CHAPTER 2

EXPERIMENTAL

2.1 Apparatus

1) Ion chromatograph DX-300, manufactured by Dionex Corp, U.S.A., consisting of :
    (a) A Rheodyne model 9126 injection valve
    (b) Anion guard column, IonPac AG4A (5x50mm)
    (c) Anion separation column, IonPac AS4A (5x250mm)
    (d) Anion guard column, IonPac AG4A-SC (5x50mm)
    (e) Anion separation column, IonPac AS4A-SC (5x250mm)
    (f) Anion separation column, a Metrosep A Supp 4 (5x250mm)
    (g) Anion self regenerating suppressor-I (4 mm)
    (h) A conductivity detector
    (i) BDS, Barspec Data System, Barspec System, Inc., Israel
2) Filter unit, Millipore, U.S.A.
3) Membrane filter, 0.45 μm cellulose acetate, Sartorius, Germany
4) Autopipette, Merck, Germany
5) Ultra-sonicator, Model 8891, Cole–Parmer Instrument Co., U.S.A.
6) Vacuum pump, Waters Associates, U.S.A.
7) Analytical Balance, Sartorius Basic BA 210s, Germany
8) Stainless steel syringe filter holder, Advantec MFS, Inc., U.S.A.
9) Glass syringe, Advantec MFS, Inc., U.S.A.

2.2 Chemicals

1) Sodium carbonate (Na_2CO_3), 99.8%, Merck, Germany
2) Sodium bicarbonate ($NaHCO_3$), 99.8%, Merck, Germany
3) Sodium chloride (NaCl), 99.5%, Carlo Erba, Italy
4) Sodium chlorite ($NaClO_2$), 80%, BDH, England
5) Sodium chlorate ($NaClO_3$), 99%, RdH, Germany
6) Sodium perchlorate ($NaClO_4$), 95%, BDH, England
7) Sodium hydroxide (NaOH), 99.0%, Merck, Germany
8) Sodium benzoate (C_6H_5OONa), 99%, BDH, England
9) Sodium oxalate ($Na_2C_2O_4$), 99.5%, Fluka, Switzerland
10) Sodium citrate ($Na_3C_6H_5O_7.2H_2O$), 99-100%, BDH, England
11) Sodium tetraborate ($Na_2B_4O_7.10H_2O$), 99.5%, Merck, Germany
12) Potassium chromate (K_2CrO_4), 99.5%, Merck, Germany
13) Potassium chloride (KCl), 99%, Merck, Germany
14) Potassium hydrogen phthalate ($C_8H_5KO_4$), 99.8-100.2%, BDH, England
15) Nitric acid (HNO_3) (~65%), Merck, Germany
16) Phthalic acid ($C_6H_4(COOH)_2$), 99%, BDH, England
17) Citric acid ($C_6H_8O_7.H_2O$), 99.0-102.0%, Carlo Erba, Italy
18) Hydrochloric acid (HCl), 37%, Merck, Germany
19) Ethylenediamine ($C_2H_4N_2H_4$), 98%, Fluka, Switzerland
20) Acetonitrile (CH_3CN), HPLC grade, Merck, Germany
21) Helium gas, 99.99% (HP), TIG, Thailand
22) Nitrogen gas, 99.99% (HP), TIG, Thailand
23) Milli-Q and Milli-RX water, Chemistry Department, Chiang Mai University

2.3 Water Samples

Chlorine-containing anions, chlorite, chloride and chlorate, are usually found in the treatment water[2], which results from the use of chlorine (Cl_2), chlorine dioxide (ClO_2) or ozone (O_3) as a disinfectant in the disinfection process. Thus, water samples from the public water supplies and the swimming pool were selected and investigated together with the natural water which was likely to be contaminated with perchlorate that resulted from the use of fireworks and matches.[34] Details of the fifteen water samples collected in Chiang Mai Province are presented in Table 2.1.

