CHAPTER 2

Experimental

2.1 Instruments and Apparatus

1. Peristaltic pump EYELA mode MP-3N, Tokyo Rikakikal Co., Ltd, Japan.
2. Flow-through cell for UV/VIS spectrometer, 10 mm light path, Perkin Elmer
3. Teflon tubing, inner diameter 1.07 mm.
4. Waterproof pH Tester 10, Eutech Instrument
5. UV/VIS Spectrophotometer model 6305, Jenway Ltd., U.K.
6. UV/VIS Spectrophotometer model 6400, Jenway Ltd., U.K.

2.2 Chemicals

1. Ammonium ferric sulphate dodecahydrate, Analytical grade, Fluka, Switzerland.
2. Aluminium nitrate, extra pure, Merck, Germany.
3. Cadmium Nitrate, Puriss, Fluka, Switzerland.
4. Calcium Nitrate, P&B, Carlo Erba, Italy.
5. Cetyltrimethylammonium bromide, pure, Serva, Germany.
6. Chromium Nitrate, Analytical grade, Merck, Germany.
7. Cobalt Nitrate, Analytical grade, BDH, England.
8. Copper nitrate, RpE, Carlo Erba, Italy.

9. Eriochrome Cyanine R, Indicator grade, Fluka, Switzerland.

10. Glacial Acetic Acid 99.8% (w/v), Commercial grade, Merck, Germany.

11. Hydrochloric Aced 35.4% (w/v), Commercial grade, Merck, Germany.

12. Magnesium Nitrate, GR grade, Merck, Germany.

13. Nickel Nitrate, GR grade, Merck, Germany.

14. Sodium Acetate, Commercial grade, Merck, Germany.

15. Sodium Bromide, pure, BDH, England.

16. Sodium Chloride, Analytical grade, Fluka, Switzerland.

17. Sodium Fluoride, Analytical grade, Merck, Germany.

18. Sodium Hydrogen Carbonate, GR, MERCK.

19. Sodium Hydroxide, Analytical grade, Merck, Germany.

20. Sodium Iodine, Analytical grade, Fluka, Switzerland.

21. Sodium Nitrate, Analytical grade, BDH, England.

22. Sodium Nitrite, RPE, Carlo Erba, Italy.

23. Sodium Sulphate, RPE, Carlo Erba, Italy.

24. Zinc Nitrate, RPE, Carlo Erba, Italy.

2.3 Preparation of Standard Solutions and Reagents

All reagents were of analytical grade. All solutions were prepared with deionized distilled water.

2.3.1 Preparation of Standard Solutions and Reagents for Fe (III) determination by FIA

2.3.1.1 Iron stock solution 1000 mg L^{-1}

Iron stock solution was prepared by dissolving 8.6350 g of ammonium ferric sulphate dodecahydrate (previously dried at 110ºC for 1·1/2 hr) in water, acidifying with 5 ml of concentrated sulphuric acid and making up to 1000 ml with 0.01 mol L^{-1} sulphuric acid solution. Working standard solutions of iron with lower concentrations were prepared from stock solutions of iron and diluted with the 0.1 mol L^{-1} of acetate buffer (pH 4.5) solution.

2.3.1.2 Eriochrome Cyanine R stock solution, 0.03 mol L^{-1}

The stock reagent was prepared by dissolving 1.6092 g of eriochrome cyanine R (ECR) in water and diluted with water in a 100 mL volumetric flask. The reagent solution of lower concentrations of ECR were prepared from stock solution of reagent and diluted with 0.1 mol L^{-1} of acetate buffer (pH 4.5) solution.

2.3.1.3 Cetytrimethylammonium bromide stock solution, 0.1 mol L^{-1}

The stock surfactant solution was prepared by dissolving 9.1125 g of cetyltrimethylammonium bromide (CTMAB) in water in a 250 mL volumetric flask. The surfactant solution were prepared from stock solution of surfactant and diluted with the 0.1 mol L^{-1} of acetate buffer (pH 4.5) solution.

2.3.1.4 A 0.1 mol L^{-1} of Acetate buffer pH 4.5

The buffer solution was prepared by dissolving 13.608 g of sodium acetate in 500 mL of deionized distilled water in a 1000 mL volumetric flask and adjusted pH to 4.5 by using a glacial acetic acid and diluted with water in a 1000 mL volumetric flask.

