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, Anachem, UK
4. Tygon tubing, inner diameter 1.52 mm, Cole-parmer, USA
5. Home-made connector Y-shape
6. CECIL 1010 series spectrophotometer, England
7. JENWAY 6400 spectrophotometer, UK
8. Six port selection valve, Valco instrument, USA
9. Waterproof pH Tester 10, Eutech Instrument

2.2 Chemicals

1. Aluminium potassium sulphate 12-hydrate, AR grade, BDH, England
2. Barium chloride, GR grade, Merck, Germany.
3. Cetyltrimethyl ammonium bromide, pure, Serva, Germany.
4. Coper sulfate, AR grade, BDH, England.
5. Cobalt sulfate, Extra pure, Merck, Germany.
6. Calcium chloride, Extra pure, Merck, Germany.
7. Cadmium nitrate, pure, BDH, England.
8. Chromium nitrate, pure, Merck, Germany.

9. EDTA di-sodium salt, GR, Merck, Germany.
10. Ethanol, GR grade, Merck, Germany.
11. Ferric nitrate, puriss, Carlo Erba, Italy.
12. Ferrous sulfate, puriss, Carlo Erba, Italy.
13. Glacial Acetic Acid 99.8% (w/v), GR grade, Merck, Germany.
14. Hydrochloric acid, AnalaR, Merck, Germany.
15. Magnesium nitrate, Extra pure, Merck, Germany.
16. Manganese sulfate, Extra pure
17. , Merck, Germany.
18. Nickel sulfate, GR grade, Carlo Erba, Italy.
19. Potassium iodide, AR grade, Sigma aldrich, Germany.
20. Potassium chloride, Extra pure, Merck, Germany.
21. Potassium hydroxide, AnR, BDH, England.
22. Quercetin dihydrate, HPLC grade, Fluka, Switzerland.
23. Sodium acetate, AR grade, Carlo Erba, Italy.
24. Sodium chloride, puriss, Fluka, Switzerland.
25. Sodium sulfate, AR grade, BDH, England
26. Sodium nitrate, AR grade, BDH, England.
27. Sodium hydrogen carbonate, AR grade, Carlo Erba, Italy.
28. Sodium bromide, pure, BDH, England.
29. Sodium iodide, RPE, Carlo Erba, Italy.
30. Sodium fluoride, GR grade Merck, Germany.
31. Sodium phosphate, LAB, M&B Ltd., England.
32. Zinc sulfate, Extra pure, 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 Aluminium (III) stock solution 1000 mg L^{-1}

Aluminium (III) 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 aluminium (III) were prepared from stock solutions of aluminium (III) and diluted with the 0.1 mol L^{-1} of acetate buffer pH 5.5.

2.3.1.2 Quercetin stock solution 1000 mg L^{-1}

The stock reagent solution was prepared by dissolving 0.1000 g of Quercetin in 63.20 mL of 95% of ethanol and diluted with water in a 100 mL volumetric flask. The reagent solution were diluted with 60% ethanol.

2.3.1.3 Cetyltrimethylammonium bromide stock solution 0.10 mol L^{-1}

The stock surfactant solution was prepared by dissolving 9.1125 g of cetyltrimethylammonium bromide (CTAB) 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 5.5) solution.

2.3.1.4 A 0.1 mol L^{-1} of Acetate buffer pH 5.5

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 5.5 by using a glacial acetic acid 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 Aluminium (III) stock solution 1000 mg L^{-1}

Aluminium (III) 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 aluminium (III) were prepared from stock solutions of aluminium (III) and diluted with water.

2.3.2.2 Quercetin stock solution 1000 mg L^{-1}

The stock reagent solution was prepared by dissolving 0.1000 g of Quercetin in 63.20 mL of 95% of ethanol and diluted with water in a 100 mL volumetric flask. The reagent solution were diluted with 60% ethanol and protected from light.

2.3.2.3 Cetyltrimethylammonium bromide stock solution 0.10 mol L^{-1}

The stock surfactant solution was prepared by dissolving 9.1125 g of cetyltrimethylammonium bromide (CTAB) 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 0.10 mol L^{-1} of Acetate buffer pH 5.5

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 5.5 by using a glacial acetic acid and diluted with water in a 1000 mL volumetric flask.

