a and b being determinated at 10% peak height

$$T = \frac{a}{b} \qquad \text{.....(1.12)}$$

The peak tailing can be the effect from any causes such as chemical effects and column overloading. The maximum loading of the column has been exceeded, broad peaks with severe tailing are obtained. The dominant separation mechanism is adversely influenced by another mechanism, e.g. adsorption phenomenon in ion exchange chromatography.

1.4.6 Column efficiency

The efficiency of column can be expressed by the approaches of chromatographic theory. The plate theory, proposed by Martin and Synge, provides a simple and convenient way to measure the performance and efficiency of column. The rate theory developed by van Deemter *et al.*, describes the contributions to band broadening.

1.4.6.1 The plate theory

The chromatographic model of the plate theory assumes that the column consists of a number of thin sections or plates and that the analytes in each

plate are equilibrated between the stationary and mobile phase. The greater the number of theoretical plates (N), the more efficient the column becomes. The number of theoretical plates can be evaluated from the analyte peak by calculation from the following equation:

$$N = 16\left(\frac{t_R}{w_b}\right)^2 = 5.54\left(\frac{t_R}{w_{0.5}}\right)^2 \quad \text{.....(1.13)}$$

Where t_R is the retention time, $w_{0.5}$ is the peak width at half height, w_b is the peak base width.

The height equivalent to a theoretical plate (H) or thickness of theoretical plate is expressed as in equation 1.14. It is evident that the efficiency of a chromatographic column increases, as the number of theoretical plates decreases.

$$H = \frac{L}{N} \quad \text{.....(1.14)}$$

Where L is the length of the column.

In general, the efficiency can be varied by changing physical column parameters such as the length, diameter and construction material of the container of the column. It can also be varied by changing chemical parameters such as the particle size of polymeric material and the mobile phase velocity.[21]

1.4.6.2 The rate theory

The rate theory is based on the rate of mass transfer between two phases, diffusion rate of solute along the column, eluent flow rate, and hydrodynamics of mobile phase. Therefore, these factors may be concluded and shown as the relationship between column efficiency and variables in column composition and analytical conditions as expressed by the van Deemter equation as follows:

$$H = A + \frac{B}{\mu} + C\mu \qquad .....(1.15)$$

Where H represents the efficiency of the column and μ represents the average linear velocity of the mobile phase. The A term represents the contribution to band broadening by eddy diffusion, the B term represents the contribution from longitudinal diffusion, and the C term represents the contribution from resistance to mass transfer.

From the equation, a graph of plate height versus the average linear velocity of the mobile phase called van Deemter plot as shown in Figure 1.8, is obtained.

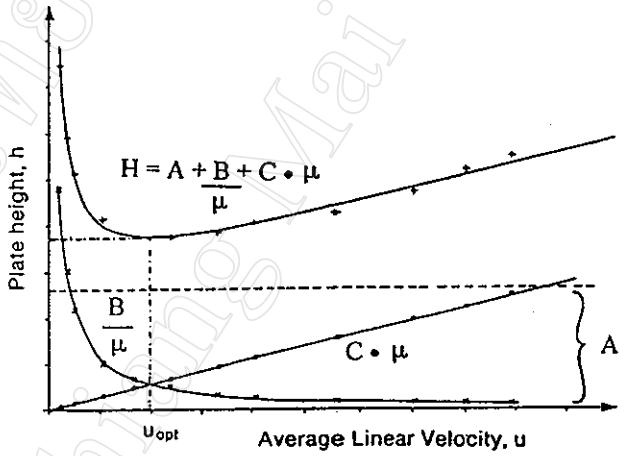

Figure 1.8 The van Deemter plot showing the relationship between efficiency and average linear velocity of the mobile phase.

According to the van Deemter plot, it is evident that at the flow rate below the optimum, the overall efficiency is dependent on the diffusion effect as the B term. At the higher flow rates, the efficiency decreases, which is dependent on the mass transfer or the C term. The A term is a constant, independent of flow rate. The best efficiency of the chromatographic system is obtained at the optimum flow rate,

which corresponds to the minimum on the curve. In practice, the system operates at high flow rate may reduce the efficiency, however it can save the analysis time and operating costs.