2.3.2 Preparation of standard solution and reagent of SIA system

2.3.2.1 Iron stock solution 1000 mg L^{-1}

Iron (III) stock solution was prepared by dissolving 4.3175.g of ammonium ferric sulphate dodecahydrate in water, acidifying with 5 ml of concentrated sulphuric acid and making up to 500 ml with 0.01 mol L^{-1} sulphuric acid solution. Working standard solutions of iron were prepared from stock solutions of iron and diluted with water.

2.3.2.2 Eriochrome Cyanine R stock solution 0.03 mol L^{-1}

The stock reagent was prepared by dissolving 1.6092 g of eriochrome cyanine R (ECR) in water and diluted with water in a 100 mL volumetric flask. The reagent solution of lower concentrations of ECR were prepared from stock solution of reagent and diluted with water.

2.3.2.3 Cetylrimethylammonium bromide stock solution 0.1 mol L^{-1}

The stock surfactant solution was prepared by dissolving 9.1125 g of cetyltrimethylammonium bromide (CTMAB) in water in a 250 mL volumetric flask.

The surfactant solution were prepared from stock solution of surfactant and diluted with water.

2.3.2.4 A 0.1 mol L^{-1} of Acetate buffer pH 4.5

The buffer solution was prepared by dissolving 6.804 g of sodium acetate in 100 mL of deionized distilled water in a 500 mL volumetric flask and adjusted pH to 5.5 by using a concentrate of acetic acid and diluted with water in a 500 mL volumetric flask.

2.4 Preliminary Studies of Spectrophotometric Determination of Iron by using Eriochrome Cyanine R as Complexing Agent

2.4.1 Absorption spectra

The absorption spectra of ECR, ECR-CTMAB and Fe-ECR-CTMAB complexes were prepared as follows:

ECR complex, A 7 mL of 5.0×10^{-4} mol L^{-1} ECR reagent solution was transferred into a 25 mL volumetric flask

ECR-CTMAB complex, A 7 mL of 5.0×10^{-4} mol L^{-1} ECR reagent solution was transferred into a 25 mL volumetric flask. A 3 mL of 4.0×10^{-3} mol L^{-1} CTMAB surfactant solutions was then added and mixed well.

ECR-CTMAB-CTMAB complex, A 0.75 mL of 10 mg L^{-1} of iron solution was transferred into a 25 mL volumetric flask. A 7 mL of 5.0×10^{-4} mol L^{-1} ECR reagent solution was added and mixed well. After that, A 3 mL of 4.0×10^{-3} mol L^{-1} CTMAB surfactant solution was added.

The contents of 3 flasks were diluted to final volume with 0.1 mol L^{-1} of acetate buffer pH 4.5, mixed thoroughly and stood for 10 minute. Finally, the absorption spectra of ECR, ECR-CTMAB and Fe-ECR-CTMAB were scanned from 400-700 nm with JENWAY 6400 and the signals were recorded with computer.

2.4.2 Study of the composition of Fe-ECR-CTMAB complex by Mole-ratio method

The mole-ratio method of Fe-ECR-CTMAB complex was defined as 2 series of solution were prepared in which iron and CTMAB concentrations were fixed while the ECR concentration was varied. Another one is prepared in which iron and ECR concentrations were fixed while the CTMAB concentration was varied.

2.5 Procedure

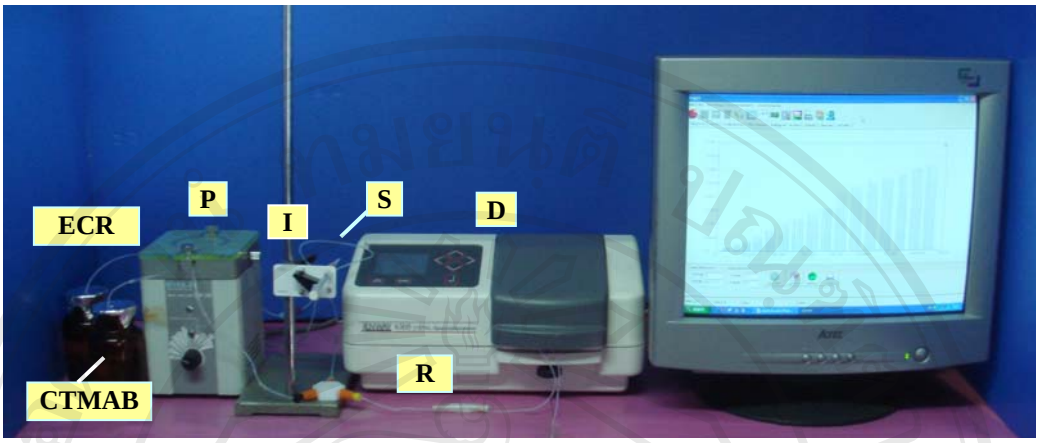
2.5.1 Procedure for collecting and treating drinking water samples for iron determination [35]