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

2.4.1 Absorption spectra

The absorption spectra of quercetin, quercetin-CTAB and Al(III)-quercetin-CTAB complexe were prepared by;

Quercetin, A 1 mL of $2x10^{-4}$ mol L^{-1} Quercetin reagent solution was transferred into a 25 mL volumetric flask.

Quercetin-CTAB complex, A 1 mL of $2x10^{-4}$ mol L^{-1} Quercetin reagent solution was transferred into a 25 mL volumetric flask. Then, add 2.5 mL of CTAB surfactant solution.

Al(III)-quercetin-CTAB complex, A 2.5 mL of 1 mg L^{-1} of aluminium (III) solution was transferred into a 25 mL volumetric flask. A 1 mL of $2x10^{-4}$ mol L^{-1} Quercetin 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 0.10 mol L^{-1} of acetate buffer pH 5.5, were mixed thoroughly and wait 10 minute. Finally, the absorption spectra of quercetin, quercetin-CTAB and Al (III)-quercetin-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(III)-Quercetin-CTAB complex by Mole-ratio method

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

2.5 Procedure

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

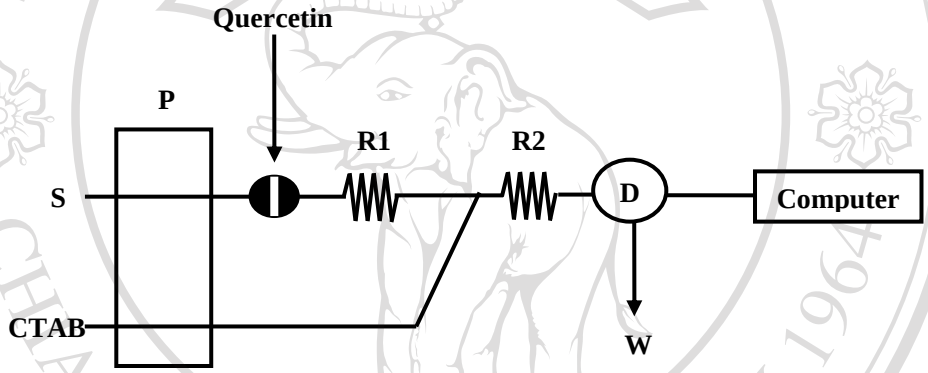
Tap water samples were collected from Aumphoe Doisaket, Hangdong, Sangpatong, Muang, Sansai, Sankumpang, Mae Rim, Mae Wang, Jomthong and in Chiang Mai University. The samples were collected in polyethylene bottles with addition of concentrated nitric acid (1 mL concentrated nitric acid per a liter of water sample) to preserve the water samples. The samples were filtered through No. 41 Whatman filter paper into a 250 mL volumetric flask and 12.5 mL of concentrated nitric acid and a few boiling chips was added. 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, 0.625 g of thiourea, 0.1 mol L^{-1} ascorbic acid (15 mL) and 0.1 mol L^{-1} of 1,10-phenanthroline (25 mL) were added as masking agents. Then, the

pH of the sample solution was adjusted to 5.5 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 aluminium (III) using Quercetin and CTAB as complexing agent

Figure 2.1 showed the experimental set-up the rFIA spectrophotometric determination of aluminium (III), 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 125 µL quercetin 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, 75 cm long) (R1). The injected reagent was merged with the sample stream at the T junction. Then the solution mixture was mixed with CTAB in a reaction coil (II) (1.07 mm diameter, 100 cm long) (R2) where the complexation of Al(III)-quercetin-CTAB took place. The resulting yellow colored complex was passed through in the flow-through cell of the spectrophotometer where the absorbance was measured at 428 nm. The signal were recorded by UT60F multimeter.

(a)



(b)

Figure 2.1 Reverse flow injection system for the determination of aluminium (III). (a) Photograph of the FI manifold and (b) Schematic diagram of (a). S, sample; CTAB, cetyltrimethylammonium bromide; R1, reaction coil (I); R2, reaction coil (II); I, injection valve; P, pump; D; detector , W; waste.