Table 2.1 Details of the water samples collected for the determination of chlorine-containing anions in this study

Sample Number	Type of the water sample
Sample No.1	Natural water of longan orchard of Mr. Boonpan Tonwanna, Moo 4*
Sample No.2	Natural water of longan orchard of Mr. Somboon Yanawongsa, Moo 4*
Sample No.3	Natural water of longan orchard of Mr. Nikom Khaya, Moo 3*
Sample No.4	Natural water of longan orchard of Mr. Boonrath Phalean, Moo 2*
Sample No.5	Natural water of longan orchard of Mr. Boonma Phokhumma, Moo 4*
Sample No.6	Natural water of longan orchard of Mr. Tha Nanti, Moo 5*
Sample No.7	Natural water of longan orchard of Mr. Sreema Tunkum, Moo 4*
Sample No.8	Public water supply in Chiang Mai University
Sample No.9	Public water supply in Providence Hotel
Sample No.10	Public water supply in Maharaj Nakorn Chiang Mai Hospital
Sample No.11	Rujirawong swimming pool water in Chiang Mai University
Sample No.12	Swimming pool water in the 103 Condominium, Chiang Mai
Sample No.13	Swimming pool water in Amari Rincome Hotel
Sample No.14	Natural water in Ang-Kaew reservoir, Chiang Mai University
Sample No.15	Natural water in Mae-Ping river

* Sarapee District, Chiang Mai Province

2.4 Preparation of Eluent Solutions

In the optimization of ion chromatographic conditions in this work the three columns used were an IonPac AS4A column (4×250 mm), an IonPac AS4A-SC column (4×250 mm) and a Metrosep A Supp 4 column (4×250 mm). The eluent system was optimized for the separation of chlorine-containing anions, namely chlorite, chloride, chlorate and perchlorate. The preparation details of each eluent system are described in the following.

2.4.1 Preparation of eluent solutions for an IonPac AS4A column

The eluent system used for the study of the separation of chlorine-containing anions with an IonPac AS4A column was a combination of sodium carbonate (Na_2CO_3) and sodium bicarbonate ($NaHCO_3$) in the isocratic system. The eluent solutions for this part were prepared as follows.

(a) Stock solution of 100 mM sodium carbonate (MW 105.99)

Sodium carbonate anhydrous (1.0599 g) was dissolved in deionized water and then the solution was diluted to 100 ml with deionized water.

(b) Stock solution of 100 mM sodium bicarbonate (MW 84.01)

Sodium bicarbonate (0.8401 g) was dissolved in deionized water and then the solution was diluted to 100 ml with deionized water.

(c) 1.0 mM sodium carbonate

The 10.0 ml volume of stock solution of 100 mM sodium carbonate was diluted to 1000 ml with deionized water.

(d) 1.0 mM sodium carbonate / 1.0 mM sodium bicarbonate

The 10.0 ml volume of 100 mM sodium carbonate plus 10.0 ml volume of 100 mM sodium bicarbonate was diluted to 1000 ml with deionized water.

(e) 1.0 mM sodium carbonate / 2.0 mM sodium bicarbonate

The 10.0 ml volume of 100 mM sodium carbonate plus 20.0 ml volume of 100 mM sodium bicarbonate was diluted to 1000 ml with deionized water.

(f) 2.0 mM sodium carbonate / 1.0 mM sodium bicarbonate

The 20.0 ml volume of 100 mM sodium carbonate plus 10.0 ml volume of 100 mM sodium bicarbonate was diluted to 1000 ml with deionized water.

(g) 2.0 mM sodium carbonate / 2.0 mM sodium bicarbonate

The 20.0 ml volume of 100 mM sodium carbonate plus 20.0 ml volume of 100 mM sodium bicarbonate was diluted to 1000 ml with deionized water.

(h) 3.0 mM sodium carbonate / 3.0 mM sodium bicarbonate

The 30.0 ml volume of 100 mM sodium carbonate plus 30.0 ml volume of 100 mM sodium bicarbonate was diluted to 1000 ml with deionized water.

(i) 4.0 mM sodium carbonate / 4.0 mM sodium bicarbonate

The 40.0 ml volume of 100 mM sodium carbonate plus 40.0 ml volume of 100 mM sodium bicarbonate was diluted to 1000 ml with deionized water.

(j) 5.0 mM sodium carbonate / 5.0 mM sodium bicarbonate

The 50.0 ml volume of 100 mM sodium carbonate plus 50.0 ml volume of 100 mM sodium bicarbonate was diluted to 1000 ml with deionized water.

2.4.2 Preparation of eluent solutions for an IonPac AS4A-SC column

The eluent system used for the study of the separation of chlorine-containing anions with an IonPac AS4A-SC column consisted of the isocratic and the gradient systems. The gradient system used two eluents with equal conductivity and different eluent strength. Thus, it was necessary to study the conductivity value and the separation of chlorine-containing anions in the isocratic system prior to the study in the gradient system. The eluent solutions of the two systems were prepared as in the following.

(a) 10.0 mM sodium hydroxide (MW 40.00)

Sodium hydroxide (0.40 g) was dissolved in deionized water and then the solution was diluted to 1000 ml with deionized water.

