CHAPTER 4

DISCUSSION AND CONCLUSIONS

4.1 Discussion

Ion chromatography is basically a chromatographic technique used for separation and determination of inorganic and organic ions. The ion chromatographic method has continuously been developed by modifying the structure of the ion exchange site on the stationary phase[39,40], the evolution of detection techniques[41] and development of the conductivity suppression[42,43] for analysis of a variety of the ions of interest. For this research, ion chromatography was employed to develop the elution system for the analysis of chlorine-containing anions, namely chlorite, chloride, chlorate and perchlorate.

The ion chromatograph employed for this work was a Dionex DX-300 system consisting of an advanced gradient pump, a Rheodyne model 9126 injection valve and a conductivity detector. Chemical suppression was achieved using Dionex ASRS-I (4mm), operated at 100 mA in the Auto-Suppression Recycle and the Chemical Suppression Modes. The anion-exchange columns, which were employed to study the separation of chlorine-containing anions, were IonPac AS4A, IonPac AS4A-SC and Metrosep A Supp 4.

For the first column, an IonPac AS4A column, sodium carbonate and sodium bicarbonate were selected as eluent because they produced low background conductivity. The eluent concentrations and the eluent flow rates were investigated in the isocratic system. The effects of variation of the eluent concentration on retention time and resolution, using a mixture of sodium carbonate/ sodium bicarbonate as the eluent at flow rate of 2.00 ml/min, are shown in Tables 3.1 and 3.2. It was found that increasing the

eluent concentration decreased retention time of chlorine-containing anions since it increased the elution power. With the same effect, the resolution between chlorite and chloride peaks and the resolution between chloride and chlorate peaks were decreased, while the resolution between chlorate and perchlorate peaks tended to increase. An explaination for this is that when increasing the eluent strength, the weakly retained ions such as chlorite, chloride and chlorate would be rapidly eluted through the column, leading to a decrease in resolution. As for perchlorate, it would still retained on the resin, suggesting that moderate strength eluents are slightly effective on the high affinity ions.[12] The effects of variation of the eluent flow rate on retention time and resolution of each anion pair are shown in Table 3.3. The results indicated that increasing the eluent flow rate could reduce the analysis time and the resolution. The optimization of conditions, which provided sufficient resolution values (≥ 1.5 for every anion pair) and reduced the analysis time, was achieved with the use of 1.0 mM sodium carbonate/ 2.0 mM sodium bicarbonate at flow rate of 2.00 ml/min.

In the analysis of real water samples, several workers have reported high concentrations of chloride ion.[44-47] Under the optimized conditions in this column, the chlorite, chloride and chlorate peaks were found to be quite close to each other. Therefore, the chloride peak may possibly overlap with the neighboring peaks in the water samples. Moreover, the analysis time for the separation of chlorine-containing anions was achieved within 35 minutes, which was not yet satisfactory.

Since the mixed solution of sodium carbonate and sodium bicarbonate could not provide the optimal condition for analysis of chlorine-containing anions, the second column, an IonPac AS4A-SC column, attempts were made to optimize the eluent types in the isocratic and gradient systems. In the gradient system using the two eluents, the variation of the eluent types at flow rate of 2.00 ml/min was investigated to study the

conductivity value and the separation of chlorine-containing anions. The results of the effects of the eluent types on retention time, resolution and peak area are shown in Tables 3.4-3.6, respectively. The results obtained indicate that the separation selectivity and sensitivity of chlorine-containing anions depend on the characteristics of the eluent types. The factors affecting the retention of chlorine-containing anions include charge or valency, size, molecular weight and concentration of the eluent ion. From the results, the retention time of anions decreased with the increasing charge of the eluent anions such as hydrogen phthalate (1-), chromate (2-) and citrate (3-) ions when the same concentration was used. The retention time of the analyte ions also tended to decrease with increasing size or molecular weight of the eluent ions, including an increase in the eluent concentration. However, these factors were all taken into consideration in studying the separation of all anions. Additionally, it was observed that chlorine-containing anions were not found with acetonitrile as eluent. It is reasonable to assume here that acetonitrile was unable to elute the analytes through the column because this eluent was a non-ionic solvent incapable of replacing the ions of interest, which have affinities with the resin.

The results in Tables 3.4 and 3.5 show the retention time and resolution data obtained with the isocratic system. It was found that the eluents could be divided into two parts. One of the eluents consisted of sodium hydroxide, sodium benzoate, sodium oxalate, phthalic acid and potassium hydrogen-phthalate that could separate chlorite, chloride and chlorate with sufficient resolution of each anion pair but with a long retention time of perchlorate. Other eluents such as sodium carbonate, sodium citrate and potassium chromate could provide a relatively shorter retention time for perchlorate but a somewhat poorer resolution of chlorite and chloride. Therefore, suitable eluents were selected to match in the gradient system with a criterion nevertheless that the equality of conductivity value is essential in the matching of these eluents.

