CHAPTER 2

Experimental

2.1 Instrument and Apparatus

1. Flow-through cell for spectrophotometer, Hellma, Germany
2. Peristaltic pump EYELA model MP-3N, Tokyo Rikakikal Co.,Ltd, Japan.
3. Teflon tubing, inner diameter 1.07 mm.
4. Waterproof pHTester 10, Eutech Instrument
5. CECIL 1010 series spectrophotometer
6. JENWAY 6400 spectrophotometer

2.2 Chemicals

1. Aluminium potassium sulphate 12-hydrate, commercial grade, BDH, England.
2. Bromopyrogallol red, indicator grade, Sigma aldrich, Germany.
3. Cetyltrimethylammonium bromide, pure, Serva, Germany.
4. Sodium Acetate, commercial grade, Merck, Germany.
5. Acetic acid, commercial grade, Merck, Germany.
6. Ethanol, commercial grade, Merck, Germany.
7. Nickel chloride, GR grade, Merck, Germany.
8. Ferric chloride, puriss, Fluka, Switzerland.
9. Ferrous chloride, commercial grade, BDH, England.
10. Coper chloride, commercial grade, BDH, England.
11. Cobalt chloride, commercial grade, Carlo Erba, Italy.

12. Magnesium chloride, commercial grade, Carlo Erba, Italy.
13. Cacium chloride, commercial grade, Carlo Erba, Italy.
14. Sodium chloride, puriss, Fluka, Switzerland.
15. Zinc nitrate, commercial grade, Carlo Erba, Italy.
16. Manganese chloride, LAB, M&B Ltd., England.
17. Cadmium nitrate, Chemika, Fluka, Switzerland.
18. Chromium chloride, pure, BDH, England.
19. Sodium sulphate, RPE, Carlo Erba, Italy.
20. Sodium nitrate, AR grade, BDH, England.
21. Sodium nitrite, RPE, Carlo Erba, Italy.
22. Sodium hydrogen carbonate, Carlo Erba, Italy.
23. Potassium chloride,Extra pure, MERCK, Germany.
24. Sodium bromide, pure, BDH, England.
25. Sodium iodide, RPE, Carlo Erba, Italy.

2.3 Preparation of Standard Solutions and Reagents

All chemicals used in this work were of analytical reagent grade. All solutions were prepared with de-ionized water.

2.3.1 Preparation of Standard Solutions and Reagents of rFI system

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

Aluminum stock solution was prepared by dissolving 0.8880 g of aluminum potassium sulphate 12-hydrate in water and diluting to 50 mL of de-ionized water.

Working standard solutions of aluminum were prepared from stock solutions of aluminum and diluted with the 0.02 mol L^{-1} of acetate buffer pH 5.0.

2.3.1.2 Bromopyrogalol red stock solution $5x10^{-4}$ mol L^{-1}

The stock reagent solution was prepared by dissolving 0.0288 g of bromopyrogalol red (BPR) in 15.80 mL of 95% of ethanol and diluted with water in a 100 mL volumetric flask. The reagent solution were diluted with 10% ethanol and protected from light.

2.3.1.3 Cetyltrimethylammonium bromide stock solution 0.25 mol L^{-1}

The stock surfactant solution was prepared by dissolving 9.1125 g of cetyltrimethylammonium bromide (CTAB) in water in a 100 mL volumetric flask. The surfactant solution were prepared from stock solution of surfactant and diluted with the 0.02 mol L^{-1} of acetate buffer pH 5.0.

2.3.1.4 0.02 mol L^{-1} of Acetate buffer pH 5.0

The buffer solution was prepared by dissolving 17.309 g of sodium acetate in water and 7.30 mL of acetic acid 1 mol L^{-1} and diluted with water in a 1000 mL volumetric flask.

2.3.2 Preparation of standard solutions and reagents of SI system

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

Aluminum stock solution was prepared by dissolving 0.8880 g of aluminium potassium sulphate 12-hydrate in water and diluting to 50 mL of de-ionized water.

Working standard solutions of aluminum were prepared from stock solutions of aluminum and diluted with water.

2.3.2.2 Bromopyrogalol red stock solution $5x10^{-4}$ mol L^{-1}

The stock reagent solution was prepared by dissolving 0.0288 g of bromopyrogalol red (BPR) in 10.53 mL of 95% of ethanol and diluted with water in a 100 mL volumetric flask. The reagent solution were diluted with 10% ethanol and protected from light.

