CHAPTER 3

RESULTS AND DISCUSSION

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

3.1.1 Absorption spectra

The absorption spectrum of BPR, BPR-CTAB, Al-BPR-CTAB complexes against water were scanned over a range from 350-700 nm, using JENWAY 6400 spectrophotometer in conjunction with the laboratory developed software connected to A PC as shown in Figure 3.1.

BPR in 15% ethanol, BPR-CTAB and Al-BPR-CTAB give red, purple and bluish-purple colors and showed a maximum at 550, 580, 583 nm, respectively.

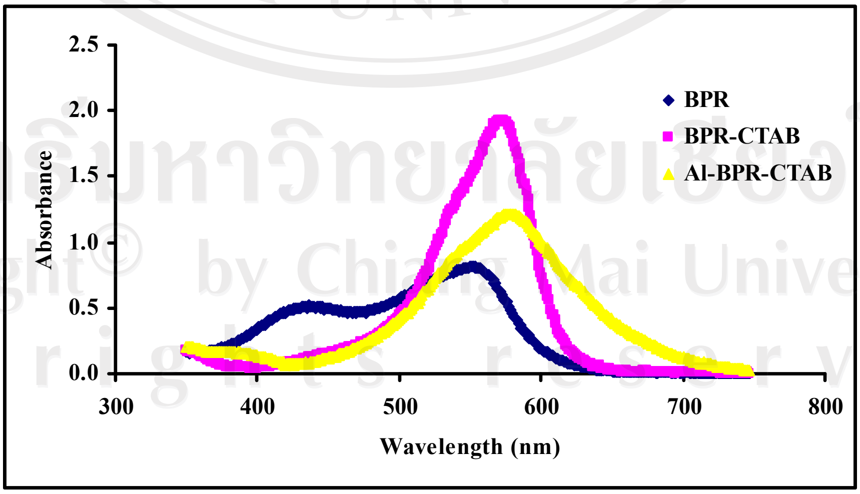


Figure 3.1 Absorption Spectra of BPR, BPR-CTAB and Al-BPR-CTAB complex against water at pH 5.0

3.1.2 Mole-ratio method

The mole-ratio methods of Al-BPR-CTAB complex of 2 series were studied at pH 5.0. Series I; the various concentrations of BPR were added to solution containing 1×10^{-5} mol L^{-1} of aluminum, 1×10^{-4} mol L^{-1} of CTAB, 10 mL of 0.20 mol L^{-1} of acetate buffer pH 5.0 and diluted with deionized water in 25 mL volumetric flasks. Absorbance of each solution was measured at 580 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 BPR was 3×10^{-5} mol L^{-1} and then it became constant and so did absorbance as AU. Therefore, 3×10^{-5} mol L^{-1} of BPR concentration was chosen for studied effect of CTAB concentration in series II.

Table 3.1 Effect of BPR concentrations for mole-ratio of Al-BPR-CTAB complex

Concentrations of BPR ($\times 10^{-5}$ mol L^{-1})	**Absorbance (AU)***
0	0.033
1	0.187
2	0.324
3	0.506
4	0.509
5	0.511
6	0.513

*average of triplicate results

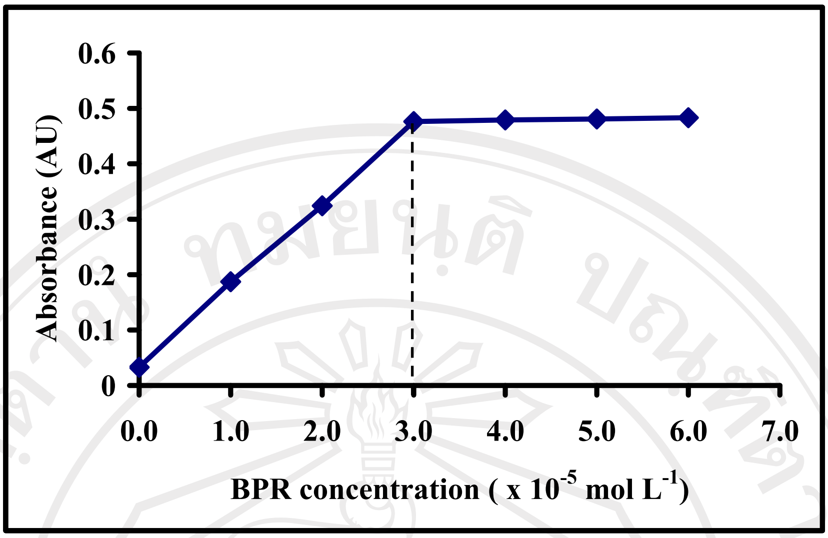


Figure 3.2 Mole-ratio study of Al-BPR-CTAB system; effect of BPR concentration. Al 1 x 10^{-5} mol L^{-1}, CTAB 1 x 10^{-4} mol L^{-1}, pH 5.0, wavelength 580 nm

Series II; the various concentration of CTAB were added to solution containing 1 x 10^{-5} mol L^{-1} of aluminum, 3 x 10^{-5} mol L^{-1} of BPR, 10 mL of 0.20 mol L^{-1} of acetate buffer pH 5.0 and diluted with deionized water in 25 mL volumetric flasks. Absorbance of each solution was measured at 580 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 at concentration of CTAB was 6 x 10^{-5} mol L^{-1} then it became constant and so did the absorbance in AU. Therefore, 6 x 10^{-5} mol L^{-1} CTAB concentration was chosen.

Table 3.2 Effect of CTAB concentrations for mole-ratio of Al-BPR-CTAB complex on the absorbance

Concentrations of CTAB (x 10^{-5} mol L^{-1})	Absorbance (AU)*
0	0.165
1	0.218
2	0.284
3	0.338
4	0.395
5	0.438
6	0.478
7	0.507
8	0.509
9	0.516
10	0.518

*average of triplicate results

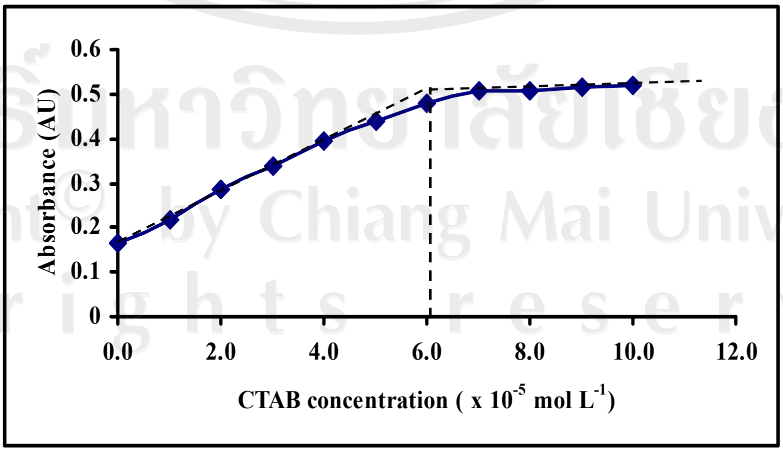


Figure 3.3 Mole-ratio study of Al-BPR-CTAB system; effect of CTAB concentration on the absorbance Al 1 x 10^{-5} mol L^{-1}, BPR 3 x 10^{-5} mol L^{-1}, pH 5.0, wavelength 580 nm

From experimental results in Tables 3.1 and 3.2 and Figures 3.2 and 3.3 gave a mole-ratio of Al : BPR : CTAB at 1 : 3 : 6. So, the reaction of Al-BPR-CTAB complex may be exactly the same as reaction of Fe-BPR-CTAB [55]. The reaction of Al-BPR-CTAB was shown in Figure 3.4.

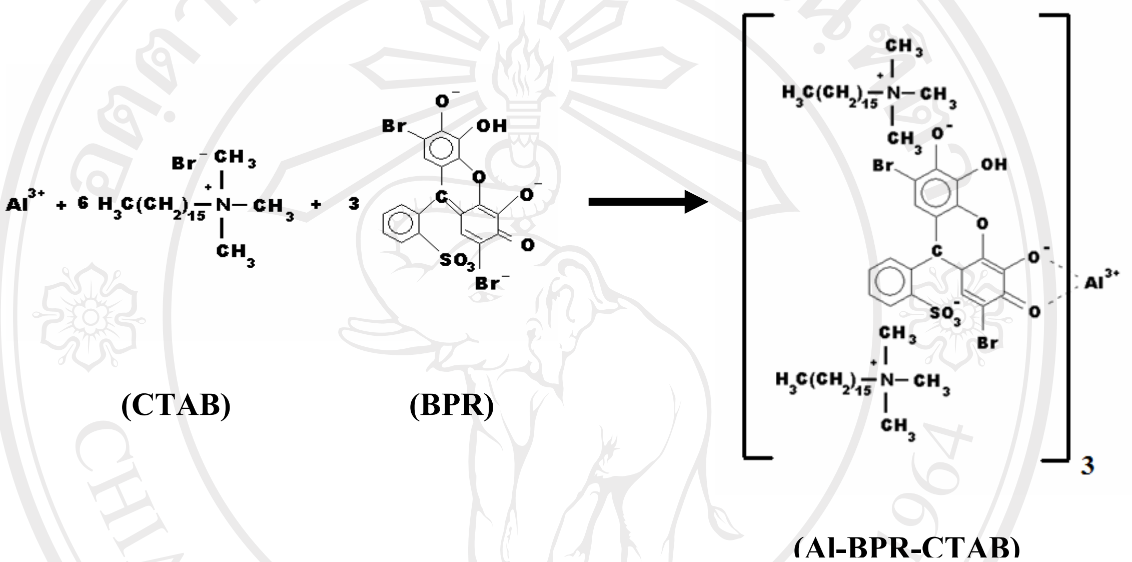


Figure 3.4 The reaction of Al-BPR-CTAB

3.2 rFIA Spectrophotometric Determination of Aluminum Using Bromopyrogalol Red 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 aluminum were optimized by studying the influences of the various parameters, such as wavelength, pH, concentration of BPR, % 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 aluminum determination was studied over the range 540 – 595 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 3.4 indicated that the highest Δpeak height (ΔP.H.) was obtained when the absorbance was measured at 580 nm. ΔPeak height (ΔP.H.) was the difference between peak height of blank and peak height of analyte. The analytical wavelength at 580 nm was selected for the further studies.