The drinking water samples were commercial drinking waters available in local markets around Chiang Mai Municipality. The samples were filtered through No. 41 Whatman filter paper into a 250 mL volumetric flask, add 5 mL concentrated nitric acid, 5 mL of 30% hydrogen peroxide and a few boiling chips. Bring to a slow boil and evaporate on a hot plate to the lowest volume as possible (about 25 mL). After standing it to cool to room temperature, 1.5 mL of 0.01 mol L^{-1} sodium fluoride was added. Then, the pH of the sample solution was adjusted to 4.5 with 0.1 mol L^{-1} of sodium hydroxide, transferred into a 250 mL volumetric flask and made up to the

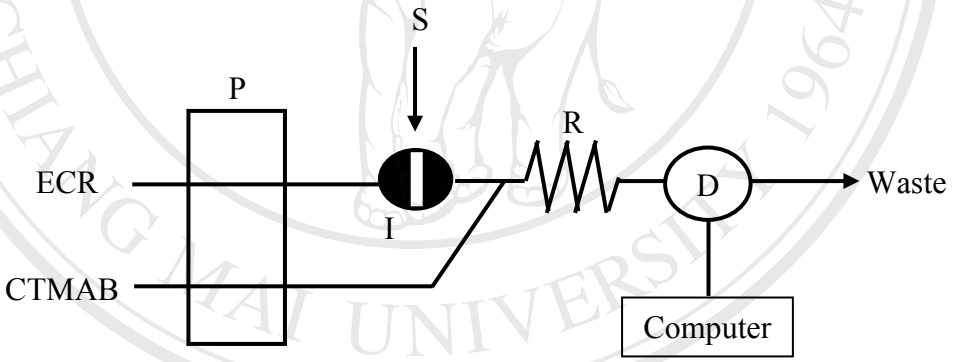
mark with deionized distilled water. Finally, it was mixed well and subsequently analysed.

2.5.2 FIA spectrophotometric determination of iron using ECR and CTMAB as complexing agent

Using the FIA-manifold as shown in Figure 2.1, this was two channels FIA manifold. Two channels consisted of a surfactant stream of cetyltrimethylammonium bromide (CTMAB) and an eriochrome cyanine R (ECR) as reagent stream, having the total flow rate of 4.5 mL min^{-1}. A 250 µL sample or standard solution was injected into the flowing stream of ECR solution. Then it was merged with CTMAB solution and formed the product of Fe-ECR-CTMAB in the reaction coil (1.07 mm inner diameter, 75 cm in length). The resulting colored complex was reached the flow-through cell of the spectrophotometer where the absorbance was measured at 610 nm.



(a)



(b)

Figure 2.1 The experimental setup of the FIA system for the determination of iron (III). (a) Photograph of the FI manifold and (b) Schematic diagram of (a). S, sample; CTMAB, cetyltrimethylammonium bromide; ECR, eriochrome cyanine r; R, reaction coil; I, injection valve; P, pump; D, detector.

2.5.2.1 Optimization of the flow system by univariate method

The univariate optimization was started with investigation of the preliminary experimental conditions obtained by loosely optimization by random (Table 2.1). Then, a studied parameter was changed while other parameters were fixed with their constant values. When the studied parameters was undergone changing to the optimized value, another parameter was varied. The other parameters were performed in the same manner through the optimized values. To optimize the conditions of the FIA manifold (Figure 2.1), the preliminary experimental conditions (Table 2.1) were used successively.