The operation of the gradient system involved the switching of valve using an advanced gradient pump. Firstly, the ion chromatographic system was equilibrated by passing the weak eluent to obtain a stable baseline signal. Then, the standard or sample solution was injected into the system. The weakly retained ions such as chlorite, chloride and chlorate were eluted through the separation column while the strongly retained perchlorate ion was still holding. At the time that chlorate was eluted at the beginning, the strong eluent was introduced into the system by switching the valve. With the strongly retained perchlorate ion, strong eluent was required. Finally, the column was reequilibrated with the weak eluent prior to the injection of the next solution.

The gradient system is accomplished by changing from a weakly eluent ion to a much more strongly eluent ion during the run. The complete separation of chlorine-containing anions such as chlorite, chloride, chlorate and perchlorate, obtained with this method, was achieved within a minimum analysis time and sufficient resolution. Each gradient method provided different selectivity and sensitivity for these anions. The results of the effects of the gradient methods on retention time, resolution and peak area are shown in Table 3.7 and Figure 3.1. The chromatograms obtained with the gradient methods A-F are presented in Figures 3.2-3.7, respectively. The separation of the four chlorine-containing anions was carried out within 20 minutes. The gradient methods A and B were established using two eluents, with different conductivity value and eluent strength. In subsequent experiments, it was found that the steps of the gradient elutions, shown in Figures 3.2 and 3.3 corresponded to the difference of conductivity values of the pair of eluent. The gradient methods C-F were established using two eluents, with equal conductivity value but different eluent strength, and are shown in Figures 3.4-3.7, respectively. Figure 3.1 shows the comparison of the sensitivity of the gradient methods in terms of peak areas. The strong eluents employed in these methods consisted of sodium

carbonate and sodium citrate. It could be seen that the citrate eluent provided the higher sensitivity for perchlorate, with the highest sensitivity in the gradient method E. For the weak anions, chlorite, chloride and chlorate, they were separated using the weak eluents such as sodium hydroxide, sodium bicarbonate, sodium benzoate and citric acid. The highest sensitivity of these weak anions was obtained when using the benzoate eluent, with the method C for chloride and with the method D for chlorite and chlorate. Although the gradient methods A and B could offer shorter analysis times, the separation between the chlorite and the chloride peaks was unsatisfactory, with the resolution values being lower than 1.5. The gradient method F, as shown in Figure 3.7, could offer high sensitivity and short analysis time, but the shift of baseline was obtained. This behavior is possible due to the excessive difference of the weak and strong eluent concentrations. Therefore, the results obtained with the gradient methods C-E, as shown in Figures 3.4-3.6, respectively, were satisfactory and acceptable.

The optimal condition, selected for the analysis of chlorine-containing anions, was the gradient method E because it could provide better resolution values between the chlorite and the chloride peaks although longer analysis time and lower sensitivity were obtained. The summary of the gradient condition of this method is given in Table 3.12. The retention times for the chlorite, chloride and chlorate peaks obtained with 1.0 mM citric acid were 2.32, 3.22 and 6.62 minutes, respectively. The retention time for the perchlorate peak obtained with 1.0 mM sodium citrate was 16.77 minutes. The chromatogram showing the separation of chlorine-containing anions is presented in Figure 3.10 (a).

In the chromatograms obtained with the gradient method, the large peak between the chlorate and the perchlorate peak is observed, as shown in Figures 3.4-3.7. This large peak occurred due to the changing of the eluent during the operation. At the

beginning, the weak eluent flowed through the columns into a conductivity detector, yielding a smooth baseline. When the strong eluent was switched to substitute the weak eluent, a rise in the conductivity value would occur while the strong eluent passed through the conductivity detector. That was the cause of appearance of the large peak.

Since the perchlorate peak appeared at the tail of large peak, the reduction of this peak was attempted by changing the detection system and using the chemical suppression. The gradient system employed for this investigation was the condition in method E. One approach was changing the detection by the replacement of a conductivity detector with the UV-VIS detector. Chlorine-containing anions chlorite, chloride, chlorate and perchlorate were detected at the wavelength 254 nm[32] and 261 nm, but no signal of all anions were obtained. This approach was not suitable for the solution. The second approach was the use of Chemical Suppression Mode of 50 mM sulfuric acid at pressure 20 psi with a conductivity detector. The chromatogram of the background signal is shown in Figure 4.1. The result obtained suggested that this condition could not reduce the large peak and it was also the cause of the increased noise. Therefore, the optimal condition in the gradient method E was employed for analysis of chlorine-containing anions. The presentation of standard/sample chromatograms could be integrated using the normal or the subtraction modes, which are shown in Figure 3.10 (a) and (b), respectively. It was found that the background signal would be erased with the integration in the subtraction mode. However, the large peak appeared in the original chromatograms, including the background and the standard/sample chromatograms.

