CHAPTER 3

RESULTS AND DISCUSSION

3.1 Preliminary Studies of Spectrophotometric Determination of Aluminium by Using Quercetin as Complexing Agent

3.1.1 Absorption spectra

The absorption spectra of Quercetin, Quercetin-CTAB, Al(III)-Quercetin and Al(III)-Quercetin-CTAB complexes in absence and presence of CTAB were recorded over a range from 350-600 nm against their reagent blank, using JENWAY 6400 spectrophotometer in conjunction with the laboratory developed software connected to a PC as shown in Figure 3.1. In the aqueous medium, Al(III)-quercetin shows its absorption maximum at 415 nm. Addition of CTAB to Al(III)-quercetin complex is accompanied by hypsochromic shift. In presence of CTAB, Al(III)-quercetin complex has absorption maximum at 428 nm.

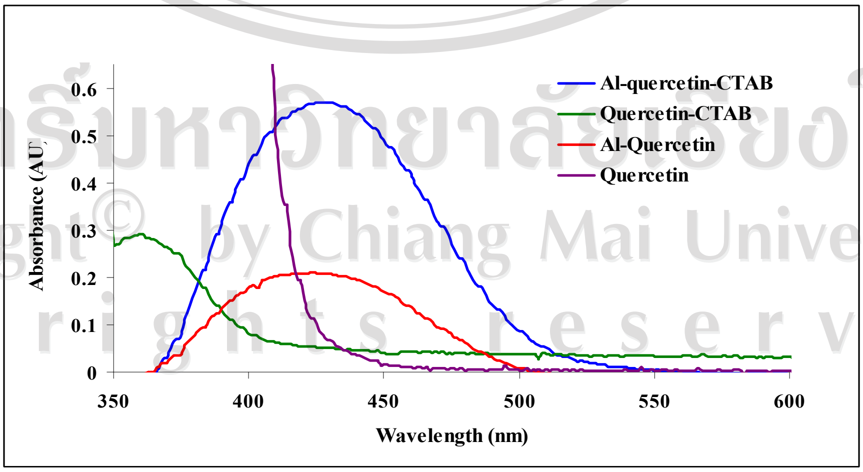

Figure 3.1 Absorption Spectra of Quercetin, Quercetin-CTAB, Al(III)- Quercetin and Al(III)- Quercetin-CTAB against reagent blank at pH 5.5

3.1.2 Stoichiometry of the complexes this was study by mole-ratio method

To study the composition of Al(III)-Quercetin-CTAB ternary complex, used the mole ratio method. In this method, a series of solution are prepared containing a fixed amount of metal ion, with varying concentrations of ligand. The absorbances of these solutions are then measured. A resulting plot of absorbance vs. ligand-to-metal ratio initially increases, then becomes constant one and the ligand to metal ratio has been achieved. The point at which the slope of the line changes corresponds to the ligand: metal ratio of the complex [80]. The mole-ratio methods of Al(III)-Quercetin-CTAB complex was defined as 2 series of solution were prepared in which Al(III) and CTAB concentrations were fixed while the Quercetin concentration was varied. Another one is prepared in which Al(III) and Quercetin concentrations were fixed while the CTAB concentration was varied.

Series I; the various concentrations of quercetin were added to solution containing 1×10^{-5} mol L^{-1} of Al(III), 1×10^{-5} mol L^{-1} CTAB, 5 mL of 0.1 mol L^{-1} of acetate buffer pH 5.5 and diluted with deionized water in 25 mL volumetric flasks. Absorbance of each solution was measured at 428 nm. The results were shown in Table 3.1 and Figure 3.2. It was found that the absorbance as peak height increased to maximum at concentration of quercetin was 3×10^{-5} mol L^{-1} and then it became constant and so did the absorbance as AU. Therefore, 3×10^{-5} mol L^{-1} of quercetin concentration was chosen for studied effect of CTAB concentration in series II.

Table 3.1 Effect of quercetin concentrations for mole-ratio Al(III)-Quercetin-CTAB complex.

[Quercetin] (x 10 $^{-5}$ mol L^{-1})	**Mole ratio (Al:Quercetin)**	**Absorbance (AU)***
1	1:1	0.119
2	1:2	0.174
3	1:3	0.229
4	1:4	0.235
5	1:5	0.240
6	1:6	0.247

*average of triplicate results

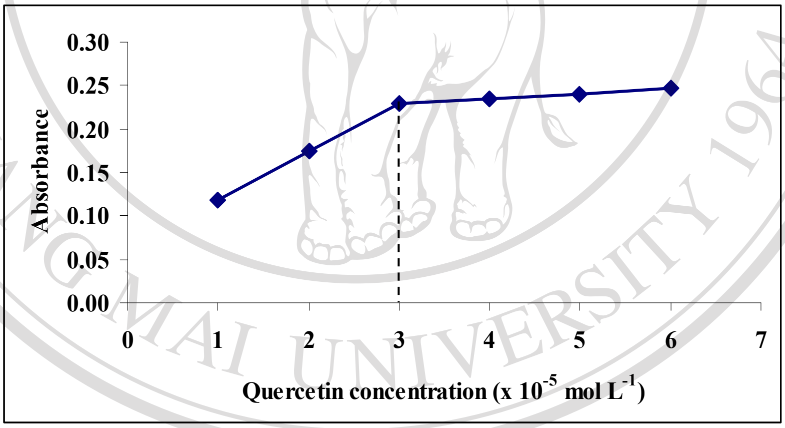

Figure 3.2 Mole-ratio study of Al-Quercetin-CTAB system; effect of Quercetin concentration. Al 1 x 10^{-5} mol L^{-1}, CTAB 1 x 10^{-5} mol L^{-1}, pH 5.5, wavelength 428 nm

Series II ; the various concentrations of CTAB were added to solution containing 1×10^{-5} mol L^{-1} of Al(III), 3×10^{-5} mol L^{-1} of Quercetin, 5 mL of 0.1 mol L^{-1} of acetate buffer pH 5.5 and diluted with deionized water in 25 mL volumetric flasks. Absorbance of each solution was measured at 428 nm. The results are shown in

Table 3.2 and Figure 3.3. It was found that the absorbance as peak height increased to maximum up to the CTAB concentration of 6×10^{-5} mol L^{-1} then it became constant and so did the absorbance in AU. Therefore, 6×10^{-5} mol L^{-1} of CTAB concentration was chosen.

Table 3.2 Effect of CTAB concentrations for mole-ratio Al(III)-Quercetin-CTAB complex.

[CTAB] (x 10 $^{-5}$ mol L^{-1})	Mole ratio (Al:Quercetin:CTAB)	Absorbance (AU)*
0	1:3:0	0.169
1	1:3:1	0.204
2	1:3:2	0.258
3	1:3:3	0.318
4	1:3:4	0.319
5	1:3:5	0.317
6	1:3:6	0.320

*average of triplicate results

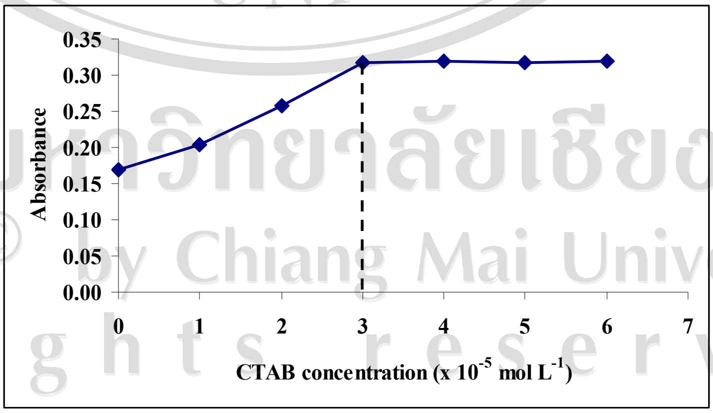

Figure 3.3 Mole-ratio study of Al(III)-Quercetin-CTAB system; effect of CTAB concentration, Al(III) 1 x 10^{-5} mol L^{-1}, Quercetin 3 x 10^{-5} mol L^{-1}, pH 5.5, wavelength 428 nm

From experimental results in Tables 3.1 and Figures 3.2 gave a mole-ratio of Al(III) : Quercetin : CTAB at 1 : 3 : 3. The complex may be exactly the same as reaction of Fe-Quercetin [81] and Al-Quercetin [38]. Moreover, W. Liu *et.al* [82] had described the reaction site of quercetin and CTAB that the most acidic phenolic OH groups of quercetin in the position 7 on the A ring can dissociate to form quercetin anions, which has electrostatic interaction with positively charged polar groups of CTAB. The complexation of Al(III)-Quercetin-CTAB was shown in Figure 3.4.

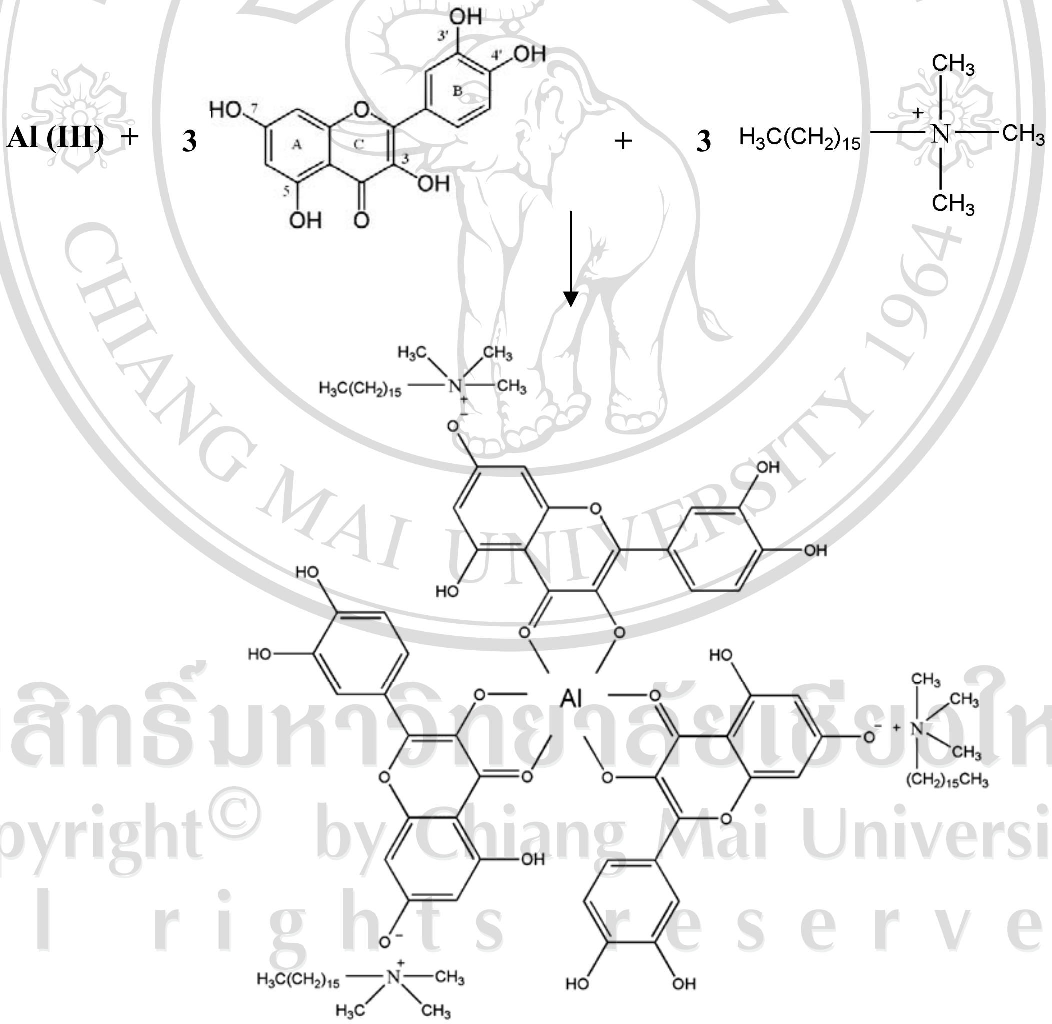

Figure 3.4 The comlexation of Al(III)-Quercetin-CTAB

3.2 rFIA Spectrophotometric Determination of Aluminium (III) Using Quercetin and Cetyltrimethyl Ammonium Bromide as A Complexing Agent

3.2.1 Optimization of the Reverse Flow System by Univariate Method

The conditions for the determination of aluminium (III) were optimized by studying the influences of the various parameters, such as wavelength, pH, concentration of Quercetin, % ethanol and CTAB, flow rate, reaction coil(I), reaction coil(II) and reagent loop, respectively. The optimum conditions obtained by means of the univariate optimization procedure (changing one variable in turn and keeping the others at their optimum values). To optimize the conditions, the rFIA manifold in Figure 2.1 and the preliminary experimental conditions (Table 2.2) were used.

3.2.1.1 Optimum wavelength

The optimum wavelength for aluminium (III) determination was studied over the range 420 – 435 nm by the proposed FIA system (Fig 2.3) using the experimental conditions as shown in Table 2.2. The results shown in Table 3.3 and Figure 3.5 indicated that the highest Δpeak height (ΔP.H.) was obtained when the absorbance was measured at 428 nm. Δpeak height (ΔP.H.) was the difference between peak height of blank and peak height of analyte. The analytical wavelength at 428 nm was selected for the further studies.

Table 3.3 Peak heights at various wavelength

Wave length (nm)	Peak Height (mV)*		ΔP.H.*
	Blank	Al (III) 0.2 ppm	
420	19.3	29.9	10.6
421	18.6	29.4	10.8
422	17.8	28.7	10.9
423	17.4	28.4	11.0
424	16.9	28.0	11.1
425	15.3	26.5	11.2
426	13.0	24.2	11.2
427	12.2	23.7	11.5
428	11.5	23.4	11.9
429	10.8	22.4	11.7
430	10.3	22.0	11.7
431	9.7	21.2	11.5
432	9.2	20.5	11.3
433	8.4	19.5	11.1
434	8.2	18.9	10.7
435	7.3	17.7	10.4

*average of triplicate results

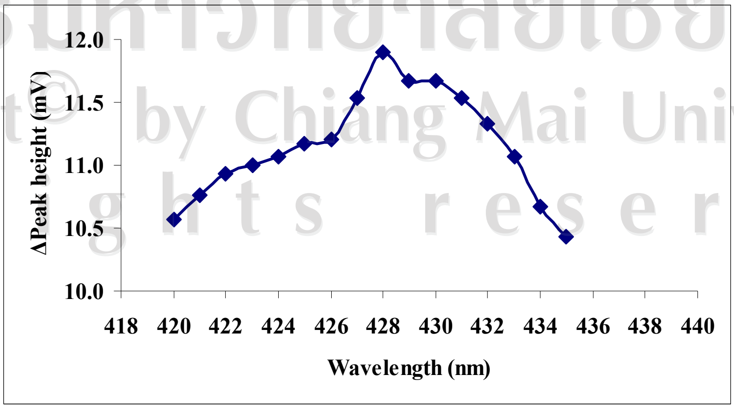

Figure 3.5 Δ Peak heights at various wavelengths

3.2.1.2 Effect of pH

The complexation of Al(III)-Quercetin-CTAB was studied pH at different values in the range of 3.0-7.0. The pH values of the solution were adjusted with acetic acid and sodium acetate. Using the manifold as shown in figure 2.1, a 0.1 mol L^{-1} concentration of acetate buffer solution was mixed in solution of aluminium (III) and CTAB. The results obtained are shown in Table 3.4 and Figure 3.6. The results indicated that the pH values below 4.5 or above 6.0 the sensitivity (slope of the calibration curve) decreased significantly. So, pH 5.5 was chosen because it provided the greatest sensitivity.