2.3.2.3 Cetyltrimethylammonium bromide stock solution 0.25 mol L^{-1}

The stock surfactant solution was prepared by dissolving 9.1125 g of cetyltrimethylammonium bromide (CTAB) in water in a 100 mL volumetric flask. The surfactant solution were prepared from stock solution of surfactant and diluted with water.

2.3.2.4 0.25 mol L^{-1} of Acetate buffer pH 5.5

The buffer solution was prepared by dissolving 7.2032 g of sodium acetate in water and 4.80 mL of acetic acid 2 mol L^{-1} and diluted with water in a 250 mL volumetric flask.

2.4 Preliminary Studies of Spectrophotometric Determination of Aluminum by Using Bromopyrogallol Red as Complexing Agent

2.4.1 Absorption spectra

The absorption spectra of BPR, BPR-CTAB and Al-BPR-CTAB complexes were prepared by;

BPR complex, A 1 mL of $1.6\text{x}10^{-4}$ mol.L^{-1} BPR reagent solution was transferred into a 25 mL volumetric flask

BPR-CTAB complex, A 1 mL of $1.6\text{x}10^{-4}$ mol.L^{-1} BPR reagent solution was transferred into a 25 mL volumetric flask. Then, add 2.5 mL of CTAB surfactant solution.

Al-BPR-CTAB complex, A 2.5 mL of 1 mg.L^{-1} of aluminum solution was transferred into a 25 mL volumetric flask. A 1 mL of $1.6\text{x}10^{-4}$ mol.L^{-1} BPR reagent solution was added and mixed well. After that, add 2.5 mL of CTAB surfactant solution.

The contents of 3 flasks were diluted to final volume with 0.02 mol L^{-1} of acetate buffer pH 5.0, were mixed thoroughly and wait 10 minute. Finally, the absorption spectra of BPR, BPR-CTAB and Al-BPR-CTAB were scanned from 350-700 nm with JENWAY 6400 and the signals were recorded with computer.

2.4.2 Study of the composition of the Al-BPR-CTAB complex by Mole-ratio method

The mole-ratio method of Al-BPR-CTAB complex was defined as 2 series of solutions were prepared in which aluminum and CTAB concentrations were fixed while the BPR concentration was varied. Another one is prepared in which aluminum and BPR concentrations were fixed while the CTAB concentration was varied.

2.5 Procedure

2.5.1 Procedure for collection and treating tap water samples for aluminum determination

Tap water samples were collected from Aumphur Hangdong, Sangpatong, Muang, Mae Jo, Sansai, Sankumpang and Mae Rim and in Chiang Mai University. The samples were collected in polyethylene bottles with addition of concentrated nitric acid (1 mL concentrated nitric acid per a liter of water sample) to preserve the water samples. The sample was transferred into a suitable volume (250 mL to 400 mL) of beaker. Add 12.5 mL concentrated nitric acid and a few boiling chips. Bring to a slow boil and evaporate on a hot plate to the lowest volumes as possible (about 50 mL). After standing it to cool to room temperature, 2.5 g of thiourea, 25% of hydroxylammonium chloride (10 mL) and 0.1 mol.L^{-1} of 1,10-phenanthroline (20 mL) were added. Then, the pH of the sample solution was adjusted to 5.0 with 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 rFIA spectrophotometric determination of aluminum using BPR and CTAB as complexing agent

Figure 2.1 showed the experimental set up the rFIA spectrophotometric determination of aluminum, which was the two channels FIA manifold. Two channels consisted of a sample(S) stream and a surfactant stream of cetyltrimethylammonium bromide (CTAB), having the total flow rate of 2.5 mL min^{-1}. A 75 µL bromopyrogallol red (BPR) in diluted ethanol solution (as reagent) was injected into the sample stream via an injection valve and mixed with sample in a reaction coil (I) (1.07 mm diameter, 100 cm long) (R_1). The injected reagent was merged with the CTAB stream at the T junction. After the mixture was mixed with CTAB in a reaction coil (II) (1.07 mm diameter, 150 cm long) (R_2) where the complexation of Al-BPR-CTAB took place. The resulting colored complex was passed through the flow cell in the flow-through cell of the spectrophotometer where the absorbance was measured at 580 nm.