Table 3.3 Peak heights at various wavelengths

Wavelength (nm)	Peak Height (mV)*		ΔP.H.* (mV)
	blank	Al (III) 0.2 ppm	
540	540	115	11
545	545	124	15
550	550	132	20
555	555	141	25
560	560	156	29
565	565	170	36
570	570	183	44
575	575	185	46
580	580	191	52
585	585	181	31
590	590	160	26
595	595	137	13

*average of triplicate results

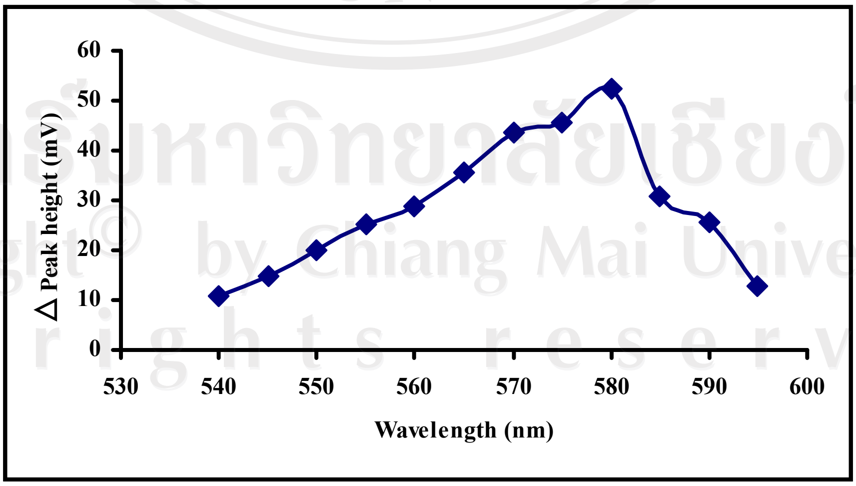


Figure 3.4 ΔPeak heights at various wavelengths

3.2.1.2 Effect of pH

The complexation of Al-BPR-CTAB was studied pH at different values in the range of 4.0-6.5. The pH values of the solution were adjusted with acetic acid and sodium acetate. Using the manifold as shown in figure 2.1, a 0.02 mol L^{-1} concentration of acetate buffer solution was mixed in solution of aluminum and CTAB. The results obtained are shown in Table 3.4 and Figure 3.5. The results indicated that the pH values below 4.5 or above 5.5 the sensitivity (slope of the calibration curve) decreased significantly. So, pH 5.0 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
	0.1	0.2	0.3	0.4		
4.0	12	24	39	50	y = 130.00x - 1.33	0.9974
4.5	37	67	93	102	y = 202.00x + 23.50	0.9862
5.0	51	90	111	119	y = 236.67x + 33.17	0.9941
5.5	47	73	81	86	y =64.00x + 61.00	0.9910
6.0	32	35	41	47	y = 46.00x + 25.17	0.9898
6.5	9	23	24	25	y = 15.33x + 19.33	0.9888

*average of triplicate results

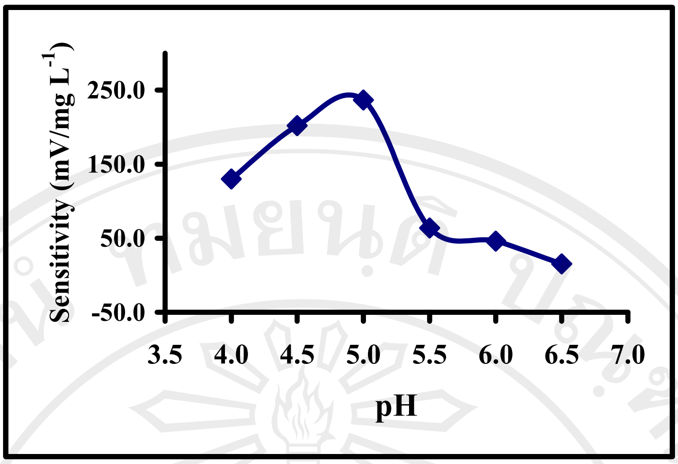


Figure 3.5 Relationship between pH and sensitivity of the calibration curve

3.2.1.3 Effect of BPR concentration

Effect of BPR concentrations on the determination of Al (III) (0.05-0.20 mg L^{-1}) was studied in the range 1.2 x 10^{-4} – 1.8 x 10^{-4} mol L^{-1}. The results are shown in Table 3.5 and Figure 3.6. It was found that sensitivity of calibration curve increased very rapidly from the BPR concentration of 1.2 x 10^{-4} to 1.6 x 10^{-4} mol L^{-1}. After that, the sensitivities were quite constant. This is due to the fact that increasing of the BPR concentration gives rise to the increase in the amounts of Al-BPR-CTAB complexation which results in a higher sensitivity of calibration curve obtained. However, beyond the BPR concentration of 1.6 x 10^{-4} mol L^{-1}, the amount of Al-BPR-CTAB complex became constant so as the Δpeak height. Consequently, a concentration of 1.6 x 10^{-4} mol L^{-1} of BPR was chosen as optimum.

Table 3.5 Effect of concentration of BPR on the sensitivity

BPR concentration ($x10^{-4}$ M)	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		
1.2	18	33	50	60	y = 289.33x – 4.17	0.9895
1.3	17	36	56	66	y = 335.33x + 2.00	0.9826
1.4	10	29	48	60	y = 340.67x + 5.67	0.9896
1.5	15	34	54	68	y =356.00x + 1.67	0.9927
1.6	23	46	68	84	y = 407.33x + 4.33	0.9925
1.7	25	42	66	84	y = 405.33x + 3.67	0.9946
1.8	23	43	67	83	y = 407.33x + 3.17	0.9935

*average of triplicate results

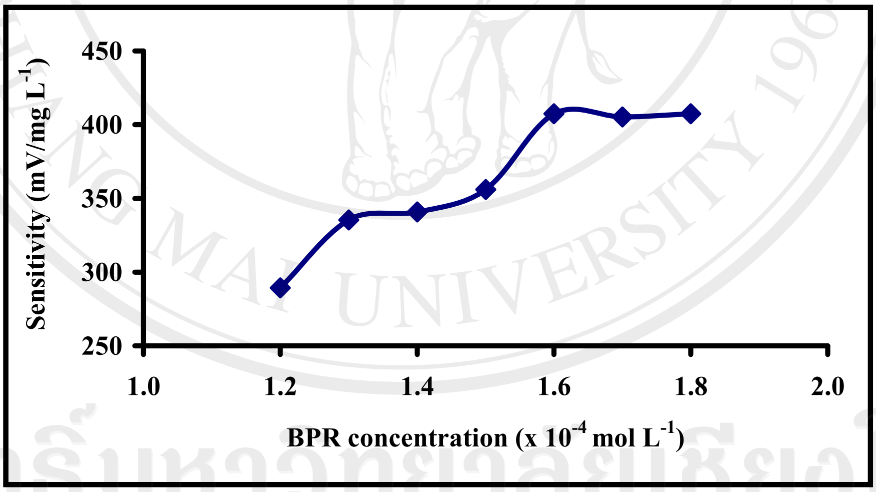


Figure 3.6 Relationship between concentration of BPR and sensitivity of the calibration curve

3.2.1.4 Effect of % ethanol in BPR solution

The effect of concentration of ethanol in BPR solution was studied in the range of 10-45% (v/v). The results are shown in Table 3.6 and Figure 3.7. It is shown that the greater sensitivity is obtained when the concentration of ethanol in BPR solution decreased. Thus, a concentration of ethanol in BPR solution of 10% (v/v) was chosen as optimum % ethanol in BPR 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		
10	17	41	62	86	y = 452.00x – 4.83	0.9994
15	16	41	61	80	y = 420.00x – 2.83	0.9947
20	22	44	66	80	y = 388.67x + 4.33	0.9898
25	20	41	63	76	y =382.67x + 2.17	0.9897
35	17	37	50	67	y = 328.67x + 1.50	0.9944
45	18	37	51	62	y = 289.33x + 6.00	0.9846

*average of triplicate results

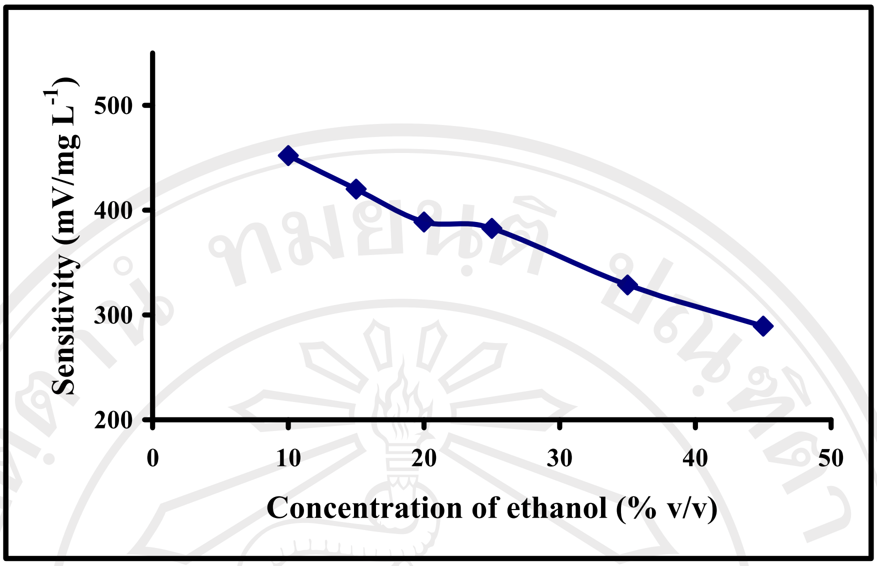


Figure 3.7 Relationship between concentration of ethanol in BPR solution and sensitivity of the calibration curve

3.2.1.5 Effect of CTAB concentration

The Effect of CTAB concentration on the determination of Al (III) (0.05-0.20 mg L^{-1}) was studied at different values in the range of 3.0 x 10^{-3} – 6.0 x 10^{-3} mol L^{-1}. The results are shown in Table 3.7 and Figure 3.8. It was found that 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 sensitivities were quite constant. This is due to the fact that increasing the CTAB concentration leading to the increase in the amounts of Al-BPR-CTAB complexation which results in a higher sensitivity of calibration curve obtained. However, beyond the CTAB concentration of 5 x 10^{-3} mol L^{-1}, the amount of Al-BPR-CTAB complexation became constant so as the Δpeak height. Consequently, a concentration of 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 ($x10^{-3}$ 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**		
3.0	30	46	66	81	y = 344.67x + 12.50	0.9979
3.5	36	50	66	77	y = 278.67x + 22.33	0.996
4.0	40	57	70	83	y = 284.67x + 27.17	0.9963
4.5	40	58	77	95	y =364.00x + 22.00	0.9998
5.0	44	65	84	99	y = 368.67x + 26.83	0.995
5.5	48	67	87	104	y = 375.33x + 29.67	0.9996
6.0	48	69	89	104	y = 379.33x + 30.17	0.9947