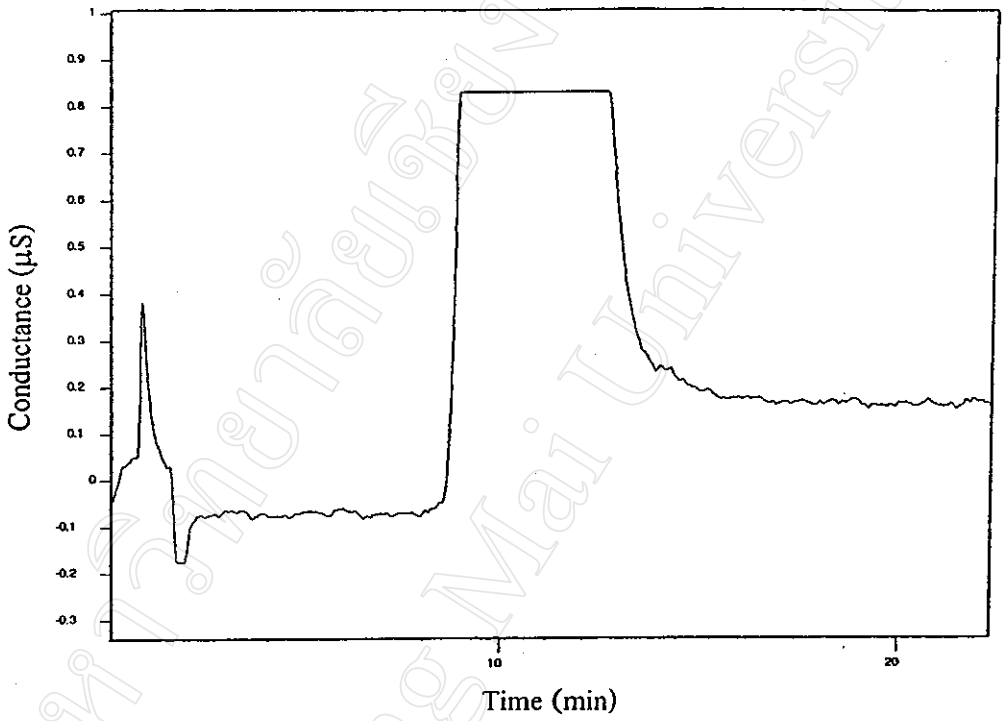

Figure 4.1 Chromatogram of the background baseline obtained with an IonPac AS4A-SC column and a conductivity detector using the gradient eluents, citric acid and sodium citrate at flow rate of 2.00 ml/min with the Chemical Suppression Mode.

With the last column, a Metrosep A Aupp 4 column, the eluent types for the separation of chlorine-containing anions were optimized using the isocratic system. The flow rate of 1.80 ml/min was selected for the operation since the maximum flow rate for this column is 2.00 ml/min according to the column manual. The variation of the eluent types was investigated and results on the effect of the eluent types on retention time, resolution and peak area are shown in Tables 3.8-3.9 and Figure 3.8, respectively. The characteristics of the eluent types, charge, size, molecular weight and concentration were found to affect the separation, selectivity and sensitivity of these anions. It can be seen that the retention time of anions decreased with increasing charge of the eluent ions, i.e. sodium benzoate (1-), sodium carbonate (2-) and sodium citrate (3-). It was noticeable that the signals of these anions were not detected when acetonitrile was used as eluent. For this study, the isocratic conditions with 2.0 mM sodium carbonate and 0.5 mM sodium oxalate could provide good resolution values ($\geq$1.5 for every anion pair) whilst the inadequate resolution values between the chlorite and chloride peaks were obtained with the use of 10 mM sodium borate, 3 mM sodium benzoate and 0.59 mM sodium citrate as the eluent. However, the elution of all anions obtained with 0.5 mM sodium oxalate was achieved in a longer analysis time. Therefore, the carbonate eluent was chosen to study the optimal concentration for the separation of chlorine-containing anions. The effects of the concentration of the carbonate eluent on retention time, peak area and resolution are shown in Tables 3.10-3.11 and Figure 3.9, respectively. It was found that decreasing retention time and resolution was obtained with an increase the eluent concentration and the eluent strength. The sensitivity of these anions decreased with increasing eluent concentration. It could be seen that the isocratic condition of 2.0 mM sodium carbonate could offer sufficient resolution and short analysis time for chlorine-containing anions

although the highest sensitivity was not obtained. Therefore, this isocratic condition was also employed for the analysis of chlorine-containing anions.