Table 2.1 Preliminary experimental conditions of FIA for studying optimum wavelength of Fe-ECR-CTMAB.

Variable	Fixed Value
0.1 mol L^{-1} of sodium acetate buffer pH 4.5	4.0
Concentration of ECR (x 10^{-4} mol L^{-1})	1.0
Concentration of CTMAB (x 10^{-3} mol L^{-1})	0.5
Flow rate (mL min^{-1})	2.5
Reaction coil length (cm)	100
Sample volume (μL)	100
Inner diameter of tubing (mm)	1.07

The studied ranges for the optimization of all parameters were studied as shown in Table 2.2.

Table 2.2 The studied range for the optimization of all parameters of FIA.

Variable	Studied range
Wavelength (nm)	595-625
pH	3.5-7.5
Concentration of ECR (x 10^{-4} mol L^{-1})	1.0-5.0
Concentration of CTMAB (x 10^{-3} mol L^{-1})	0.1-3.5
Flow rate (mL min^{-1})	1.0-5.5
Reaction coil length (cm)	25-125
Sample volume (μL)	100-400

2.5.2.2 Linearity of calibration graph

Using the FIA manifold (Figure 2.1) and the optimum conditions, linear range of calibration graph was obtained from the results for several iron (III) standards in the concentration ranging from 0-1.50 mg L^{-1}. Concentrations of iron were measured by FIA method and recorded as peak heights. A typical calibration graph was obtained by plotting the peak heights against various concentrations of iron (III).

2.5.2.3 Precision

The precision of the proposed method was verified by injecting 11 replicates of 0.1 mg L^{-1} standard iron (III) solution, and calculated % RSD from the equations as follows;

$$\%RSD = \frac{SD \times 100}{\overline{X}} \qquad (2.1)$$

Where $\%RSD$ = percentage relative standard

SD = standard deviation

$\overline{X}$ = mean

2.5.2.4 Detection limit

Using the FIA manifold (Figure 2.1) and the optimum conditions, the detection limit was determined from the regression equation with the calculated parameters of the intercept of the straight line and three-times the standard deviation of the regression time [67].

2.5.2.5 Accuracy of the proposed method

The accuracy of the proposed method were verified by spiking the water samples with various concentrations of iron standard solution (0, 0.10, 0.15, 0.20 and 0.25 mg L^{-1}) respectively using the recommended procedure. Then, iron (III) concentrations were calculated from linear regression equation obtained from the calibration graph. Finally, the percentage recovery was calculated from the equation as follows;

$$\%\text{Recovery} = \frac{(\text{total Fe (III) concentration-Fe (III) concentration in sample}) \times 100}{\text{Spiked Fe (III) concentration}} \quad (2.2)$$

2.5.2.6 Interference studies

The interference effects of some possible foreign ions in FIA system for iron determination were studied by the proposed FIA procedure using the optimum conditions. A systematic study to check for the effects of some possible foreign ions (Al^{3+}, Ca^{2+}, Cu^{2+}, Co^{2+}, Cr^{3+}, Fe^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Na^{+}, Ni^{2+}, Br^{-}, Cl^{-}, HCO_3^{-}, I^{-}, NO_2^{-}, NO_3^{-}, PO_4^{3-}, SO_4^{2-}) on the determination of ion was undertaken up the maximum w/w ratio of iron to foreign ions at 1: 1000.

2.5.2.7 Validation method

In order to validate the FIA method for iron (III) determination, a comparative determination of iron by the ICP-MS method was carried out. Results obtained by both methods were verified by using student *t*-test. The calculated t_{cal} value was obtained from the equation as follows [67];