*average of triplicate results

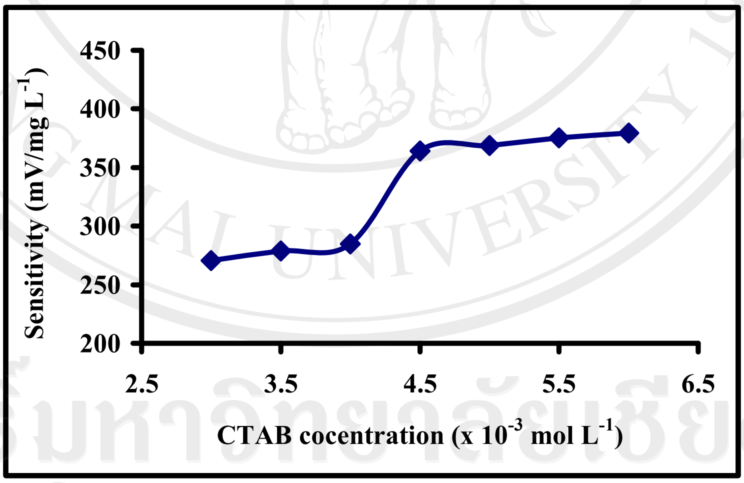


Figure 3.8 Relationship between concentration of CTAB and sensitivity of the calibration curve

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 aluminum solutions (0.05-0.20 mg L^{-1}) which were flowed into the rFI manifold as shown in Figure 2.1. The flow rates were varied from 1.5 to 4.0 ml min^{-1}. The effect of flow rate on the sensitivity was shown in Table 3.8 and Figure 3.9. 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}. This is cause 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**		
1.5	33	56	78	99	y = 439.33x + 11.67	0.9996
2.0	39	61	88	106	y = 460.00x + 16.17	0.9946
2.5	35	64	91	111	y = 511.33x + 11.33	0.9927
3.0	26	51	76	96	y = 459.33x + 3.67	0.9983
3.5	29	53	74	97	y = 450.00x + 7.00	0.9994
4.0	24	41	63	80	y = 380.00x + 4.50	0.9972

*average of triplicate results

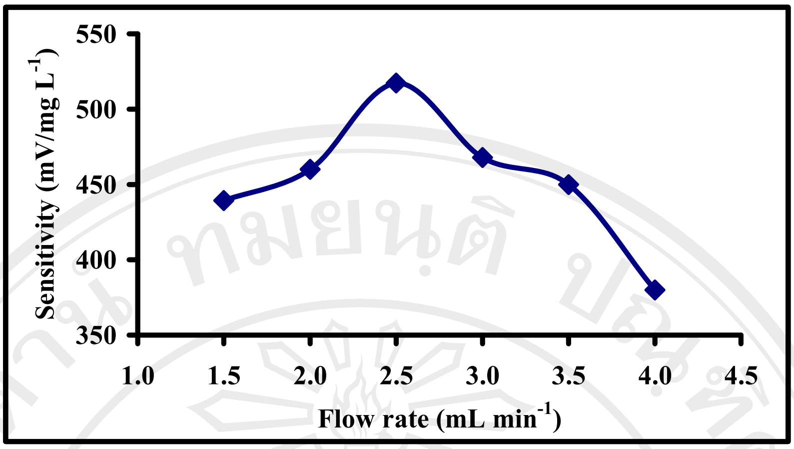


Figure 3.9 Relationship between flow rate and sensitivity of the calibration curve

3.2.1.7 Effect of reaction coil (I) length

The effect of reaction coil (I) length on the determination of Al (III) (0.05-0.20 mg L^{-1}) was studied by using Tygon tubing with diameter of 1.07 mm i.d. and length of reaction coil (I) length 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 100 cm. It can be explained that increasing the reaction coil (I) length up to 100 cm give rise to an increase in the residence time allowing well mixing between aluminum and BPR. On the other hand, the sensitivity of the calibration curve decreased when the reaction coil (I) length was longer than 100 cm. This is due to dispersion occurred at the reaction coil (I) length longer than 100 cm. The reaction coil (I) length 100 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		
25	40	57	77	94	y = 367.33x + 21.00	0.9994
50	37	59	79	97	y = 402.00x + 18.00	0.9977
75	40	64	85	105	y = 432.67x + 19.33	0.9985
100	39	62	85	107	y =457.33x + 16.00	0.9997
125	38	60	82	102	y = 427.33x + 17.17	0.9994
150	40	62	84	102	y = 414.00x + 20.00	0.998

*average of triplicate results

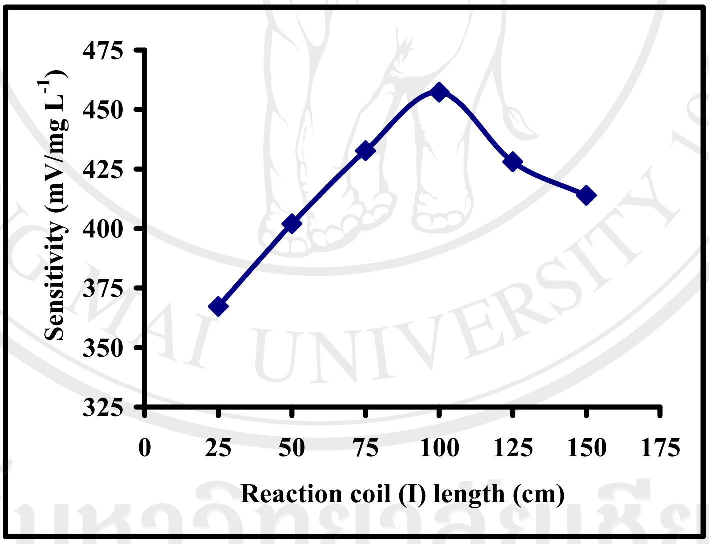


Figure 3.10 Relationship between reaction coil (I) length and sensitivity of the calibration curve

3.2.1.8 Effect of reaction coil (II) length

The effect of reaction coil (II) length on the determination of Al (III) (0.05-0.20 mg L^{-1}). The 1.07 mm i.d. Tygon tubing was examined for using as a reaction coil (II). The reaction coils (II) lengths were varied from 50 to 200 cm. The results are shown in Table 3.10 and Figure 3.11. The sensitivity increased to a maximum at a reaction coil (II) length of 150 cm. It can be explained that increasing the reaction coil (II) length up to 150 cm an increase in the residence time is obtained, allowing well mixing between Al-BPR and CTAB. On the other hand, the sensitivity of the calibration curve decreased when the reaction coil (II) length was longer than 150 cm. This is due to the fact that dispersion occurred at the reaction coil (II) length longer than 150 cm. The reaction coil (II) length 150 cm 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**		
50	33	57	81	102	y = 463.33x + 10.33	0.9987
75	38	65	87	109	y = 470x + 16.00	0.9977
100	34	60	83	106	y = 477.33x + 10.83	0.9985
125	42	67	93	116	y =495.33x + 17.50	0.9997
150	32	59	85	109	y = 510.67x + 7.50	0.9994
175	38	64	90	109	y = 470.00x + 15.83	0.9983
200	38	61	80	98	y = 396.00x + 19.83	0.9970

*average of triplicate results

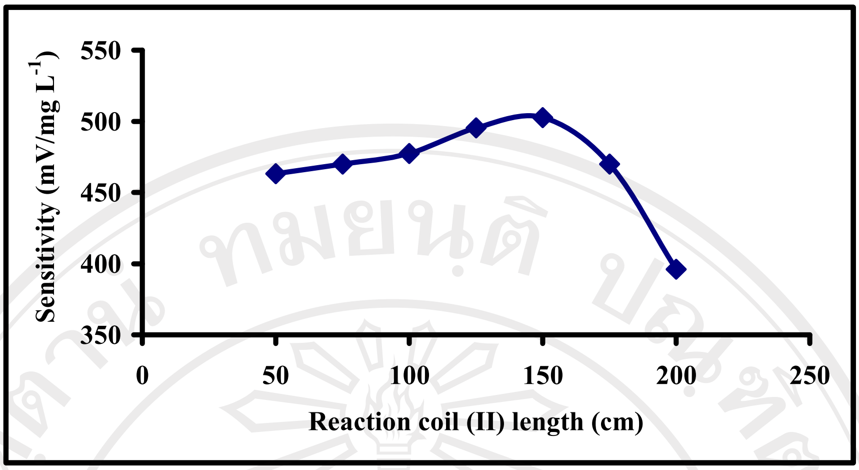


Figure 3.11 Relationship between reaction coil (II) length and sensitivity of the calibration curve

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 0.05-0.20 mg L^{-1} aluminum was studied by varying reagent volume of 50, 75, 100, 125 and 150 μL. As shown in Table 3.11 and Figure 3.12, the sensitivity increases with increasing reagent volume up to 75 μL because the increase in reagent volume increase the number of complex (Al-BPR-CTAB), that cause increases in Δpeak height. A reagent volume of 75 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		
50	29	49	70	88	y = 412.00x + 6.83	0.9987
75	32	59	83	107	y = 502.67x + 7.33	0.9993
100	30	57	79	100	y = 460.67x + 9.00	0.9964
125	34	60	83	102	y =450.67x + 13.33	0.9997
150	30	54	76	96	y = 438.00x + 9.5	0.9980

*average of triplicate results

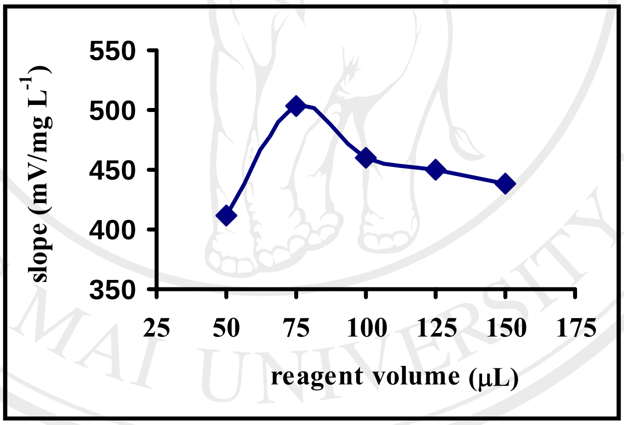


Figure 3.12 Relationship between reagent volume and sensitivity of the calibration curve

A diagram in the 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 Optimum conditions for aluminum determination

Variable	Studied range	Optimum value
Wavelength (nm)	540 - 595	580
pH	4.0 – 6.5	5.0
Concentration of BPR (x 10^{-4} mol L^{-1})	1.2 – 1.8	1.6
Concentration of ethanol in BPR solution (% v/v)	10 - 45	15
Concentration of CTAB (x 10^{-3} mol L^{-1})	3.0 – 6.0	5.0
Flow rate (mL min^{-1})	1.5- 4.0	2.5
Reaction coil (I) length (cm)	25-150	100
Reaction coil (II) length (cm)	50-200	150
Reagent volume (μL)	50-125	75

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 aluminum standard solution into rFI system under the suitable conditions as shown in Table 3.12, linear range of the calibration graph was obtained for aluminum standards at the concentration ranging 0.05 – 0.11 mg L^{-1} and 0.13 – 0.14 mg L^{-1}. All measurements were made in pentaplicate injections. The results obtained are shown in Table 3.13 and Figure 3.13.