The optimum condition in the isocratic system is summarized in Table 3.13. The retention times of the chlorite, chloride, chlorate and perchlorate peaks obtained with 2.0 mM sodium carbonate at flow rate of 1.80 ml/min were 2.60, 2.92, 3.95 and 19.32 minutes, respectively. The chromatogram obtained with this isocratic condition is presented in Figure 3.11.

The elution system was developed for the analysis of chlorine-containing anions under the ion chromatographic method. The weakly and the strongly retained anions could be separated in the single injection using the gradient and isocratic systems which provided short analysis times. Recently, a single column ion chromatography (SCIC) method for the determination of the anions, including chloride, chlorite, chlorate, nitrate, sulfate and perchlorate was developed by M.Biesaga et al.[32] This method was based on Vydac 302 IC column with 1.0 mM potassium hydrogen-phthalate at a flow rate of 2.5 ml/min and these species were detected with UV detection at 254 nm. The separation was achieved with an analysis time longer than 40 minutes, of which the results are shown in Figure 4.2. It could be concluded that the established conditions in this research enabled the complete separation of chlorine-containing anions within a relatively short analysis time under the ion chromatographic method. Moreover, this gradient system could decrease a limitation of the isocratic system, yielding better resolution between adjacent peaks and shorter analysis time.

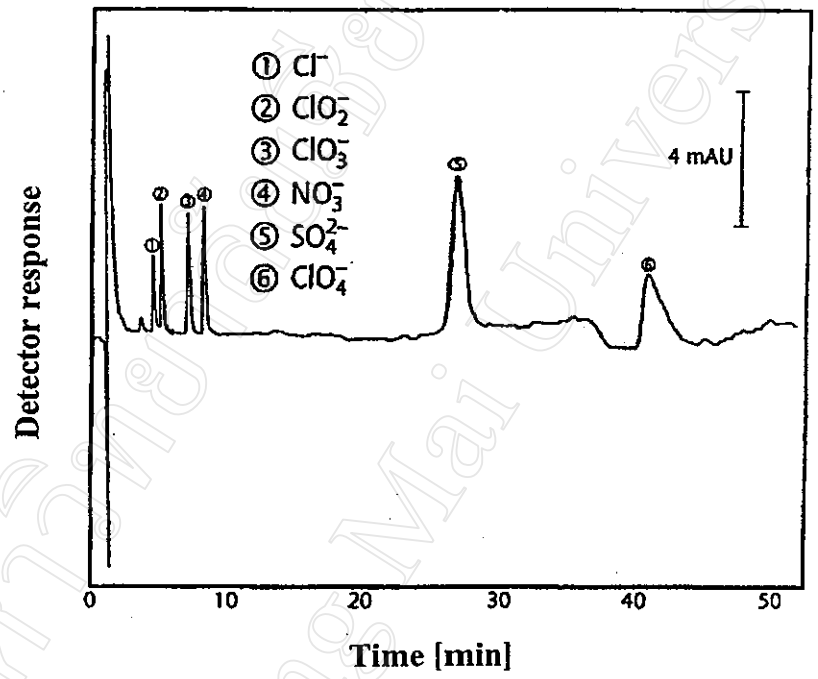

Figure 4.2 Chromatogram of standard mixture of anions obtained with Vydac 302 IC column, 1.0 mM potassium hydrogen-phthalate at flow rate of 2.5 ml/min and UV detection at 254 nm.[32]

The retention mechanism is the interaction between chlorine-containing anions and the resin matrix[48],which involves the electrostatic force and the displacement of counter ions to the fixed sites with similar charge of the analyte anions. The ion-exchange separation relies on the different affinities of the anions to the fixed ions in consequence of the differences of the anions such as charge, size and structure of the different ionic solutes.[21]

$$\underset{\text{ion exchange}}{\text{Resin}\text{—}NR_3^+A^-} + \underset{\text{solution}}{E^-} \longleftrightarrow \underset{\text{ion exchange}}{\text{Resin}\text{—}NR_3^+E^-} + \underset{\text{solution}}{A^-}$$

The anion exchange process is represented by the above equation, where the subscripts A and E stand for the analyte anions and the eluent anions, respectively. Firstly, the anion exchange column is equilibrated by passing the eluent through the ion exchanger to replace all of the exchangeable sites on the polymeric materials with the eluent anions; this would affect the ion exchange process and can occur at the equilibrium. Then, the standard anion solution is injected onto the column and the analyte anions replace the eluent anions by exchanging with an equivalent number. After that the eluent ions, which are continually pumped through the system, compete with the analyte anions to retain the exchange sites of the polymeric materials.[49] The analyte anions are then eluted through the column into the suppressor and a conductivity detector, respectively.

The eluents employed in this investigation were mostly inorganic salts because salts can control the pH and still perform a duty as the competing ion, which affected the distribution coefficient of the analyte ion.[50] Therefore, the retention and the selectivity of the separation depended on the type of competing ion. In addition, the changing concentration of the competing ion affected the amount of displacement of the

analyte anions, as mass action effect.[21] It is related to a shift of the ion exchange equilibrium constant. The competing ion with a large size, high molecular weight and high charge would have high eluotropic strength such as sodium citrate.