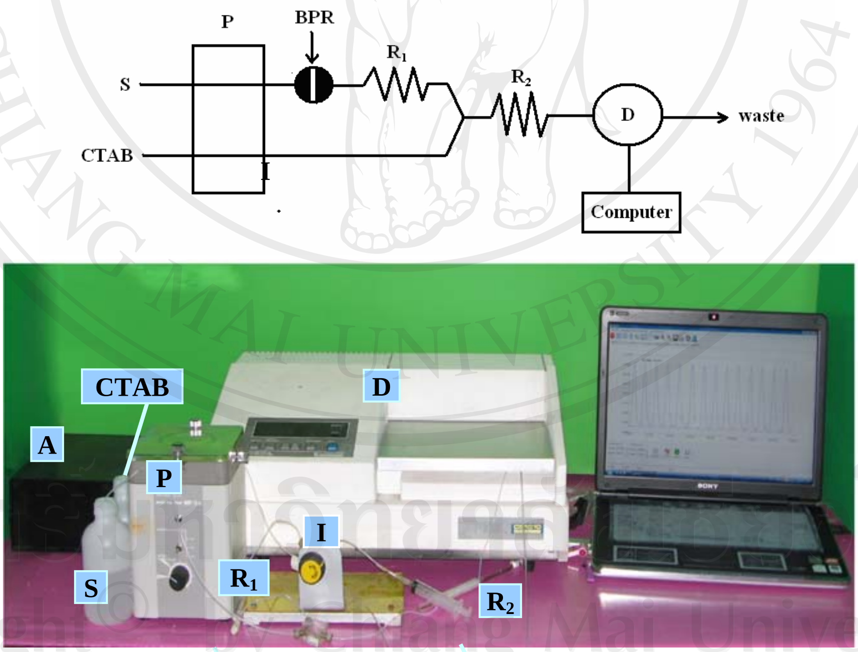


Figure 2.1 Reverse flow injection system for the determination of aluminum. S, sample; CTAB, cetyltrimethylammonium bromide; BPR, bromopyrogallol red; R_1, reaction coil (I); R_2, reaction coil (II); A, analog to digital converter; I, injection valve; P, pump; D, detector (CECIL).

2.5.2.1 Optimization of the reverse flow system

The studied range for the optimization of development of reverse flow injection to determinination of aluminum was shown in table 2.1. The univariate optimization was started with the selection of the preliminary experimental conditions. 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 rFIA manifold (Figure 2.1), the preliminary experimental conditions (Table 2.2) were proposed.

Table 2.1 The studied range for the optimization of all parameters of rFIA

Variable	**Studied range**
wavelength (nm)	540 - 595
pH	4.0 – 6.5
Concentration of BPR (x 10^{-4} mol L^{-1})	1.2 – 1.8
Concentration of ethanol in BPR solution (% v/v)	10 - 45
Concentration of CTAB (x 10^{-3} mol L^{-1})	3.0 – 6.0
Flow rate (mL min^{-1})	1.5- 4.0
Reaction coil (I) length (cm)	25-150
Reaction coil (II) length (cm)	50-200
Reagent volume (μL)	50-125

Table 2.2 Preliminary experimental conditions of rFIA for studying optimum wavelength of Al-BPR-CTAB

Experimental parameters	**Pretested conditions**
0.2 M of sodium acetate buffer pH	6.0
Concentration of BPR (x 10^{-4} mol L^{-1})	1.5
Concentration of ethanol in BPR solution (% v/v)	25
Concentration of CTAB (x 10^{-3} mol L^{-1})	3.0
Flow rate (mL min^{-1})	4.0
Reaction coil (I) length (cm)	100
Reaction coil (II) length (cm)	100
Reagent volume (µL)	75
Inner diameter of tubing (mm)	1.07

2.5.2.2 Linearity of calibration graph

Working standard solutions of aluminum over the ranges of 0.01-1.20 mg L^{-1} were prepared from the stock solution (10 mg L^{-1}). The series of aluminum standard solutions with different concentrations were flowed into the rFI system (Figure 2.1) by means of pentaplicate results. Concentrations of aluminum were measured by rFI method and recorded as peak heights. A typical calibration graph was obtained by plotting the peak heights against various concentrations of aluminum.