(b) 100 mM sodium benzoate (MW 144.11)

Sodium benzoate (1.4411 g) was dissolved in deionized water and then the solution was diluted to 100 ml with deionized water.

The solution of 0.2, 1.0 and 3.0 mM sodium benzoate were prepared by diluting 2.0, 10.0 and 30.0 ml of 100 mM sodium benzoate to 1000 ml with deionized water, respectively.

(c) 100 mM sodium oxalate (MW 134.00)

Sodium oxalate (1.3400 g) was dissolved in deionized water and then the solution was diluted to 100 ml with deionized water.

The solution of 0.5 mM sodium oxalate was prepared by diluting 5.0 ml of 100 mM sodium oxalate to 1000 ml with deionized water.

(d) 1.0 mM phthalic acid (MW 164.13)

Phthalic acid (0.1641 g) was dissolved with deionized water in a beaker. The beaker was placed in a boiling water bath while stirring the solution until the crystals of phthalic acid had been dissolved. This solution was diluted to 1000 ml with deionized water.

(e) 1.0 mM potassium hydrogen phthalate (MW 204.22)

Potassium hydrogen phthalate (0.2042 g) was dissolved in deionized water and then the solution was diluted to 1000 ml with deionized water.

(f) 12.6 mM and 12.8 mM sodium carbonate (MW 105.99)

Sodium carbonate anhydrous (1.3355 and 1.3567 g) were dissolved in deionized water and then the solutions were diluted to 1000 ml with deionized water, respectively.

(g) 1.0 mM citric acid (MW 210.14)

Citric acid (0.2101 g) was dissolved in deionized water and then the solution was diluted to 1000 ml with deionized water.

(h) 100 mM sodium citrate (MW 294.10)

Sodium citrate (2.9410 g) was dissolved in deionized water and then the solution was diluted to 100 ml with deionized water.

The solution of 0.275, 0.59 and 1.0 mM sodium citrate were prepared by diluting 2.75, 5.9 and 10.0 ml of 100 mM sodium citrate to 1000 ml with deionized water, respectively.

(i) 1.0 mM potassium chromate (MW 194.20)

Potassium chromate (0.1942 g) was dissolved in deionized water and the solution was diluted to 1000 ml with deionized water.

2.4.3 Preparation of eluent solutions for a Metrosep A Supp 4 column

The isocratic system was used for a Metrosep A Supp 4 column in this work. Some of the eluent solutions were the same as the eluent solutions that used for an IonPac AS4A-SC column. The preparation details of the eluent solutions consisting of 3 mM sodium benzoate, 0.59 mM sodium citrate and 0.5 mM sodium oxalate are described in Section 2.4.2. For other eluent solutions, they were prepared as follows.

(a) 10.0 mM sodium tetraborate (MW 381.37)

Sodium tetraborate (3.8137 g) was dissolved in deionized water and the solution was diluted to 1000 ml with deionized water.

(b) 1.0 M sodium carbonate (MW 105.99)

Sodium carbonate anhydrous (10.5989 g) was dissolved in deionized water and then the solution was diluted to 100 ml with deionized water.

The solutions of 0.6, 1.2, 2.0, 6.0 and 12.8 mM sodium carbonate were prepared by diluting 0.6, 1.2, 2.0, 6.0 and 12.8 ml of 100 mM sodium carbonate to 1000 ml with deionized water.

2.5 Preparation of Reagent Solutions for Cleaning System

Since an ion chromatograph was used throughout in this research, it was necessary to clean the system. Cleaning the ion chromatograph involved four parts, (1) advance gradient pump, (2) analytical columns and guard columns, (3) anion self-regenerating suppressor and (4) conductivity detector. The advance gradient pump always required deionized water for cleaning, enabling the inorganic salts of eluent solutions to be washed before and after every operation to avoid the accumulation of salts in the composition of pump. The preparation details of the reagent solutions for cleaning the other parts are described as in the following.

2.5.1 Preparation of reagent solutions for columns

(a) The reagent solutions for cleaning an IonPac AG4A guard column, an IonPac AS4A analytical column, an IonPac AG4A-SC guard column and an IonPac AS4A-SC analytical column in this work were prepared from 500 mM sodium hydroxide and 1.0 M hydrochloric acid as follows.

A 500 mM sodium hydroxide (MW 40.00) solution was used for cleaning up the contaminants soluble in base which become accumulated on the column. The reagent solution was prepared by dissolving 10.0 g of sodium hydroxide in deionized water and diluting the solution to the mark of a 500 ml volumetric flask with deionized water.