1.5 Optimization of Chromatographic System

In the analytical process, the optimization has the purpose to obtain the optimum condition, which can separate the components of interest in the minimum analysis time. The three important parameters of the optimization process are resolution, speed and capacity. The relationship of these parameters is shown in Figure 1.9. The optimization for one of all parameters can have an effect to each other.

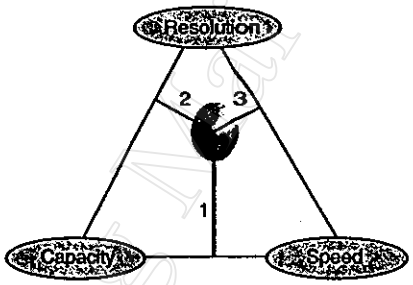

Figure 1.9 The chromatographer's triangle.[8]

The chromatographic condition can be optimized using all the resources of the instrumentation, software or the other modification. The optimized analytical separation uses the potential of the most efficient parameter as selectivity.

1.6 Isocratic and Gradient Chromatography

In the isocratic system, all parameters of conditions and setting of the separation are held constant. Conversely, in the gradient system, one or more parameters are varied continuously. The most typical gradient chromatography is a variation of the mobile phase composition from low elution strength to high elution

strength, which is a powerful technique. The main reason for using gradient elution is a widely different retention of the interested components.

Gradient elution has generally not been used in ion chromatography with conductimetric detection. Mainly the problem is associated with a severe baseline shift, which is a major change of background conductivity and can greatly limit the operation. However, the gradient elution has been used for special applications.[22-25] The gradient elution of ion chromatographic method is usually accomplished by step rather than by a continuous gradient. The gradient system often leads to satisfactory separation of all components of a mixture in minimum analysis time.

The major equipment of isocratic system is the pump which delivers only one eluent system, with the pulse dampener and pressure controller being used to keep the smoothness and constant flow throughout the analysis. The equipment necessary for gradient elution consists of a second eluent reservoir, an additional pump (for a high-pressure-mixing gradient system), a mixing chamber and a proportioning valve that is regulated using a gradient control system. The gradient system can occur in one of two types which are the low-pressure mixing gradient system and the high-pressure mixing gradient system, as shown in Figure 1.10.

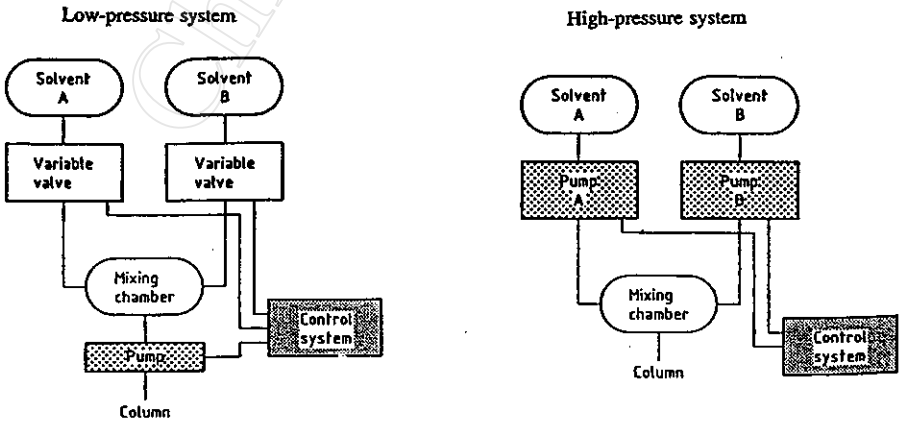

Figure 1.10 Schematic of the low-pressure and high-pressure mixing gradient system.[26]

In the configuration, the eluents are blended at atmospheric pressure using the mixing chamber under the low-pressure mixing gradient system. Then the mixture is pumped into the column with a single high-pressure eluent delivery system. The high-pressure mixing gradient system required the two eluent delivery systems, one for each eluent, the eluents are mixed after leaving the pumps. The amount of each eluent in the gradient is controlled by the relative differences in the flow rates using control system.