Table 3.4 Effect of pH on the sensitivity

pH	**ΔP.H.* (mV) obtained from the standard Al(III) (mg L^{-1})**					**y = mx + c**	**r^2**
	0.05	**0.10**	**0.15**	**0.20**	**0.25**		
3.0	0.6	1.2	1.9	2.4	3.6	3.61x - 0.22	0.9703
3.5	2.6	3.8	5.8	10.1	10.6	11.11x - 0.07	0.9413
4.0	2.4	5.2	8.0	11.1	13.3	13.91x - 0.35	0.9979
4.5	3.8	11.3	17.3	24.0	29.5	32.08x - 2.08	0.9974
5.0	8.9	13.8	23.2	30.7	37.3	36.91x + 0.65	0.9932
5.5	11.3	22.9	30.3	39.9	47.0	44.20x + 3.75	0.9928
6.0	9.0	18.0	26.9	33.9	41.6	40.58x + 1.53	0.9973
6.5	9.3	15.9	19.1	23.5	28.6	23.11x + 5.41	0.9890
7.0	6.8	12.1	15.4	20.0	23.9	21.01x + 3.02	0.9955

* average of triplicate results

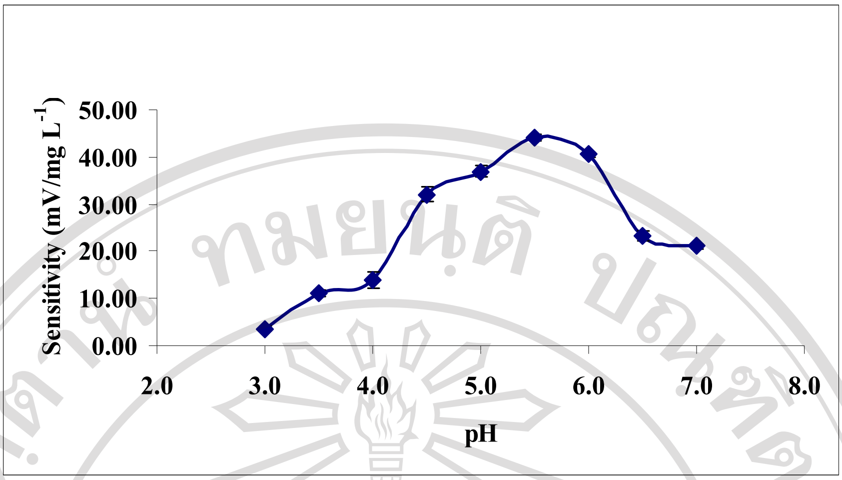

Figure 3.6 Relationship between pH and sensitivity of the calibration curve

3.2.1.3 Effect of Quercetin concentration

Effect of Quercetin concentrations on the determination of Al (III) (0.05-0.25 mg L^{-1}) was studied in the range 100 – 500 mg L^{-1}. The results are shown in Table 3.5 and Figure 3.7. It was found that sensitivity of calibration curve increased very rapidly from the Quercetin concentration of 200 – 300 mg L^{-1}. After that, the sensitivities were quite constant. This is due to the fact that increasing of the Quercetin concentration gives rise to the increase in the amounts of Al(III)-Quercetin-CTAB complexation which results in a higher sensitivity of calibration curve obtained. However, beyond the Quercetin concentration of 300 mg L^{-1}, the amount of Al(III)-Quercetin-CTAB complex became constant so as the Δpeak height. Consequently, a concentration of 300 mg L^{-1} of Quercetin was chosen as optimum.

Table 3.5 Effect of concentration of quercetin on the sensitivity

Quercetin Concentration (mg L^{-1})	ΔP.H.* (mV) obtained from the standard Al(III) (mg L^{-1})					y = mx + c	r^2
	0.05	0.10	0.15	0.20	0.25		
100	7.0	14.3	23.1	27.9	33.8	33.567x + 1.0933	0.9890
200	10.7	18.4	29.6	36.7	42.5	40.933x + 3.0133	0.9878
300	7.2	19.8	20.3	38.6	47.1	49.300x - 1.1867	0.9942
400	8.7	20.3	30.9	40.3	50.9	52.167x - 1.0933	0.9989
500	9.6	19.8	29.9	41.7	51.1	52.483x - 1.0633	0.9990

* average of triplicate results

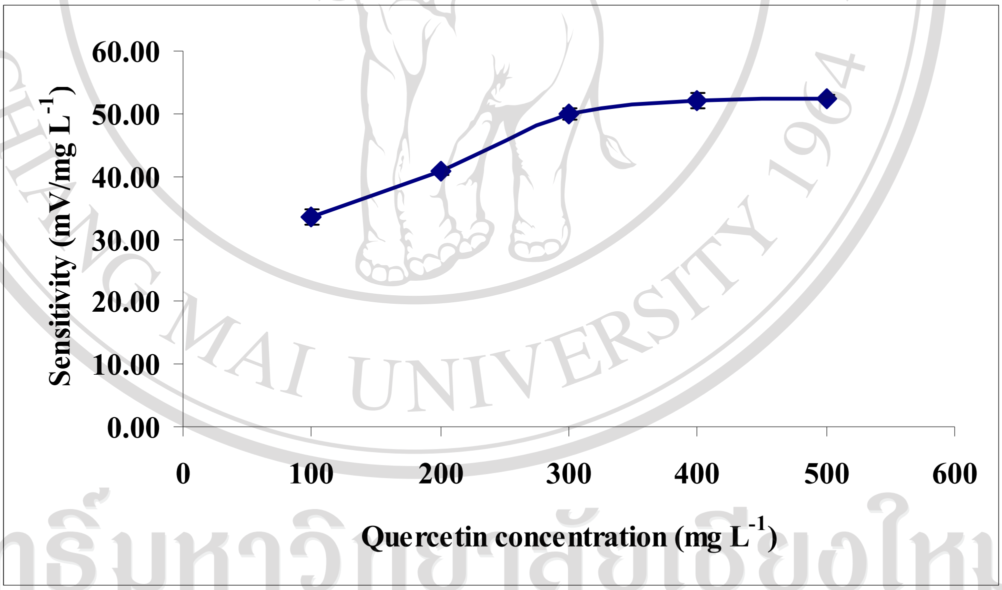

Figure 3.7 Relationship between concentration of Quercetin and sensitivity of the calibration curve

3.2.1.4 Effect of % ethanol in quercetin solution

The effect of concentration of ethanol in quercetin solution was studied in the range of 40-90% (v/v). The results are shown in Table 3.6 and Figure 3.8. It is shown that the greater sensitivity is obtained when the concentration of ethanol in quercetin solution increased. Thus, a concentration of ethanol in quercetin solution of 60% (v/v) was chosen as optimum % ethanol in quercetin solution.

Table 3.6 Effect of concentration of ethanol on the sensitivity

Concentration of ethanol (% v/v)	ΔP.H.* (mV) obtained from The standard Al(III) (mg L^{-1})					y = mx + c	r^2
	0.05	0.10	0.15	0.20	0.25		
40	11.0	18.6	27.7	38.0	48.1	38.55x + 0.71	0.9967
50	9.2	19.3	28.1	37.5	47.2	49.83x + 0.74	0.9946
60	9.0	19.8	31.0	40.3	48.8	56.01x + 2.05	0.9919
70	9.7	19.8	30.7	41.2	51.1	57.91x + 0.59	0.9956
80	10.9	20.6	28.2	38.7	46.2	58.13x - 0.07	0.9906
90	9.7	18.5	28.0	36.6	44.0	59.35x - 0.78	0.9937

* average of triplicate results

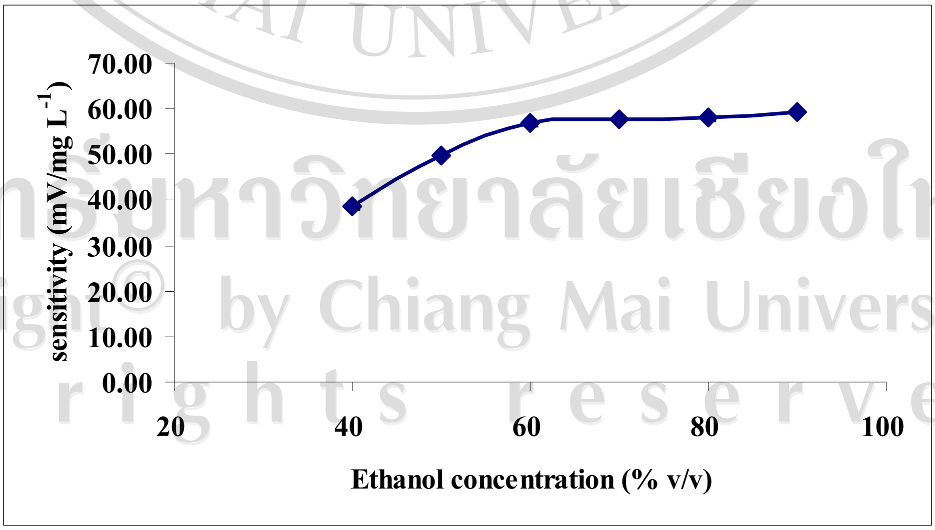

Figure 3.8 Relationship between concentration of ethanol in Quercetin solution and sensitivity of the calibration curve

3.2.1.5 Effect of CTAB concentration

The Effect of CTAB concentration on the determination of Al(III) (0.05-0.25 mg L^{-1}) was studied at different values in the range of 2.0 x 10^{-3} – 4.5 x 10^{-3} mol L^{-1}. The results are shown in Table 3.7 and Figure 3.9. It was found that sensitivity increased very rapidly from the CTAB concentration of 2.5 x 10^{-3} – 3.5 x 10^{-3} mol L^{-1}. After that, the sensitivities were quite constant. This is due to the fact that increasing the CTAB concentration leading to the increase in the amounts of Al(III)-Quercetin-CTAB complexation which results in a higher sensitivity of calibration curve obtained. However, beyond the CTAB concentration of 3.5 x 10^{-3} mol L^{-1}, the amount of Al(III)-Quercetin-CTAB complexation became constant so as the Δpeak height. Consequently, a concentration of 3.5x 10^{-3} mol L^{-1} of CTAB was chosen as optimum.

Table 3.7 Effect of concentration of CTAB on the sensitivity

CTAB concentration (x 10^{-3} mol L^{-1})	**ΔP.H.* (mV) obtained from the standard Al(III) (mg L^{-1})**					**y = mx + c**	**r^2**
	0.05	**0.10**	**0.15**	**0.20**	**0.25**		
2.0	11.0	18.6	27.7	38.0	48.1	46.850x + 0.5633	0.9965
2.5	9.2	19.3	28.1	37.5	47.2	47.117x - 0.0100	0.9996
3.0	9.0	19.8	31.0	40.3	48.8	50.050x - 0.2700	0.9967
3.5	9.7	19.8	30.7	41.2	51.1	52.133x - 0.7733	0.9998
4.0	10.7	20.6	31.9	42.3	52.7	52.883x - 0.0967	0.9997
4.5	14.5	26.9	37.2	47.8	57.4	53.383x + 4.7433	0.9980

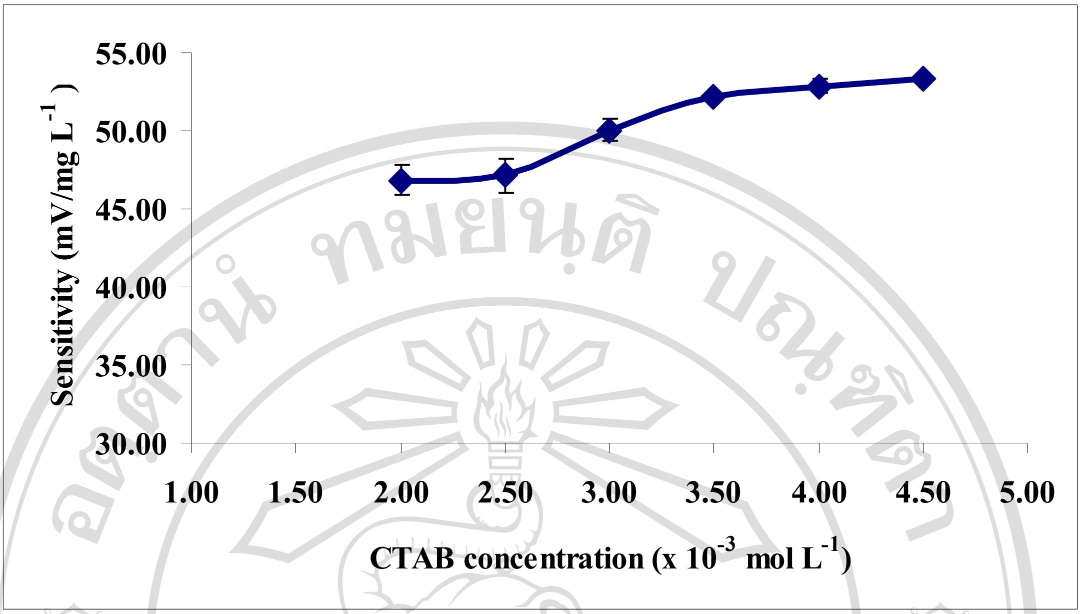

Figure 3.9 Relationship between concentration of CTAB and sensitivity of the calibration curve

3.2.1.6 Effect of flow rate

Effect of flow rate of standard and CTAB solutions were studied, by determination of various concentrations of standard Al(III) solutions (0.05-0.25 mg L^{-1}) which were flowed into the rFI manifold as shown in Figure 2.1. The flow rates were varied from 2.0 to 6.0 ml min^{-1}. The effect of flow rate on the sensitivity was shown in Table 3.8 and Figure 3.10. It can be seen that the optimum flow rate was 2.5 ml min^{-1}. In addition, the sensitivity of the calibration curve decreased, when the flow rate was lower than 2.5 ml min^{-1}. Because the low flow rate increased dispersion. On the other hand, the sensitivity of the calibration curve was lower, when the flow rate was higher than 2.5 ml min^{-1}. This is due to the fact that the higher flow rate reduced the reaction time and hence reduced complex formation.

Table 3.8 Effect of flow rate on the sensitivity

Flow rate (mL min^{-1})	**ΔP.H.* (mV) obtained from the standard Al(III) (mg L^{-1})**					**y = mx + c**	**r^2**
	0.05	**0.10**	**0.15**	**0.20**	**0.25**		
2.0	13.2	25.4	34.9	44.7	54.4	50.93x + 3.95	0.9977
2.5	11.8	24.9	35.6	46.1	55.5	54.26x + 2.22	0.9964
3.0	11.9	25.0	35.6	44.8	53.7	51.68x + 3.18	0.9934
3.5	11.3	20.3	31.1	40.1	48.6	47.25x + 1.92	0.9983
4.0	8.1	17.4	27.9	35.2	46.2	47.05x - 1.27	0.9971
4.5	8.1	18.2	26.1	36.3	43.5	44.45x - 0.22	0.9973
5.0	12.7	24.7	31.8	38.8	44.7	39.10x + 7.08	0.9800
5.5	11.5	23.0	31.4	38.0	43.1	39.08x + 5.93	0.9752
6.0	10.4	20.1	26.3	32.4	36.7	32.45x + 5.70	0.9789

*average of triplicate results

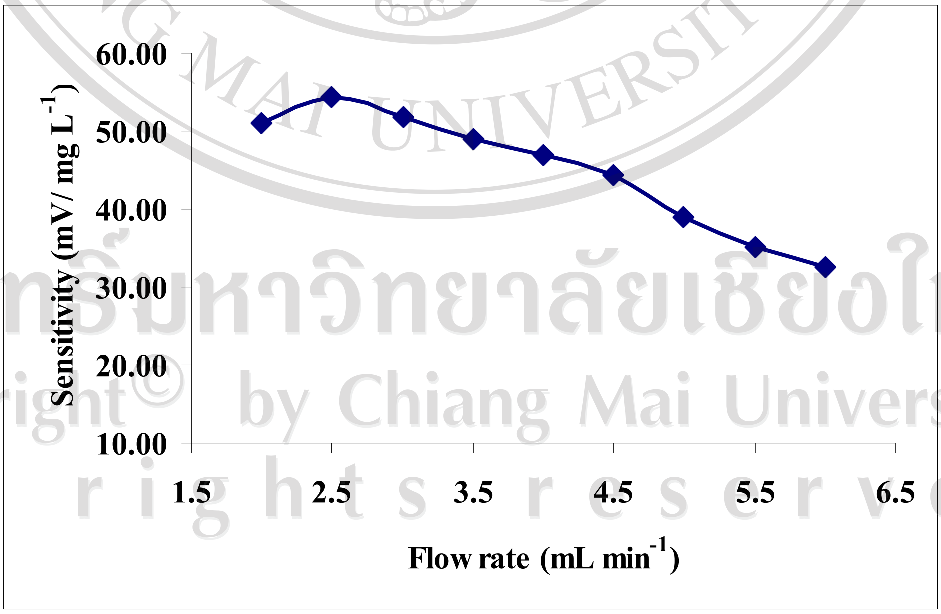

Figure 3.10 Relationship between flow rate and sensitivity of the calibration curve

3.2.1.7 Effect of reaction coil (I) length

The effect of reaction coil (I) length on the determination of Al (III) (0.05-0.25 mg L^{-1}) was studied by using Tygon tubing with diameter of 1.07 mm i.d. and length of reaction coil (I) were varied from 25 to 150 cm. The results are shown in Table 3.9 and Figure 3.10. The sensitivity increased to a maximum at a reaction coil (I) length of 75 cm. It can be explained that increasing the reaction coil (I) length up to 75 cm give rise to an increase in the residence time allowing well mixing between aluminium (III) and QC. On the other hand, the sensitivity of the calibration curve decreased when the reaction coil (I) length was longer than 75 cm. This is due to dispersion occurred at the reaction coil (I) length longer than 75 cm. The reaction coil (I) length 75 cm was chosen as optimum since it provided the greatest sensitivity.