The elution order of chlorine-containing anions obtained with the anion-exchange columns, an IonPac AS4A-SC and a Metrosep A Supp 4, was found to be chlorite, chloride, chlorate and perchlorate ions. All of chlorine-containing anions possessed charge 1-, but they are different in terms of structure and molecular weight. The structure (molecular weight) of chlorite, chloride, chlorate and perchlorate ions are angular (67.45), sphere (35.45), trigonal pyramidal (83.45) and tetrahedral (99.45), respectively. An ion with a high molecular weight that has a low charge density would be poorly solvated. For a small radius of the solvated ion, the ion could be retained onto the ion exchanger with great affinity. It was observed that the perchlorate ion was strongly retained on the anion- exchange column with appearance of a broad and tailing peak. Perchlorate is a polarizable anion, which has the highly delocalized charge and the large molecular volume.[51,52] Therefore, the strong interaction between this analyte ion and the active site on the polymer material occurred, which made the elution of perchlorate very difficult, leading to a band broadening.

In this research, three anion-exchange columns were employed for the development of the elution system. Firstly, the specific characteristics of an IonPac AS4A columns of 250×4.0 mm dimension, a 16 micron diameter microporous resin bead consisted of polystyrene crosslinked with 0.5% divinylbenzene and functionalized with quaternary ammonium groups. The ion exchange capacity is 20 μequiv/column and stable in the pH range 0-14. The second column is an IonPac AS4A-SC column of 250×4.0 mm

dimension, substrated with a 13 micron diameter microporous resin bead of the crosslinking ethylvinylbenzene with 55% divinylbenzene. The anion exchange layer is functionalized with quaternary ammonium groups. The ion exchange capacity and stability are the same as the first column. Moreover, this column is compatible with the conventional organic solvents.[53] The last column, a Metrosep A Supp 4 column of 250× 4.0 mm dimension, substrated with a 9 micron diameter of particle size, consisted of polyvinyl alcohol with quaternary ammonium groups. The column is stable in the pH range 3-12 and also compatible with the organic solvents.

It would be seen that all anion-exchange columns have the same strong anion exchanger as quaternary ammonium groups. An IonPac AS4A and IonPac AS4A-SC columns have the same capacity, but the degrees of cross-linking are different. An IonPac AS4A column has 0.5% degrees of cross-linking while an IonPac AS4A-SC column has 55% degrees of cross-linking, which makes the second column could be contained up to 100% of the conventional organic solvents. However, both columns are of similar selectivity[53] although the particle sizes have a slight difference. With a Metrosep A Supp 4 column, differences in selectivity, when compared to an IonPac AS4A and IonPac AS4A-SC columns, were obtained. It was found that the third column provided higher sensitivity of chlorine-containing anions as shown in Table 3.5 and Figure 3.8. This could be contributed to the smaller particle size. Additionally, this column can improve the resolution between the system peak and the weakly retained ions. However, it was observed that longer retention times of the weakly retained ions were obtained, but with no increase in the resolution of these anions, as shown in Tables 3.4, 3.6, 3.8 and 3.9.

The signals of chlorine-containing anions were detected using a conductivity detector whereby the background signal of the eluent ion was decreased with

the neutralization reaction in the suppressor system.[54,28] The suppressor cell consisted of the cation exchange membranes and the electrodes placed along the length of the regenerant channels. The electrical current is continuously applied across the electrodes during the operation, in which there is the eluent flow through the cell. The water in the regenerant channels is electrolyzed as follows in the equation.

$$\text{At the anode} : \quad 2H_2O \rightarrow 4H^+ + O_{2(g)} + 4e- \qquad .....(4.1)$$

$$\text{At the cathode} : \quad 2H_2O + 2e- \rightarrow 2OH^- + H_{2(g)} \qquad .....(4.2)$$

The hydronium ions and oxygen gas are generated at the anode, while the cathode generated the hydroxide ions and hydrogen gas. The hydronium ions would be moved from the anode chamber by passing through the cation exchange membrane into the eluent chamber to neutralize the eluent ions as the carbonate and the citrate ions. The sodium ions in the eluent passed through this membrane into the cathode chamber to maintain the electronic neutrality with the hydroxide ions. The solution of carbonic or citric acid, which continually flows into a conductivity detector, would produce the background conductivity. The conductivity detection is based on the electrical conductivity of the ionic solution when placed between two oppositely charged electrodes. [55] Therefore, the presence of any anions in the solution allowed the electrical current to flow between the electrodes. The signal of the conductivity value is directly proportional to the concentration of the conductive species in the solution.