1.7 Instrumentation for Ion Chromatography

The basic components of ion chromatographic system are the eluent containers for the mobile phase, an eluent delivery system or pump(s) to move the eluent and sample through the system, an injection to allow sample introduction, a column(s) to provide solute separation, a suppressor to reduce the background conductivity, a conductivity detector to detect the separated components, a data collection or recorder to assist in interpretation and storage of results. The schematic diagram of an ion chromatograph is shown in Figure 1.11.

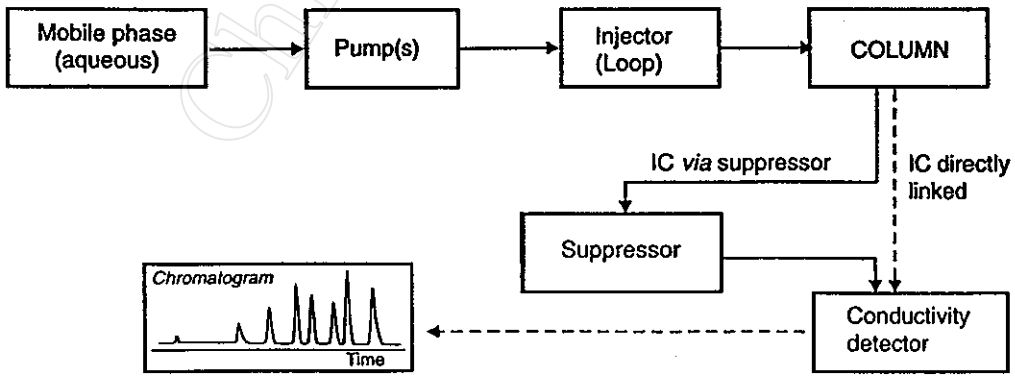

Figure 1.11 The schematic diagram of an ion chromatograph.

1.7.1 Eluent delivery system

The function of the eluent delivery system is to deliver the mobile phase through the chromatographic system. Delivery of the mobile phase requires the reproducible flow rate and pulse free to obtain the minimal baseline noise. Dual-reciprocating-piston pumps are used for the eluent delivery system of ion chromatograph which the arrangement is controlled using a motor.[27]

1.7.2 Sample introduction

The sample or standard solution is introduced into the ion chromatograph via an injection with automatic injection of the six-port valve, which can reduce the dispersion and the broadening of peaks. The valve comprises the two flow paths such as the load position and the inject position. Firstly, in the load position, the sample or standard solution is injected into a loop using a syringe. The valve is switched to the inject position, the solution in the loop will be directly inserted to the flow path of the eluent into the chromatographic system. The highly reproducible injections are obtained with the automatic injection.

1.7.3 Column

The separation of a mixture into components can be achieved with the important factor which is the analytical column. An ion exchanger column is usually used for the ion chromatographic system, which consists of the three important parts, an insoluble matrix of organic or inorganic material, a fixed ionic part that is incorporated into the insoluble matrix and an ion exchanger that is capable of replacing reversibly its ions with ions of the same charge in the solution. The selectivity, capacity and efficiency of the analytical column depended on the nature of the packing material. A guard column is often placed in front of the analytical column to protect the particulate matter and contaminants from the solvents or the samples into the analytical column, and it can increase the lifetime of the analytical column.

1.7.4 Suppressor

In the highly ionic eluent system, the suppressor is used to reduce the conductivity value and allowing the detection of analyte species. The Anion Self-Regenerating Suppressor (ASRS-I) with AutoSuppression Recycle Mode was employed in this research, is shown in Figure 1.12.