Table 3.13 Linearity of aluminum determination

Aluminum ($mg\ L^{-1}$)	Peak height (mV)						ΔP.H.* (mV)
	1	2	3	4	5	$\bar{x}$	
0	227	227	228	228	228	228	0
0.01	227	229	232	232	233	231	-3
0.02	225	225	226	226	226	226	2
0.03	223	221	223	225	224	223	4
0.04	220	221	220	220	221	220	7
0.05	218	216	215	217	219	217	11
0.06	213	210	211	213	211	212	16
0.07	205	206	203	205	205	205	23
0.08	198	200	200	198	197	199	29
0.09	193	193	193	193	193	193	35
0.10	185	187	187	188	187	187	41
0.11	180	180	180	179	180	180	48
0.13	172	171	171	171	171	171	56
0.15	168	168	166	168	168	168	60
0.20	156	156	156	156	156	156	72
0.25	144	144	145	145	145	145	83
0.30	135	135	135	134	134	135	93
0.35	125	125	124	125	124	125	103
0.40	116	114	116	116	115	115	112
0.45	112	113	112	100	114	110	117
0.50	104	104	106	105	105	105	123
0.80	96	96	97	96	96	96	131
1.20	93	92	91	90	90	91	136

*average of pentaplicate results

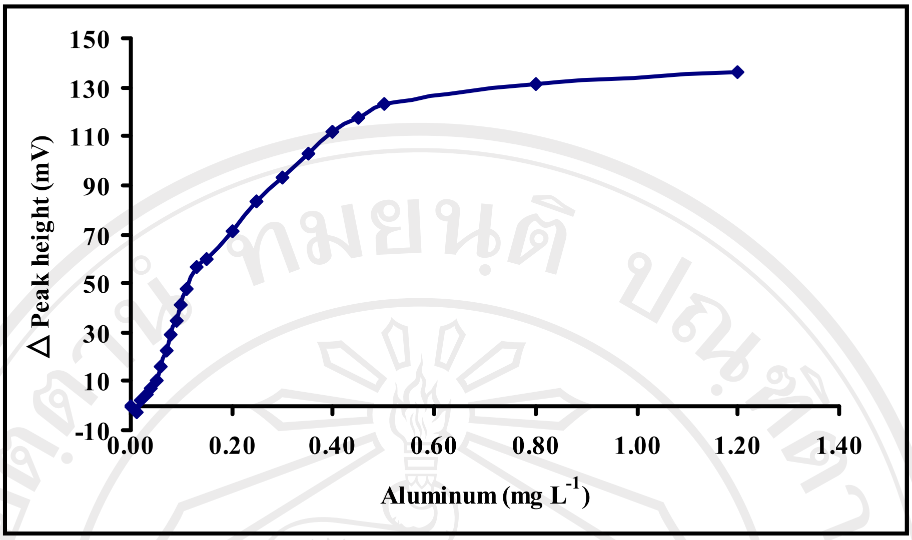


Figure 3.13 Relationship between Δpeak height and concentration of aluminum

3.2.2.2 Precision of the flow injection system

The precision of the proposed method was verified by 11 replicated determination of 0.1 mg L^{-1} standard aluminum, under the optimum conditions listed in Table 3.12. The relative standard deviation was found to be 0.33% (Table 3.14).

Table 3.14 Precision verification using standard 0.1 mg L^{-1} aluminum

Experimental number	Peak height (mV)
1	206
2	206
3	205
4	206
5	206
6	206
7	206
8	205
9	205
10	204
11	205
$\bar{x}$	205
S.D.	0.69
% R.S.D.	0.33

*average of triplicate results

3.2.2.3 Calibration curve

As depicted in Figure 2.1, the standard solutions containing 0.05-0.11 and 0.15-0.40 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 3.14 and 3.15 were established by plotting Δpeak heights versus the various aluminum concentrations. Correlation coefficients (r^2) and the regression equation are as follows:

$y = 617.86x – 20.629$ ($r^2 = 0.9992$) (Al 0.05-0.11 mg L^{-1})

$y = 209.64x – 29.435$ ($r^2 = 0.9986$) (Al 0.15-0.40 mg L^{-1})

Where y is Δpeak height in mV

x is concentration of aluminum in mg L^{-1}

Table 3.15 ΔPeak height for calibration curve

Aluminum (mg L^{-1})	**Peak height (mV)**						**ΔP.H.*** **(mV)**
	1	**2**	**3**	**4**	**5**	**$\bar{x}$**	
0	227	227	228	228	228	228	0
0.05	218	216	215	217	219	217	11
0.06	213	210	211	213	211	212	16
0.07	205	206	203	205	205	205	23
0.08	198	200	200	198	197	199	29
0.09	193	193	193	193	193	193	35
0.10	185	187	187	188	187	187	41
0.11	180	180	180	179	180	180	48
0.15	168	168	166	168	168	168	60
0.20	156	156	156	156	156	156	72
0.25	144	144	145	145	145	145	83
0.30	135	135	135	134	134	135	93
0.35	125	125	124	125	124	125	103
0.40	116	114	116	116	115	115	112

*average of pentaplicate results

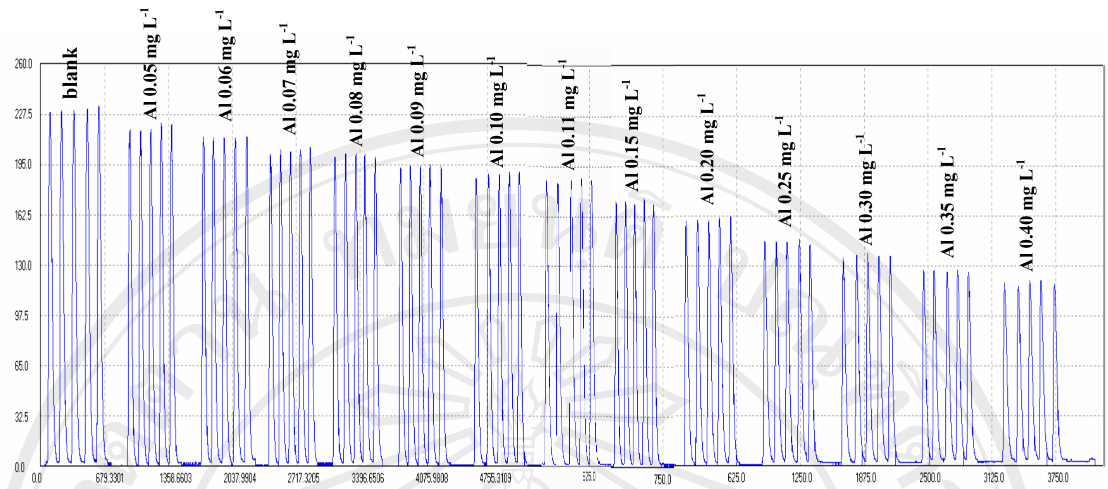
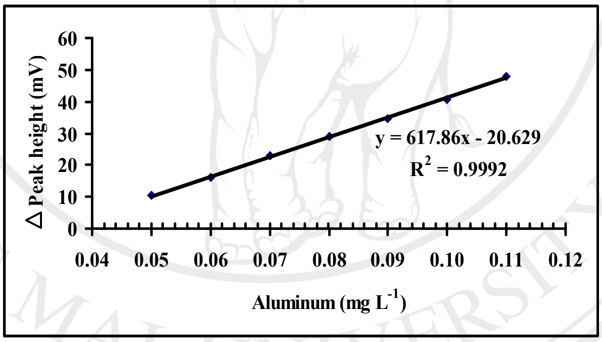
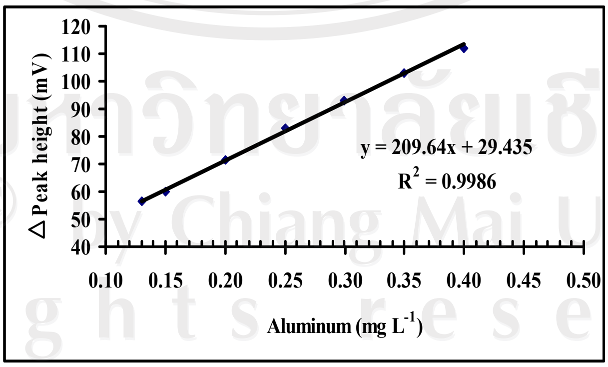


Figure 3.14 Calibration signal of rFIA spectrophotometric determination of aluminum 0.05-0.11mg L^{-1} and 0.15-0.40 mg L^{-1}



(a)



(b)

Figure 3.15 The Calibration curve of rFIA spectrophotometric determination of aluminum: (a) aluminum 0.05-0.11 mg L^{-1}; (b) aluminum 0.15-0.40 mg L^{-1}

3.2.2.4 Detection limit

The detection limit was determined by the method reported by Miller and Miller [61], which was calculated from the linear regression line of twice calibration curves. The results are giving in Tables 3.16 and 3.17. The detection limit of the proposed method was found to be 0.002 and 0.014 mg L^{-1} from aluminum 0.05-0.11 and 0.15-0.40 mg L^{-1}. The concentration at limit of detection (C_L) can be calculated from equation 2.2-2.3.