Method performance of the gradient system obtained with an IonPac AS4A-SC anion-exchange column, the eluents of 1.0 mM citric acid with 1.0 mM sodium citrate at flow rate of 2.00 ml/min and a conductivity detector was improved in terms of

linearity range, reproducibility and detection limit for chlorine-containing anions. The linearity range for chlorite, chloride, chlorate and perchlorate were studied in the range of 0.4-200, 0.2-100, 0.2-200 and 0.4-200 ng/µl, respectively. The results were evaluated using the external calibration curves and the correlation coefficient (r^2). Table 3.14 lists the concentration ranges investigated and the linearity curves are presented in Figure 3.12. The correlation coefficients of chlorite, chloride, chlorate and perchlorate ions were 0.9890, 0.9998, 0.9999 and 0.9999, respectively. These results provided excellent linear regressions for chloride, chlorate and perchlorate except for chlorite, which is possibly due to the effect of system peak. The chlorite peak appeared just after the system peak. At low concentration of chlorite ion, 0.2 ng/µl, the peak could not be integrated due to the sudden drop of system peak and the limited separation between these two peaks occurred at a high concentration of chlorite, resulting in an error in the integration of peak area. However, the linear regression is sufficient for the analysis of this anion. The reproducibility of all anions was investigated using seven replicate injections of the chlorine-containing anion solution. The retention time and peak area precisions expressed in terms of the relative standard deviation (RSD) were between 0.30-0.61% and 1.21-3.68%, which are shown in Tables 3.15 and 3.16, respectively. The detection limit and the minimum detectable quantity for the analyte ions were improved using the seven replicate injections of the lowest concentration of chlorine-containing anions. The results obtained in this investigation are summarized in Tables 3.17 and 3.18. The detection limits for chlorite, chloride, chlorate and perchlorate were found to be 0.03, 0.01, 0.02 and 0.06 ng, whereas the minimum detectable quantities were 0.0043, 0.0012, 0.0062 and 0.0122 ng.sec, respectively. The chromatograms are presented in Figure 3.13.

The accuracy of quantitative analysis for chlorine-containing anions in the developed system was improved using the spiked recovery method. The water samples

selected in this investigation consisted of the public water supplies in Maharajh Nakorn Chiang Mai Hospital, Rujirawong swimming pool water and the natural water of Mae-Ping river. The three-concentration levels of the standard solution were spiked into these samples, and then preserved with the ethylenediamine solution, as described in Section 2.8. The quantitative recoveries for all anions were 85.40% at the 4.41%RSD, 112.97% at the 5.46%RSD, 85.91% at the 3.06%RSD and 99.27% at the 1.05%RSD, as shown in Tables 3.19-3.22, respectively. Figures 3.14-3.15 show the comparison of the chromatograms between the spiked and non-spiked samples for the public water supplies in Maharaj Nakorn Chiang Mai Hospital and Rujirawong swimming pool water in Chiang Mai University, respectively.

The nine sources of the natural water, three sources of the public water supplies and three sources of the swimming pool water were selected for the determination of chlorine-containing anions under this gradient method. The external standard method was employed for the quantitative analysis by the construction of the calibration curves, obtained by plotting the average peak area against the concentration of each anion. The calibration curves are shown in Figure 3.16. The chlorine-containing anions, found in the water samples were chloride and chlorate ions. The results obtained are summarized in Table 3.23. The chromatograms of some water samples obtained with the natural water in a longan orchard, the public water supplies in Chiang Mai University, the swimming pool water in Amari Rincome Hotel and the natural water in Ang-Kaew reservoir are presented in Figures 3.17-3.20, respectively.

Method performance of the isocratic system obtained with the Metrosep A Supp 4 anion-exchange column, the sodium carbonate eluent at flow rate of 1.80 ml/min and a conductivity detector improved the linearity range, reproducibility and detection

limit for chlorine-containing anions. The linearity ranges for chlorite, chloride, chlorate and perchlorate were studied in the ranges of 0.1-100, 0.1-100, 0.1-100 and 0.2-100 ng/μl, respectively. The results were evaluated using the external calibration curves and the correlation coefficient (r^2). Table 3.24 shows the linear concentration ranges investigated and the linearity curves are presented in Figure 3.21. The correlation coefficients of these anions were 0.9990, 0.9998, 0.9989 and 0.9999, respectively. These results provided excellent linear regressions for chlorite, chloride, chlorate and perchlorate. The reproducibility of all anions was investigated using seven replicate injections of the chlorine-containing anion solution. The retention time and peak area precisions expressed in terms of the relative standard deviation (RSD) were between 0.25-1.00% and 0.56-2.14%, which are shown in Tables 3.25 and 3.26, respectively. The detection limit and the minimum detectable quantity for the analyte ions were improved using the seven replicate injections of the lowest concentration of chlorine-containing anions. The results obtained in this investigation are summarized in Tables 3.27 and 3.28. The detection limits for chlorite, chloride, chlorate and perchlorate were found to be 0.005, 0.002, 0.007 and 0.07 ng, whereas the minimum detectable quantities were 0.0006, 0.0002, 0.0013 and 0.0185 ng.sec, respectively. The chromatograms are presented in Figure 3.22.

