

CHAPTER 2

EXPERIMENTAL

2.1 Chemicals and Materials

1. Alizarin red S, $C_{14}H_7NaO_7S$, A.R., Carlo Erba, Italy
2. Calcium chloride dihydrate, $CaCl_2 \cdot 2H_2O$, A.R., BDH, England
3. Copper nitrate trihydrate, $Cu(NO_3)_2 \cdot 3H_2O$, RPE, Carlo Erba, Italy
4. Eriochrome cyanine R, $C_{23}H_{15}Na_3O_9S$, A.R., Fluka, Switzerland
5. Glacial acetic acid, CH_3COOH , 99.8%, A.R., Carlo Erba, Italy
6. Hydrochloric acid, HCl , 37%, A.R., Merck, Germany
7. Magnesium chloride, $MgCl_2$, A.R., Fluka, Switzerland
8. Nitric acid, HNO_3 , 65%, A.R., Carlo Erba, Italy
9. Sodium chloride, $NaCl$, A.R., Carlo Erba, Italy
10. Sodium fluoride, NaF , Lab Grade, Merck, Germany
11. Sodium hydroxide, $NaOH$, A.R., Labscan, Ireland
12. Sodium nitrate, $NaNO_3$, A.R., BDH, England
13. Sodium 2-(parasulfophenylazo)-1,8-dihydroxy-3,6-naphthalene disulfonate (SPADNS), $C_{16}H_9N_2Na_3O_{11}S_3$, A.R., Fluka, Switzerland
14. Sodium sulfate, Na_2SO_4 , A.R., Carlo Erba, Italy
15. Sulfuric acid, H_2SO_4 , 98%, A.R., BDH, England
16. Tran-1,2 diaminocyclohexane-N,N,N',N'-tetraacetic acid (CDTA), $C_{14}H_{22}N_2O_8$, A.R., Fluka, Switzerland
17. Zirconyl chloride octahydrate, $ZrOCl_2 \cdot 8H_2O$, A.R., Carlo Erba, Italy
18. Zirconyl nitrate dihydrate, $ZrO(NO_3)_2 \cdot 2H_2O$, Pure, Prolabo, France

2.2 Instruments and apparatus

1. Fluoride combination electrode, Cole-Parmer, Model 800-323-4340, USA
2. Hotplate & Stirrer, Bibby, Model CB162, UK
3. pH meter, Metrohm, Model 1744, Switzerland
4. Spectrophotometer, Jenway, Model 6400, UK

2.3 Preparation of the standard solutions and reagents

2.3.1 Fluoride stock solution (100 mg/l)

A 0.2210 g NaF was dissolved in distilled water to produce 1000 ml solution.

2.3.2 Alizarin red S solutions

The Alizarin red S solutions with concentrations of 6.80, 8.50, 10.20, 13.60, 15.30, 17.00 and 18.70 g/l were prepared by weighing 0.68, 0.85, 1.02, 1.36, 1.53, 1.70 and 1.87 g of Alizarin red S respectively, then dissolving each of Alizarin red S in 100 ml of distilled water.