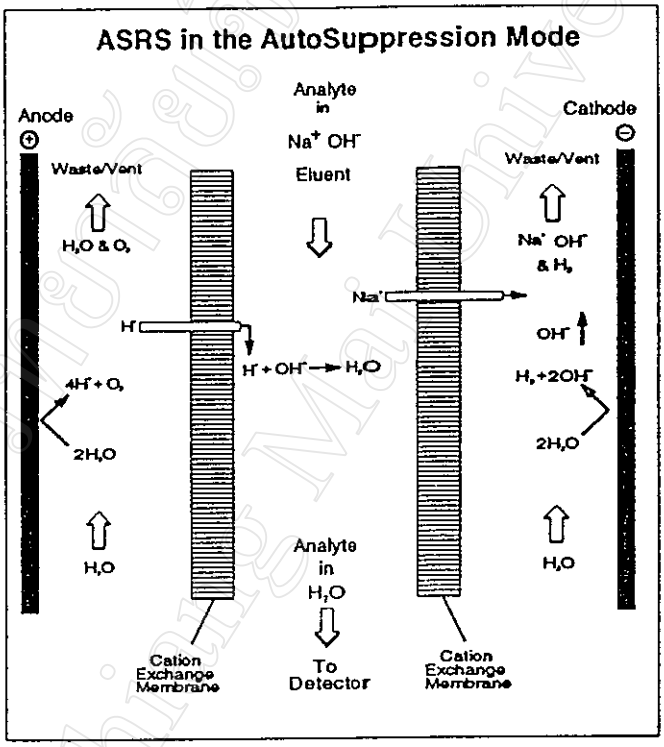

Figure 1.12 The AutoSuppression Recycle Mode.[28]

The suppressor system uses the neutralized conductivity cell effluent as the source of water for the regenerant chamber water. The water regenerant undergoes electrolysis to form hydrogen gas and hydroxide ions in the cathode chamber while oxygen gas and hydronium ions are formed in the anode chamber. The cation exchange membranes allow hydronium ions to move from the anode chamber into the eluent chamber to neutralize hydroxide. Sodium ions in the eluent, attracted by the electrical

potential applied to the cathode, move across the membrane into the cathode chamber to maintain electronic neutrality with hydroxide ions at the electrode.

1.7.5 Conductivity

The conductivity detection is based upon the electrical conductivity of an ionic solution when placed between two oppositely charged electrodes. The presence of ions in the solution allows electrical current to flow between the electrodes, whereby negatively charged ions migrate to the anode and positively charged ions migrate to the cathode. The conductance (G) is measured between two electrodes inserted into a conducting medium. The conductance of the solution varies with the concentration and the relationship obtained is linear for very dilute solutions. The conductance is related to the specific conductance or conductivity (k, in $Smol^{-1}$) and the equivalent ionic conductance (Λ_0, Sm^2mol^{-1}) as expressed in equation 1.16.

$$k = G K_{cell} = \Lambda_0 \frac{Cz}{1000} \qquad \text{.....(1.16)}$$

Where K_{cell} is cell constant.

The equivalent ionic conductance refers to the conductivity of an ion that has a valence of z and molar concentration (C, mol/l) tends towards 0 in a solution at 25°C. Therefore, the conductance (G, μS) can be expressed as equation 1.17.

$$G = \Lambda_0 \frac{Cz}{10^{-3} K_{cell}} \qquad \text{.....(1.17)}$$

Temperature directly affects the conductivity of a solution. A change in room temperature causes a regular oscillation in the baseline and also affects the reproducibility and linearity of the determination.

1.8 Analysis with Ion Chromatography

1.8.1 Qualitative analysis

The retention data is the most commonly used for the identification of peaks by matching the retention time of the sample component to the standard reference compound in the two chromatograms. However, it can only be used for preliminary identification because there are so many variables in a chromatographic system and the samples may contain unexpected compounds with the same retention time.

The standard addition method is used to confirm the results. The small amount of a known component is added to the sample, which is analyzed first, and this sample is reanalyzed. If a quantitative increase is obtained in the peak of the component corresponding the standard, the component representing the peak is identified.

1.8.2 Quantitative analysis

The external standard method can be employed in the quantitative analysis. The standard solutions are then analyzed, and calibration curves are established by plotting the peak area versus concentration for each analyte ion. The unknown concentrations of analyte in the sample can be determined. In a multilevel calibration, several different amounts of the standard solution are prepared and analyzed. A linear least-square regression method is used and it can lead to a more precise value for unknown concentrations.