The accuracy of quantitative analysis for chlorine-containing anions in the developed system was improved using the spiked recovery method. The water samples were selected in this investigation consisted of the public water supplies in Maharaj Nakorn Chiang Mai Hospital, Rujirawong swimming pool water and the natural water of Mae-Ping river. The three-concentration levels of the standard solution were spiked into these samples, and then preserved with the ethylenediamine solution, as described in Section 2.8. The quantitative recoveries for all anions were 90.07% at the 5.32%RSD, 115.47% at the 4.75%RSD, 84.13% at the 3.44%RSD and 98.63% at the 0.74%RSD, as

shown in Tables 3.29-3.32, respectively. Figures 3.23-3.24 show the comparison of the chromatograms between the spiked and non-spiked samples for Rujirawong swimming pool water in Chiang Mai University and the natural water in Mae-Ping river, respectively.

The nine sources of the natural water, three sources of the public water supplies and three sources of the swimming pool water were selected for the determination of chlorine-containing anions under this gradient method. The external standard method was employed for the quantitative analysis by the construction of the calibration curves, obtained from plotting the average peak area against the concentration of each anion. The calibration curves are illustrated in Figure 3.25. The chlorine-containing anions, found in the water samples were chloride and chlorate ions. The results obtained are summarized in Table 3.33. The chromatograms of some water samples obtained with the natural water in a longan orchard, the public water supplies in Maharaj Nakorn Chiang Mai Hospital, the swimming pool water in Chiang Mai University and the natural water in Mae-Ping river, are presented in Figures 3.26-3.29, respectively.

In the analysis of chlorine-containing anions using the gradient and the isocratic conditions, the mixtures of standards and the common anions such as fluoride, nitrate, phosphate and sulfate were also injected into the ion chromatograph. Chlorine-containing anions were identified by matching the retention time of each peak in the chromatogram of sample with each peak in the chromatogram of the standard solution. However, the results were further confirmed with the addition method by adding the individual standard anions into the water samples. Figures 3.30-3.33 show the comparison between the chromatograms of the sample and the sample added with chlorite, chloride, chlorate and perchlorate, respectively.

Different concentrations of chlorine-containing anions were found in several water samples using the gradient and isocratic systems. In the analysis of seven natural water samples from the longan orchard that had used potassium chlorate as the chemical fertilizer, the results showed no appearance of all species except the chloride ion in the range of 3.77-6.39 ng/μl. Two water samples from natural sources, Ang-Kaew reservoir and Mae-Ping river, were found to contain only chloride ion in the range of 2.68-3.70 ng/μl. In the analysis of three samples of public water supplies in Chiang Mai University, Providence Hotel and Maharaj Nakorn Chiang Mai Hospital, all samples were found to contain chloride ion in the range of 9.15-18.87 ng/μl and one sample from Maharaj Nakorn Chiang Mai Hospital was found to contain chlorate ion at 0.89 ng/μl. Analysis of three water samples from Rujirawong swimming pool, the swimming pool of 103 Condominium and the swimming pool of Amari Rincome Hotel, revealed that these samples contained chloride and chlorate ions in the ranges of 259.50-430.80 ng/μl and 17.60-45.50 ng/μl, respectively. Especially high concentrations of these ions were found in the swimming pool water samples due to the fact that the swimming pool had been affected by the disinfection process, involving the use of chlorine, chlorine dioxide or ozone as a disinfectant.[35,56] In this process various anions are formed, including chlorite, chloride and chlorate.[57,58] These anions were likely to remain in the water. However, the chlorite ion was not found because this anion could react with the metal ions and the organic materials in the water.[2] As for the chlorate ion, its detected quantities in the swimming pool water were higher than a Maximum Contaminant Level (MCL) of 1.0 ng/μl for this anion according to a recommendation of the US Environmental Protection Agency (EPA).[32]