2.5.2.1 Optimization of the reverse flow system

The studied range for the optimization of development of reverse flow injection to determination of aluminium (III) 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)	420 – 435
pH	3-7
Concentration of quercetin (mg L^{-1})	100 – 500
Concentration of ethanol in quercetin solution (% v/v)	40 – 90
Concentration of CTAB (x 10^{-3} mol L^{-1})	2.0 – 4.5
Flow rate (mL min^{-1})	2.0- 6.0
Reaction coil (I) length (cm)	25-150
Reaction coil (II) length (cm)	25-150
Reagent volume (μL)	50-200

2.5.2.2 Linearity of calibration graph

Working standard solutions of aluminium (III) over the ranges of 0.005 - 1.5 mg L^{-1} were prepared from the intermediate aluminium (III) stock solution (10 mg L^{-1}). The series of aluminium (III) standard solutions with different concentrations were flowed into the rFI system (Figure 2.1) by means of pentaplicate results. Concentrations of aluminium (III) 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 aluminium (III).

2.5.2.3 Precision

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

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

When %RSD = percentage relative standard

SD = standard deviation

$\overline{X}$ = mean

2.5.2.4 Detection limit [79]

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).

$$C_L = 3 \times \frac{S_{y/x}}{b} \quad (2.2)$$

$$Sy/x = \left\{ \frac{\Sigma(Y_i - \hat{Y}_i)^2}{n-2} \right\}^{\frac{1}{2}} \quad (2.3)$$

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

$\hat{Y}_i$ = 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 aluminium (III) standard solutions (0, 0.10, 0.15, and 0.20 mg L^{-1}) respectively using the recommended procedure. Then, aluminium (III) concentrations were calculated from linear regression equation obtained from the calibration graph. Finally, the percentage recovery was calculated from the equation as follows;

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

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 aluminium (III) standard solution.

2.5.2.7 Validation method

In order to validate the rFI method for aluminum determination, a comparative determination of aluminium (III) 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 [79];

$$t = \frac{\bar{x}_d \sqrt{n}}{S_d} \quad (2.5)$$

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

$$\bar{x}_d = \frac{\sum x_d}{n} \quad (2.7)$$

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 aluminium (III) using quercetin 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 (aluminium (III) solution).

Valve port 3 was connected to a reagent solution (Quercetin 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(III)-Quercetin-CTAB complex was monitored at 428 nm through a 1 cm path length flow cell.

Figure 2.2 The SIA system for determination of aluminium (III). 1, holding coil; 2, syringe pump; 3, selection valve; 4, detector; 5, computer; 6, waste.

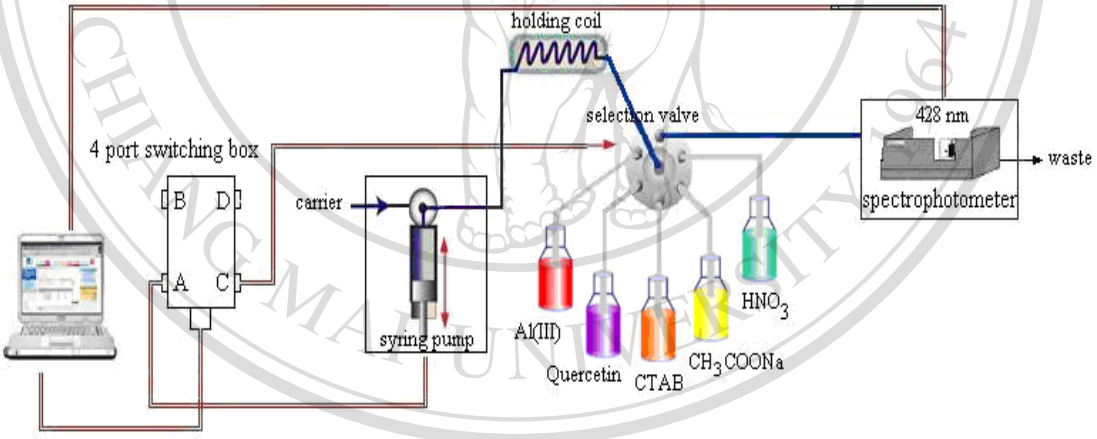


Figure 2.3 SI manifold for the determination of aluminium (III) in water samples

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 2.2. 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 428 nm and displayed in this process. The time required to analyze one sample was approximately 1.5 min. Table 2.3 lists the steps of the experimental entered to the FIAlab 5.0 for windows software (Figure 2.5).