Table 3.16 Calculation of detection limit of rFIA spectrophotometric determination of aluminum 0.05-0.11 mg L^{-1}

Aluminum (mg L^{-1})	**Y_i^***	**$\hat{Y}_i$**	**$\lvert Y_i-\hat{Y}_i\rvert$**	**$\lvert Y_i-\hat{Y}_i\rvert^2$**
0.05	10.60	10.26	0.34	0.11
0.06	16.00	16.44	0.44	0.20
0.07	22.80	22.62	0.18	0.03
0.08	29.00	28.80	0.20	0.04
0.09	34.60	34.98	0.38	0.14
0.10	40.80	41.16	0.36	0.13
0.11	47.80	47.34	0.46	0.22
$\Sigma(\lvert Y_i-\hat{Y}_i\rvert^2)$				0.87
S_y/x				0.42
C_L , LOD				0.002
LOQ				0.007

*average of triplicate results

The linear regression equation is Y = 617.86x-20.629

$$S_y/x = [0.87/(7-2)]^{1/2}$$
$$= 0.42$$
$$C_L\text{ , LOD} = (3 \times 0.42)/617.86$$
$$= 0.002\text{ mg L}^{-1}\text{ Al(III)}$$
$$\text{LOQ} = (10 \times 0.42)/617.86$$
$$= 0.007\text{ mg L}^{-1}\text{ Al(III)}$$

Table 3.17 Calculation of detection limit of rFIA spectrophotometric determination of aluminum 0.15-0.40 mg L^{-1}

Aluminum (mg L^{-1})	**Y_i^***	**$\hat{Y}_i$**	**$\lvert Y_i-\hat{Y}_i\rvert$**	**$\lvert Y_i-\hat{Y}_i\rvert^2$**
0.15	60.00	61.05	1.05	1.10
0.20	71.60	71.48	0.12	0.01
0.25	83.00	81.92	1.08	1.17
0.30	93.00	92.35	0.65	0.42
0.35	103.00	102.79	0.21	0.05
0.40	112.20	113.22	1.02	1.04
$\Sigma(\lvert Y_i-\hat{Y}_i\rvert^2)$				3.79
S_y/x				0.97
C_L , LOD				0.014
LOQ				0.046

*average of triplicate results

The linear regression equation is $Y = 208.69x+29.745$

$$S_y/x = [3.79/(6-2)]^{1/2}$$

$$= 0.97$$

$$C_L \text{ , LOD} = (3 \times 0.97)/208.69$$

$$= 0.014 \text{ mg L}^{-1} \text{ Al(III)}$$

$$\text{LOQ} = (10 \times 0.97)/208.69$$

$$= 0.046 \text{ mg L}^{-1} \text{ Al(III)}$$

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 aluminum 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} aluminum 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 3.18.

Table 3.18 Interference studies for 0.20 mg L^{-1} standard aluminum by rFIA method

Interference	Aluminum : Interference	Peak height* (mV)	% Recovery
Cu^{2+}	1 : 0	72	-
	1 : 1	77	107
	1 : 2	80	111
	1 : 10	124	173
Fe^{2+}	1 : 0	72	-
	1 : 0.5	75	104
	1 : 1	78	110
	1 : 1.5	86	120
Fe^{3+}	1 : 0	72	-
	1 : 1	76	106
	1 : 2	94	132
	1 : 3	103	143
Ni^{2+}	1 : 0	72	-
	1 : 1	75	104
	1 : 2	78	109
	1 : 5	82	113
Co^{2+}	1 : 0	72	-
	1 : 5	74	103
	1 : 15	78	108
	1 : 30	80	119
Cr^{3+}	1 : 0	72	-
	1 : 5	69	95
	1 : 15	64	89
	1 : 20	58	81

*average of triplicate results

Table 3.18 (Continued).

Interference	Aluminum : Interference	Peak height* (mV)	% Recovery
Cd^{2+}	1 : 0	72	
	1 : 10	74	102
	1 : 40	76	106
	1 : 100	81	112
Zn^{2+}	1 : 0	72	-
	1 : 20	75	104
	1 : 60	78	107
	1 : 100	81	113
Mn^{2+}	1 : 0	72	-
	1 : 100	73	102
	1 : 300	74	103
	1 : 500	73	101
Mg^{2+}	1 : 0	72	-
	1 : 300	70	98
	1 : 400	76	105
	1 : 500	77	106
Na^{+}	1 : 0	72	-
	1 : 500	73	100
	1 : 1500	70	96
	1 : 2500	69	96
Ca^{2+}	1 : 0	72	-
	1 : 500	71	98
	1 : 1500	73	102
	1 : 2500	70	97

*average of triplicate results

Table 3.18 (Continued).

Interference	Aluminum : Interference	Peak height* (mV)	% Recovery
NO_2^-	1 : 0	72	-
	1 : 100	72	100
	1 : 300	75	104
	1 : 500	73	101
SO_4^{2-}	1 : 0	72	-
	1 : 100	71	100
	1 : 300	69	97
	1 : 500	68	96
HCO_3^-	1 : 0	72	-
	1 : 100	72	100
	1 : 300	72	101
	1 : 500	75	105
Br^-	1 : 0	72	-
	1 : 100	73	100
	1 : 300	74	102
	1 : 500	75	103
I^-	1 : 0	72	-
	1 : 100	73	101
	1 : 300	73	102
	1 : 500	75	104
Cl^-	1 : 0	72	-
	1 : 500	71	99
	1 : 1500	70	97
	1 : 2500	69	97

*average of triplicate results

The interference effects of some possible foreign ions on aluminum determination using rFIA system for were summarized in Table 3.19. It was found that Fe^{2+}, Fe^{3+} and Cu^{2+} interfered determination aluminum in tap water, which refered standard quality of tap water defined by metropolitan waterworks authority (Appendix A).

Table 3.19 Summary of interference effects of some ions on the response obtained from aluminum 0.2 mg L^{-1}

Interference ion	Tolerable concentration ratio*
Na^{+}, Ca^{2+}, Cl^{-}	2500
Mn^{2+}, Mg^{2+}, NO_2^{-}, HCO_3^{-}, SO_4^{2-}, Br^{-}, I^{-}	500
Cd^{2+}, Zn^{2+}	80
Co^{2+}	20
Cr^{3+}	11
Ni^{2+}	4
Cu^{2+}, Fe^{2+}, Fe^{3+}	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 aluminum 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 3.20. It was found that Fe^{2+} was masked with 0.008 mol L^{-1} of 1,10-phenanthroline [62]. Fe^{3+} was reduced to Fe^{2+} with 1% v/v of hydroxylammonium chloride and masked as the same manner as Fe^{2+} [62] and Cu^{2+} was masked with 0.5% v/v of thiourea [63].

Table 3.20 Effect of masking agent for mask Fe^{2+}, Fe^{3+} and Cu^{2+} the response obtained from aluminum 0.2 mg L^{-1}

Interference	Concentration of masking agent	Aluminum :Interference	Peak height* (mV)	% Recovery
Fe^{2+}	0.004 mol L^{-1} PHT	1 : 0	72	-
		1 : 10	78	108
		1 : 15	83	115
		1 : 20	89	124
	0.008 mol L^{-1} PHT	1 : 0	72	-
		1 : 10	78	108
		1 : 15	79	110
		1 : 20	85	118
	0.012 mol L^{-1} PHT	1 : 0	72	-
		1 : 5	78	108
		1 : 10	79	110
		1 : 15	81	113

*average of triplicate results

PHT was 1,10-phenanthroliene

Table 3.20 (continued).

Interference	Concentration of masking agent	Aluminum :Interference	Peak height* (mV)	% Recovery
Fe^{3+}	0.5% HAC + 0.008 mol L^{-1} PHT	1 : 0	72	-
		1 : 10	75	104
		1 : 15	79	110
		1 : 20	85	118
	1.0% HAC + 0.008 mol L^{-1} PHT	1 : 0	72	-
		1 : 10	75	104
		1 : 15	78	108
		1 : 20	82	114
	1.5% HAC + 0.008 mol L^{-1} PHT	1 : 0	72	-
		1 : 10	75	104
		1 : 15	78	108
		1 : 20	81	113
Cu^{2+}	0.25% thiourea	1 : 0	72	-
		1 : 30	78	108
		1 : 50	80	111
		1 : 70	85	118
	0.50% thiourea	1 : 0	72	-
		1 : 30	78	108
		1 : 50	79	110
		1 : 70	81	113
	0.75% thiourea	1 : 0	72	-
		1 : 30	77	108
		1 : 50	78	110
		1 : 70	80	113

*average of triplicate results

PHT was 1,10-phenanthroliene

HAC was hydroxylammonium chloride

3.2.2.7 Determination of aluminum in waters

The proposed rFI spectrophotometric method was applied to the simultaneous determination of aluminum in tap water samples which were collected from several Aumphur in Chiang Mai and boiling water from aluminum ware. The peak heights from each sample were compared with standard calibration curve. The results were given in Table 3.21

Table 3.21 Determination of aluminum in water samples by rFIA method

Water samples	Peak heights				SD	Aluminum concentration* (mg L^{-1})	% Recovery*
	1	2	3	$\bar{x}$			
Hangdong	ND**	ND**	ND**	-	-	ND**	-
Sangpatong	0.116	0.113	0.115	0.115	0.002	0.115 ± 0.002	100.20
Muang	0.077	0.070	0.075	0.073	0.004	0.073 ± 0.004	101.23
Mae Jo	0.123	0.133	0.128	0.128	0.005	0.128 ± 0.005	103.53
Sansai	ND**	ND**	ND**	-	-	ND**	-
Sankumpang	ND**	ND**	ND**	-	-	ND**	-
Mae Rim	0.369	0.357	0.363	0.363	0.06	0.363 ± 0.006	99.84
Chiang Mai University	0.234	0.248	0.212	0.231	0.018	0.231 ± 0.018	99.76
Boil water 3 h	0.116	0.108	0.112	0.112	0.004	0.112 ± 0.004	99.73
Boil water 6 h	0.023	0.025	0.023	0.023	0.001	0.023 ± 0.001	98.69
Boil water 9 h	ND**	ND**	ND**	-	-	ND**	-

*average of triplicate results

**not detected

The aluminum contents in the water samples were in the range of 0.023-0.363 mg L^{-1} and 0.011-0.350 mg L^{-1} using the proposed method and ICP-OES respectively. The results obtained by the proposed rFI spectrohotometric method compared favorably with those obtained by ICP-OES using the student t-test (Table 3.22 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 rFI spectrohotometric with those obtained by ICP-OES was 0.509. It was seen that the experimental t-value for Al(III) assay(0.509) which was smaller than the theoretical t-value at a confidence interval of 95% (2.45) indicating that results obtained by both methods were in excellent agreement.