1.8.3 Reproducibility

Precision of the analysis can be defined as the concordance of a series of measurements of the equal quantity. The number of measurements depends 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 the following equations.

$$SD = \sqrt{\frac{(x_i - \bar{x})^2}{n-1}} \quad .....(1.18)$$

$$\%RSD = \left(\frac{SD}{\bar{x}}\right)^2 100 \quad .....(1.19)$$

Where x_i is the individual value in data, $\bar{x}$ is the mean of data and n is the number of measurement.

1.8.4 Detection limit and minimum detectable quantity (MDQ)

The detection limit (L) can be defined as the weight of substance to give a signal twice the standard deviation of the noise level. The detection limit of each ion is determined by injecting the lowest concentration of the mixed standard solution under the established condition. It can be calculated as follows.[29]

$$L = \frac{2nm_x}{R} \quad .....(1.20)$$

Where L is the detection limit, R is the peak signal response, n is the noise level and m_x is the amount of compound injected. In practice, the noise level is obtainable by means of integrating the zoomed baseline of chromatogram of each ion.

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.

$$MDQ = Lw_{0.5} \quad .....(1.21)$$

Where $w_{0.5}$ is the width at half height (sec).

1.8.5 The percent recovery

The percent recovery of each ion in the water sample is quantitatively confirmed by using the spiked recovery method. The known amount of standard solution is spiked into the sample at various concentrations. The percent recovery (%R) can be calculated as follows.

$$\% \text{ Recovery} = \left(\frac{A-B}{C}\right)100 \qquad \text{.....(1.22)}$$

Where A is the amount of ion in the sample obtained with standard solution spiked at various concentrations, B is the amount of ion in the sample obtained without spiking ion solution and C is the concentration of standard ion solution spiked to the sample.

1.8.6 Test of significance

The development of a new analytical method often requires the comparison of results obtained with an accepted method. The F test and t test are commonly applied to indicate whether there is a significant difference between two methods.[30]

The F test is designed to indicate whether there is a significant difference between two methods based on their standard deviation. F test is defined in terms of the variances of the two methods, where the variance is the square of the standard deviation.

$$F = \left(\frac{S_{d,1}}{S_{d,2}}\right)^2 \qquad \text{.....(1.23)}$$

Where $S_{d,1} > S_{d,2}$. There are two different degrees of freedom, ν_1 and ν_2, where degrees of freedom defined as N-1 of each case. If the calculated F value

according to the equation 1.23 exceeds a tabulated F value at selected confidence level, then there is a significant difference between the variances of the two methods. A list of F values at the 95% confidence level is given in Table A-1.

The t test is designed to test a new method with an accepted method by analyzing several different samples. An average difference ($\overline{D}$) is calculated and the individual deviations of each from $\overline{D}$ are used to compute a standard deviation, S_d. The t value for the multiple samples is calculated, as in the following equations.

$$t = \frac{\overline{D}}{S_d}\sqrt{N} \qquad \text{.....(1.24)}$$

$$S_d = \sqrt{\frac{\sum (D_i - \overline{D})^2}{N-1}} \qquad \text{.....(1.25)}$$

Where D_i is the individual difference between the two methods for each sample and $\overline{D}$ is the mean of all the individual differences. Usually, a test at the 95% confidence level is considered significant. If the calculated t value according to the equation 1.24 exceeds a tabulated t value at selected confidence level, then there is a significant difference between the two methods. A list of t values at the 95% confidence level is given in Table A-2.

1.9 The Analysis of Anions Using Ion Chromatographic Method

An ion chromatographic method has been widely applied for the analysis of inorganic and organic species since it was first introduced by Hamish *et al.* in 1975. The chlorine-containing anions were analyzed using several techniques under ion chromatographic method, as in the following paragraphs.