$$t = \frac{\overline{X_d}}{S_d}\sqrt{n} \qquad (2.3)$$

$$S_d = \sqrt{\frac{\sum (X_d - \overline{X}_d)^2}{n-1}} \qquad (2.4)$$

$$\overline{X}_d = \frac{\sum X_d}{n} \qquad (2.5)$$

Where; X_d the difference between two method

$\overline{X}_d$ the mean difference

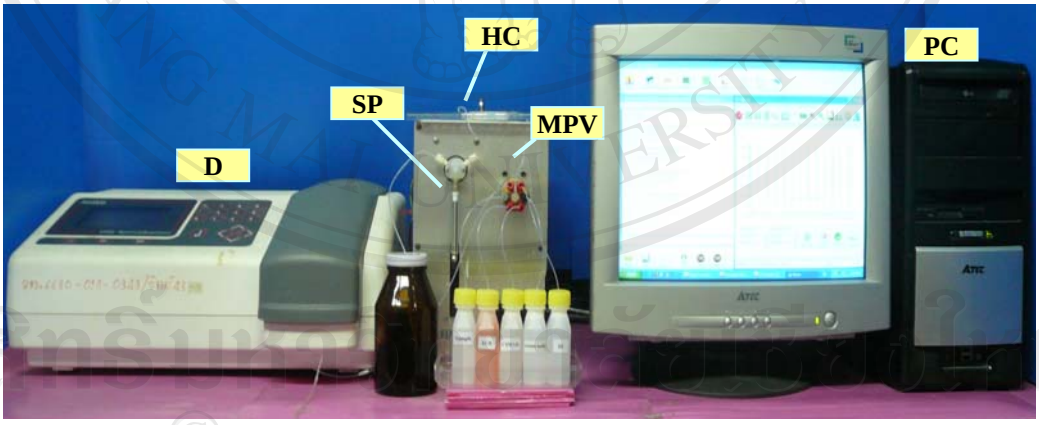
S_d the standard deviation

n number of sample

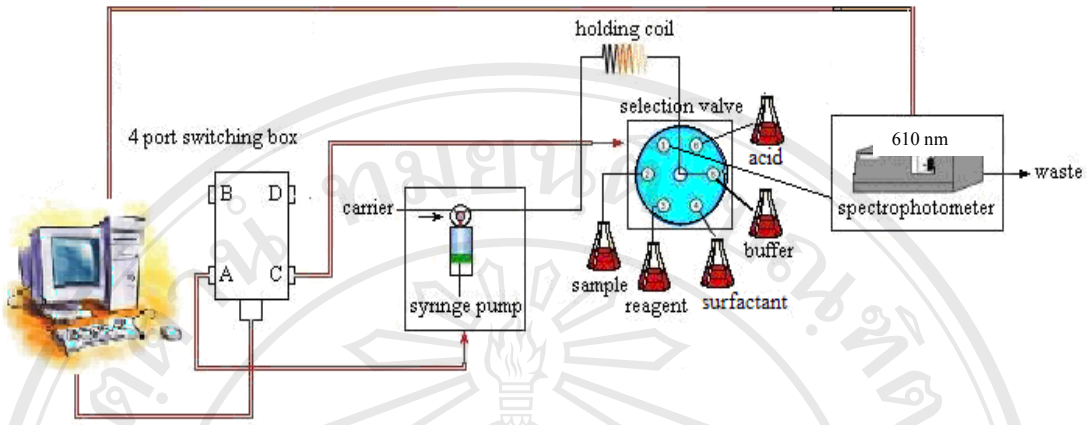
$n-1$ number of degree of freedom

2.5.3 SIA spectrophotometric determination of iron using ECR and CTMAB as complexing agent

The SIA analysis system, FIAlab® 3000 used in this study consists of the following components (Fig. 2.2): a 2.5 ml syringe pump (CAVRO XL 3000), a multiposition 6-port selection valve and a JENWAY 6400 spectrophotometer with a 1 cm path length cell for absorbance measurements. The Fe-ECR-CTMAB complex was monitored by measuring the absorbance at 610 nm. A personal computer was used for fluid control, data collection and analysis using FIALAB for WINDOWS (version 5.0) (Fig 2.3). The SI-integrated fluidic system is connected to the computer via an RS-232 interface. The 1.07 mm of inner diameter Tygon tubing was used for the holding coils.



(a)



(b)

Figure 2.2 The experimental setup of the SIA system for determination of iron (III). (a) Photograph of the SIA manifold and (b) Schematic diagram of (a). HC, holding coil; SP, syringe pump; MPV, multiposition 6-port selection valve; D, detector; PC, personal computer.