A 1.0 M hydrochloric acid (MW 36.46) solution was used for cleaning up the contaminants soluble in acid which become accumulated on the column. The reagent solution was prepared by pipetting 49.27 ml of conc. hydrochloric acid 37% w/w into a 500 ml volumetric flask containing deionized water and the solution was diluted to the mark with deionized water.

(b) Cleaning of a Metrosep A Supp 4 column always required 5% of acetonitrile, deionized water and the eluent solution. By flushing these reagent solutions through the analytical and guard column, respectively, the column could be considered clean.

2.5.2 Preparation of reagent solutions for suppressor

A 0.1 M potassium chloride (MW 74.55) in 1.0 M hydrochloric acid solution used for cleaning the membrane of the anion self-regenerating suppressor was prepared by dissolving 3.7275 g of potassium chloride with deionized water in the beaker. The solution was transferred to a 500 ml of volumetric flask and 50.0 ml conc. hydrochloric acid 37% w/w was pipette into this volumetric flask. Then this solution was diluted to the mark with deionized water.

2.5.3 Preparation of reagent solutions for conductivity detector

A 3.0 M nitric acid (MW 63.01) used for cleaning the cell electrodes was prepared by pipetting 70.0 ml of conc. nitric acid 65 % w/w into a 250 ml volumetric flask and diluting the solution to the mark with deionized water.

A 1.0 mM potassium chloride (MW 74.55) used to calibrate the cell constant of a conductivity detector was prepared by pipetting 50.0 ml of stock solution 10.0 mM potassium chloride into a 500 ml volumetric flask and diluting the solution to the mark with deionized water.

It should be noted that the stock solution 10.0 mM potassium chloride was prepared by dissolving 0.1864 g of potassium chloride with deionized water and diluting to the mark of a 250 ml volumetric flask.

In addition to cleaning systems, anion-exchange column, anion self-regenerating suppressor and conductivity detector, the part was required to clean must be disconnected from each other, otherwise the damage due to contaminants and incompatible reagent solutions could occur.

On the daily basis, the solutions were prepared, filtered through a 0.45 μ m cellulose acetate membrane (Millipore) and degassed in an ultrasonic bath for about 15-20 min to remove micro-particles and dissolved gases, respectively, from the solutions. This could ensure that micro-particles and dissolved gases could not affect the system performance and it is a practice that can lengthen the life-time of column.

2.6 Preparation of Standard Solutions of Chlorine-containing Anions

Stock standard solutions of chlorine-containing anions such as chlorite, chloride, chlorate and perchlorate were prepared by dissolving the corresponding mass of the inorganic salts for each of chlorine-containing anions in Table 2.2 with deionized water. These standard solutions were diluted to the mark of a 100 ml volumetric flask of each anion with deionized water.

Table 2.2 Masses of the inorganic salts of stock standard solutions of chlorine-containing anions, 1000 ng/μl

Chlorine-containing anion	Inorganic salt	Mass (g)
ClO_2^-	Sodium chlorite ($NaClO_2$)	0.1676
Cl^-	Sodium chloride (NaCl)	0.1648
ClO_3^-	Sodium chlorate ($NaClO_3$)	0.1275
ClO_4^-	Sodium perchlorate ($NaClO_4$)	0.1231

Standard stock solutions, 1000 ng/μl of most individual chlorine-containing anions, are stable for at least one month when stored at 4°C, except for the chlorite standard solution which is only stable for two weeks when stored from light at 4°C.[37]

Diluted working standard solutions of chlorine-containing anions consisting of chlorite, chloride, chlorate and perchlorate were freshly prepared from the stock standard solutions by dilution everyday.

2.7 Preparation of Reagent Solution for Preservation of Water Samples

A 100 g/l ethylenediamine preservative solution[37] was prepared by pipetting 5.6 ml of conc. ethylenediamine 98.0% into a 50 ml volumetric flask and diluting the solution to the mark with deionized water. Ethylenediamine solution should be prepared every month.

2.8 Water Sample Collection, Preservation and Storage

The water samples used in this work were collected from local areas in Chiang Mai Province. The samples must be collected in brown glass-bottles or opaque bottles, which could protect the samples from light. The samples still required the preservation for chlorite which may react with the natural organic matter in an untreated water by adding 0.5 ml of the 100 g/l ethylenediamine preservative solution per 1.0 litre of the water samples and stored at 4°C.