In the development of the novel method, the gradient system with two eluents, the validity of this method was studied by comparing the results of the quantitative analysis in the samples with the accepted method. The F test and t test were employed to indicate whether there was a significant difference between the two methods based on the standard deviations and the mean, respectively. The results of the comparison of the variance for the quantity of chloride and chlorate between the gradient and isocratic systems are shown in Tables 3.34 and 3.35, respectively. For the analysis of chloride, the tabulated F value at 95% confidence level for ν_1 and ν_2 = 10 is 2.98, whereas the calculated value was lower. Therefore, it could be concluded that there is no significant difference in the precision for the determination of the chloride ion using the two methods. However, the results of the analysis of chlorate showed that the calculated F value was higher than the tabulated value at 95% confidence level for $\nu_1 = 6$ and $\nu_2 = 5$, then there is a significant difference between the variance of the two method. The mean values of the quantity of chlorite and chlorate using both methods were compared with the T test. The results of the analysis of chloride and chlorate are shown in Tables 3.36 and 3.37, respectively. In the analysis of chloride, the tabulated t value at 95% confidence level for four degrees of freedom is 2.78, whereas the calculated value was lower than this. In the analysis of chlorate, the calculated t value was lower than the tabulated value at 95% confidence level for two degrees of freedom. Therefore, it could be concluded that there is no significant difference in the results obtained for the determination of the chloride and chlorate ions between the two methods at this confidence level.

The comparisons of the performance and accuracy obtained with the gradient and the isocratic systems are summarized in Table 3.38. The linearity was

improved in the wide concentration ranges, which provided a good linear relationship between the peak area and the concentration of chlorine-containing anions with the satisfactory correlation coefficients for both systems. Good precisions of retention times and peak areas were obtained for all anions with relative standard deviations (RSD) of 0.30-3.68% in the gradient system and 0.25-2.14% in the isocratic system. Furthermore, the developed methods could offer high sensitivity for the analysis of chlorine-containing anions. Although the detection limits obtained with the gradient system were higher than those obtained with the isocratic system, both methods proved to give sufficient efficiency for the quantitative analysis of chlorine-containing anions in the public water supply samples. These methods can also be applied to the analysis of all anions in the drinking water samples except the species of perchlorate due to the inadequate sensitivity of detection. The derivatization[59] or the use of a large loop injection[60,52] could enhance the sensitivity of analytical method. The accuracy of the quantitative analysis for chlorine-containing anions in the water samples was improved using the spiked recovery method. The results obtained for the spiked anions were between 84.13-115.47% with the relative standard deviation values of 0.74-5.46% in both methods. The spike method was employed to evaluate the sample preparation procedure, in which the samples were preserved with the ethylenediamine (EDA) solution to prevent the degradation of chlorite from the metal-catalytic reactions and the reactivity towards free chlorine as a residual disinfectants.[37] The results showed that the acceptable recoveries for chlorine-containing anions were obtained with the preservation process in the gradient and isocratic methods.

It was observed that water with high purity was very important for the analysis of the inorganic species, especially in the gradient system. Since the quality of

water could have the effect on the shape of peak, especially the perchlorate peak, ultra pure water, i.e. Milli-Q and Milli-RX water, was used throughout in this research.

The gradient system established in this work using the two eluents as citric acid and sodium citrate could provide both high performance and good accuracy in the analysis of chlorine-containing anions. Only the detection limits were still worse than those in the isocratic system. Although the analysis time for the complete separation of all anions was 18.0 minutes, the total time for the operation in the single injection, including the time of restabilization of the baseline with the weakly eluent, was approximately 23 minutes. However, a gradient system obtained with two eluent types with equal conductivity value and different eluent strength would be an alternative technique for the application using the ion chromatographic method.

4.2 Conclusions

A sensitive ion chromatographic method was developed for the analysis of chlorine-containing anions such as chlorite, chloride, chlorate and perchlorate in the natural and the treated water samples using the gradient and isocraic elutions. The gradient method was based on the use of an IonPac AS4A-SC column with the gradient condition of 1.0 mM citric acid and 1.0 mM sodium citrate as eluent at flow rate of 2.00 ml/min. The isocratic method was based on the use of a Metrosep A Supp 4 column with 2.0 mM sodium carbonate as eluent at flow rate of 1.80 ml/min. Both systems employed a conductivity detector. These methods provided high precision and good linearity for all species over the concentration range investigated and low detection limits were obtained with a 100 μl injection, which were enough for analysis of these species in the water

samples. The accuracy of analytical method was improved using the spiked recovery method, which provided the acceptable recoveries for these species in the range of 84.13-115.47%. The complete separation of all species was achieved in the short analysis time and with no interference from the common anions using the two methods.

Analysis of chlorine-containing anions using both methods was applied to the analysis of fifteen water samples. Nine water samples from natural sources were found to contain only chloride ion in the range of 2.68-6.39 ng/μl. Three samples from public water supplies were found to contain chloride ion in the range of 9.15-18.87 ng/μl and one sample from these sources was found to contain chlorate ion at 0.89 ng/μl. The three water samples from swimming pool water were found to contain chloride and chlorate ions in the ranges of 259.50-430.80 ng/μl and 17.60-45.50 ng/μl, respectively. The gradient system was developed using the two eluent types with equal conductivity value but different eluent strength. Furthermore, the better efficiency of separation and the shorter analysis time were obtained with this gradient method using the ion chromatographic method.