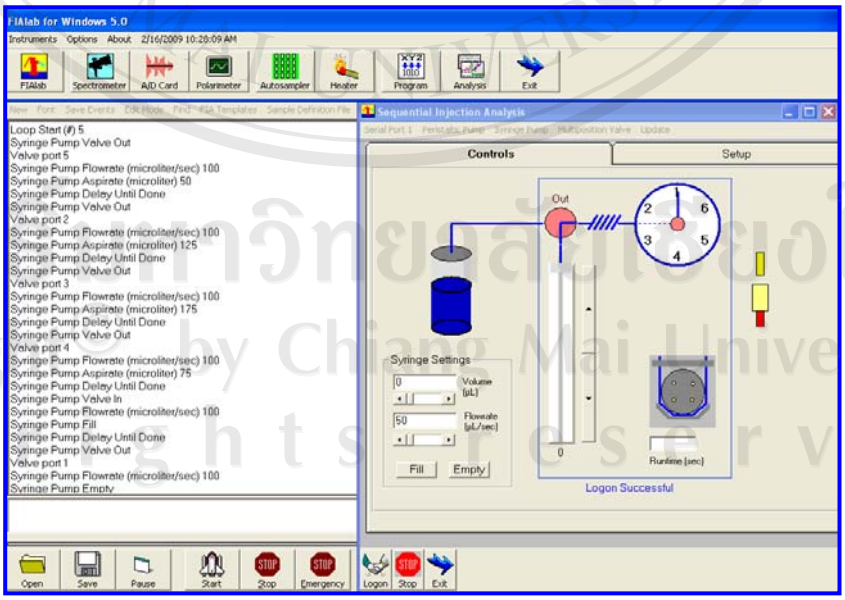


Figure 2.3 FIA labs 5.0 for windows software.

2.5.3.1 Sequential injection method

Table 2.3 lists the flow procedure selected for the sequential determination of iron (III). For the running of the automatic system, 50 µl of 0.1 mol L^{-1} of acetate buffer pH 5.5, 125 µl of sample solutions, 175 µl of 3.5×10^{-4} mol L^{-1} ECR, 75 µl of 2.5×10^{-3} mol L^{-1} CTMAB were aspirated sequentially through the valve port 5, 2, 3 and 4 respectively into the holding coil at the same flow rate of 100 µl.sec^{-1}. Then the aspirated sample and reagents zones were held for 15 seconds in the 150 cm of holding coil to complete reaction. A flow reversal was used to forward the composite zone through port 1 to the detector. The absorbance of the resultant Fe-ECR-CTMAB complex was then monitored at λ_{max} of 610 nm with the optimized spectrometer. Figure 2.4 shows schematic diagram of device sequence for one cycle of the SIA system.

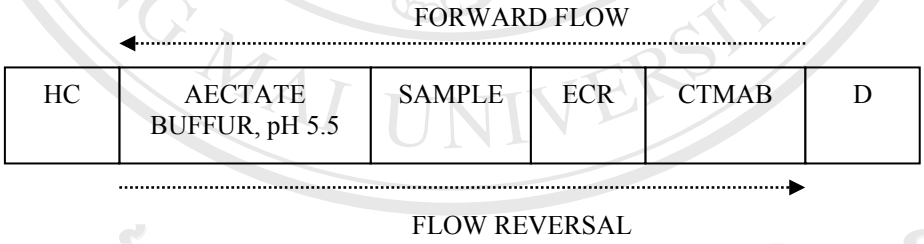


Figure 2.4 Schematic diagram of the device sequence for one cycle of the SIA system. HC, holding coil; CTMAB, cetyltrimethylammonium bromide; ECR, eriochrome cyanine r; D, detector.

Table 2.3 The device sequence for one full cycle of the sequential injection system used for iron (III) determination.

Step	Syringe Pump	Valve port	Description
1	Out	Acetate buffer, pH 5.5	Pump stops, select acetate buffer pH 5.5 solutions (vale position 5)
2	Reverse		50 μL of acetate buffer pH 5.5 was aspirated into holding coil by using flow rate 100 μL s^{-1}.
3	Out	Iron solutions	Pump stops, select iron solutions (vale position 2)
4	Reverse		125 μL of iron solution was aspirated into holding coil by using flow rate 100 μL s^{-1}.
5	Out	ECR	Pump stops, select ECR reagent solutions (vale position 3)
6	Reverse		175 μL of ECR solution was aspirated into holding coil by using flow rate 100 μL s^{-1}.
7	Out	CTMAB	Pump stops, select CTMAB solutions (vale position 4)

Table 2.3 (Continued).