The preserved water samples were filtered through a 0.45 μm cellulose acetate membrane and were diluted to a suitable concentration (for some water samples) prior to analysis by using the ion chromatograph. However, these samples should be analyzed within 14 days because the ethylenediamine could preserve chlorite for 14 days.

2.9 Optimization of Ion Chromatographic Conditions

The ion chromatographic conditions were optimized for a study of the separation of chlorine-containing anions such as chlorite, chloride, chlorate and perchlorate using the following three anion-exchange columns: an IonPac AS4A, an IonPac AS4A-SC and a Metrosep A Supp 4. The optimization was investigated in the isocratic and the gradient systems to obtain the conditions that would yield good efficiency of separation and short analysis time.

A mixed standard solution used in this investigation consisted of 5.0, 1.0, 5.0 and 5.0 ng/µl of chlorite, chloride, chlorate and perchlorate, respectively. In the analysis, these solutions were introduced onto the ion chromatograph and the separation of the chlorine-containing anions occurred within the analytical column. Then these anions were detected using a conductivity detector with a temperature compensation at 1.7 %/°C and an output range at 10 µS. The details of optimization for each column are described as follows.

2.9.1 Optimization of conditions for an IonPac AS4A column

For this column, the optimization was carried out to study the effects of the eluent concentrations and the eluent flow rates in the isocratic system, using a mixture of sodium carbonate and sodium bicarbonate as the eluents.

2.9.1.1 The effects of the eluent concentration

A 100 µl mixed standard solution of chlorine-containing anions was injected onto the IonPac AS4A column at a flow rate of 2.00 ml/min, employing the following eluent concentrations.

(a) 1.0 mM sodium carbonate

(b) 1.0 mM sodium carbonate/1.0 mM sodium carbonate

(c) 1.0 mM sodium carbonate/2.0 mM sodium bicarbonate

(d) 2.0 mM sodium carbonate/1.0 mM sodium bicarbonate

(e) 2.0 mM sodium carbonate/2.0 mM sodium bicarbonate

The results of these experiments regarding the retention time and resolution of each anion pair are shown in Tables 3.1 and 3.2.

2.9.1.2 The effects of the eluent flow rate

A 100 μl mixed standard solution of chlorine-containing anions was injected onto the IonPac AS4A column at flow rates of 1.00, 1.50 and 2.00 ml/min by using the following eluent concentrations.

(a) 2.0 mM sodium carbonate/2.0 mM sodium bicarbonate

(b) 3.0 mM sodium carbonate/3.0 mM sodium bicarbonate

(c) 4.0 mM sodium carbonate/4.0 mM sodium bicarbonate

(d) 5.0 mM sodium carbonate/5.0 mM sodium bicarbonate

The results of these experiments regarding the retention time and resolution of each anion pair are shown in Table 3.3.

2.9.2 Optimization of conditions for an IonPac AS4A-SC column

The optimization for this column was carried out to study the effects of the eluent type in the isocratic system in order to select the suitable eluents for use in the gradient system.

2.9.2.1 The effects of the eluent type in isocratic system

A 100 μl mixed standard solution of chlorine-containing anions was injected onto the IonPac AS4A-SC column at a flow rate of 2.00 ml/min, employing the following eluent concentrations.

(a) 10.0 mM sodium hydroxide

(b) 3.0 mM sodium benzoate

(c) 0.5 mM sodium oxalate

(d) 1.0 mM phthalic acid

(e) 1.0 mM potassium hydrogen phthalate

(f) 12.8 mM sodium carbonate

(g) 1.0 mM sodium citrate

(h) 1.0 mM potassium chromate

(i) acetonitrile

The obtained results of the retention time, resolution and peak area of each anion pair are shown in Tables 3.4-3.6, respectively.

2.9.2.2 The effects of the eluent type in gradient system

A pair of the eluent types with equal conductivity value but different of eluent strength were used by injection of a 100 μl mixed standard solution of chlorine-containing anions onto the IonPac AS4A-SC column at a flow rate of 2.00 ml/min. The gradient eluent methods were investigated as follows.