2.5.2.3 Precision

The precision of the proposed method was verified by injecting 11 replicates of 0.2 mg L^{-1} standard aluminum solution, and calculated % RSD from the equation as follows;

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

When %RSD = percentage relative standard

SD = standard deviation

$\overline{X}$ = mean

2.5.2.4 Detection limit [52]

The detection limit was determined by the method reported by Miller and Miller, which was calculated from the linear regression line of the calibration curve. The concentration at limit of detection (C_L) can be calculated from the equation (2.2).

$$C_L = 3 \times \frac{(S_y/x)}{b} \qquad (2.2)$$

$$S_y/x = \left\{ \frac{\sum (Y_i - \hat{Y}_i)^2}{(n-2)} \right\}^{\frac{1}{2}} \qquad (2.3)$$

When Y_i = response value from the instrument corresponding to the individual x-values

$\hat{Y}$ = value of y on the calculated regression line corresponding to the individual x-values

n = number of points on the calibration line

b = slope of the straight line

2.5.2.5 Accuracy of the proposed method

The accuracy of the proposed method were verified by spiking the treated water samples with various concentrations of aluminum standard solutions (0, 0.16, 0.18, 0.20 and 0.22 mg.L^{-1}) respectively using the recommended procedure. Then, aluminum concentrations were calculated from linear regression equation obtained from the calibration graph. Finally, the percentage recovery was calculated from the equation as follows;

$$\%\text{Recovery} = \frac{(\text{total Al(III) concentration- Al(III) concentration in sample}) \times 100}{\text{Spiked Al(III) concentration}} \qquad (2.4)$$

2.5.2.6 Interference studies

The interference effects of some possible foreign ions in rFIA system for aluminum determination were studied by the proposed rFIA procedure under the optimum conditions. A systematic study to check for the effects of some possible foreign ions (Cu^{2+}, Fe^{2+}, Fe^{3+}, Ni^{2+}, Co^{2+}, Cr^{3+}, Cd^{2+}, Zn^{2+}, Mn^{2+}, Mg^{2+}, Na^{+}, Ca^{2+}, NO$_2^-$, SO$_4^{2-}$, HCO$_3^-$, Br$^-$, I$^-$, Cl$^-$, Na$^+$) by adding known amounts of each interference to 0.2 mg L^{-1} of aluminum standard solution.

2.5.2.7 Validation method

In order to validate the rFI method for aluminum determination, a comparative determination of aluminum by the ICP-OES 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 [61];

$$t = \frac{\bar{x}_d \sqrt{n}}{S_d} \tag{2.5}$$

$$S_d = \sqrt{\frac{\sum (x_d - \bar{x}_d)^2}{n - 1}} \tag{2.6}$$

$$\bar{x}_d = \frac{\sum x_d}{n} \tag{2.7}$$

Where;	x_d	the difference between two method
	$\bar{x}_d$	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 aluminum using BPR and CTAB as complexing agent

The SIA system (Figure 2.2 and 2.3) was arranged using the following equipment: FIAlab® 3000 system consists of a syringe pump (syringe reservoir 2.5 mL) and a 6-port selection valve which is connected to a 4-port switching box.

The 6-port selection valve under the following functions:

Valve port 1 was connected to a detector.

Valve port 2 was connected to a sample solution (aluminum solution).

Valve port 3 was connected to a reagent solution (BPR solution).

Valve port 4 was connected to a surfactant solution (CTAB solution).

Valve port 5 was connected to a buffer solution (acetate buffer pH 5.5).

Valve port 6 was connected to an acid (nitric acid solution).

The 4 ports switching box under the following functions:

Port A was connected to a syringe control (CAVRO XL 3000).

Port C was connected to a valve control unit.

Port B and D weren't available.

A Jenway 6400 spectrophotometer equipped with a 1 cm path length cell over the wavelength range 360-800 nm. The flow system used Teflon tubes as the liquid channels. The holding coil was constructed by winding the teflon tubing around the small test tubes (1.5 cm o.d.). An absorbance signal can be retrieved directly from a Jenway 6400 spectrophotometer via the RS-232 interface. The absorbance of Al-BPR-CTAB complex in alkaline solution was monitored at 580 nm through a 1 cm path length flow cell.