Table 3.22 Comparative determination of aluminum in water sample by proposed rFIA method and ICP-OES

Water samples	**Concentrations (mg L^{-1})**		x_d
	rFIA*	**ICP-OES***	
Hangdong	ND**	0.011	-
Sangpatong	0.115	0.127	-0.012
Muang	0.073	0.072	0.001
Mae Jo	0.128	0.128	0.000
Sansai	ND**	0.011	-
Sankumpang	ND**	0.014	-
Mae Rim	0.363	0.350	0.013
Chiang Mai University	0.231	0.252	-0.007
Boil water 3 hour	0.112	0.110	0.002
Boil water 6 hour	0.023	0.031	-0.008
Boil water 9 hour	ND**	0.014	-
Σ			-0.011
S_d			0.00816
T			0.509

*average of triplicate results
**not detected

3.3 SIA Spectrophotometric Determination of Aluminium Using Bromopyrogalol Red and Cetyltrimethyl Ammonium Bromide as A Complexing Agent

The conditions for the determination of aluminum 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 2.5 were used. The range of variables studied and the optimal values chosen are shown in Table 2.4.

3.3.1 Study aspiration order

The complexation of Al-BPR-CTAB was studied at different aspiration orders. The sensitivities obtained are shown in Table 3.23. 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 3.23 Sensitivity at various aspiration orders

Series	Aspiration order	Sensitivity (AU/mg L^{-1})
1	A-B-C	0.3157
2	A-C-B	0.2062
3	B-A-C	0.2558
4	B-C-A	ND*
5	C-A-B	0.2155
6	C-B-A	ND*

*not detected

A was 0.1 mg L^{-1} aluminum standard solution

B was 1.6 x 10^{-4} mol L^{-1} bromopyrogallol red

C was 5 x 10^{-3} mol L^{-1} cetyltrimethylammonium bromide

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 2.5) 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 aluminum determination by measuring the absorbance at the wavelength 430 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, 6.0 and 5.5) on the determination of aluminum in the mixture solutions. The results are shown in Table 3.24 and Fig 3.16. 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 3.24 Effect of pH on the sensitivity

pH	**Sensitivity (AU/mg L^{-1})**				**SD**
	1	**2**	**3**	$\bar{x}$*	
4.0	0.1883	0.1963	0.2030	0.1959	0.0074
4.5	0.2667	0.2713	0.2713	0.2698	0.0027
5.0	0.2930	0.2907	0.3163	0.3035	0.0142
5.5	0.4213	0.4130	0.4303	0.4215	0.0087
6.0	0.3103	0.3007	0.3140	0.3083	0.0069

*average of triplicate results

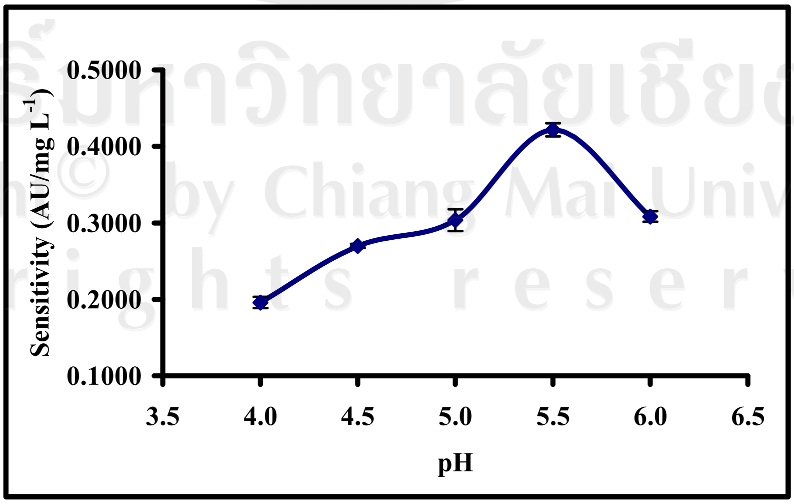


Figure 3.16 Relationship between various pH and sensitivity of the calibration curve

3.3.2.2 Effect of pH concentration

The effect of acetate buffer concentration at pH 5.5 on the reactions of aluminum, BPR and CTAB were studied in the range of 0.20 – 0.40 mol L^{-1}. The results are shown in Table 3.25 and Fig 3.17. It was found that the sensitivity of the calibration curve increased very rapidly from the pH 5.5 concentration of 0.20-0.25 mol L^{-1}. After that, the sensitivities of were quite constant when increases pH concentration. Therefore, a concentration of 0.25 mol L^{-1} acetate buffer at pH 5.5 was chosen as optimum.

Table 3.25 Effect of concentration of acetate buffer pH 5.0 on the sensitivity

Concentration of acetate buffer pH 5.5 (mol L^{-1})	**Sensitivity (AU/mg L^{-1})**				**SD**
	1	**2**	**3**	**$\bar{x}$***	
0.20	0.3867	0.3780	0.3833	0.3827	0.0044
0.25	0.5037	0.4963	0.5003	0.5001	0.0037
0.30	0.4990	0.4927	0.4947	0.4955	0.0032
0.35	0.4963	0.5080	0.5057	0.5033	0.0062
0.40	0.4987	0.5000	0.4973	0.4987	0.0014

*average of triplicate results

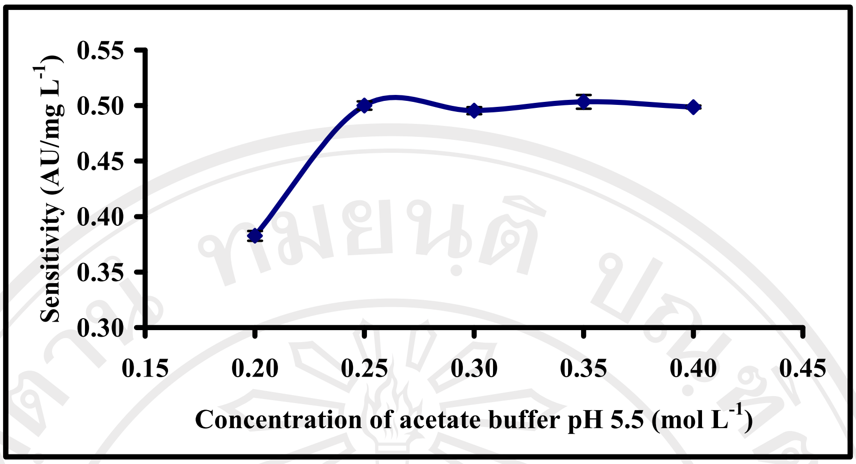


Figure 3.17 Relationship between various concentration of acetate buffer pH 5.5 and sensitivity of the calibration curve

3.3.2.3 Effect of BPR concentration

Effect of BPR concentrations on the determination of Al (III) (0.05-0.20 mg L^{-1}) was studied with the following SI system in Figure 2.2 or 2.3, the concentration of BPR solutions were varied from 1.2 x 10^{-4} – 1.6 x 10^{-4} mol L^{-1} of BPR in 50% ethanol solution. The results are shown in Table 3.26 and Figure 3.18. It was found that the sensitivity increased very rapidly from the BPR concentration of 1.2 x 10^{-4} – 1.4 x 10^{-4} mol L^{-1}. This is due to the fact that increasing the BPR concentration results in increasing the amounts of Al-BPR-CTAB complexation that cause higher sensitivity. So, a concentration of 1.4 x 10^{-4} mol L^{-1} of BPR was chosen as optimum.

Table 3.26 Effect of various concentration of BPR on the sensitivity

Concentration of BPR solutions (x 10^{-4} mol L^{-1})	Sensitivity (AU/mg L^{-1})				SD
	1	2	3	$\bar{x}$ *	
1.2	0.3150	0.3390	0.3203	0.3248	0.0126
1.3	0.5307	0.5300	0.5107	0.5238	0.0114
1.4	0.6483	0.6470	0.6487	0.6480	0.0009
1.5	0.4630	0.4643	0.4550	0.4608	0.0050
1.6	0.4530	0.4603	0.4620	0.4584	0.0048

*average of triplicate results

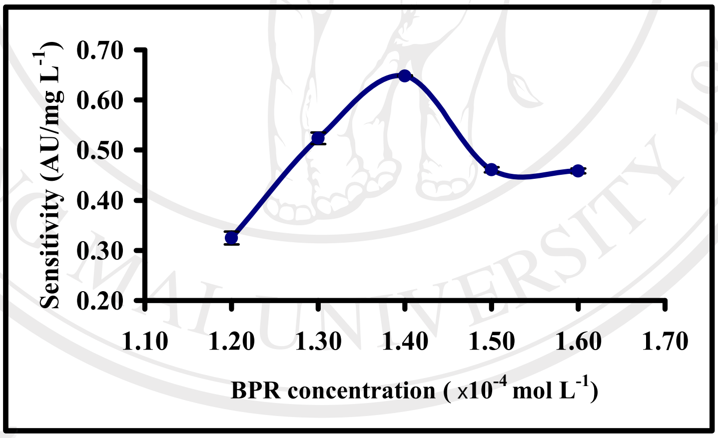


Figure 3.18 Relationship between various concentration of BPR in 50 % ethanol solution and sensitivity of the calibration curve

3.3.2.4 Effect of % ethanol in BPR solution

The effect of ethanol concentration in BPR solution was studied in the range of 10-50% (v/v). The results are shown in Table 3.27 and Figure 3.19. It is shown that the greater slope is obtained when the concentration of ethanol in BPR solution decreased. Thus, a concentration of ethanol in BPR solution of 10% (v/v) was chosen as optimum % ethanol in BPR solution.