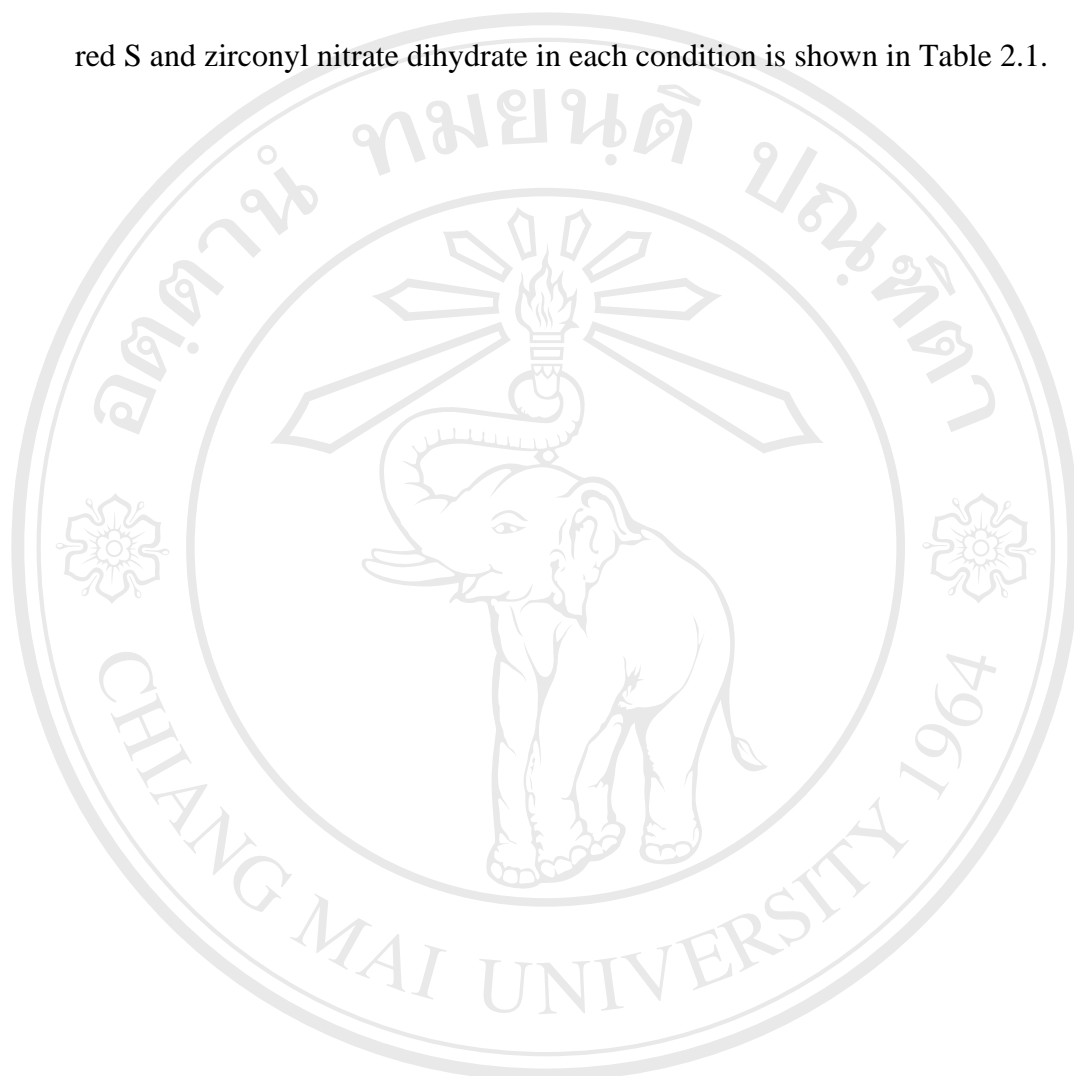
2.3.3 Zirconyl nitrate dihydrate solutions

Prepared the zirconyl nitrate dihydrate solutions with concentrations of 6.50, 7.60, 8.20, 8.70, 9.30, 9.80, 10.90, 13.10, 17.40, 26.10 and 43.50 g/l by weighing 0.65, 0.76, 0.82, 0.87, 0.93, 0.98, 1.09, 1.31, 1.74, 2.61, 4.35 g of zirconyl nitrate dihydrate respectively, and dissolving each of zirconyl nitrate dihydrate in 100 ml of distilled water.

2.3.4 Alizarin red S - Zirconyl nitrate dihydrate solution

The mixed solution of Alizarin red S and zirconyl nitrate dihydrate was prepared by adding 100 ml of Alizarin red S solution to 100 ml of zirconyl nitrate dihydrate solution slowly with constant stirring. Shaked occasionally and allowed to

stand overnight. Diluted 20 ml of this solution to 100 ml to serve as indicator. When not in use, the solutions were best kept in dark closet. The concentrations of Alizarin red S and zirconyl nitrate dihydrate in each condition is shown in Table 2.1.



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Table 2.1 The concentrations of Alizarin red S and zirconyl nitrate dihydrate were mixed in each condition

Concentration of Alizarin red S (g/l)	Concentration of zirconyl nitrate dihydrate (g/l)
6.80	8.70
8.50	8.70
10.20	8.70
13.60	8.70
15.30	8.70
17.00	8.70
18.70	8.70
18.70	6.50
18.70	7.60
18.70	8.20
18.70	9.30
18.70	9.80
18.70	10.90
18.70	13.10
18.70	17.40
18.70	26.10
18.70	43.50

2.3.5 Total ionic strength adjustment buffer: (TISAB)

A 250 ml of distilled water was added into a 500 ml beaker. Placed the beaker on a magnetic stirrer. 29.00 g of NaCl, 28.50 ml of CH₃COOH and 2.00 g of CDTA were dissolved. After the solid dissolved, the solution was cooled and adjusted the pH to 5-5.5 with 6 M NaOH. The solution was cooled down further to the room temperature, transferred it to a 500 ml volumetric flask and diluted to the volume with distilled water.