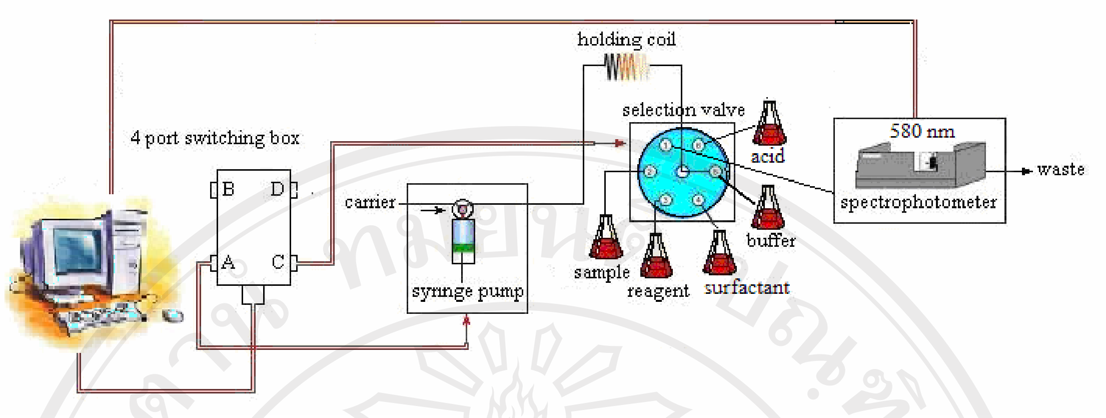


Figure 2.2 SI manifold for the determination of aluminum in water samples

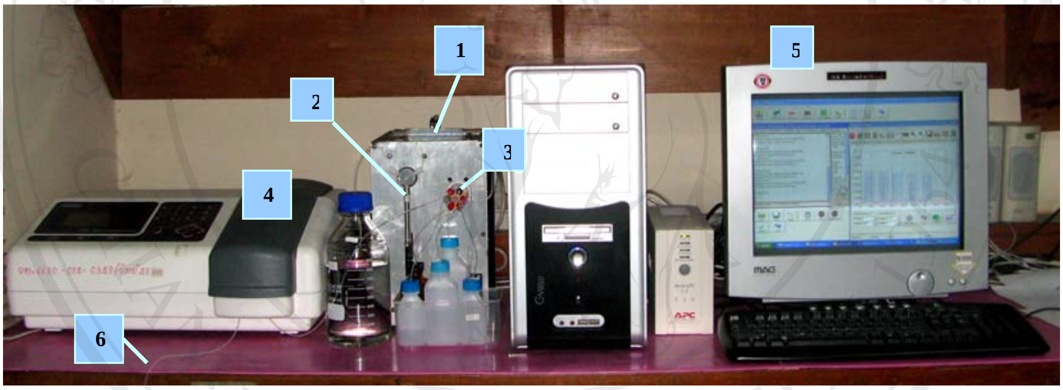


Figure 2.3 The SIA system for determination of aluminum. 1, holding coil; 2, syringe pump; 3, selection valve; 4, detector; 5, computer; 6, waste.

2.5.3.1 Sequential injection method

The 4 – port RS-232 switching box received an activation command from the PC through master port. When the system was initialized, it activated port a move the piston of the syringe to zero position. It also activated port C to actuate with the valve at position 5. Then, it activated port A to drive the syringe to aspirate the buffer with the desired volume. After that, it activated port C to actuate the valve at position 2 (sample) and it activated port A to drive the syringe to aspirate the desired volume of solution. The method was shown in Table 2.3. Finally, the PC was sending the empty syringe command through port A. It received an absorbance signals from the spectrophotometer and drove the plot module to plot the SIA grams on Senee SIA software (Figure 2.4). The maximum peak heights were detected at 580 nm and displayed in this process. The time required to analyze one sample was approximately 1.57 min. Table 2.3 lists the steps of the experimental entered to the FIAlab 5.0 for windows software (Figure 2.5).