Table 3.9 Effect of reaction coil (I) length on the sensitivity

Reaction coil (I) length (cm)	**ΔP.H.* (mV) obtained from the standard Al(III) (mg L^{-1})**					**y = mx + c**	**r^2**
	0.05	**0.10**	**0.15**	**0.20**	**0.25**		
25	8.5	19.3	29.1	36.0	43.6	43.41x + 1.25	0.9910
50	11.6	22.7	32.6	40.8	48.3	45.81x + 3.73	0.9930
75	9.5	22.7	33.8	43.1	52.7	53.33x + 0.35	0.9945
100	10.7	22.7	33.8	42.4	50.6	49.68x + 2.25	0.9923
125	10.1	22.4	31.5	41.4	49.4	48.80x + 1.68	0.9945
150	9.4	20.3	31.5	38.7	45.5	45.23x + 1.92	0.9861

* average of triplicate results

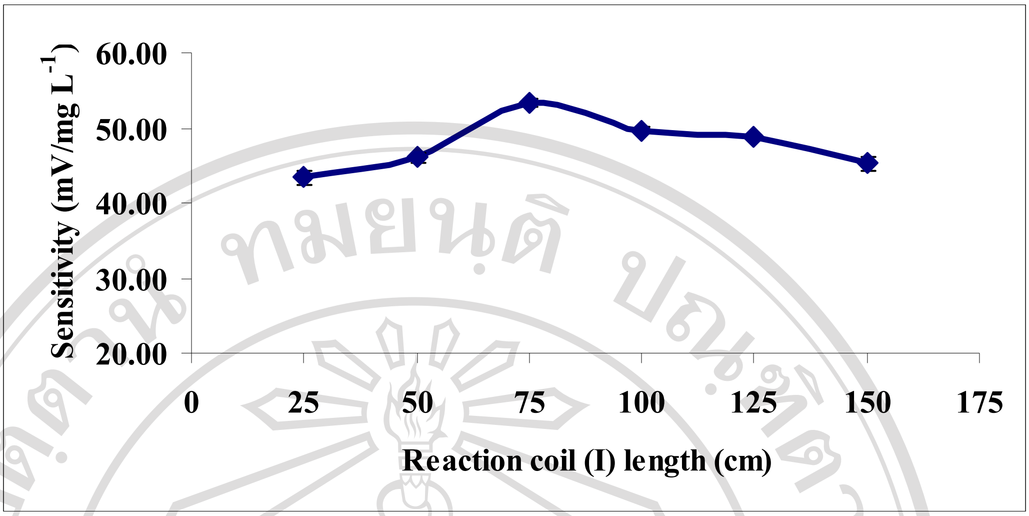

Figure 3.11 Relationship between reaction coil (I) length and sensitivity of the calibration curve

3.2.1.8 Effect of reaction coil (II) length

The effect of reaction coil (II) length on the determination of Al (III) (0.05-0.25 mg L^{-1}). The 1.07 mm i.d. Tygon tubing was examined for using as a reaction coil (II). The reaction coils (II) were varied from 25 to 150 cm. The results are shown in Table 3.10 and Figure 3.12. The sensitivity increased to a maximum at a reaction coil (II) length of 100 cm. It can be explained that increasing the reaction coil (II) length up to 100 cm an increase in the residence time is obtained, allowing well mixing between Al(III)-Quercetin and CTAB. On the other hand, the sensitivity of the calibration curve decreased when the reaction coil (II) length was longer than 100 cm. This is due to the fact that dispersion occurred at the reaction coil (II) length longer than 100 cm. The 100 cm reaction coil (II) length was chosen as optimum since it provided the greatest sensitivity.

Table 3.10 Effect of reaction coil (II) length on the sensitivity

Reaction coil (II) length (cm)	ΔP.H.* (mV) obtained from the standard Al(III) (mg L^{-1})					y = mx + c	r^2
	0.05	0.10	0.15	0.20	0.25		
25	14.9	25.9	32.5	41.4	47.1	40.03x + 8.32	0.9891
50	11.2	22.0	31.9	41.2	49.7	48.13x + 2.34	0.9979
75	12.8	25.1	36.4	44.7	55.0	52.03x + 3.56	0.9954
100	11.0	25.7	37.6	49.2	59.7	60.36x + 0.42	0.9960
125	14.8	28.6	39.9	50.5	60.5	56.61x + 4.89	0.9958
150	11.6	26.6	36.4	47.5	56.8	55.70x + 2.36	0.9921

* average of triplicate results

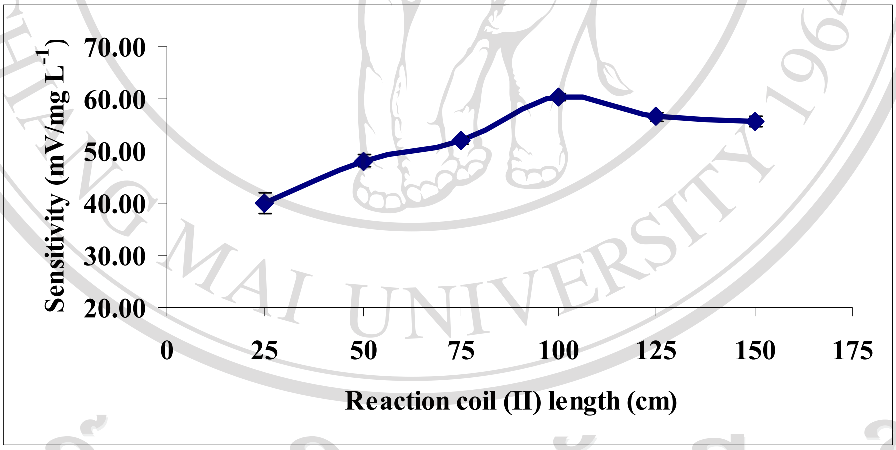

Figure 3.12 Relationship between reaction coil (II) length and sensitivity of the calibration curve

3.2.1.9 Effect of reagent volume

The reagent volume injected into the aluminum stream has a significant effect on peak height. The effect of reagent volume on the determination of 0.05-0.25 mgL^{-1} Al(III) was studied by varying reagent volume of 50, 75, 100, 125 and 150 µL. As shown in Table 3.11 and Figure 3.13, the sensitivity increases with increasing reagent volume up to 125 μL because the increase in reagent volume increase the number of complex (Al(III)-Quercetin-CTAB), that cause increases in Δpeak height. A reagent volume of 125 μL was chosen as a compromise between good sensitivity and reagent consumption.

Table 3.11 Effect of reagent volume on the sensitivity

Reagent loop (μL)	**ΔP.H.* (mV) obtained from the standard Al(III) (mg L^{-1})**					**y = mx + c**	**r^2**
	0.05	**0.10**	**0.15**	**0.20**	**0.25**		
50	10.8	20.8	28.2	34.5	39.7	35.71x + 5.39	0.9837
75	12.0	24.8	36.4	43.8	51.8	49.26x + 4.22	0.9849
100	13.1	27.5	39.2	48.6	57.1	54.58x + 4.34	0.9881
125	13.2	27.2	40.4	51.7	61.9	60.91x + 2.33	0.9956
150	12.3	25.8	38.1	49.8	58.7	58.41x + 1.87	0.9947
175	13.9	26.8	37.6	48.0	57.7	54.41x + 4.13	0.9969
200	13.6	26.1	37.8	46.9	56.9	53.68x + 4.04	0.9959

* average of triplicate results

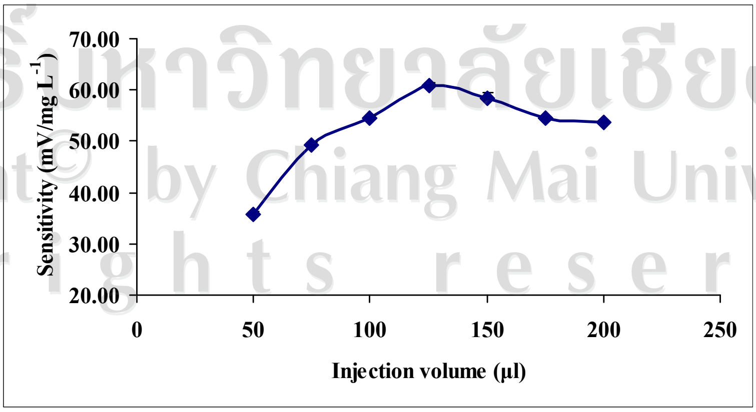

Figure 3.13 Relationship between reagent volume and sensitivity of the calibration curve

3.2.1.8 Summary of the Studied Range and Optimum Conditions

A diagram of the recommended rFIA manifold is displayed in Figure 2.1. Table 3.12 shows the ranges over which the variables involved in the rFIA system were studied and their optimum values.

Table 3.12 Univariate optimization of chemical and rFIA variables.

Variable	Studied range	Optimum value
Wavelength (nm)	420-435	428
pH	3.0 - 7.0	5.5
Concetration of Quercetin (mg L^{-1})	100 - 500	300
Concentration of ethanol in quercetin solution (% v/v)	40 - 90	60
Concentration of CTAB (x 10^{-3} mol L^{-1})	2.5 - 4.5	3.5
Flow rate (mL min^{-1})	2.0 – 6.0	2.5
Reaction Coil (I) length (cm)	25 -150	75
Reaction Coil (II) length (cm)	25 -150	100
Sample volume (µL)	50 - 200	125

3.2.2 Analytical Characteristics of the method

3.2.2.1 Linear range

The linear range of the proposed method was studied by flowing Al (III) standard solution into rFI system under the suitable conditions as shown in Table 3.12, linear range of the calibration graph was obtained for Al (III) standards at the concentration ranging from 0.02–0.50 mg L^{-1}. All measurements were made in pentaplicate injections. The results obtained are shown in Table 3.13 and Figure 3.14.

Table 3.13 Peak height at various aluminium (III) concentrations for linearity check of the calibration graph

Aluminium	**Peak height (AU)**						**ΔP.H.***
(mg L^{-1})	**1**	**2**	**3**	**4**	**5**	**$\bar{x}$**	**(mV)**
0	16.3	16.3	16.4	16.3	16.2	16.3	0.0
0.005	16.4	16.4	16.3	16.4	16.4	16.4	0.1
0.01	16.7	16.7	16.9	16.8	16.9	16.8	0.5
0.02	17.6	17.6	17.8	17.7	17.6	17.7	1.4
0.03	18.2	18.1	18.2	18.2	18.4	18.2	1.9
0.04	18.9	18.9	19	18.7	18.8	18.9	2.6
0.05	19.6	19.5	19.4	19.6	19.6	19.5	3.2
0.06	20.2	20	20.1	20.2	20.1	20.1	3.8
0.07	20.8	20.7	20.7	20.7	20.7	20.7	4.4
0.08	21.3	21.5	21.4	21.5	21.4	21.4	5.1
0.09	21.9	22	21.9	22.1	22	22.0	5.7
0.10	22.6	22.6	22.7	22.8	22.6	22.7	6.4
0.15	25.4	25.6	25.6	25.6	25.6	25.6	9.3
0.20	28.6	28.6	28.7	28.9	28.6	28.7	12.4
0.25	31.2	31.2	31.2	31.2	31.2	31.2	15.0
0.30	34.3	34.3	34.3	34.3	34.3	34.3	18.0
0.35	36.9	36.7	36.9	36.8	36.6	36.8	20.5
0.40	39.7	39.7	39.7	39.7	39.7	39.7	23.4
0.45	42.8	43	42.7	42.5	42.7	42.7	26.4
0.50	45.1	45.3	45.3	45.2	45.3	45.2	28.9
0.60	48.9	48.9	48.9	48.9	48.9	48.9	32.6
0.70	52.7	52.8	52.7	52.7	52.7	52.7	36.4
0.80	56.6	58.1	58.1	58.1	58.1	57.8	41.5
0.90	60.2	60.1	60.4	60.5	60.4	60.3	44.0
1.00	60.9	61.4	61.2	61.2	61.3	61.2	44.9
1.5	65.3	65.2	65.6	65.4	65.9	65.5	49.2

* average of triplicate results

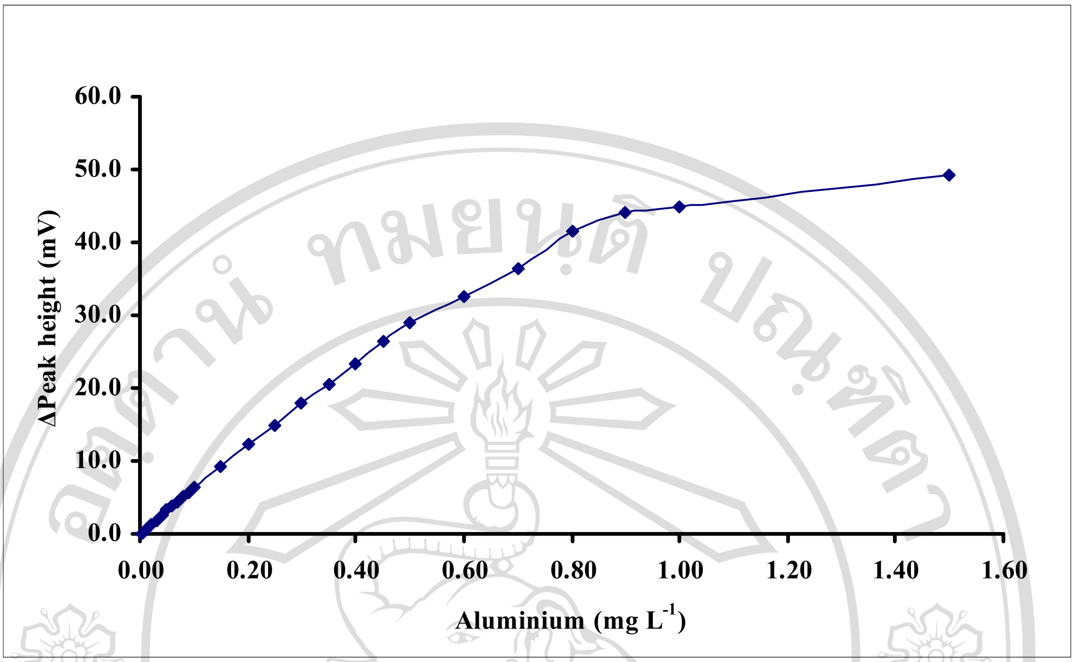

Figure 3.14 Relationship between Δpeak height and concentrations of Al (III) 0.005-1.5 mg L^{-1}

3.2.2.2 Precision of the flow injection system

The precision of the proposed method was verified by 11 replicated determination of 0.2 mg L^{-1} standard Al(III), under the optimum conditions listed in Table 3.12. The relative standard deviation was found to be 1.10% (Table 3.14).