2.3.6 Hydrochloric acid solutions (1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 mol/l)

Hydrochloric acid solutions with concentrations of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 mol/l were prepared by pipetting 8.30, 16.60, 24.90, 33.20, 41.50, 49.70 and 58.00 ml of concentrated hydrochloric acid into 100 ml volumetric flask and diluting to the volume with distilled water.

2.3.7 Sulfuric acid solutions (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 mol/l)

Prepared 100 ml of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 mol/l sulfuric acid solutions by pipetting corresponding volume of 2.70, 5.40, 8.20, 10.90, 13.60, 16.30, 19.00 ml of concentrated sulfuric acid and making up to the volume with distilled water.

2.3.8 Eriochrome cyanine R solutions

Prepared 500 ml of 8.4×10^{-4} , 1.7×10^{-3} , 3.4×10^{-3} , 6.7×10^{-3} , 1.0×10^{-2} mol/l Eriochrome cyanine R by weighing accurately 0.23, 0.45, 0.90, 1.80 and 2.70 g of Eriochrome cyanine R respectively, dissolving and making up the volume to the mark with distilled water.

2.3.9 Zirconyl chloride octahydrate solutions

The zirconyl chloride octahydrate solutions with different concentrations of 2.0×10^{-4} , 2.2×10^{-4} , 4.0×10^{-4} , 4.2×10^{-4} , 8.0×10^{-4} , 8.3×10^{-4} , 1.6×10^{-3} , 3.3×10^{-3} mol/l were prepared by weighing 0.066, 0.070, 0.130, 0.135, 0.258, 0.266, 0.520, 1.060 g of zirconyl chloride octahydrate respectively, then dissolving each of zirconyl chloride octahydrate in 50 ml distilled water and the solution is called “zirconyl solution”. Adding the appropriate volume of hydrochloric acid and diluting to 1000 ml with distilled water.

2.3.10 SPADNS solutions

Prepared 500 ml of 8.4×10^{-4} , 1.7×10^{-3} , 3.4×10^{-3} , 6.7×10^{-3} and 1.3×10^{-2} mol/l SPADNS solutions by weighing 0.24, 0.48, 0.96, 1.92 and 3.83 g of SPADNS respectively, dissolving and making up to the volume with distilled water.

2.3.11 Sodium hydroxide, 6 mol/l

Prepared 1000 ml of 6 mol/l sodium hydroxide by weighing 240 g of NaOH, dissolving and making up the volume to the mark with distilled water.

2.3.12 Stock solution of interfering ions (calcium, chloride, magnesium, nitrate, phosphate, sulfate and copper), 2000 mg/l

2000 mg/l solutions of interfering ion (Ca^{2+} or Cl^- or Mg^{2+} or NO_3^- or PO_4^{3-} or SO_4^{2-} or Cu^{2+}) was prepared using the compounds indicated in Table 2.2. Weighed the compound with appropriate weight, dissolved and quantitatively transferred into a 1000 ml volumetric flask, then diluted to the volume with distilled water.

Table 2.2 Form and weight of chemicals used in preparation of interfering ion solutions

Stock Solution of	Compound Used	Accurate Weight of, g
Ca ²⁺	CaCl ₂ .2H ₂ O	7.35
Cl ⁻	NaCl	3.29
Mg ²⁺	MgCl ₂ .6H ₂ O	8.36
NO ₃ ⁻	NaNO ₃	2.75
PO ₄ ³⁻	Na ₃ PO ₄ .12H ₂ O	8.00
SO ₄ ²⁻	Na ₂ SO ₄	2.96
Cu ²⁺	Cu(NO ₃) ₂ .3H ₂ O	7.60