(a) Method A : 10.0 mM sodium hydroxide [1] and 12.6 mM sodium carbonate[2]

Condition : time 1.4 – 1.5 min %[1] 100 to %[2] 100

(b) Method B : 3.0 mM sodium bicarbonate [1] and 12.8 mM sodium carbonate[2]

Condition : time 2.5 – 2.8 min %[1] 100 to %[2] 100

(c) Method C : 3.0 mM sodium benzoate [1] and 0.59 mM sodium citrate[2]

Condition : time 1.8 – 2.0 min %[1] 100 to %[2] 100

(d) Method D : 1.0 mM sodium benzoate [1] and 0.28 mM sodium citrate[2]

Condition : time 2.0 – 2.5 min %[1] 100 to %[2] 100

(e) Method E : 1.0 mM citric acid [1] and 1.0 mM sodium citrate[2]

Condition : time 7.0 – 8.0 min %[1] 100 to %[2] 100

(f) Method F : 0.2 mM sodium benzoate [1] and 12.8 mM sodium carbonate[2]

Condition : time 4.5 – 5.0 min %[1] 100 to %[2] 100

The obtained results, including retention time, resolution of each anion pair and peak area, are shown in Table 3.7 and Figure 3.1. The chromatograms obtained from each method are presented in Figures 3.2-3.7.

2.9.3 Optimization of conditions for a Metrosep A Supp 4 column

The optimization on the third column was carried out to study the effects of the eluent type and the concentration of sodium carbonate in the isocratic system.

2.9.3.1 The effects of the eluent type

A 100 μl mixed standard solution of chlorine-containing anions was injected onto the Metrosep A Supp 4 column at a flow rate of 1.80 ml/min, using the eluent types as follows:

(a) 2.0 mM sodium carbonate
(b) 10.0 mM sodium tetraborate
(c) 3.0 mM sodium benzoate
(d) 0.59 mM sodium citrate
(e) 0.5 mM sodium oxalate
(f) acetonitrile

The obtained results, including the retention time, resolution of each anion pair and peak area, are shown in Tables 3.8-3.9 and Figure 3.8, respectively.

2.9.3.2 The effects of the concentration of sodium carbonate

A 100 μl mixed standard solution of chlorine-containing anions was injected onto the Metrosep A Supp 4 column at a flow rate of 1.80 ml/min, using the concentration of sodium carbonate as follows:

(a) 0.6 mM sodium carbonate
(b) 1.2 mM sodium carbonate
(c) 2.0 mM sodium carbonate
(d) 6.0 mM sodium carbonate
(e) 12.8 mM sodium carbonate

The obtained results, the retention time, resolution of each anion pair and peak area, are shown in Tables 3.10-3.11 and Figure 3.9, respectively.

2.10 Optimal Conditions for Chlorine-containing Anions

From the investigation of the isocratic and gradient systems for analysis of chlorite, chloride, chlorate and perchlorate using three anion exchange columns and a conductivity detector, the optimal conditions were selected to obtain a minimum analysis time and sufficient resolution between the adjacent peaks. These conditions were based on an IonPac AS4A-SC column with the gradient system and a Metrosep A Supp 4 column with the isocratic system. Both of the optimal conditions are described as in the following:

2.10.1 Gradient conditions obtained with an IonPac AS4A-SC column

The optimal gradient conditions for analysis of chlorite, chloride, chlorate and perchlorate obtained with an IonPac AS4A-SC anion-exchange column are summarized in Table 3.12.

For the gradient system, either normal integration or subtraction integration could be used for the operation on a chromatogram.[38] The chromatograms of chlorine-containing anions under this gradient condition using both integration modes are presented in Figure 3.10.

2.10.2 Isocratic conditions obtained with a Metrosep A Supp 4 column

The optimal isocratic conditions for analysis of chlorite, chloride, chlorate and perchlorate obtained with a Metrosep A Supp 4 anion-exchange column are summarized in Table 3.13. The chromatogram of this isocratic eluent method is presented in Figure 3.11.

To apply the method of analysis for the samples, the optimal conditions in the gradient and the isocratic systems were studied in terms of performance and accuracy. The two methods were then directed to determine the chlorine-containing anions, chlorite, chloride, chlorate and perchlorate, in the water samples, which the investigations were established in the following:

2.11 Gradient System Using an IonPac AS4A-SC Anion-exchange Column

Investigation on linearity, reproducibility and detection limit for the determination of chlorine-containing anions was attempted using an IonPac AS4A-SC column and a conductivity detector, the gradient eluents consisting of 1.0 mM citric acid and 1.0 mM sodium citrate at flow rate of 2.00 ml/min with a temperature compensation at 1.7 %/°C and an output range of 10 µS. Details of this part are described as follows.

2.11.1 Linearity of gradient system

The concentrations of chlorite, chloride, chlorate and perchlorate were investigated in the range of 0.2-200.0 ng/µl. A 100 µl mixed standard solution was injected onto an IonPac AS4A-SC column under the established condition. The external calibration curves were constructed from the experimental data, average peak areas and the concentration of chlorine-containing anions. The obtained results of average peak area and the calibration curves are shown in Table 3.14 and Figure 3.12, respectively.