Table 3.14 Precision verification using standard 0.2 mg L^{-1} aluminium (III)

Experimental number	**Peak height (mV)**
1	34.8
2	35.1
3	35.0
4	35.4
5	34.6
6	35.4
7	35.8
8	35.4
9	35.2
10	35.6
11	35.8
$\bar{x}$	35.3
S.D.	0.39
% R.S.D.	1.10

3.2.2.3 Calibration curve

As depicted in Figure 2.1, the standard solutions containing 0.02-0.50 mg L^{-1} were flowed into the rFI system under the established optimum conditions (Table 3.12). The results are shown in Table 3.15. The calibration curve as shown in Figure 3.15 and 3.16 were established by plotting Δpeak heights versus the various aluminum concentrations. Correlation coefficients (r^2) and the regression equation is as follows:

$y = 61.363x - 0.0246$ ($r^2 = 0.9998$)

Where y is Δpeak height in mV

x is concentration of aluminium (III) in mg L^{-1}

Table 3.15 ΔPeak height for various Al (III) concentrations (0-0.50 mg L^{-1})

Aluminium	Peak height (mV)						ΔP.H.*
(mg L^{-1})	1	2	3	4	5	$\bar{x}$	(mV)
0	10.90	11.00	10.90	10.90	11.00	10.94	0.00
0.02	12.10	12.00	11.90	12.20	12.30	12.10	1.16
0.06	14.40	14.50	14.70	14.50	14.50	14.52	3.58
0.10	17.10	17.10	16.90	17.00	16.80	16.98	6.04
0.15	20.10	20.30	20.20	20.10	20.10	20.16	9.22
0.20	23.10	22.90	23.20	23.20	23.50	23.18	12.24
0.25	26.20	26.40	26.10	26.40	26.50	26.32	15.38
0.30	29.10	29.40	29.50	29.60	30.10	29.54	18.60
0.35	32.70	32.40	32.60	32.70	32.70	32.62	21.68
0.40	35.40	35.40	35.30	35.30	35.50	35.38	24.44
0.45	38.10	38.20	38.50	38.30	38.20	38.26	27.32
0.50	41.40	41.50	41.40	41.90	41.70	41.60	30.66

*average of pentaplicate results

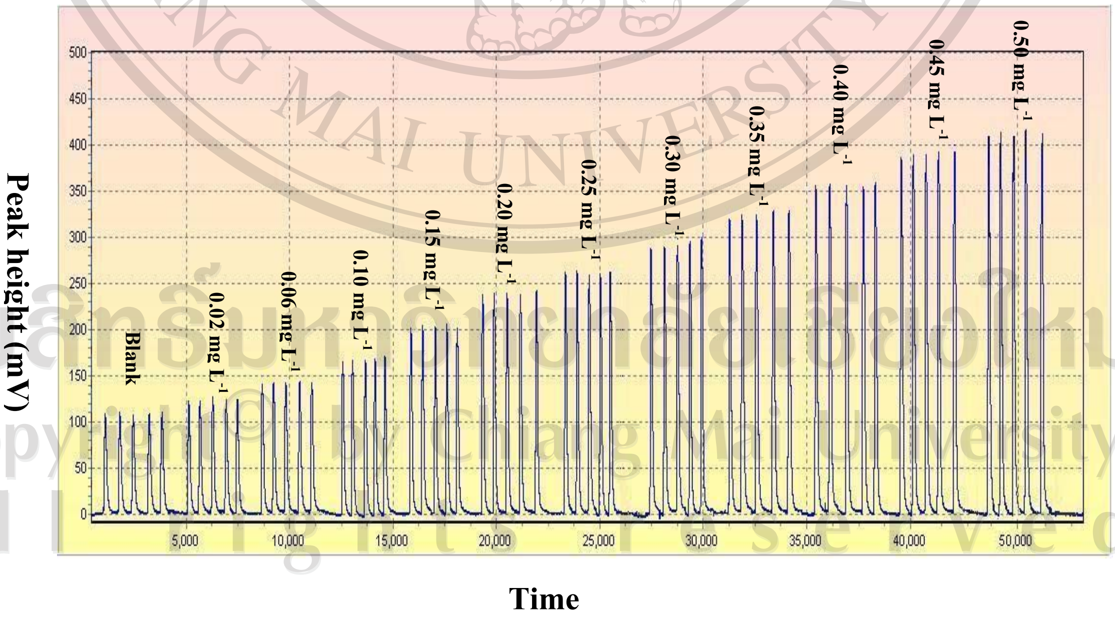

Figure 3.15 Calibration signal of rFIA spectrophotometric determination of Al(III) 0.02-0.50 mg L^{-1}

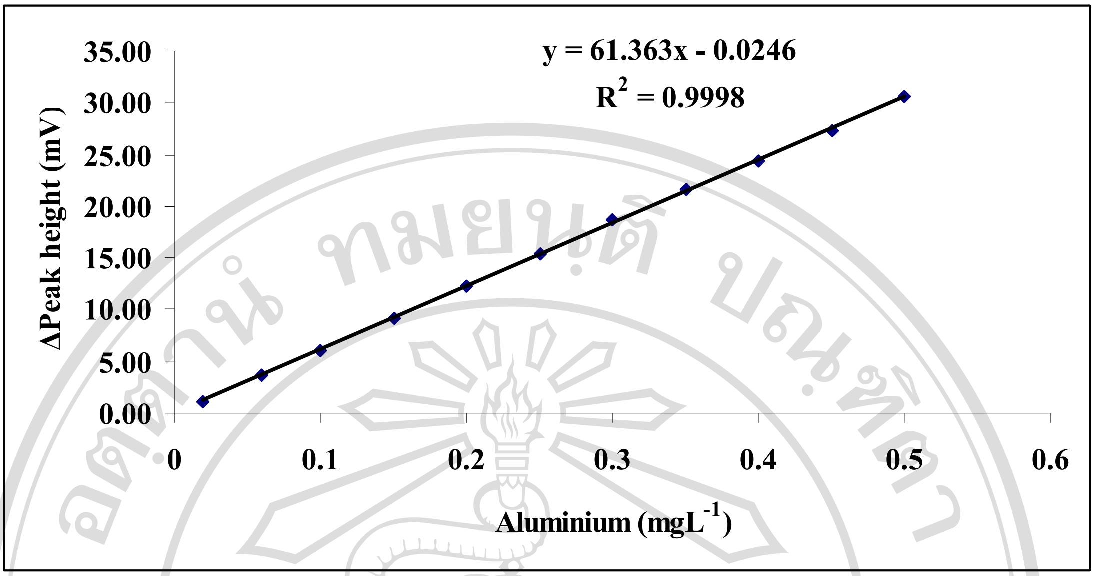

Figure 3.16 The Calibration curve of rFIA spectrophotometric determination of Al(III) 0.02-0.5 mg L^{-1}

3.2.2.4 Detection limit

The detection limit was determined by the method reported by Miller and Miller [81], which was calculated from the linear regression line of calibration curve. The results are giving in Tables 3.16. The detection limit of the proposed method was found to be 0.007 mg L^{-1}. The concentration at limit of detection (*CL*) can be calculated from equation 2.2-2.3.

Table 3.16 Calculation of detection limit of rFIA spectrophotometric determination of aluminium (III)

Aluminium (mgL^{-1})	Y_i^*	$\hat{Y}_i$	$\lvert Y_i^* - \hat{Y}_i \rvert$	$\lvert Y_i^* - \hat{Y}_i \rvert^2$
0.02	1.16	1.18	0.02	0.0068
0.06	3.58	3.64	0.06	0.0049
0.10	6.04	6.10	0.06	0.0018
0.15	9.22	9.17	0.05	0.0003
0.20	12.24	12.24	0.00	0.0089
0.25	15.38	15.31	0.07	0.0025
0.30	18.60	18.38	0.22	0.0007
0.35	21.68	21.45	0.23	0.0201
0.40	24.44	24.52	0.08	0.0333
0.45	27.32	27.59	0.27	0.0022
0.50	30.66	30.67	0.01	0.0001
$\sum(\lvert Y_i^* - \hat{Y}_i \rvert^2)$				0.1968
S_y/x				0.1479
C_L,LOD				0.007
LOQ				0.024

The linear regression equation is Y = 61.685x - 0.0514

Sy/x = $[0.1968/(11-2)]^{1/2}$

= 0.1479

C_L, LOD = (3 x 0.1479)/61.363

= 0.007 mg L^{-1} Al(III)

LOQ = (10 x 0.1479)/61.363

= 0.024 mg L^{-1} Al(III)

3.2.2.5 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 obtained (Table 3.12). A systematic study to check for the effects of some possible foreign ions on the determination of aluminium (III) was undertaken for the maximum w/w ratio of aluminum to foreign ions up to 1 : 500. The solutions of a 0.2 mg L^{-1} aluminium (III) standard containing varying concentrations of diverse ions were determined using the rFIA system. The tolerance is defined as the largest foreign-ion concentration causing % recovery between 90-110 % for determining the analyte of interest. The tolerance values for the ions studied are given in Table 3.17.

Table 3.17 Interference studies for 0.20 mg L^{-1} standard aluminum by rFIA method

Interference	Aluminium : Interference	Peak height* (mV)	% Recovery
Cu^{2+}	1 : 0	12.6	-
	1 : 0.2	12.0	95
	1 : 0.4	13.1	104
	1 : 0.8	14.1	112
Fe^{2+}	1 : 0	12.6	-
	1 : 0.1	12.6	100
	1 : 0.5	13.6	108
	1 : 1.0	15.5	123
Fe^{3+}	1 : 0	12.6	-
	1 : 0.05	12.6	100
	1 : 0.1	12.6	100
	1 : 0.5	16.0	127

* average of triplicate result

Table 3.17 (Continued)

Interference	Aluminium : Interference	Peak height* (mV)	% Recovery
NO_2^-	1 : 0	12.6	-
	1 : 50	12.6	100
	1 : 80	12.1	96
	1 : 100	11.7	93
Na^+	1 : 0	12.6	-
	1 : 500	12.6	100
	1 : 1000	13.0	103
	1 : 2500	12.7	101
K^+	1 : 0	12.6	-
	1 : 500	12.6	100
	1 : 2500	12.9	102
	1 : 5000	12.7	101
Mg^{2+}	1 : 0	12.6	-
	1 : 50	12.6	100
	1 : 100	13.1	104
	1 : 250	12.0	95
Ca^{2+}	1 : 0	12.6	-
	1 : 50	12.9	102
	1 : 80	12.1	96
	1 : 100	10.3	82
Ba^{2+}	1 : 0	12.6	-
	1 : 50	12.9	102
	1 : 100	13.0	103
	1 : 500	13.2	105
Mn^{2+}	1 : 0	12.6	-
	1 : 100	11.8	94
	1 : 500	13.6	108
	1 : 1000	15.6	124

* average of triplicate result

Table 3.17 (Continued)

Interference	Aluminium : Interference	Peak height* (mV)	% Recovery
Co^{2+}	1 : 0	12.8	-
	1 : 100	12.4	97
	1 : 500	12.9	101
	1 : 600	12.7	99
Zn^{2+}	1 : 0	12.8	-
	1 : 10	12.8	100
	1 : 100	13.6	106
	1 : 125	15.2	119
Ni^{2+}	1 : 0	12.8	-
	1 : 10	12.9	101
	1 : 100	13.3	104
	1 : 500	13.3	104
Cr^{3+}	1 : 0	12.8	-
	1 : 100	12.8	100
	1 : 200	13.1	102
	1 : 500	14.6	114
Cd^{2+}	1 : 0	12.8	-
	1 : 10	12.7	99
	1 : 100	12.8	100
	1 : 1000	12.3	96
F^-	1 : 0	12.8	-
	1 : 100	12.5	98
	1 : 200	12.5	98
	1 : 500	12.3	96
PO_4^{3-}	1 : 0	12.8	-
	1 : 10	13.3	104
	1 : 500	13.8	108
	1 : 1000	14.0	109

* average of triplicate result

Table 3.17 (Continued)

Interference	Aluminium : Interference	Peak height* (mV)	% Recovery
Cl^-	1 : 0	12.6	-
	1 : 100	12.4	98
	1 : 500	12.8	101
	1 : 1000	11.6	92
NO_3^-	1 : 0	12.6	-
	1 : 100	12.5	99
	1 : 250	13.2	105
	1 : 500	12.1	96
SO_4^{2-}	1 : 0	12.6	-
	1 : 100	12.2	97
	1 : 500	13.1	104
	1 : 1000	12.9	102
HCO_3^-	1 : 0	12.6	-
	1 : 1	12.9	102
	1 : 5	12.7	101
	1 : 10	12.5	99
Br^-	1 : 0	12.6	-
	1 : 500	12.9	102
	1 : 1000	12.9	102
	1 : 1500	12.0	95
I^-	1 : 0	12.6	-
	1 : 100	12.9	102
	1 : 500	12.9	102
	1 : 1000	12.2	97
CN^-	1 : 0	12.6	-
	1 : 5	12.3	98
	1 : 10	12.1	96
	1 : 20	11.7	93

* average of triplicate result

The interference effects of some possible foreign ions on aluminum determination using rFIA system were summarized in Table 3.18. It was found that Fe^{2+}, Fe^{3+} and Cu^{2+} interfered seriously with the determination aluminium (III) in tap water, which referred standard quality of tap water defined by metropolitan waterworks authority (Appendix A).

Table 3.18 Summary of interference effects of some ions on the response obtained from aluminium (III) 0.2 mg L^{-1}

Interference ions	**Tolerable concentration ratio*(mg L^{-1}) of ion/Al (III)**
Na^+, Cd^{2+}, PO_4^{3-}, Cl^-, Br^-, I^-, SO_4^{2-}	≥1000
Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, F^-, NO^{3-},	500
Mg^{2+}	250
PO_4^{3-}, Cr^{3+}	200
Zn^{2+}	100
Ca^{2+},	80
CN^-	20
NO_2^-, HCO_3^-	10
Fe^{2+}	0.5
Cu^{2+}	0.4
Fe^{3+}	0.1

The concentration of interference ion is considered to be interfered when causing % recovery less than 90% and more than 110% with respect of the signal of aluminium alone.

3.2.2.6 Effect of masking agents and interference

The effect of masking agents and interference was studied by the proposed rFIA procedure under the optimum conditions. In order to overcome interference effects of Fe^{2+}, Fe^{3+} and Cu^{2+} on Al (III) determination various masking agents were investigated. The results are shown in Table 3.19. It was found that Fe^{2+} was masked with 0.01 mol L^{-1} of 1,10-phenanthroline [83]. Fe^{3+} was reduced to Fe^{2+} with 0.006 mol L^{-1} of ascorbic acid and masked in the same manner as Fe^{2+} [83] and Cu^{2+} was masked with 0.25% v/v of thiourea [83].