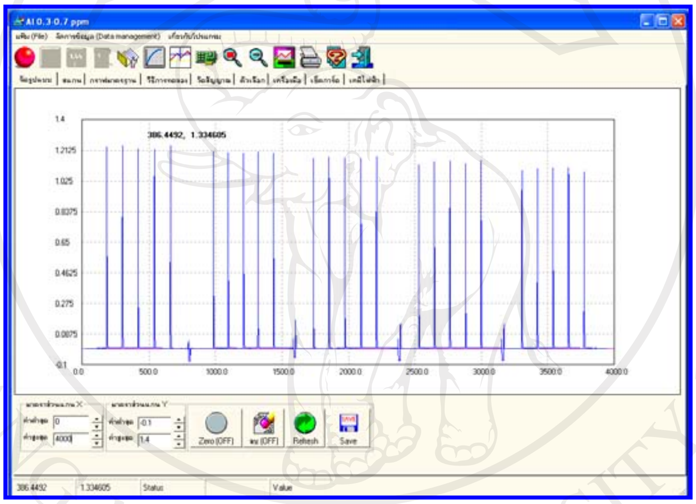
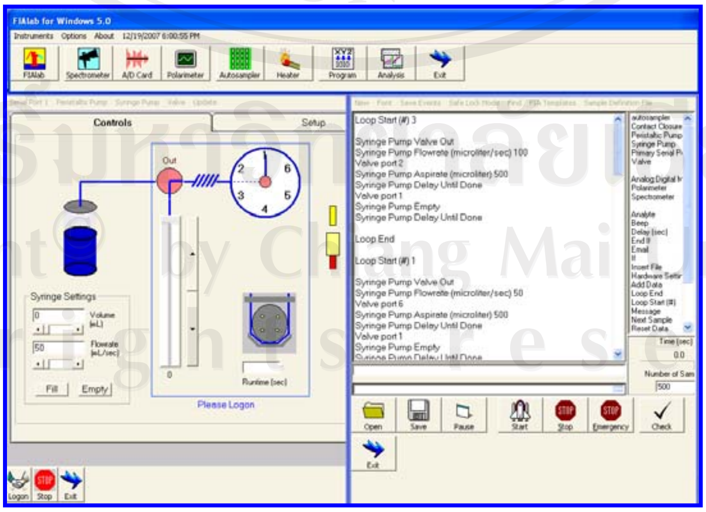


Figure 2.4 Senee SIA software for plot the SIA grams



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Loop Start (#) 3

Syringe Pump Valve Out
Syringe Pump Flowrate (microliter/sec) 100
Valve port 2
Syringe Pump Aspirate (microliter) 500
Syringe Pump Delay Until Done
Valve port 1
Syringe Pump Empty
Syringe Pump Delay Until Done

Loop End

Loop Start (#) 1

Syringe Pump Valve Out
Syringe Pump Flowrate (microliter/sec) 50
Valve port 6
Syringe Pump Aspirate (microliter) 500
Syringe Pump Delay Until Done
Valve port 1
Syringe Pump Empty
Syringe Pump Delay Until Done
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Figure 2.5 FIAlab 5.0 for windows software

Table 2.3 Experimental protocol as shown in the FIAlab for windows software

Command	Description
Loop Start (#) 5	The experimental was done 5 repeat.
Syringe Pump Valve Out Valve port 5 Syringe Pump Flowrate (microliter/sec) 150 Syringe Pump Aspirate (microliter) 150 Syringe Pump Delay Untill Done	150 mL of buffer pH 5.5 was aspirated into holding coil by using flow rate 150 μL s^{-1}.
Syringe Pump Valve Out Valve port 2 Syringe Pump Flowrate (microliter/sec) 150 Syringe Pump Aspirate (microliter) 150 Syringe Pump Delay Untill Done	150 mL of aluminum solution was aspirated into holding coil by using flow rate μL s^{-1}.
Syringe Pump Valve Out Valve port 3 Syringe Pump Flowrate (microliter/sec) 150 Syringe Pump Aspirate (microliter) 75 Syringe Pump Delay Untill Done	75 mL of BPR solution was aspirated into holding coil by using flow rate 150 μL s^{-1}.

Table 2.3 (Continued)

Command	Description
Syringe Pump Valve Out Valve port 1 Syringe Pump Flowrate (microliter/sec) 150 Syringe Pump Dispense (microliter) 100 Syringe Pump Delay Untill Done Syringe Pump Valve Out Valve port Syringe Pump Flowrate (microliter/sec) 150 Syringe Pump Aspirate (microliter) 100 Syringe Pump Delay Untill Done	Buffer, aluminum and BPR were mixed well by using flow rate 150 μL s^{-1}.
Syringe Pump Valve Out Valve port 4 Syringe Pump Flowrate (microliter/sec) 150 Syringe Pump Aspirate (microliter) 100 Syringe Pump Delay Untill Done	100 mL of CTAB solution was aspirated into holding coil by using flow rate 150 μL s^{-1}.