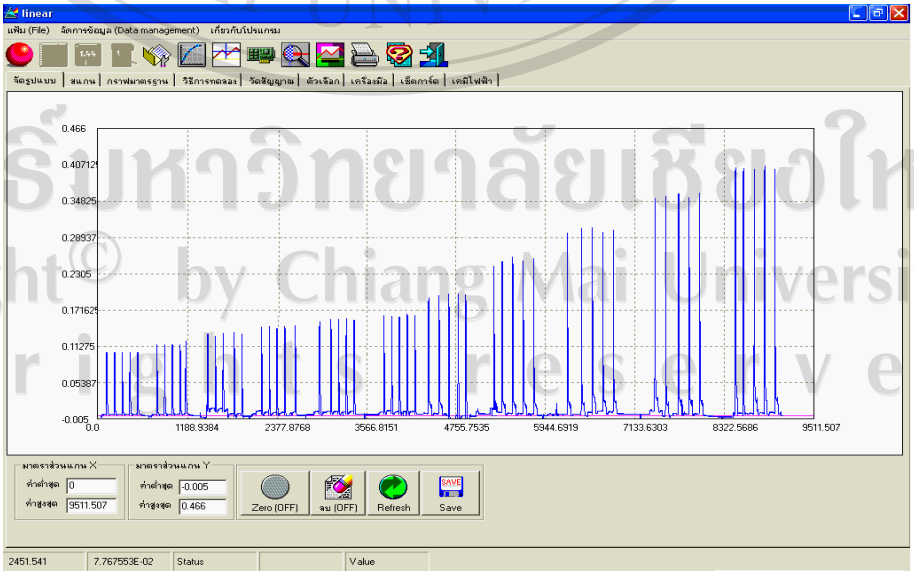


Figure 2.4 Senee SIA software for plot the SIA grams

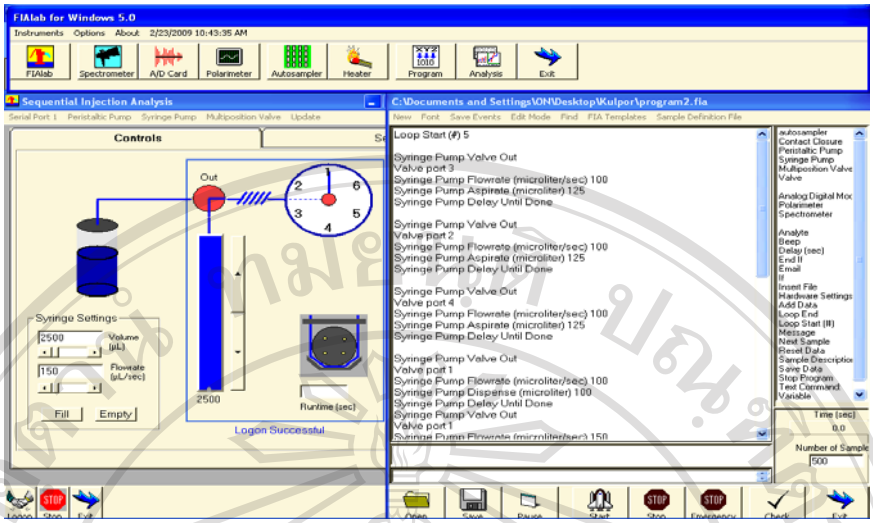


Figure 2.5 FIAlab 5.0 for windows software

Table 2.2 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) 125 Syringe Pump Aspirate (microliter) 125 Syringe Pump Delay Untill Done	125 µL of acetate buffer was aspirated into holding coil by using flow rate 125 µL s^{-1}.
Syringe Pump Valve Out Valve port 2 Syringe Pump Flowrate (microliter/sec) 125 Syringe Pump Aspirate (microliter) 175 Syringe Pump Delay Untill Done	100 µL of aluminium solution was aspirated into holding coil by using flow rate 125 µL s^{-1}.