Table 3.19 Effect of masking agent for mask Fe^{2+}, Fe^{3+} and Cu^{2+} the response obtained from Al(III) 0.2 mg L^{-1}

Interference	Concentration of masking agent	Aluminium :Interference	Peak height* (mV)	% Recovery
Cu^{2+}	0.10% Thiourea	1 : 0	12.4	-
		1 : 10	12.6	103
		1 : 15	13.0	105
		1 : 20	13.7	108
	0.25% Thiourea	1 : 0	12.1	-
		1 : 20	12.4	102
		1 : 30	12.7	105
		1 : 50	13.0	107
	0.50% Thiourea	1 : 0	12.3	-
		1 : 30	12.6	102
		1 : 50	12.9	105
		1 : 70	13.7	111

* average of triplicate result

Table 3.19 (Continued)

Interference	**Concentration of masking agent**	**Aluminium :Interference**	**Peak height[*] (mV)**	**% Recovery**
Fe^{2+}	0.005 mol L^{-1} 1,10-phenanthroline	1 : 0	12.4	-
		1 : 1	12.7	103
		1 : 5	13.9	112
		1 : 10	16.8	137
	0.008 mol L^{-1} 1,10-phenanthroline	1 : 0	12.4	-
		1 : 5	12.5	101
		1 : 10	12.8	103
		1 : 15	15.5	125
	0.01 mol L^{-1} 1,10-phenanthroline	1 : 0	12.4	-
		1 : 5	12.2	98
		1 : 10	12.8	103
		1 : 15	13.3	107
Fe^{3+}	0.002 mol L^{-1} Ascorbic acid + 0.01 mol L^{-1} 1,10-phenanthroline	1 : 0	12.4	-
		1 : 5	13.0	105
		1 : 10	14.0	113
		1 : 15	15.3	123
	0.004 mol L^{-1} Ascorbic acid + 0.01 mol L^{-1} 1,10-phenanthroline	1 : 0	12.4	-
		1 : 10	13.0	105
		1 : 15	13.4	108
		1 : 20	14.4	116
	0.006 mol L^{-1} Ascorbic acid + 0.01 mol L^{-1} 1,10-phenanthroline	1 : 0	12.4	-
		1 : 10	12.8	103
		1 : 15	12.4	100
		1 : 20	13.3	107

* average of triplicate result

3.2.2.7 Determination of aluminium (III) in waters

The proposed rFI spectrophotometric method was applied to the simultaneous determination of aluminium (III) in tap water samples which were collected from several Aumphur in Chiang Mai. The peak heights from each sample were compared with standard calibration curve. The results were given in Table 3.20

Table 3.20 Determination of aluminium (III) in water samples by rFIA method

Water samples	**Peak heights**				**SD**	**Aluminium concentration* (mg L^{-1})**	**% Recovery***
	1	**2**	**3**	$\bar{x}$			
Chom Thong	0.271	0.268	0.271	0.270	0.002	0.270 ± 0.002	98.7
Doi Saket	0.081	0.076	0.079	0.079	0.003	0.079 ± 0.003	99.9
Hangdong	0.075	0.078	0.077	0.077	0.002	0.077 ± 0.002	102.2
Mae Rim	0.181	0.183	0.185	0.183	0.002	0.183 ± 0.002	99.9
Mae Wang	0.118	0.120	0.121	0.120	0.002	0.120 ± 0.002	102.3
Mueang	0.081	0.082	0.085	0.083	0.002	0.083 ± 0.002	99.4
Sanpathong	ND**	ND**	ND**	-	-	ND**	98.3
Sansai	0.051	0.049	0.053	0.050	0.002	0.050 ± 0.002	100.8
Sankumphaeng	ND**	ND**	ND**	-	-	ND**	101.4
Chaing Mai University	0.221	0.227	0.225	0.224	0.003	0.224 ± 0.003	98.8

* average of triplicate result

The Al(III) contents in the water samples were in the range of 0.050-0.270 mg L^{-1} and 0.011-0.273 mg L^{-1} using the proposed method and ICP-MS respectively. The results obtained by the proposed rFI spectrohotometric method compared favorably with those obtained by ICP-MS using the student *t*-test (Table 3.21 and Appendix B in Table B.1). It was seen that experimental *t*-value for Al(III) assay, which was smaller than the theoretical *t*-value at a confidence interval of 95% (4.30) indicating that results obtained by both methods were in excellent agreement.

Table 3.21 Comparative determination of aluminium (III) in tap water samples by proposed rFIA method and ICP-MS

Water samples	**Concentrations (mg L^{-1})**		$t_{calculated}$
	rFIA*	**ICP-MS***	
Jomthong	0.270	0.273	-3.000
Doi Saket	0.079	0.081	-1.606
Hangdong	0.077	0.075	1.890
Mae Rim	0.183	0.189	-5.196
Mae Wang	0.120	0.120	-0.378
Muang	0.083	0.087	-3.606
Sanpathong	ND**	0.011	-
Sansai	0.050	0.048	2.598
Sankumpang	ND**	0.014	-
Chaing Mai University	0.224	0.228	-2.079

*average of triplicate results
**not detected

3.3 SIA Spectrophotometric Determination of Aluminium (III) Using Quercetin and as A Complexing Agent

The conditions for the determination of aluminium (III) were optimized by studying the influences of the various parameters, such as sample and reagent volumes, reagent/carrier flow rates, and reagent concentrations of the respective measurements. The optimum conditions obtained by means of the univariate optimization procedure (changing one variable in turn and keeping the others at their optimum values). The optimal value for each parameter was judged from maximum response of the detector, minimum noise of the baseline and relative standard deviation. To optimize the conditions, the SIA manifold in Figure 2.2 and 2.3 and preliminary experimental conditions in Table 2.4 were used. The range of variables studied and the optimal values chosen are shown in Table 2.3.

3.3.1 Study aspiration order

The complexation of Al(III)-Quercetin-CTAB was studied at different aspiration orders. The sensitivities obtained are shown in Table 3.22. It was found that the aspiration order of first series provides a highest sensitivity. So, aspiration order of first series was chosen for further optimization of SIA method.

Table 3.22 Sensitivity at various aspiration orders

Series	Aspiration order	Sensitivity (AU/mg L^{-1})
1	A-B-C	0.1605
2	A-C-B	0.1247
3	B-A-C	0.1412
4	B-C-A	ND*
5	C-A-B	0.0820
6	C-B-A	0.1118

A was 0.1 mg L^{-1} aluminium (III) standard solution

B was 300 mg L^{-1} Quercetin

C was 3.5 x 10^{-3} mol L^{-1} cetyltrimethylammonium bromide

*ND is not detected.

3.3.2 Optimization of the sequential injection system by univariate method

To optimize the experimental conditions, the SIA manifold in Figure 2.2-2.3 was employed and the preliminary experimental conditions (Table 2.4) were re-investigated.

3.3.2.1 Effect of pH

In general, the complex equilibrium of any complex formation reaction is pH dependent. Selectivity of certain ions can be achieved by altering the pH of the solution to an appropriate pH value. It is necessary to examine the suitable pH to assess the best selectivity for aluminium (III) determination by measuring the absorbance at the wavelength 428 nm and use the preliminary experimental conditions in Table 2.5. A systematic study was carried out to check the influence of various pH values (4.0, 4.5, 5.0, 5.5 and 6.0) on the determination of aluminium (III) in the mixture solutions. The results are shown in Table 3.23 and Fig 3.17. The results indicated that at pH value below 5.5 or above 5.5 the slope decreased significantly. So, pH 5.5 was chosen because it provided the greatest sensitivity.

Table 3.23 Effect of pH on the sensitivity

pH	Sensitivity (AU/mg L^{-1})				SD
	1	2	3	$\bar{x}^*$	
4.0	0.1376	0.1391	0.1348	0.1372	0.002
4.5	0.1471	0.1454	0.1464	0.1463	0.001
5.0	0.1526	0.1542	0.1558	0.1542	0.002
5.5	0.1647	0.1631	0.1623	0.1634	0.001
6.0	0.1443	0.1415	0.1428	0.1429	0.001

*average of triplicate results

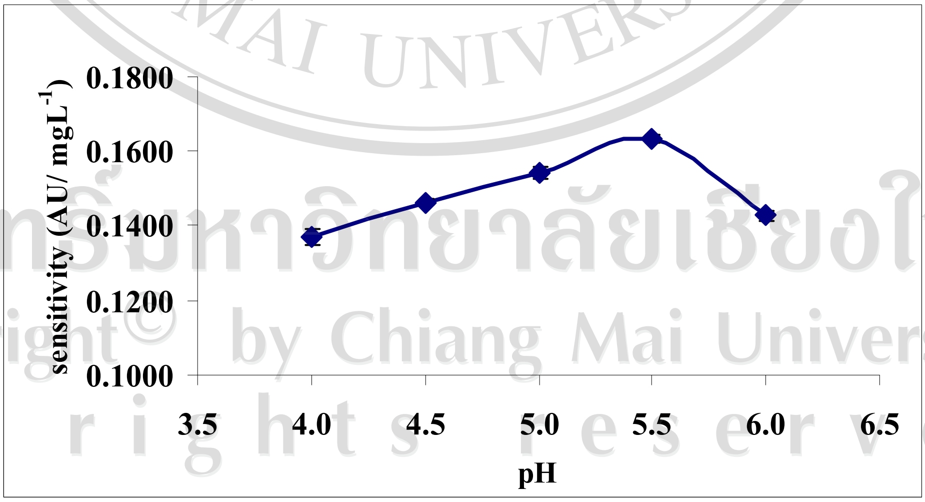

Figure 3.17 Relationship between various pH and sensitivity of the calibration curve

3.3.2.2 Effect of concentration of acetate buffer on the sensitivity

The effect of the concentration of buffer solution was investigated within the range 0.01-0.2 mol L^{-1}. The results are shown in Table 3.24 and Figure 3.18. It was found that the sensitivity of the SI method increased from the acetate buffer concentration of 0.01-0.1 mol L^{-1}. After that, the sensitivity was decreased. Therefore, 0.1 mol L^{-1} of acetate solution was chosen for subsequent experiments since it gave the highest sensitivity.

Table 3.24 Effect of concentration of acetate buffer pH 5.5 on the sensitivity.

Concentration of acetate buffer pH 5.5 (mol L^{-1})	**Sensitivity (AU/mg L^{-1})**				**SD**
	1	**2**	**3**	**$\bar{x}^*$**	
0.01	0.1121	0.1246	0.1180	0.1182	0.0063
0.05	0.1566	0.1512	0.1619	0.1566	0.0054
0.10	0.1753	0.1811	0.1728	0.1764	0.0043
0.15	0.1655	0.1620	0.1689	0.1655	0.0035
0.20	0.1572	0.1481	0.1537	0.1530	0.0046

*average of triplicate results

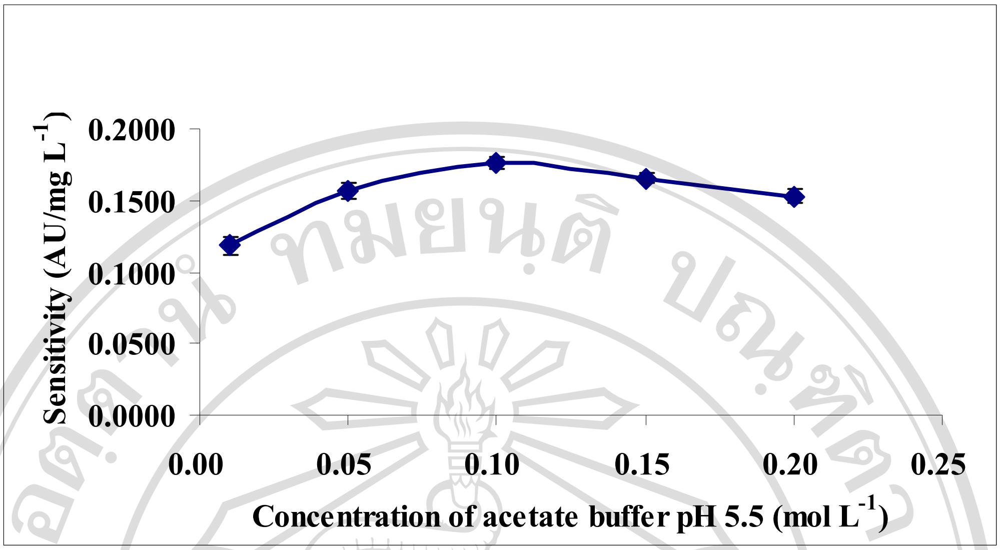

Figure 3.18 Relationship between various concentrations of acetate buffer pH 5.5 and sensitivity of the calibration curve

3.3.2.3 Effect of Quercetin concentration

Effect of Quercetin concentrations on the determination of Al (III) (0.1-0.5 mg L^{-1}) was studied with the following SI system in Figure 2.2 or 2.3, the concentration of Quercetin solutions were varied from 200 – 500 mg L^{-1} of Quercetin in 60% ethanol solution. The results are shown in Table 3.25 and Figure 3.19. It was found that the sensitivity increased very rapidly from the Quercetin concentration of 250 – 350 mg L^{-1}. This is due to the fact that increasing the Quercetin concentration results in increasing the amounts of Al(III)-Quercetin-CTAB complexation that cause higher sensitivity. So, a concentration of 350 mg L^{-1} of Quercetin was chosen as optimum.

Table 3.25 Effect of various concentration of Quercetin on the sensitivity

Concentration of Quercetin solutions (mg L^{-1})	**Sensitivity (AU/mg L^{-1})**				**SD**
	1	**2**	**3**	**$\bar{x}^*$**	
200	0.1431	0.1451	0.1481	0.1454	0.0025
250	0.1505	0.1512	0.1539	0.1519	0.0018
300	0.1813	0.1788	0.1796	0.1799	0.0013
350	0.1842	0.1835	0.1851	0.1843	0.0008
400	0.1769	0.1797	0.1805	0.1790	0.0019
450	0.1781	0.1710	0.1657	0.1716	0.0062
500	0.1631	0.1658	0.1614	0.1634	0.0022

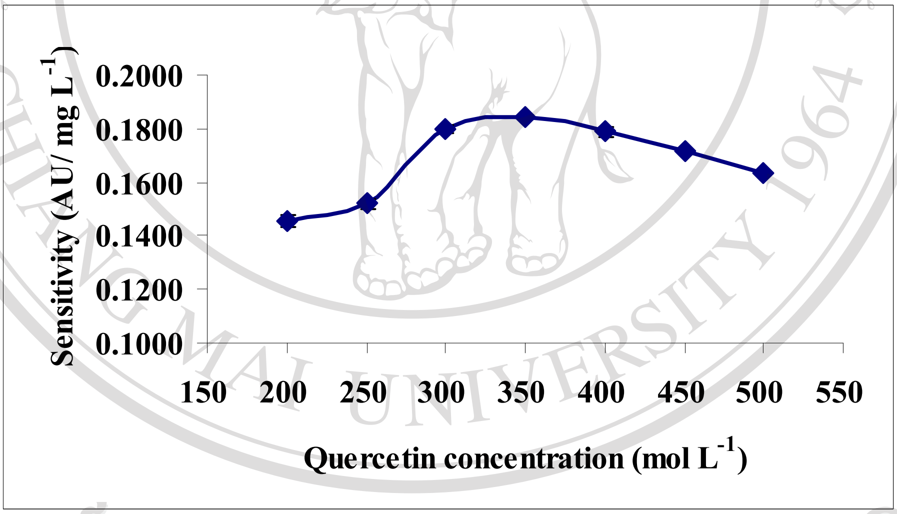

Figure 3.19 Relationship between various concentration of Quercetin in 60 % ethanol solution and sensitivity of the calibration curve

3.3.2.4 Effect of % ethanol in Quercetin solution

The effect of ethanol concentration in Quercetin solution was studied in the range of 40-90% (v/v). The results are shown in Table 3.26 and Figure 3.20. It is shown that the greater slope is obtained when the concentration of ethanol in

Quercetin solution increased. Thus, a concentration of ethanol in Quercetin solution of 70% (v/v) was chosen as optimum % ethanol in Quercetin solution.