Table 2.3 (Continued)

Command	**Description**
Syringe Pump Valve Out Valve port 1 Syringe Pump Flowrate (microliter/sec) 150 Syringe Pump Dispense (microliter) 100 Syringe Pump Delay Untill Done Syringe Pump Valve Out Valve port 1 Syringe Pump Flowrate (microliter/sec) 150 Syringe Pump Aspirate (microliter) 100 Syringe Pump Delay Untill Done	CTAB was mixed in the solution (Al-BPR in buffer).
Delay (sec) 40	Wait for 40 s to complete the reaction.
Syringe Pump Valve In Syringe Pump Flowrate (microliter/sec) 150 Syringe Pump Fill Syringe Pump Delay Untill Done Syringe Pump Valve Out Valve port 1 Syringe Pump Flowrate (microliter/sec) 150 Syringe Pump Empty Syringe Pump Delay Untill Done	The syringe pump to aspirate filled a carrier solution. Then, it pushed a carrier in syringe pump to holding coil and the complex solution moved to a detector by using flow rate 150 µL s^{-1}.
Loop End	

2.5.3.2 Optimization of the sequential injection system

The studied range for the optimization of development of sequential injection to determination of aluminum was shown in table 2.4. The optimization was started with the selection of the preliminary experimental conditions. 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 and 2.3), the preliminary experimental conditions (Table 2.5) were proposed.

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

Variable	Studied range
pH	4.0 – 6.0
Concentration of pH (mol L^{-1})	0.2 - 0.4
Concentration of BPR (x 10^{-4} mol L^{-1})	1.2 – 1.6
Concentration of ethanol in BPR solution (% v/v)	10 – 50
Concentration of CTAB (x 10^{-3} mol L^{-1})	1.0 – 6.0
Aspiration volume of buffer (μL)	100 - 200
Aspiration volume of BPR (μL)	50 -150
Aspiration volume of CTAB (μL)	50 -150
Aspiration volume of sample (μL)	100 - 200
Flow rate (μL s^{-1})	50 - 175
Holding time (s)	20 - 60

Table 2.5 Preliminary experimental conditions of SIA for studying optimum pH of Al-BPR-CTAB

Experimental parameters	Pretested conditions
Wavelength (nm)	580
Concentration of BPR (x 10^{-4} mol L^{-1})	1.5
Concentration of ethanol in BPR solution (% v/v)	50
Concentration of CTAB (x 10^{-3} mol L^{-1})	3.0
Aspiration volume of buffer (µL)	100
Aspiration volume of BPR (µL)	100
Aspiration volume of CTAB (µL)	100
Length of holding coil (cm)	300
Inner diameter of tube (mm)	1.07
Aspiration volume of sample (µL)	100
Flow rate (µL s^{-1})	150
Holding time (s)	60

2.5.3.3 Linearity of calibration graph

Working standard solutions of aluminum over the ranges of 0.02-5.00 mg L^{-1} was prepared from the stock solution (10 mg L^{-1}). A series of aluminum 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 aluminum.

2.5.3.4 Precision

The precision of the proposed method was verified by injecting 11 replicates of 0.08, 0.2, 0.4 and 1 mg L^{-1} standard aluminum solution, and calculated %RSD from equation 2.1.

2.5.3.5 Detection limit

Detection limit of the proposed method for aluminum 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 aluminum standard solutions (0, 0.4, 0.5, 0.6 and 0.7 mg.L^{-1}) respectively using the recommended procedure. Then, the results were plotted standard addition curve. Al (III) concentration in sample was calculated from y = mx + c (y is signal of spiking the treated water samples with concentrations of aluminum standard solutions 0 mg.L^{-1}). Total Al (III) concentration was calculated from y = mx + c (y is signal of spiking the treated water samples with various concentrations of aluminum standard solutions 0.4, 0.5, 0.6 and 0.7 mg.L^{-1} mg.L^{-1}). Finally, the percentage recovery was calculated from equation 2.4.

2.5.3.7 Interference studies

The interference effects of some possible foreign ions in the SIA system for aluminum 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 aluminum. The results obtained by SIA were confirmed by comparison with those obtained by ICP-OES using the student t-test as described earlier.