Step	Syringe Pump	Valve port	Description
8	Reverse	CTMAB	75 µL of CTMAB solutions was aspirated into holding coil by using flow rate 100 µL s^{-1}. Wait for 15 s to complete the reaction.
9	In	Water carrier solution	Pump on, select water carrier solution, water solution was filled into syringe pump using flow rate 100 µL s^{-1}.
10	Out		Pump stops, select vale position 1
11	Forward		The carrier solution in syringe pump was pushed into holding coil and the mixture (Fe, ECR and CTMAB) solution moved to a detector by using flow rate 100 µL s^{-1}.
12	Out		Pump stops, valve turns to position 5

2.5.3.2 Optimization of the sequential injection system

The studied range for the optimization of development of sequential injection to determination of iron was shown in Table 2.4. The optimization was started with investigation of the preliminary experimental conditions obtained by loosely optimized by random. Then, a studied parameter was varied, while others parameters

were fixed with their constant values. When the studied parameter was undergone changing to the optimized value, another parameter was varied. The other parameters were performed in the same manner through the optimized values. To optimize the conditions of the SI system (Figure 2.2), the preliminary experimental conditions (Table 2.5) were applied.

Table 2.4 The studied range for the optimization of all parameters of SIA.

Variable	Studied range
pH	4.0-6.0
Concentration of acetate buffer (mol L^{-1})	0.01-0.2
Concentration of ECR (x 10^{-4} mol L^{-1})	2.5-4.5
Concentration of CTMAB (x 10^{-3} mol L^{-1})	1.0-3.0
Aspiration volume of buffer (μL)	25-125
Aspiration volume of ECR (μL)	100-225
Aspiration volume of CTMAB (μL)	50-150
Aspiration volume of sample (μL)	75-175
Flow rate (μL s^{-1})	50-175
Holding time (s)	5-25
Holding coil length (cm)	75-200
Reaction coil length (cm)	0-100

Table 2.5 Preliminary experimental conditions of SIA for studying optimum pH of Fe-ECR-CTMAB.

Experimental parameters	Pretested conditions
Wavelength (nm)	610
Concentration of ECR (x 10^{-4} mol L^{-1})	3.0
Concentration of CTMAB (x 10^{-3} mol L^{-1})	1.0
Aspiration volume of buffer (μL)	100
Aspiration volume of ECR (μL)	100
Aspiration volume of CTMAB (μL)	100
Aspiration volume of sample (μL)	100
Inner diameter of tube (mm)	1.07
Flow rate (μL s^{-1})	100
Holding time (s)	5
Holding coil length (cm)	125
Reaction coil length (cm)	25

2.5.3.3 Linearity of calibration graph

Working standard solutions of iron (III) over the ranges of 0-2.0 mg L^{-1} was prepared from the stock solution (10 mg L^{-1}). A series of iron standard solutions with different concentrations were injected into the finally proposed SIA manifold by means of a syringe pump in triplicate. The resulting peak heights were measured.

A typical calibration graph was obtained by plotting the peak heights against various concentrations of iron (III).

2.5.3.4 Precision

The precision of the proposed method was verified by injecting 12 replicates of 0.1 mg L^{-1} standard iron (III) solution, and calculated %RSD from equation 2.1.

2.5.3.5 Detection limit

Detection limit of the proposed method for iron determination was studied using the same procedure as described in section 2.5.2.4.

2.5.3.6 Accuracy of the proposed method

The accuracy of the proposed method were verified by spiking the treated water samples with various concentrations of iron (III) standard solution (0, 0.10, 0.15, 0.20 and 0.25 mg L^{-1}) respectively using the recommended procedure. Then, the results were plotted standard addition curve. Fe (III) concentration in sample was calculated from the calibration graph. Finally, the percentage recovery was calculated from the equation 2.2.

2.5.3.7 Interference studies

The interference effects of some possible foreign ions in the SIA system for iron (III) determination were studied using the same procedures as described in 2.5.2.6.

2.5.3.8 Validation method

The proposed SIA instrumentation has been tested to the determination of iron (III). The results obtained by SIA were confirmed by comparison with those obtained by ICP-MS using the student *t*-test as described earlier.