Table 3.26 Effect of various concentration of ethanol on the sensitivity

Ethanol Concentration (%v/v)	Sensitivity (AU/mg L^{-1})				SD
	1	2	3	$\bar{x}^*$	
40	0.1165	0.1115	0.1207	0.1162	0.0046
50	0.1453	0.1380	0.1435	0.1423	0.0038
60	0.1624	0.1555	0.1618	0.1599	0.0038
70	0.1885	0.1814	0.1959	0.1886	0.0073
80	0.1971	0.1956	0.2012	0.1980	0.0029
90	0.1949	0.2003	0.2096	0.2016	0.0074

*average of triplicate results

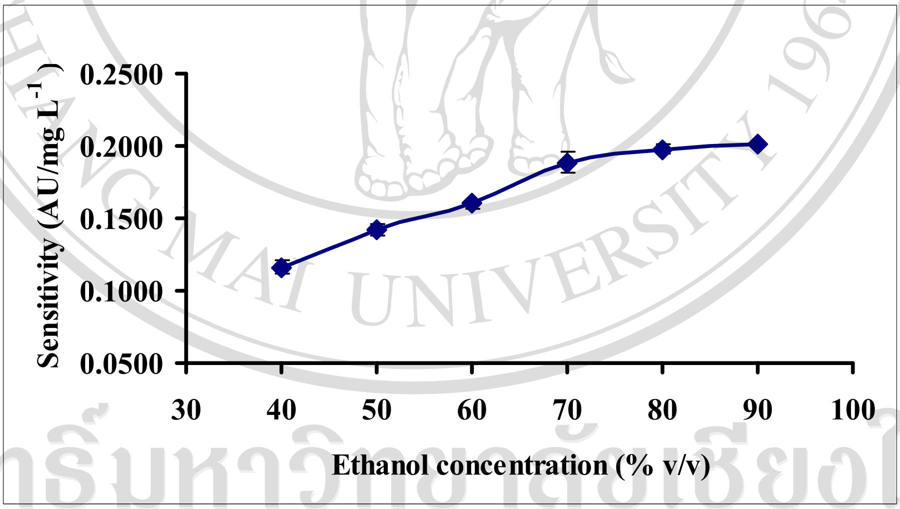

Figure 3.20 Relationship between various concentrations of ethanol on the sensitivity of the calibration curve

3.3.2.5 Effect of CTAB concentration

The Effect of CTAB concentration on the determination of Al(III) (0.1-0.5 mg L^{-1}) was studied at different values in the range of $3.0 \times 10^{-3} – 5.5 \times 10^{-3}$

mol L^{-1}. The results are shown in Table 3.27 and Figure 3.21. It was found that the sensitivity increased very rapidly from the CTAB concentration of 4.0 x 10^{-3} – 4.5 x 10^{-3} mol L^{-1}. After that, the sensitivity became quite constant. This is due to the increasing in the CTAB concentration causes an increase the amounts of Al(III)-Quercetin-CTAB complexation that gave higher sensitivity. Beyond the highest sensitivity, the amount of Al(III)-Quercetin-CTAB became constant so as the Δpeak height. Consequently, a concentration of 4.5 x 10^{-3} mol L^{-1} of CTAB was chosen as optimum.

Table 3.27 Effect of various concentration of CTAB on the sensitivity

CTAB of concentration (x10^{-3} mol L^{-1})	**Sensitivity (AU/mg L^{-1})**				**SD**
	1	**2**	**3**	**$\bar{x}^*$**	
3.0	0.1452	0.1417	0.1409	0.1426	0.0023
3.5	0.1552	0.1537	0.1572	0.1554	0.0018
4.0	0.1568	0.1586	0.1572	0.1575	0.0009
4.5	0.1962	0.1948	0.1939	0.1950	0.0012
5.0	0.1782	0.1733	0.1809	0.1775	0.0039
5.5	0.1601	0.1684	0.1665	0.1650	0.0043

*average of triplicate results

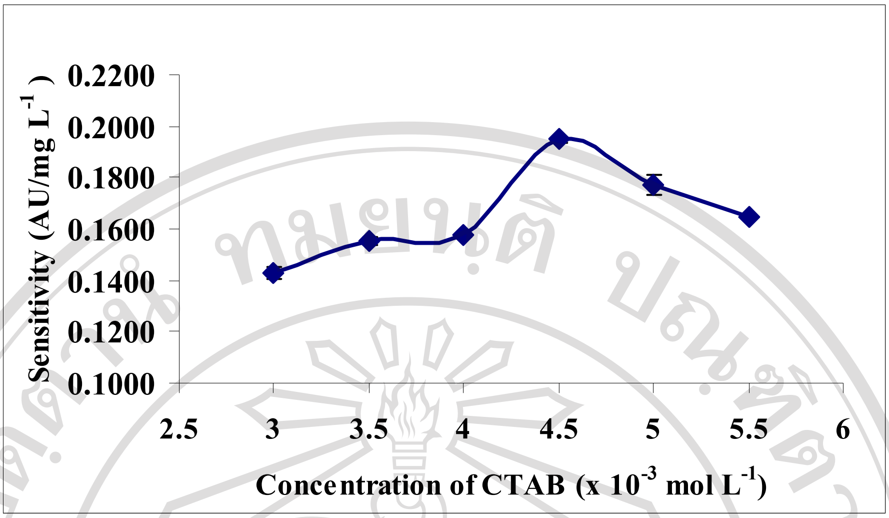

Figure 3.21 Relationship between concentrations of CTAB on the sensitivity of the calibration curve

3.3.2.6 Effect of aspiration volumes of acetate buffer

The aim for optimization of this parameter is to minimize the consumption of reagent while maintaining the best sensitivity, accuracy and reproducibility of the procedure for the analyte of interest. The procedure adopted for optimizing this parameter was to keep the volumes of other reagents and the sample at constant values (chosen by trial and error) while varying the reagent to be optimized at different volumes. This was done by changing the period during which the specific sample and/or reagent volume was aspirated into the holding coil. In this investigation, the effect of aspirated volumes of the 0.10 mol L^{-1} of acetate buffer pH 5.5 was studied over the range 75 – 175 µL at every 25 µL interval (Table 3.28 and Fig 3.22). It was found that the sensitivity increased when the aspiration volume of acetate buffer 0.10 mol L^{-1} at pH 5.5 was increased and reached a maximum sensitivity at 125 µL, above which the sensitivity started to decline. So, a volume of 125 µL was chosen as an optimum for subsequent measurements.

Table 3.28 Effect of various aspiration volume of 0.10 mol L^{-1} of acetate buffer pH 5.5 on the sensitivity

Aspiration volume of buffer (µL)	**Sensitivity (AU/mg L^{-1})**				**SD**
	1	**2**	**3**	**$\bar{x}$***	
75	0.1640	0.1700	0.1675	0.1672	0.0030
100	0.1776	0.1735	0.1750	0.1754	0.0021
125	0.2085	0.2019	0.2025	0.2043	0.0036
150	0.1899	0.1875	0.1901	0.1892	0.0014
175	0.1685	0.1590	0.1525	0.1600	0.0080

*average of triplicate results

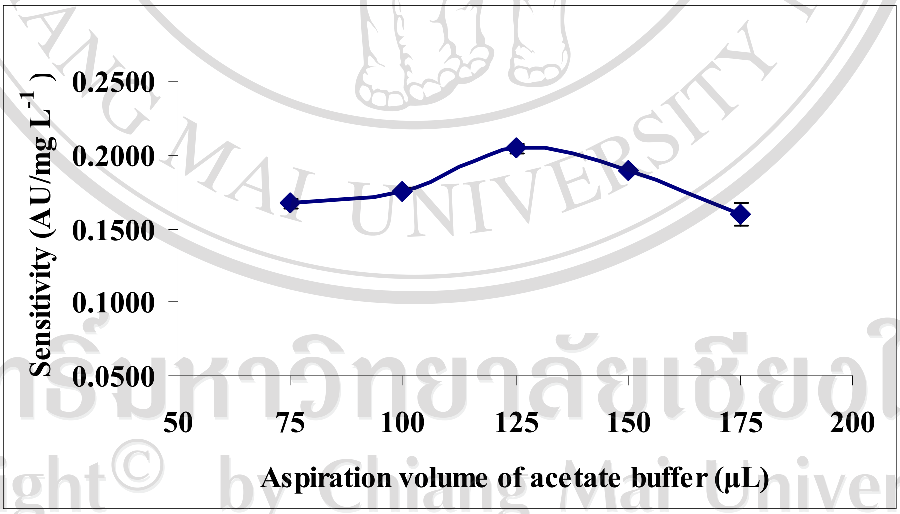

Figure 3.22 Relationship between various aspiration volumes of 0.10 mol L^{-1} of acetate buffer pH 5.5 on the sensitivity of the calibration curve

3.3.2.7 Effect of aspiration volumes of Quercetin

To minimize the consumption of reagent volume, the effect of the Quercetin aspirated volume was considered. When the volumes of Quercetin solution were varied from 75 to 175 µL at every 25 µL interval, maximum sensitivity was obtained at a volume of 125 µL (Table 3.29 and Fig 3.23). Thus, a 125 µL of Quercetin solution was chosen as an optimum volume for subsequent measurements.

Table 3.29 Effect of various aspiration volumes of 350 mg L^{-1} of Quercetin in 70% ethanol on the sensitivity

Aspiration volume of Quercetin (µL)	**Sensitivity (AU/mg L^{-1})**				**SD**
	1	**2**	**3**	**$\bar{x}^*$**	
75	0.1780	0.1675	0.1785	0.1747	0.0062
100	0.1945	0.2000	0.1945	0.1963	0.0032
125	0.2545	0.2650	0.2675	0.2623	0.0069
150	0.2355	0.2320	0.2425	0.2367	0.0053
175	0.2005	0.2150	0.2070	0.2075	0.0073

*average of triplicate results

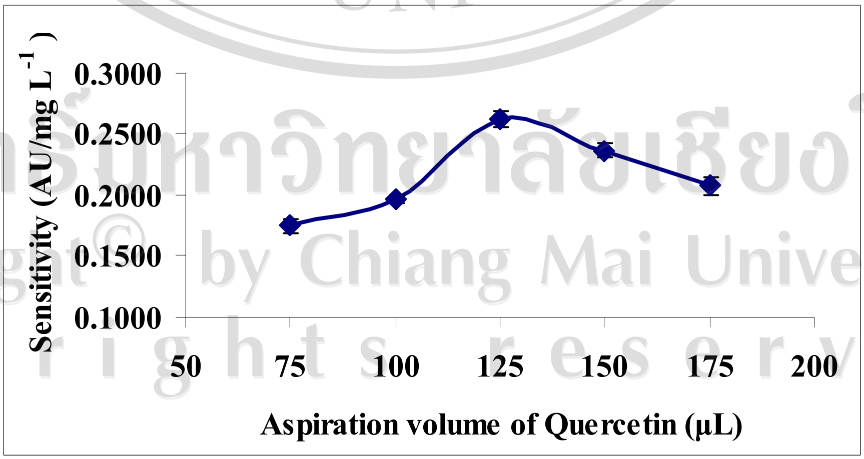

Figure 3.23 Relationship between various aspiration volumes of 350 mg L^{-1} of Quercetin in ethanol 70% on the sensitivity of the calibration curve

3.3.2.8 Effect of aspiration volumes of CTAB

The Effect of aspiration volumes of CTAB on the determination of Al(III) (0.1-0.5 mg L^{-1}) was studied at different concentrations in the range of 50-150 µL. The results are shown in Table 3.30 and Figure 3.24. It was found that the sensitivity decreased, when the aspiration volume of CTAB lower and higher than 100 µL. Thus, the optimum aspiration volume of CTAB was 100 µL because it was highest sensitivity.

Table 3.30 Effect of various aspiration volume of 4.5 x 10^{-3} mol L^{-1} of CTAB on the Sensitivity

Aspiration volume of CTAB (µL)	**Sensitivity (AU/mg L^{-1})**				**SD**
	1	**2**	**3**	**$\bar{x}^*$**	
50	0.1765	0.1895	0.1720	0.1793	0.0091
75	0.1825	0.1780	0.1835	0.1813	0.0029
100	0.2240	0.2255	0.2215	0.2237	0.0020
125	0.2205	0.2135	0.2085	0.2142	0.0060
150	0.2070	0.2160	0.2135	0.2122	0.0046

*average of triplicate results

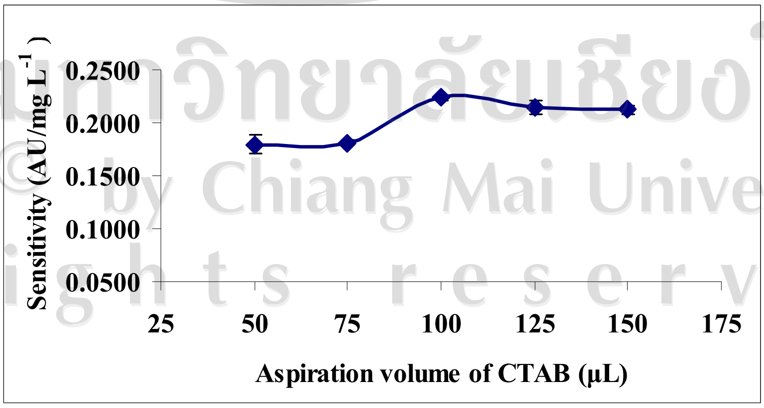

Figure 3.24 Relationship between various aspiration volumes of 4.5 x 10^{-3} mol L^{-1} of CTAB on the sensitivity of the calibration curve

3.3.2.9 Effect of aspiration volumes of sample

The influence of the sample volumes were examined between 100 to 225 µL at every 25 µL interval and it was found that the sensitivity increased markedly up to 175 µL, above which the sensitivity started to decline (Table 3.31 and Fig 3.25). So, a volume of 175 µL was chosen as an appropriate sample volume for further investigations.

Table 3.31 Effect of various aspiration volume of sample on the sensitivity

Aspiration volume of sample (µL)	**Sensitivity (AU/mg L^{-1})**				**SD**
	1	**2**	**3**	$\bar{x}^*$	
100	0.1848	0.1802	0.1788	0.1813	0.003
125	0.2033	0.2073	0.2076	0.2061	0.002
150	0.2582	0.2579	0.2598	0.2586	0.001
175	0.3032	0.3149	0.3182	0.3121	0.008
200	0.2667	0.2666	0.2692	0.2675	0.001
225	0.2439	0.2415	0.2447	0.2434	0.002

*average of triplicate results

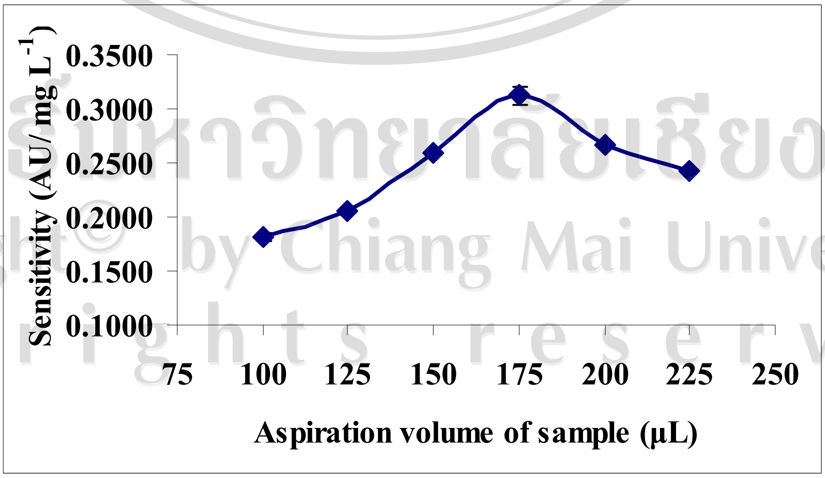

Figure 3.25 Relationship between various aspiration volumes of sample on the sensitivity of the calibration curve

3.3.2.9 Effect of flow rate

The flow rate is one of the most important parameters to be optimized because it regulates the amount of final product (yellow color product as chromophore) formed and hence the sensitivity together with the sample throughput. The effect of the flow rate on the absorbance was investigated from 50–175 µL s^{-1}. The sample, Quercetin, CTAB and buffer volumes aspirated were kept constant at their optimum values by changing the flow rate over the range 50–175 µL s^{-1}. The effect of flow rate is shown in Table 3.32 and Figure 3.26. Maximum sensitivity was obtained at a flow rate of 125 µL s^{-1}. Therefore, a flow rate of 125 µL s^{-1} was chosen for further investigations. Increasing the flow rates above 125 µL s^{-1} does not significantly enhance the sensitivity, but they increase the pressure in the tubing, resulting in the more consumption of the reagents.