Table 2.2 (Continued)

Command	Description
Syringe Pump Valve Out Valve port 3 Syringe Pump Flowrate (microliter/sec) 125 Syringe Pump Aspirate (microliter) 125 Syringe Pump Delay Untill Done	125 μL of Quercetin solution was aspirated into holding coil byusing flow rate 125 μL s^{-1}.
Syringe Pump Valve Out Valve port 1 Syringe Pump Flowrate (microliter/sec) 125 Syringe Pump Dispense (microliter) 100 Syringe Pump Delay Untill Done Syringe Pump Valve Out Valve port 1 Syringe Pump Flowrate (microliter/sec) 125 Syringe Pump Aspirate (microliter) 100 Syringe Pump Delay Untill Done	Buffer, aluminium and quercetin were mixed well by using flow rate 125 μL s^{-1}.
Syringe Pump Valve Out Valve port 4 Syringe Pump Flowrate (microliter/sec) 125 Syringe Pump Aspirate (microliter) 100 Syringe Pump Delay Untill Done	100 μL of CTAB solution was aspirated into holding coil by using flow rate 125 μL s^{-1}.

Table 2.2 (Continued)

Command	Description
Syringe Pump Valve Out Valve port 1 Syringe Pump Flowrate (microliter/sec) 125 Syringe Pump Dispense (microliter) 100 Syringe Pump Delay Untill Done Syringe Pump Valve Out Valve port 1 Syringe Pump Flowrate (microliter/sec) 125 Syringe Pump Aspirate (microliter) 100 Syringe Pump Delay Untill Done	CTAB was mixed in the solution (Al-quercetin in buffer).
Delay (sec) 40	Wait for 40 s to complete the reaction
Syringe Pump Valve In Syringe Pump Flowrate (microliter/sec) 125 Syringe Pump Fill Syringe Pump Delay Untill Done Syringe Pump Valve Out Valve port 1 Syringe Pump Flowrate (microliter/sec) 125 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 125 $\mu L\ s^{-1}$.
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 aluminium(III) was shown in table 2.3. 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 2.4) were proposed.

Table 2.3 The studied range for the optimization of all parameters of SIA

Variable	Studied range
pH	4.0 – 6.0
Concentration of quercetin solution (mg L^{-1})	200 – 500
Concentration of ethanol in quercetin solution (% v/v)	40 – 90
Concentration of CTAB(x 10^{-3} mol L^{-1})	3.0 – 5.5
Aspiration volume of buffer (µL)	75 – 175
Aspiration volume of quercetin (µL)	75 – 175
Aspiration volume of CTAB (µL)	50 – 150
Aspiration volume of sample (µL)	75 – 225
Flow rate (µL s^{-1})	50 – 175
Holding time (s)	10 – 50

Table 2.4 Preliminary experimental conditions of SIA for studying optimum pH of Al(III)-Quercetin-CTAB

Experimental parameters	Pretested conditions
Wavelength (nm)	428
Concentration of quercetin (mg L^{-1})	350
Concentration of ethanol in quercetin solution (% v/v)	70
Concentration of CTAB (x 10^{-3} mol L^{-1})	4.5
Aspiration volume of buffer (μL)	125
Aspiration volume of quercetin (μL)	125
Aspiration volume of CTAB (μL)	100
Length of holding coil (cm)	125
Inner diameter of tube (mm)	1.07
Aspiration volume of sample (μL)	175
Flow rate (μL s^{-1})	125
Holding time (s)	40

2.5.3.3 Linearity of calibration graph

Working standard solutions of aluminium (III) over the ranges of 0.01-1.00 mg L^{-1} was prepared from the intermediate aluminium (III) stock solution (10 mgL^{-1}). A series of aluminium (III) 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 aluminium (III).

2.5.3.4 Precision

The precision of the proposed method was verified by injecting 11 replicate of 0.05, 0.1, 0.4 and 0.6 mg L^{-1} standard aluminium (III) solution, and calculated %RSD from equation 2.1.

2.5.3.5 Detection limit

Detection limit of the proposed method for aluminium (III) 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 known concentrations of Al (III) standard solutions (0, 0.1, 0.15, 0.2 and 0.25 mg L^{-1}) respectively using the recommended procedure. The standard addition curve was obtained by plotting peak heights against various Al (III) concentrations added. 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}).

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

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