2.11.2 Reproducibility of gradient system

A mixed standard solution for studying the reproducibility consisted of 8.0, 0.8, 8.0 and 8.0 ng/µl of chlorite, chloride, chlorate and perchlorate, respectively. The seven replicates of this standard solution were performed with 100 µl injection volume onto the IonPac AS4A-SC column under the established condition. The retention time and average peak area of each chlorine-containing anion were used to calculate the relative standard deviation in accordance with the equations 1.18 and 1.19. The obtained results are shown in Tables 3.15 and 3.16.

2.11.3 Detection limit of gradient system

The lowest concentration of chlorite, chloride, chlorate and perchlorate, used for determination of detection limit, were 0.05, 0.01, 0.05 and 0.05 ng/μl, respectively. Under the same experimental conditions, the triplicate runs of blank solution with a 100 μl injection volume, was introduced onto the IonPac AS4A-SC column to investigate the noise level (n) of each chlorine-containing anion. The seven replicates of mixed standard solution were carried out to investigate the peak signal response (R) and the width at half height of peak ($w_{0.5}$); the data obtained for each anion are shown in Table 3.17. The detection limit (L) and minimum detectable quantity (MDQ) of chlorine-containing anions could be calculated from these data using equations 1.20 and 1.21, respectively. The obtained results and the chromatograms of chlorine-containing anions are shown in Table 3.18 and Figure 3.13, respectively.

2.11.4 Recovery of chlorine-containing anions in the water samples using gradient system

The accuracy for quantification of chlorine-containing anions with the IonPac AS4A-SC column was improved by the spiked recovery method. The different types of water samples, which were selected for this investigation, consisted of the water samples from (1) Maharaj Nakorn Chiang Mai Hospital (2) Rujirawong swimming pool in Chiang Mai University and (3) Mae-Ping river. The three concentration levels of mixed standard solution were spiked into the water samples, preserved with ethylenediamine solution at 4.0, 8.0 and 16.0 ng/μl. These samples were filtered through 0.45 μm cellulose acetate membrane and then a 100 μl sample was introduced onto the ion chromatograph under the established condition. The percent recovery of chlorine-containing anions was calculated using equation 1.22. The obtained results of percent recoveries of each chlorine-containing anion are shown in Tables 3.20-3.22 and the chromatograms are presented in Figures 3.14 and 3.15.

2.11.5 Determination of chlorine-containing anions in the water samples using gradient system

In this work, the optimized method was established for determination of chlorine-containing anions in the water samples. The natural water, the public water supplies and the swimming pool water were selected to be analyzed by using the optimized method. The external standard method was used in the investigation by the calibration curves of chlorine-containing anions, constructed from average peak area and concentration of chlorite, chlorate and perchlorate in the range of 0.4-50.0 ng/μl and chloride in the range of 0.2-25.0 ng/μl.

For the analysis, the 100 μl mixed standard solution, containing chlorine-containing anions in the above concentration, was injected onto the ion chromatograph under the established condition for constructing the external calibration curves, as shown in Figure 3.16. Then the water samples, which had undergone the appropriate preparation, were injected under the same condition. The quantitative analysis of chlorine-containing anions was carried out by making use of the linear equations of each external calibration curve. The obtained results of the analysis are shown in Table 3.23 and the chromatograms of some water samples are presented in Figures 3.17-3.20.

2.12 Isocratic System Using a Metrosep A Supp 4 Anion-exchange Column

Investigation on linearity, reproducibility and detection limit for the determination of chlorine-containing anions was attempted using a Metrosep A Supp 4 column and a conductivity detector using 2.0 mM sodium carbonate at flow rate of 1.80 ml/min with a temperature compensation at 1.7 %/°C and an output range of 10 μS. Details of this part are described as follows.

2.12.1 Linearity of isocratic system

The concentrations of chlorite, chloride, chlorate and perchlorate were investigated in the range of 0.1-100.0 ng/μl. A 100 μl mixed standard solution was injected onto a Metrosep A Supp 4 column using 2.0 mM sodium carbonate at flow rate of 1.80 ml/min. The external calibration curve was constructed from the experimental data, average peak areas and the concentration of chlorine-containing anions. The obtained results of average peak area and the calibration curves are shown in Table 3.24 and Figure 3.21, respectively.