Table 3.32 Effect of various flow rate on the sensitivity

Flow rate (µL s^{-1})	**Sensitivity (AU/mg L^{-1})**				**SD**
	1	**2**	**3**	**$\bar{x}^*$**	
50	0.1285	0.1280	0.1255	0.1273	0.0016
75	0.1770	0.1695	0.1655	0.1707	0.0058
100	0.2410	0.2305	0.2395	0.2370	0.0057
125	0.3220	0.3060	0.3145	0.3142	0.0080
150	0.2865	0.2870	0.2850	0.2862	0.0010
175	0.1325	0.1450	0.1490	0.1422	0.0086

*average of triplicate results

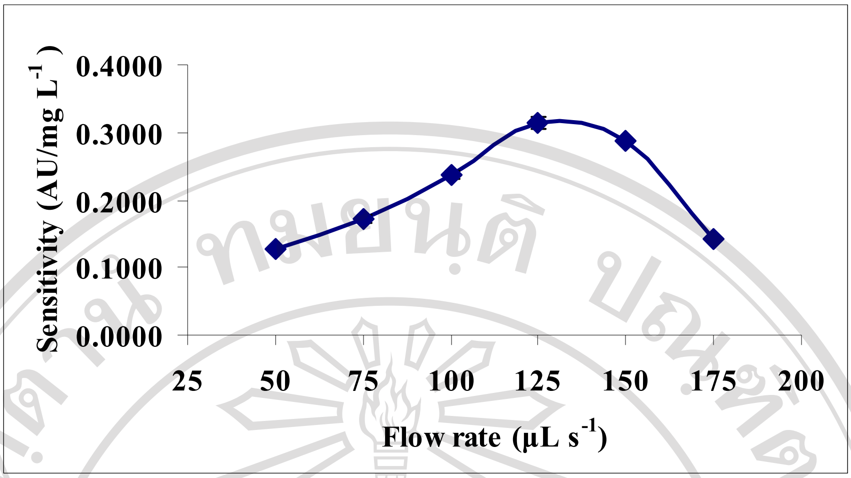

Figure 3.26 Relationship between various flow rates on the sensitivity of the calibration curve.

3.3.2.10 Effect of holding time

The effect of holding time on the determination of Al (III) (0.10-0.50 mg L^{-1}) was studied in the range of 10-50 s .The results are shown in Table 3.33 and Figure 3.27. The sensitivity increased to a maximum at holding time 30 s. It can be explained that increasing the holding time results in an increase in Al(III)-Quercetin-CTAB complex because its mixing is better than that occurred at the holding time below 30 s. The sensitivity decreased when holding time greter than 30 s because of dispersion. So, a holding time of 30 s was chosen as optimum since it provided the greatest sensitivity.

Table 3.33 Effect of various holding time on the sensitivity

Holding time (s)	**Sensitivity (AU/mg L^{-1})**				**SD**
	1	**2**	**3**	$\bar{x}^*$	
10	0.1045	0.1015	0.0975	0.1012	0.0035
20	0.1635	0.1655	0.1640	0.1643	0.0010
30	0.1895	0.1910	0.1930	0.1912	0.0018
40	0.1785	0.1795	0.1775	0.1785	0.0010
50	0.1790	0.1750	0.1810	0.1783	0.0031

*average of triplicate results

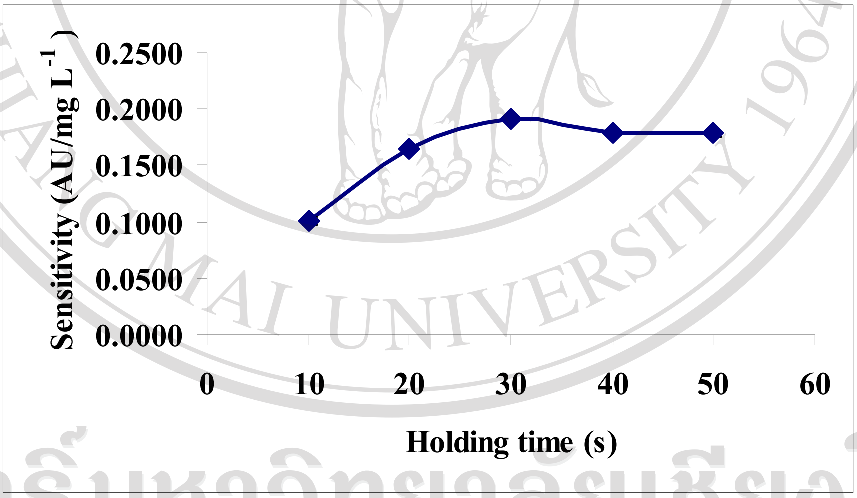

Figure 3.27 Relationship between various holding time on the sensitivity of the calibration curve

A diagram in the SI manifold is displayed in Figure 2.2 and 2.3. Table 3.34 shows the ranges over which the variables involved in the SIA system were studied and their optimum values.

Table 3.34 Optimum conditions for aluminium (III) determination

Variable	Studied range	Optimum value
pH	4.0 – 6.0	5.5
Concentration of acetate buffer pH 5.5 (mol L^{-1})	0.01-0.2	0.1
Concentration of Quercetin (mg L^{-1})	200 – 500	350
Concentration of ethanol in Quercetin solution (% v/v)	40 – 90	70
Concentration of CTAB (x 10^{-3} mol L^{-1})	3.0 – 5.0	4.5
Aspiration volume of acetate buffer (μL)	75 - 175	125
Aspiration volume of Quercetin (μL)	75 - 175	125
Aspiration volume of CTAB (μL)	50 – 150	100
Aspiration volume of sample (μL)	50 – 225	175
Flow rate (μL s^{-1})	50 – 175	125
Holding time (s)	10 – 60	30

3.3.3 Analytical Characteristics of the method

3.3.3.1 Linear range

The linear range of the proposed method was studied by aspiration of appropriate volume of aluminium (III) standard solution into the SI system under the suitable conditions as shown in Table 3.34. Linear calibration graphs were obtained for aluminium (III) standards over the concentration ranges of 0.02 – 0.60 mg L^{-1}. All measurements were made in pentaplicate injections. The results obtained are shown in Table 3.35 and Figure 3.28.

Table 3.35 Linearity of aluminium determination

Aluminium (mg L^{-1})	**Peak height (AU)**						**ΔP.H.* (AU)**
	1	**2**	**3**	**4**	**5**	$\bar{x}$	
0.00	0.104	0.102	0.100	0.101	0.105	0.102	0.000
0.01	0.103	0.103	0.103	0.103	0.103	0.103	0.001
0.02	0.113	0.114	0.114	0.113	0.112	0.113	0.011
0.04	0.124	0.125	0.124	0.121	0.124	0.124	0.021
0.06	0.133	0.130	0.131	0.134	0.132	0.132	0.030
0.08	0.144	0.145	0.142	0.145	0.143	0.144	0.041
0.10	0.152	0.153	0.151	0.151	0.153	0.152	0.050
0.15	0.173	0.172	0.172	0.171	0.174	0.172	0.070
0.20	0.202	0.201	0.200	0.203	0.204	0.202	0.100
0.25	0.220	0.221	0.220	0.219	0.221	0.220	0.118
0.30	0.248	0.248	0.243	0.244	0.242	0.245	0.143
0.35	0.270	0.268	0.267	0.270	0.27	0.269	0.167
0.40	0.296	0.294	0.296	0.294	0.295	0.295	0.193
0.45	0.316	0.314	0.314	0.315	0.313	0.314	0.212
0.50	0.343	0.344	0.342	0.341	0.341	0.342	0.240
0.60	0.391	0.390	0.387	0.388	0.387	0.389	0.286
0.70	0.414	0.411	0.413	0.411	0.41	0.412	0.309
0.80	0.442	0.449	0.450	0.442	0.448	0.446	0.344
0.90	0.472	0.470	0.473	0.473	0.471	0.472	0.369
1.00	0.485	0.487	0.485	0.482	0.484	0.485	0.382

*average of pentaplicate results

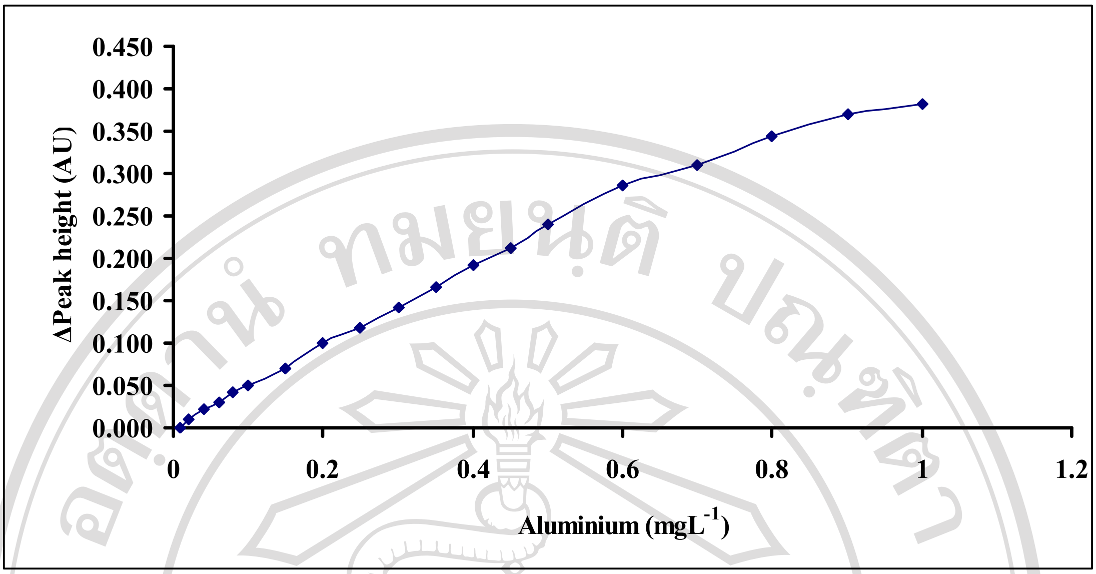

Figure 3.28 Relationship between Δpeak height and concentration of aluminium(III) 0.01-1 mg L^{-1}

3.3.3.2 Precision of the flow injection system

The precision of the proposed method was verified by 11 replicated determination of standard aluminium (III) solution, using the optimum conditions (Table 3.34). Table 3.36 as shown the relative standard deviation was found to be 1.23, 1.14, 0.67 and 0.76 % of aluminium (III) 0.05, 0.10, 0.40 and 0.60 mg L^{-1}, respectively.

Table 3.36 Precision verification using various concentrations of aluminium standard

Experimental number	**Peak height (AU) obtained from the standard Al(III) (mg L^{-1})**			
	0.05	**0.1**	**0.4**	**0.6**
1	0.131	0.154	0.293	0.387
2	0.131	0.156	0.291	0.390
3	0.133	0.153	0.295	0.395
4	0.132	0.155	0.296	0.389
5	0.130	0.152	0.295	0.394
6	0.134	0.153	0.293	0.390
7	0.135	0.151	0.293	0.392
8	0.132	0.154	0.296	0.394
9	0.134	0.153	0.294	0.391
10	0.134	0.157	0.29	0.388
11	0.131	0.155	0.292	0.386
$\bar{x}$	0.132	0.154	0.293	0.391
S.D.	0.002	0.002	0.002	0.003
% R.S.D.	1.23	1.14	0.67	0.76

3.3.3.3 Calibration curve

As depicted in Figures 2.2 and 2.3, the standard solutions containing 0.02-0.60 mg L^{-1} were aspirated with appropriate volume into the SI system under the established optimum conditions (Table 3.34). The results are shown in Table 3.37. The calibration curve as shown in Figure 3.30 was established by plotting Δpeak height versus the various aluminium (III) concentrations. A correlation coefficients (r^2) and the regression equation are as follows:

$y = 0.4828x + 0.0023$ ($r^2 = 0.9996$)

Where y is Δpeak height in AU

x is concentration of aluminium (III) in mg L^{-1}

Table 3.37 ΔPeak height for calibration curve

Aluminium (mg L^{-1})	Peak height (AU)						ΔP.H.* (AU)
	1	2	3	4	5	$\bar{x}$	
0.00	0.104	0.108	0.107	0.107	0.109	0.107	0.000
0.02	0.119	0.117	0.117	0.115	0.118	0.117	0.010
0.04	0.128	0.127	0.130	0.130	0.128	0.129	0.022
0.06	0.135	0.138	0.135	0.136	0.137	0.136	0.029
0.08	0.148	0.150	0.151	0.150	0.149	0.150	0.043
0.10	0.161	0.160	0.158	0.158	0.160	0.159	0.052
0.20	0.207	0.209	0.211	0.209	0.208	0.209	0.102
0.30	0.251	0.254	0.256	0.254	0.255	0.254	0.147
0.40	0.298	0.301	0.301	0.300	0.298	0.300	0.193
0.50	0.349	0.35	0.351	0.348	0.351	0.350	0.243
0.60	0.401	0.400	0.399	0.402	0.401	0.401	0.294

*average of pentaplicate results

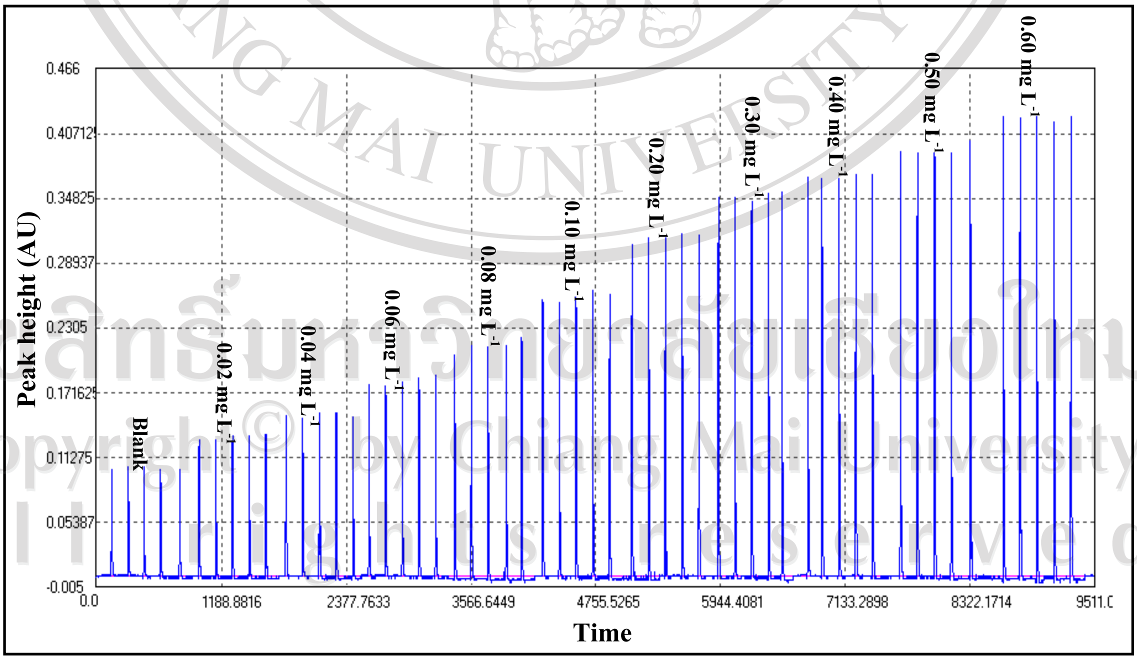

Figure 3.29 Calibration signal of SIA spectrophotometric determination of aluminium(III) 0.02-0.60 mg L^{-1}.