Table 3.27 Effect of various concentration of ethanol on the sensitivity

Ethanol (% v/v)	**Sensitivity (AU/mg L^{-1})**				**SD**
	1	**2**	**3**	$\bar{x}$ *	
10	0.5307	0.5333	0.5303	0.53143	0.00163
20	0.5037	0.5127	0.515	0.51047	0.00597
30	0.4907	0.493	0.4977	0.4938	0.00357
40	0.473	0.4667	0.463	0.46757	0.00506
50	0.4483	0.4443	0.4447	0.44577	0.0022

*average of triplicate results

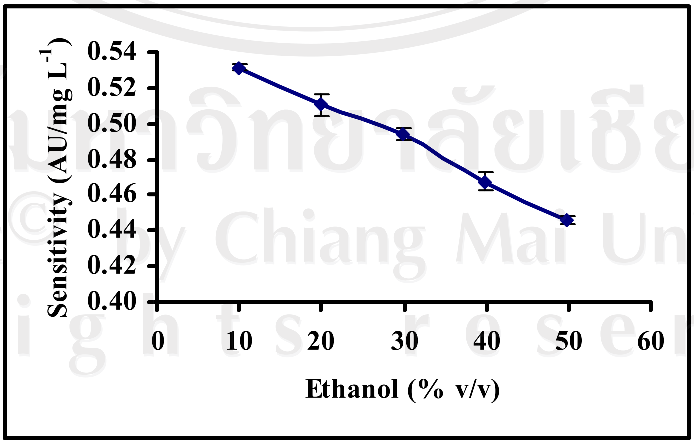


Figure 3.19 Relationship between various concentration of ethanol on the sensitivity of the calibration curve

3.3.2.5 Effect of CTAB concentration

The Effect of CTAB concentration on the determination of Al (III) (0.05-0.20 mg L^{-1}) was studied at different values in the range of 1.0 x 10^{-3} – 6.0 x 10^{-3} mol L^{-1}. The results are shown in Table 3.28 and Figure 3.20. It was found that the sensitivity increased very rapidly from the CTAB concentration of 1.0 x 10^{-3} – 4.0 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-BPR-CTAB complexation that gave higher sensitivity. Beyond the highest sensitivity, the amount of Al-BPR-CTAB became constant so as the Δpeak height. Consequently, a concentration of 5 x 10^{-3} mol L^{-1} of CTAB was chosen as optimum.

Table 3.28 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	$\bar{x}^*$	
1	0.4920	0.4930	0.4800	0.4883	0.0072
2	0.4983	0.5103	0.4847	0.4978	0.0128
3	0.5140	0.5067	0.5197	0.5135	0.0065
4	0.5697	0.5743	0.5797	0.5746	0.0050
5	0.5873	0.5900	0.5883	0.5885	0.0014
6	0.5920	0.5950	0.5917	0.5929	0.0018

*average of triplicate results

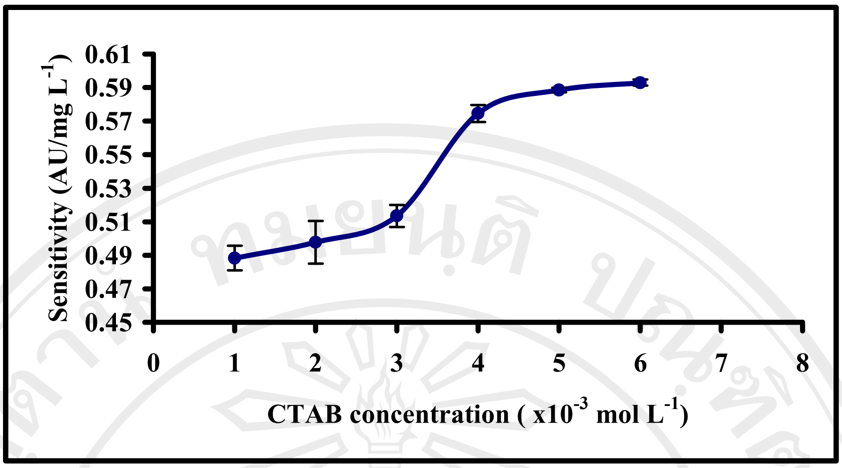


Figure 3.20 Relationship between concentration of CTAB on the sensitivity of the calibration curve

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 0.25 mol L^{-1} of acetate buffer pH 5.5 was studied over the range 100 – 200 μL at every 25 μL interval (Table 3.29 and Fig 3.21). It was found that the sensitivity increased when the aspiration volume of acetate buffer 0.25 mol L^{-1} at pH 5.5 was increased and reached a maximum sensitivity at 150 μL, above which the sensitivity started to decline. So, a volume of 150 μL was chosen as an optimum for subsequent measurements.

Table 3.29 Effect of various aspiration volume of 0.25 mol L^{-1} of acetate buffer pH 5.5 on the sensitivity

Aspirate buffer (μL)	Sensitivity (AU/mg L^{-1})				SD
	1	2	3	$\bar{x}^{*}$	
100	0.4977	0.4940	0.4920	0.4946	0.0029
125	0.5920	0.6003	0.5807	0.5910	0.0098
150	0.6797	0.6847	0.6817	0.6820	0.0025
175	0.6260	0.6227	0.6303	0.6263	0.0038
200	0.5350	0.5360	0.5233	0.5314	0.0090

*average of triplicate results

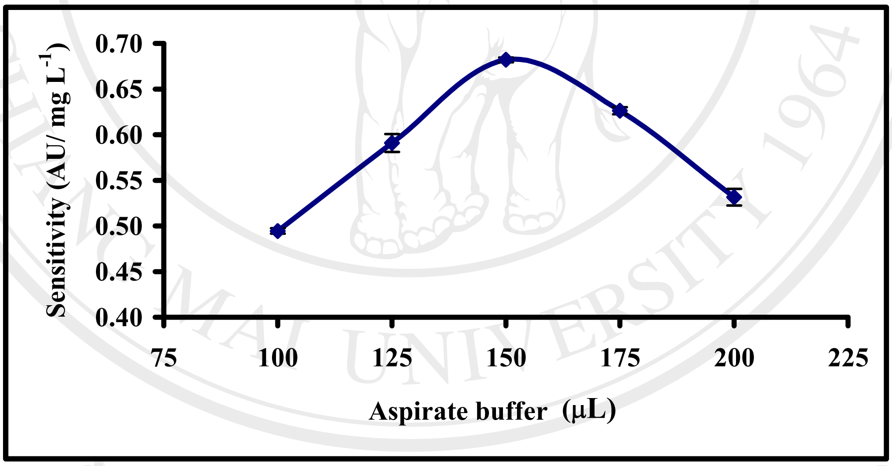


Figure 3.21 Relationship between various aspiration volume of 0.25 mol L^{-1} of acetate buffer pH 5.5 on the sensitivity of the calibration curve

3.3.2.7 Effect of aspiration volumes of BPR

To minimize the consumption of reagent volume, the effect of the BPR aspirated volume was considered. When the volumes of BPR solution were varied from 50 to 150 µL at every 25 µL interval, maximum sensitivity was obtained at a volume of 75 µL (Table 3.30 and Fig 3.22). Thus, a 75 µL of BPR solution was chosen as an optimum volume for subsequent measurements.

Table 3.30 Effect of various aspiration volumes of 1.40 x 10^{-4} L of BPR in 10% ethanol on the sensitivity

Aspirate BPR (µL)	**Sensitivity (AU/mg L^{-1})**				**SD**
	1	**2**	**3**	**$\bar{x}$**	
50	0.5643	0.5593	0.5793	0.5676	0.0104
75	0.7450	0.7407	0.7353	0.7403	0.0049
100	0.5463	0.5600	0.5587	0.5550	0.0076
125	0.4083	0.4010	0.4100	0.4064	0.0048
150	0.2573	0.2767	0.2600	0.2647	0.0118

*average of triplicate results

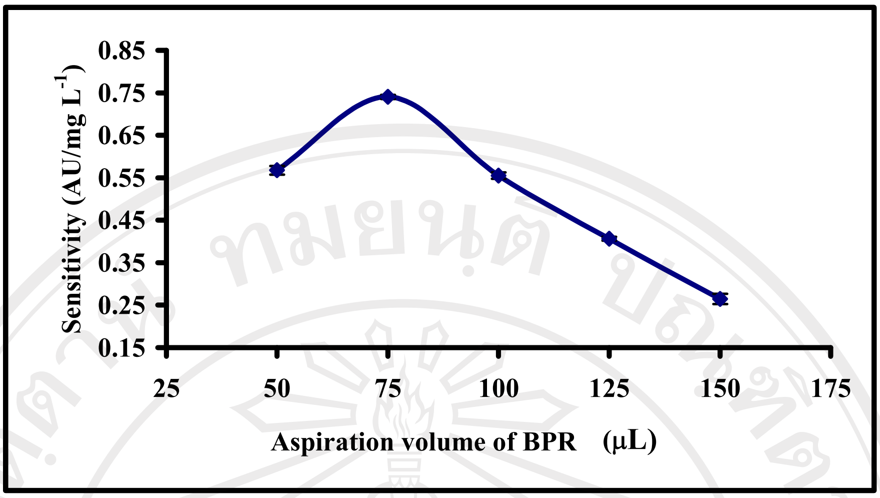


Figure 3.22 Relationship between various aspiration volumes of 1.40 x 10^{-4} L of BPR in ethanol 10% on the sensitivity of the calibration curve

3.3.2.8 Effect of aspiration volumes of CTAB

The Effect of aspiration volumes of CTAB on the determination of Al (III) (0.1-0.4 mg L^{-1}) was studied at different concentrations in the range of 50-150 µL. The results are shown in Table 3.31 and Figure 3.23. 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 3.31 Effect of various aspiration volume of 5.0 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	0.3320	0.3073	0.3390	0.3261	0.0167
75	0.6473	0.6327	0.6577	0.6459	0.0126
100	0.6697	0.6623	0.6703	0.6674	0.0045
125	0.5663	0.5693	0.5797	0.5718	0.0070
150	0.4977	0.4863	0.4797	0.4879	0.0091