2.4 Experimental method

2.4.1 Optimization of Alizarin red S method

To determine the concentration of fluoride by this method, this can be done by preparing a series of standard fluoride solution and samples, adding Alizarin red S-zirconyl nitrate dihydrate solution, hydrochloric and sulfuric acid, then heating the standard fluoride solutions and samples; stopping heating when the solutions boil. Four hours after cooling or on the following day comparing the color of the samples with the standard fluoride solutions. The parameter optimization in this method is needed to find out which parameter will be involved. Therefore, the following parameters namely; concentrations of Alizarin red S, zirconyl nitrate dihydrate, hydrochloric and sulfuric acid will be studied as follows:

a) Concentration of Alizarin red S

Prepared 10 ml of 0.00, 0.50, 1.00, 1.50, 2.00, 2.50 and 3.00 mg/l standard fluoride solutions by serial dilution of 100 mg/l fluoride stock solution. Each of standard fluoride solution was placed in an Erlenmeyer flask. Added 0.20 ml of the mixed solution of 6.80 g/l Alizarin red S and 8.70 g/l zirconyl nitrate dihydrate, added 0.20 ml of 3.0 mol/l hydrochloric acid and 0.20 ml of 1.5 mol/l sulfuric acid. Heated the standard fluoride solutions on a hot plate. Brought the contents rapidly to the boiling point and removed soon after boiling began. Four hours after cooling or the following day, the solution color was observed and measured by a spectrophotometer. The procedure used in this study following that described above but the concentration of Alizarin red S was varied as shown in Table 2.1.

b) Concentration of zirconyl nitrate dihydrate

When 18.70 g/l of Alizarin red S was selected. The effect of zirconyl nitrate dihydrate concentration on the formation of the solution color was studied by varying the concentration of zirconyl nitrate dihydrate as shown in Table 2.1. Each of zirconyl nitrate dihydrate concentration was studied following the procedure **a)**.

c) Concentration of hydrochloric acid

Once the appropriate concentration and the volume of Alizarin red S-zirconyl nitrate dihydrate solution were selected. The concentration of hydrochloric acid which affects the color of the solution was studied by using 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 mol/l of hydrochloric acid. The procedure used in this study following that described in **a)**.

d) Concentration of sulfuric acid

The concentrations of sulfuric acid were studied by using 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 mol/l sulfuric acid, then the experiment was carried out by following the procedure as previously done in a).

2.4.2 Optimization of Eriochrome cyanine R method

The procedure of this method done by adding Eriochrome cyanine R solution and zirconyl chloride octahydrate solution to a series of standard fluoride solutions and samples; measuring the absorbances of the standard fluoride solutions and samples with the spectrophotometer; constructing the calibration curve and determining the amount of fluoride in the samples from the calibration curve. In this method, the intensity of the solution color depends on the acidity and the quantity of dye present. By these reasons, the effects of Eriochrome cyanine R concentration, zirconyl chloride octahydrate concentration and the volume of hydrochloric acid were studied as follows:

a) Concentration of Eriochrome cyanine R

Prepared 10 ml of 0.00, 0.30, 0.60, 0.90, 1.20 mg/l standard fluoride solutions by serial dilution of 100 mg/l fluoride stock solution. Each of standard fluoride solution, 1.00 ml of 8.4×10^{-4} mol/l Eriochrome cyanine R solution was added, followed by 1.00 ml of 8.0×10^{-4} mol/l zirconyl chloride octahydrate solution and mixed well. Measured the absorbance of the standard fluoride solutions and constructed the calibration curve. The procedure used in this study followed that described above but the concentrations of Eriochrome cyanine R were changed from 8.4×10^{-4} mol/l to 1.7×10^{-3} , 3.4×10^{-3} , 6.7×10^{-3} and 1.0×10^{-2} mol/l.

b) Concentration of zirconyl chloride octahydrate

The procedure for determining fluoride ion under this stress was similar to that done previously in a), but the concentrations of zirconyl chloride octahydrate were changed from 8.0×10^{-4} mol/l to 2.0×10^{-4} , 4.0×10^{-4} , 1.6×10^{-3} and 3.3×10^{-3} mol/l, respectively.

c) The volume of hydrochloric acid

The appropriate concentrations of Eriochrome cyanine R and zirconyl chloride octahydrate were selected. The volume of concentrated hydrochloric acid was varied from 700 ml to 500, 600, 800 and 900 ml. In this study, the volume of hydrochloric acid was added to the zirconyl solution before diluting to 1000 ml with distilled water. The procedure was again similar to those as done before.

Under the optimized condition, limit of detection, sensitivity and precision of the method were studied.