2.12.2 Reproducibility of isocratic system

A mixed standard solution for studying the reproducibility consisted of 10.0, 1.0, 10.0 and 10.0 ng/μl of chlorite, chloride, chlorate and perchlorate, respectively. The seven replicates of this standard solution were performed with 100 μl injection volume onto the Metrosep A Supp 4 column obtained with 2.0 mM sodium carbonate at flow rate of 1.80 ml/min. The retention time and average peak area of each chlorine-containing anion were used to calculate the relative standard deviation using equations 1.18 and 1.19. The obtained results are shown in Tables 3.25 and 3.26.

2.12.3 Detection limit of isocratic system

The lowest concentration chlorite, chloride, chlorate and perchlorate, used for determination of detection limit, was 0.05 ng/μl of each anion. Under the same experimental conditions, the triplicate runs of blank solution with a 100 μl injection volume, was introduced onto the Metrosep A Supp 4 column to investigate the noise level (n) of each chlorine-containing anion. The seven replicates of mixed standard solution were carried out to investigate the peak signal response (R) and the width at half height of peak ($w_{0.5}$), the data for each anion are shown in Table 3.27. The detection limit (L) and minimum detectable quantity (MDQ) of chlorine-containing anions could be calculated from these data using equations 1.20 and 1.21, respectively. The obtained results and the chromatograms of chlorine-containing anions are shown in Table 3.28 and Figure 3.22, respectively.

2.12.4 Recovery of chlorine-containing anions in the water samples using isocratic system

The accuracy for quantification of chlorine-containing anions with the Metrosep A Supp 4 column was improved by the spiked recovery method. The different type of water samples, were selected for this investigation, consisted of the water samples from (1) Maharaj Nakorn Chiang Mai Hospital (2) Rujirawong swimming pool in Chiang Mai University and (3) Mae-Ping river. The three concentration levels of mixed standard solution were spiked into the water samples, preserved with ethylenediamine solution. The three concentration levels of chlorite and chlorate were 2.0, 4.0 and 8.0 ng/μl, chloride were 1.0, 2.0 and 4.0 ng/μl and perchlorate were 4.0, 8.0 and 16.0 ng/μl. These water samples were filtered through 0.45 μm cellulose acetate membrane and then a 100 μl water sample was introduced onto a Metrosep A Supp 4 column under the established condition. The percent recovery of chlorine-containing anions was calculated using to the equation 1.22. The obtained results of percent recoveries of each chlorine-containing anion are shown in Tables 3.29-3.32 and the comparison of chromatograms between the non-spiked sample and the spiked sample is illustrated in Figures 3.23 and 3.24.

2.12.5 Determination of chlorine-containing anions in the water samples using isocratic system

The water samples selected for analysis using isocratic system were natural water, the public water supplies and the swimming pool water, in the same manner as the samples for gradient system in order to compare the results of the quantitative analysis of the two systems using the ion chromatographic method. The external standard method was used in the investigation. The calibration curves of chlorine-containing anions were constructed from average peak area and concentration of chlorite, chlorate and perchlorate in the range of 0.4-50.0 ng/μl and chloride in the range of 0.2-25.0 ng/μl.

For the analysis, the 100 μl mixed standard solution containing chlorine-containing anions in the above concentration was injected onto the ion chromatograph under the established condition for construction of the external calibration curves, as shown in Figure 3.25. Then the water samples, which had undergone the appropriate preparation, were injected under the same condition. The quantitative analysis of chlorine-containing anion was carried out by calculating from the linear equations of each external calibration curve. The obtained results of the analysis are shown in Table 3.33 and the chromatograms of some water samples are presented in Figures 3.26-3.29.

2.13 Identification of Chlorine-containing Anions in the Water Samples

To identify chlorine-containing anions as the interested anions, the addition method was employed by adding the individual standard anions into the water samples, and then a 100 μl mixed standard solution was injected onto the ion chromatograph using both of the established conditions. Furthermore, this method was still used to confirm the established conditions that could be used to analyze the chlorine-containing anions not found in the water samples. The chromatograms are presented in Figures 3.30-3.33.

2.14 Comparison of the Quantitative Analysis of the Water Samples Using Gradient and Isocratic Systems

The quantitative analysis of chlorine-containing anions in the water samples used gradient and isocratic systems under the optimal ion chromatographic method. To establish the validity of the experimental data, the two sets of these data were proved by comparison of a difference of two experimental data employing the precision and the experimental means to get along in the same way. These procedures were carried out using the F test and the t test, which could calculate using equations 1.23 and 1.24, respectively. The obtained results are shown in Tables 3.34-3.37.

The obtained results of performance and accuracy in the gradient and isocratic systems were directly compared, as shown in Table 3.38.