G. Schminke and A. Seubert[31] have developed ion chromatographic method for the simultaneous determination of the disinfection by-products bromate,

chlorite, chlorate and the so-called seven standard anions, fluoride, chloride, nitrite, sulfate, bromide, nitrate and phosphate. The separation of the ten anions was carried out using a high-capacity PS/DVB anion exchanger column. The quantification of fluoride, chloride, nitrite, sulfate, bromide, nitrate, phosphate and chlorate was detected after chemical suppression. The post column reaction based on chlorpromazine (CHP) was optimized for the determination of chlorite and bromate using spectrophotometric detection at 530 nm. The method was applied for analysis of several water samples, such as drinking water, mineral and swimming pool water.

M. Biesaga *et al.*[32] have developed a single column ion chromatography (SCIC) and capillary electrophoresis (CE) for the determination of chlorine-containing anions. The SCIC method was based on the use of a Vydac column 302 IC and hydrogen-phthalate eluent at 2.5 ml/min with UV detection at 254 nm. The separation of all anions was achieved in long analysis time. The CE method consisted of a fused-silica capillary, sodium chromate as electrolyte with applied voltage in negative polarity and detected the signal using UV detector at 254 nm. This method can provide the complete separation below 5 min. Both methods were applied for the analysis of tap water, swimming pool water and bleaching preparation samples.

L.K. Jackson *et al.*[33] have developed ion chromatographic method using a Dionex AS4-HC anion exchange column with a carbonate eluent and suppressed conductivity for the determination of disinfection by-product anions such as bromate, chlorite, bromide and chlorate at low ppb levels in drinking water. The detection limits were calculated to be 2.38, 1.73, 1.78 and 1.07 μg/l for chlorite, bromate, bromide and chlorate, respectively. The method was linear for these anions over the typical concentration range and acceptable recoveries were obtained for anions spiked in drinking water.

P.E. Jackson *et al.*[34] have developed ion chromatographic method for the determination of perchlorate in drinking and ground waters. The method was based

on a Dionex IonPac AS11 column, hydroxide eluent, large loop (1000 μl) injection and suppressed conductivity detection. This method is free of interference from common anions and it provides the elution of the perchlorate ion within 10 min. The linearity was obtained over the range of 2.5-100 μg/l perchlorate and quantitative recoveries were obtained for low μg/l levels of perchlorate in spiked samples.

B. Nowack and U. Gunten[35] have developed ion chromatographic method for the determination of low concentration of chlorate in natural waters. The method was based on an osmate-catalyzed postcolumn reaction of chlorate with iodide and UV detection of triiodide. The eluent consisted of sodium tetraborate decahydrate and sodium formate at flow rate 1.0 ml/min. The osmate catalysis allows the oxidation of iodide by chlorate at pH 3 instead of 6 M HCl for the uncatalyzed reaction. The method also allows the simultaneous determination of chlorite, bromate and nitrite at the low μg/l level.

J.J. Ellington and J.J. Evans[36] have developed ion chromatographic method on a microbore AS16 anion exchange column and a conductivity detector for separation and detection of perchlorate from the ionic plant extract. The extract was heated to precipitate proteins, centrifuged, exposed to alumina, and filtered through a cartridge filled with divinylbenzene to yield a water clear extract for IC analysis. Heating the extract and treatment with alumina reduced substantially the ionic content of the extracts without loss of perchlorate.

1.10 The Scope and Aims of This Research

The ion chromatographic conditions of two elution systems, namely the gradient and isocratic systems were optimized for analysis of chlorine-containing anions such as chlorite, chloride, chlorate and perchlorate and detection was achieved using a conductivity detector. The new method of gradient system was successfully

developed using the two eluents for the separation of all species. The obtained conditions were applied to determine these anions in water samples, including public water supplies, swimming pool water and natural water. The obtained results were quantitatively compared between the two elution systems.

The aims of this research are as follows.

(1) To investigate and obtain optimum ion chromatographic conditions for analysis of chlorine-containing anions such as chlorite, chloride, chlorate and perchlorate.

(2) To apply the obtained conditions in the quantitative analysis of chlorine-containing anions in water samples, including public water supplies, swimming pool water and natural water.