2.4.3 Optimization of SPADNS method

To determine the amount of fluoride by SPADNS method, it was done by adding SPADNS solution and zirconyl chloride octahydrate solution to a series of standard fluoride solution and samples; measuring the absorbances of the standard fluoride solutions and samples with the spectrophotometer; constructing the calibration curve and determining the amount of fluoride in the samples from the calibration curve. This method, like the Eriochrome cyanine R method, involves complex equilibria in which the acidity, zirconyl ions and SPADNS pose similar effects. Therefore, the following parameters namely; concentrations of SPADNS, zirconyl chloride octahydrate and the volume of hydrochloric acid were optimized as follows:

a) Concentration of SPADNS

Prepared 10 ml of 0.00, 0.30, 0.60, 0.90, 1.20 and 1.40 mg/l of standard fluoride solutions by serial dilution of 100 mg/l fluoride stock solution. To each of standard fluoride solution, 1.00 ml of 3.4×10^{-3} mol/l SPADNS solution was added and followed by 1.00 ml of 8.3×10^{-4} mol/l zirconyl chloride octahydrate solution, mixed them well. The absorbances of the standard fluoride solutions were then measured with a spectrophotometer and the calibration curve was constructed. Then the experiment was carried out by following the procedure as previously done above but the concentrations of SPADNS were changed from 3.4×10^{-3} mol/l to 8.4×10^{-4} , 1.7×10^{-3} , 6.7×10^{-3} and 1.3×10^{-2} mol/l.

b) Concentration of zirconyl chloride octahydrate

The procedure for determining fluoride ion under this effect was similar to the previously done in a) but the concentrations of zirconyl chloride octahydrate were varied from 8.3×10^{-4} mol/l to 2.2×10^{-4} , 4.2×10^{-4} , 1.6×10^{-3} and 3.3×10^{-3} mol/l respectively.

c) The volume of hydrochloric acid

Once the appropriate concentrations of SPADNS and zirconyl chloride octahydrate solution were selected, the next parameter, the volume of concentrated hydrochloric acid was varied from 700 ml to 500, 600, 800 and 900 ml, respectively.

In this study, the volume of hydrochloric acid was added to the zirconyl solution before diluting the zirconyl solution to 1000 ml with distilled water. After that, the experiment of fluoride determination was carried out using the same procedure as done with other parameters.

Under the optimized condition, limit of detection, sensitivity and precision of the method were studied.

2.4.4 Interference study in Eriochrome cyanine R and SPADNS methods

Seven ions, i.e. Ca^{2+} , Cl^- , Mg^{2+} , NO_3^- , PO_4^{3-} , SO_4^{2-} and Cu^{2+} , were studied by preparing 1.00 mg/l of standard fluoride solution plus the increasing amounts of interfering ions. Tolerant concentration is regarded as a foreign ion concentration which causes $\pm 5\%$ error in an instrument response compared to a response of 1.00 mg/l standard fluoride solution without a foreign ion. The concentrations of seven ions used were in the range of 0 to 500 mg/l.

2.5 Analysis of fluoride in water samples by Eriochrome cyanine R method

Prepared 10 ml of 0.00, 0.30, 0.60, 0.90, 1.20 mg/l standard fluoride solution by serial dilution of the fluoride stock solution. Added 1.00 ml of 3.4×10^{-3} mol/l Eriochrome cyanine R solution followed by 1.00 ml of 8.0×10^{-4} mol/l zirconyl chloride octahydrate solution. Mixed thoroughly, measured the absorbance of each solution and constructed the calibration curve for determining the amount of fluoride in water samples. Water samples were collected in Mueang district of Chiang Mai and Lamphun provinces.

2.6 Analysis of fluoride in water samples by SPADNS method

Prepared 10 ml of 0.00, 0.30, 0.60, 0.90, 1.20 and 1.40 mg/l standard fluoride solution by serial dilution of 100 mg/l fluoride stock solution. Added 1.00 ml of 1.3×10^{-2} mol/l SPADNS solution and 1.00 ml of 8.3×10^{-4} mol/l zirconyl chloride octahydrate solution. Mixed well, measured the absorbance of each solution and

constructed the calibration curve for determining the amount of fluoride in the water samples.

2.7 Analysis of fluoride in water samples by fluoride ion selective electrode method

Prepared 0.10, 0.50, 1.00, 3.00, 5.00 mg/l of standard fluoride solution by serial dilution of 100 mg/l fluoride stock solution. Added 10 ml of TISAB to each 10 ml of the standard. Measured the potential of the fluoride standard solutions by fluoride ion selective electrode against a reference electrode. Constructed the calibration curve for further analysis of the water samples.

All experiments in this study were performed at least three times in order to obtain accurate results.