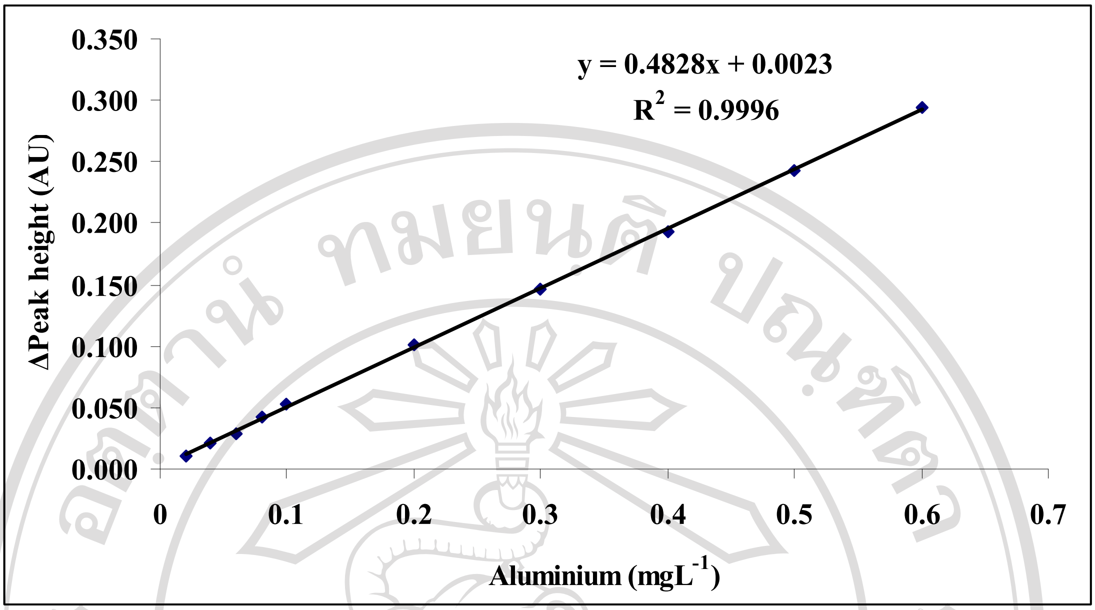

Figure 3.30 Calibration signal of SIA spectrophotometric determination of aluminium (III) 0.02-0.6 mgL^{-1}

3.3.3.4 Detection limit

The detection limit was determined by the method reported by Miller and Miller [79], which was calculated from the linear regression line of twice calibration curves. The results are given in Tables 3.38. The detection limit of the proposed method was found to be 0.013 mg L^{-1}. The concentration at limit of detection (*CL*) can be calculated from equation 2.2-2.3.

Table 3.38 Calculation of detection limit of SIA spectrophotometric determination of aluminum

Aluminium (mgL^{-1})	Y_i^*	$\hat{Y}_i$	$\|Y_i^* - \hat{Y}_i\|$	$\|Y_i^* - \hat{Y}_i\|^2$
0.02	0.010	0.012	0.002	4.0×10^{-6}
0.04	0.022	0.022	0.000	0
0.06	0.029	0.031	0.002	4.0×10^{-6}
0.08	0.043	0.041	0.002	3.0×10^{-6}
0.10	0.052	0.051	0.002	3.0×10^{-6}
0.20	0.102	0.099	0.003	9.0×10^{-6}
0.30	0.147	0.147	0.000	0
0.40	0.193	0.195	0.003	9.0×10^{-6}
0.50	0.243	0.244	0.001	1.0×10^{-6}
0.60	0.294	0.292	0.002	3.0×10^{-6}
$\sum(\|Y_i^* - \hat{Y}_i\|^2)$				3.5×10^{-5}
S_y/x				0.002
C_L,LOD				0.013
LOQ				0.044

The linear regression equation is $Y = 0.4828x + 0.0023$

$$\begin{aligned}
Sy/x &= [3.5 \times 10^{-5}/(10-2)]^{1/2} \\
&= 0.002 \\
C_L,\ \text{LOD} &= (3 \times 0.002)/0.4828 \\
&= 0.013\ \text{mg L}^{-1}\ \text{Al(III)} \\
\text{LOQ} &= (10 \times 0.002)/0.4828 \\
&= 0.044\ \text{mg L}^{-1}\ \text{Al(III)}
\end{aligned}$$

3.3.3.5 Interference Studies

The interference effects of some possible foreign ions on the determination of aluminum were studied by the proposed SIA procedure under the optimum conditions obtained (Table 3.34). A systematic study to check for the effects of some possible foreign ions on the determination of aluminium (III) was undertaken for the maximum w/w ratio of aluminum to foreign ions up to 1 : 500 The solutions of a 0.2 mg L^{-1} aluminium (III) standard containing varying concentrations of diverse ions were determined using the SIA system. The tolerance is defined as the largest foreign-ion concentration causing % recovery between 90-110% for determining the analyte of interest. The tolerance values for the ions studied are given in Table 3.39.

Table 3.39 Interference studies for 0.20 mg L^{-1} standard aluminum by SIA method

Interference	Aluminium : Interference	Peak height* (AU)	% Recovery
Cu^{2+}	1 : 0	0.152	-
	1 : 0.1	0.154	101
	1 : 0.5	0.164	108
	1 : 1.0	0.175	115
Fe^{2+}	1 : 0	0.152	-
	1 : 0.5	0.155	102
	1 : 1.0	0.161	106
	1 : 2.0	0.175	115
Fe^{3+}	1 : 0	0.152	-
	1 : 0.05	0.158	104
	1 : 0.1	0.165	109
	1 : 0.2	0.176	116

* average of triplicate results.

Table 3.39 (Continued)

Interference	Aluminium : Interference	Peak height* (AU)	% Recovery
NO_2^-	1 : 0	0.152	-
	1 : 50	0.150	99
	1 : 80	0.147	97
	1 : 100	0.140	92
Na^+	1 : 0	0.152	-
	1 : 500	0.153	101
	1 : 1000	0.157	103
	1 : 2500	0.160	105
K^+	1 : 0	0.152	-
	1 : 500	0.152	100
	1 : 2500	0.154	101
	1 : 5000	0.157	103
Ca^{2+}	1 : 0	0.152	-
	1 : 50	0.151	99
	1 : 80	0.144	95
	1 : 100	0.140	92
Ba^{2+}	1 : 0	0.152	-
	1 : 50	0.155	102
	1 : 100	0.159	105
	1 : 500	0.166	109
Mn^{2+}	1 : 0	0.152	-
	1 : 100	0.157	103
	1 : 500	0.161	106
	1 : 600	0.170	112
Co^{2+}	1 : 0	0.152	-
	1 : 100	0.151	99
	1 : 500	0.153	101
	1 : 1000	0.169	111

* average of triplicate results.

Table 3.39 (Continued)

Interference	Aluminium : Interference	Peak height* (AU)	% Recovery
Zn^{2+}	1 : 0	0.152	-
	1 : 10	0.152	100
	1 : 100	0.162	107
	1 : 200	0.171	113
Ni^{2+}	1 : 0	0.152	-
	1 : 10	0.152	100
	1 : 100	0.154	101
	1 : 500	0.157	103
Cr^{3+}	1 : 0	0.152	-
	1 : 100	0.153	101
	1 : 200	0.157	103
	1 : 500	0.168	111
Cd^{2+}	1 : 0	0.152	-
	1 : 10	0.151	99
	1 : 100	0.15	99
	1 : 1000	0.148	97
F^-	1 : 0	0.152	-
	1 : 100	0.15	99
	1 : 250	0.148	97
	1 : 500	0.145	95
PO_4^{3-}	1 : 0	0.152	-
	1 : 10	0.151	99
	1 : 500	0.147	97
	1 : 1000	0.144	95
Cl^-	1 : 0	0.152	-
	1 : 100	0.152	100
	1 : 500	0.15	99
	1 : 1000	0.146	96

* average of triplicate results.

Table 3.39 (Continued)

Interference	Aluminium : Interference	Peak height* (AU)	% Recovery
NO_3^-	1 : 0	0.152	-
	1 : 100	0.151	99
	1 : 250	0.147	97
	1 : 500	0.143	94
SO_4^{2-}	1 : 0	0.152	-
	1 : 100	0.154	101
	1 : 500	0.157	103
	1 : 1000	0.162	107
HCO_3^-	1 : 0	0.152	-
	1 : 5	0.150	99
	1 : 10	0.144	95
	1 : 20	0.138	91
Br^-	1 : 0	0.152	-
	1 : 500	0.154	101
	1 : 1000	0.153	101
	1 : 1500	0.150	99
I^-	1 : 0	0.152	-
	1 : 100	0.152	100
	1 : 500	0.149	98
	1 : 1000	0.142	93
CN^-	1 : 0	0.152	-
	1 : 10	0.149	98
	1 : 20	0.143	94
	1 : 50	0.142	93

* average of triplicate results.

The interference effects of some possible foreign ions in the SIA system for aluminum were summarized in Table 3.40. It was found that Fe^{2+}, Fe^{3+} and Cu^{2+} interfered determination aluminium (III) in tap water, which refered standard quality of tap water defined by metropolitan waterworks authority (Appendix A).

Table 3.40 Summary of interference effects of some ions on the response obtained from aluminium (III) 0.2 mg L^{-1} by SIA method

Interference ions	**Tolerable concentration ratio*(mg L^{-1}) of ion/Al (III)**
Na^+, Cd^{2+}, PO_4^{3-}, Cl^-, Br^-, I^-,	≥1000
Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, F^-, NO^{3-}, SO_4^{2-}	500
Mg^{2+}	250
PO_4^{3-} Cr^{3+}	200
Ca^{2+}, Zn^{2+}	100
CN^-	50
NO_2^- HCO_3^-	20
Fe^{2+}	1
Cu^{2+}, Fe^{3+}	0.5

*The concentration of interference ion is considered to be interfered when causing % recovery less than 90% and more than 110% with respect of the signal of aluminum alone.

3.3.3.6 Effect of masking agents and interference

The effect of masking agents and interference was studied by the proposed SIA procedure using the optimum conditions. Interference effects of Fe^{2+}, Fe^{3+} and Cu^{2+} on Al^{3+} determination could be overcome by using suitable masking agents. The results are shown in Table 3.41. It was found that Fe^{2+} was masked with 0.008 mol L^{-1} of 1,10-phenanthroline and Fe^{3+} was reduced to Fe^{2+} with 0.006 mol L^{-1} of ascorbic acid and was masked in the same manner as Fe^{2+} [81]. Cu^{2+} was masked with 0.25% v/v of thiourea [81].

Table 3.41 Effect of masking agent for mask Fe^{2+}, Fe^{3+} and Cu^{2+} the response obtained from aluminium (III) 0.2 mg L^{-1} by SIA method

Interference	**Concentration of masking agent**	**Aluminium :Interference**	**Peak height* (AU)**	**% Recovery**
Cu^{2+}	0.10% Thiourea	1 : 0	0.148	-
		1 : 10	0.154	104
		1 : 15	0.171	111
		1 : 20	0.205	120
	0.25% Thiourea	1 : 0	0.148	-
		1 : 20	0.152	103
		1 : 30	0.163	107
		1 : 50	0.186	114
	0.50% Thiourea	1 : 0	0.148	-
		1 : 30	0.149	101
		1 : 50	0.158	106
		1 : 70	0.179	113
Fe^{2+}	0.004 mol L^{-1} 1,10-phenanthroline	1 : 0	0.148	-
		1 : 1	0.161	109
		1 : 5	0.170	115
		1 : 10	0.181	122
	0.006 mol L^{-1} 1,10-phenanthroline	1 : 0	0.148	-
		1 : 5	0.151	102
		1 : 10	0.156	105
		1 : 15	0.161	109
	0.008 mol L^{-1} 1,10-phenanthroline	1 : 0	0.148	-
		1 : 10	0.150	101
		1 : 15	0.154	104
		1 : 20	0.160	108

*average of triplicate results

Table 3.41 (Continued)

Interference	Concentration of masking agent	Aluminium :Interference	Peak height* (AU)	% Recovery
Fe^{3+}	0.002 mol L^{-1} Ascorbic acid + 0.008 mol L^{-1} 1,10-phenanthroline	1 : 0	0.148	-
		1 : 5	0.158	107
		1 : 10	0.176	119
		1 : 15	0.183	124
	0.004 mol L^{-1} Ascorbic acid + 0.008 mol L^{-1} 1,10-phenanthroline	1 : 0	0.148	-
		1 : 5	0.151	102
		1 : 10	0.162	109
		1 : 15	0.171	116
	0.006 mol L^{-1} Ascorbic acid + 0.008 mol L^{-1} 1,10-phenanthroline	1 : 0	0.148	-
		1 : 10	0.149	101
		1 : 15	0.152	103
		1 : 20	0.159	107

*average of triplicate results

3.3.3.7 Determination of aluminium (III) in waters

The proposed SIA spectrophotometric method was applied to the simultaneous determination of aluminium (III) in tap water samples which were collected from several districts in Chiang Mai. The peak heights from each sample were compared with standard calibration curve. The results were given in Table 3.42

Table 3.42 Determination of aluminium (III) in water samples by SIA method

Water samples	**Aluminium found (mg L^{-1})**				**SD**	**Aluminium concentration* (mg L^{-1})**	**% Recovery***
	1	**2**	**3**	**$\bar{x}$**			
Jomthong	0.261	0.267	0.265	0.264	0.003	0.264 ± 0.003	99.7
Doi Saket	0.077	0.079	0.078	0.078	0.001	0.078 ± 0.001	100.5
Hangdong	0.077	0.079	0.074	0.077	0.003	0.077 ± 0.003	101.8
Mae Rim	0.184	0.188	0.184	0.185	0.002	0.185 ± 0.002	100.1
Mae Wang	0.125	0.121	0.121	0.122	0.002	0.122 ± 0.002	98.3
Muang	0.087	0.085	0.084	0.085	0.002	0.085 ± 0.002	99.2
Sanpathong	ND**	ND**	ND**	-	-	ND**	100.9
Sansai	0.045	0.050	0.048	0.048	0.003	0.048 ± 0.003	99.0
Sankumpang	ND**	ND**	ND**	-	-	ND**	101.1
Chaing Mai University	0.223	0.227	0.220	0.223	0.004	0.223 ± 0.004	102.7

The Al(III) contents in the water samples were in the range of 0.048-0.264 mg L^{-1} and 0.011-0.273 mg L^{-1} using the proposed method and ICP-MS respectively. The results obtained by the proposed SIA spectrohotometric method compared favorably with those obtained by ICP-MS using the student *t*-test (Table 3.43 and Appendix B in Table B.1). It was evident that the *t*-value for Al(III) contents in water samples determined by comparison the results obtained by SIA spectrophotometric method with those obtained by ICP-MS were shown in table 3.43. It was seen that the experimental *t*-value for Al(III) assay which was smaller than the theoretical *t*-value at a confidence interval of 95% (4.30) indicating that results obtained by both methods were in excellent agreement.

Table 3.43 Comparative determination of aluminium (III) in water sample by proposed SIA method and ICP-MS

Water samples	**Concentrations (mg L^{-1})**		$t_{\text{calculated}}$
	SIA[*]	**ICP-MS**[*]	
Chom Thong	0.264	0.273	-4.914
Doi Saket	0.078	0.081	-5.196
Hangdong	0.077	0.075	1.147
Mae Rim	0.185	0.189	-2.750
Mae Wang	0.122	0.120	1.750
Mueang	0.085	0.087	-1.890
Sanpathong	ND[**]	0.011	-
Sansai	0.048	0.048	-0.229
Sankumphaeng	ND[**]	0.014	-
Chaing Mai University	0.223	0.228	-2.302

[*] average of triplicate results
[**] not detected