*average of triplicate results

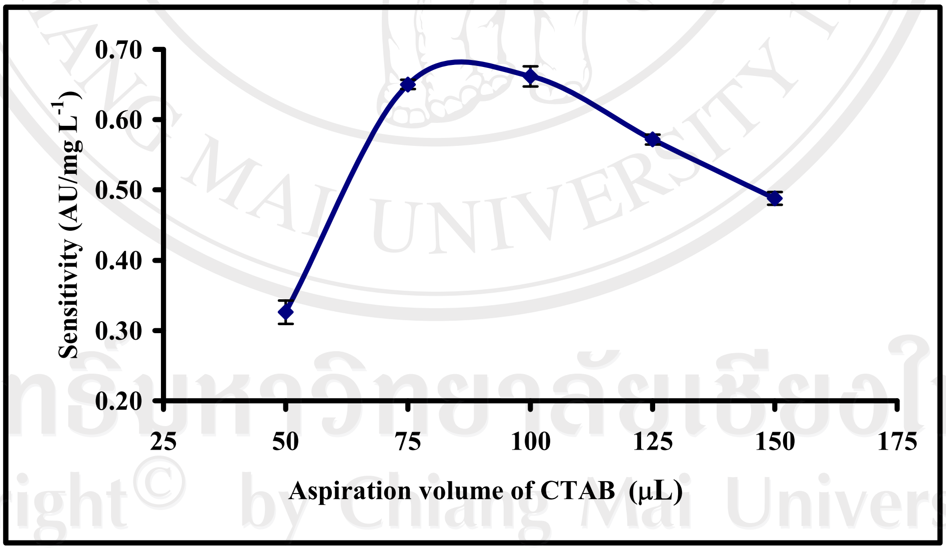


Figure 3.23 Relationship between various aspiration volumes of 5 x 10^{-3} mol L^{-1} of CTAB on the sensitivity of the calibration curve

3.3.2.9 Effect of aspiration volumes of sample

The influence of the sample volumes were examined between 100 to 200 µL at every 25 µL interval and it was found that the sensitivity increased markedly up to 150 µL, above which the sensitivity started to decline (Table 3.32 and Fig 3.24). So, a volume of 150 µL was chosen as an appropriate sample volume for further investigations.

Table 3.32 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	0.4957	0.4987	0.5027	0.4990	0.0035
125	0.5697	0.5623	0.5740	0.5687	0.0059
150	0.6280	0.6367	0.6327	0.6325	0.0044
175	0.5523	0.5643	0.5580	0.5582	0.0060
200	0.5310	0.5213	0.5103	0.5209	0.0078

*average of triplicate results

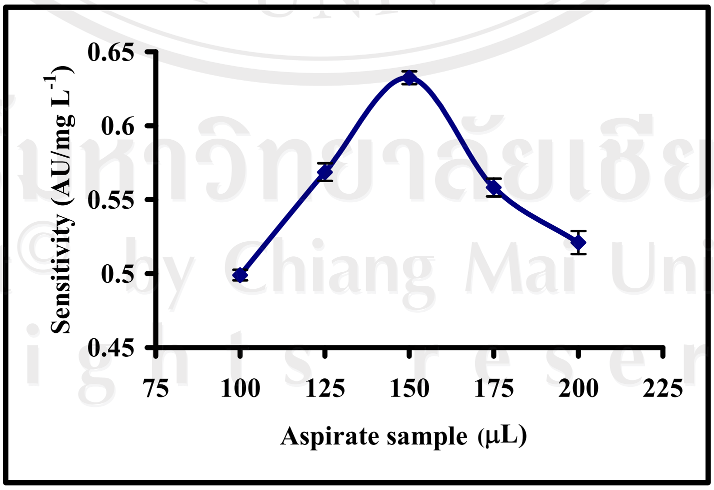


Figure 3.24 Relationship between various aspiration volumes of 5 x 10^{-3} mol L^{-1} of CTAB on the sensitivity of the calibration curve

3.3.2.10 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-orange 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, BPR, 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 3.33 and Figure 3.25. Maximum absorbance 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 3.33 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	0.4007	0.3883	0.3820	0.3903	0.0095
75	0.4167	0.4210	0.4337	0.4238	0.0088
100	0.4820	0.4897	0.4910	0.4876	0.0049
125	0.6867	0.6773	0.6873	0.6838	0.0056
150	0.6687	0.6233	0.6323	0.6414	0.0064
175	0.3973	0.3753	0.3700	0.3809	0.0145

*average of triplicate results

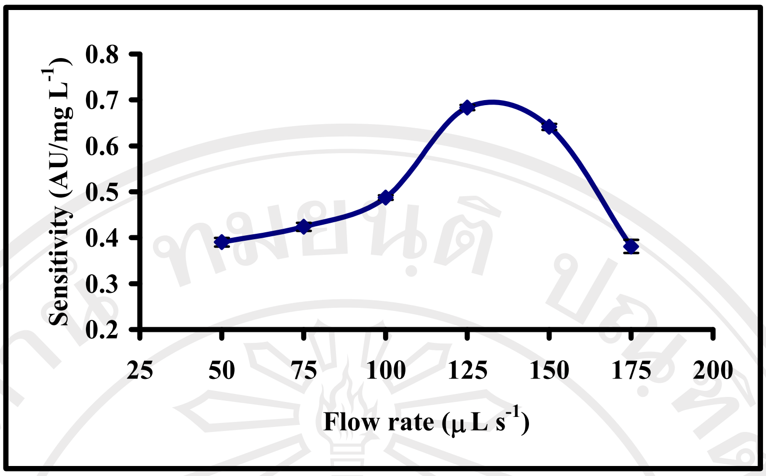


Figure 3.25 Relationship between various flow rate on the sensitivity of the calibraton curve

3.3.2.11 Effect of holding time

The effect of holding time on the determination of Al (III) (0.10-0.40 mg L^{-1}) was studied in the range of 20-60 s .The results are shown in Table 3.34 and Figure 3.26. The sensitivity increased to a maximum at holding time 40 s. It can be explained that increasing the holding time results in an increase in Al-BPR-CTAB complex because its mixing is better than that occurred at the holding time below 40 s. The sensitivity decreased when holding time greter than 40 s because of dispersion. So, a holding time of 40 s was chosen as optimum since it provided the greatest sensitivity.

Table 3.34 Effect of various holding time on the sensitivity

Holding time (s)	Sensitivity (AU/mg L^{-1})				SD
	1	2	3	$\bar{x}^*$	
20	0.4027	0.415	0.409	0.4089	0.00615
30	0.4313	0.4383	0.4373	0.43563	0.00379
40	0.684	0.6807	0.672	0.6789	0.0062
50	0.6763	0.6787	0.6727	0.6759	0.00302
60	0.5857	0.5957	0.5767	0.58603	0.0095

*average of triplicate results

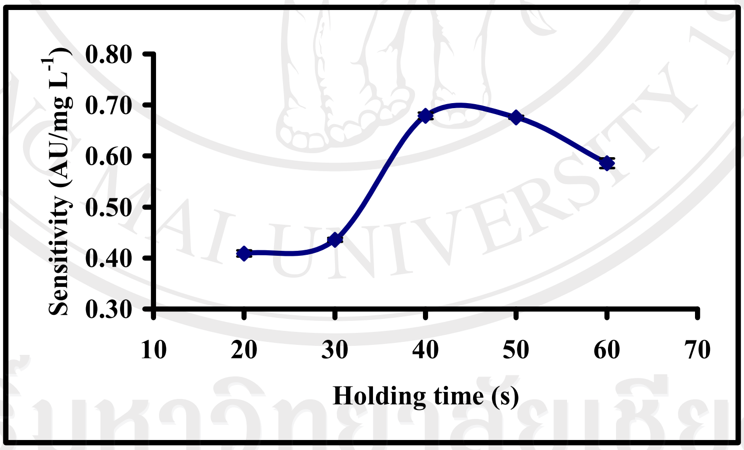


Figure 3.26 Relationship between various holding time on the sensitivity of the calibration curve

A diagram in the SI manifold is displayed in Figure 2.2 and 2.3. Table 3.35 shows the ranges over which the variables involved in the SIA system were studied and their optimum values.

Table 3.35 Optimum conditions for aluminum determination

Variable	Studied range	Optimum value
pH	4.0 – 6.0	5.5
Concentration of pH (mol L^{-1})	0.2-0.4	0.25
Concentration of BPR (x 10^{-4} mol L^{-1})	1.2– 1.6	1.4
Concentration of ethanol in BPR solution (% v/v)	10 – 50	10
Concentration of CTAB (x 10^{-3} mol L^{-1})	1.0 – 6.0	5.0
Aspiration volume of buffer (µL)	100-200	150
Aspiration volume of BPR (µL)	50-150	75
Aspiration volume of CTAB (µL)	50-150	100
Aspiration volume of sample (µL)	100-200	150
Flow rate (µL.s^{-1})	50-175	125
Holding time (s)	20-60	40

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 aluminum standard solution into the SI system under the suitable conditions as shown in Table 3.32. Linear calibration graphs were obtained for aluminum standards over the concentration ranges of 0.02 – 0.30 mg L^{-1} and 0.30 – 1.00 mg L^{-1}. All measurements were made in pentaplicate injections. The results obtained are shown in Table 3.36 and Figure 3.27.

Table 3.36 Linearity of aluminum determination

Aluminum ($mg\ L^{-1}$)	Peak height (AU)						ΔP.H.* (mV)
	1	2	3	4	5	$\bar{x}$	
0.00	1.431	1.437	1.439	1.405	1.427	1.428	0.000
0.02	1.404	1.396	1.397	1.393	1.413	1.401	0.027
0.04	1.395	1.395	1.387	1.382	1.382	1.388	0.040
0.06	1.373	1.381	1.371	1.377	1.375	1.375	0.052
0.08	1.357	1.368	1.356	1.362	1.363	1.361	0.067
0.10	1.347	1.351	1.345	1.348	1.347	1.348	0.080
0.20	1.282	1.284	1.287	1.289	1.283	1.285	0.143
0.30	1.210	1.226	1.212	1.220	1.209	1.215	0.212
0.40	1.190	1.192	1.192	1.193	1.194	1.192	0.236
0.50	1.166	1.174	1.170	1.168	1.169	1.169	0.258
0.60	1.148	1.149	1.136	1.151	1.129	1.143	0.285
0.70	1.125	1.117	1.117	1.120	1.122	1.120	0.308
0.80	1.101	1.100	1.097	1.094	1.094	1.097	0.331
0.90	1.079	1.071	1.073	1.080	1.062	1.073	0.355
1.00	1.052	1.051	1.055	1.051	1.044	1.051	0.377
1.50	0.971	0.980	0.971	0.977	0.970	0.974	0.454
2.00	0.927	0.934	0.931	0.932	0.929	0.931	0.497

*average of pentaplicate results

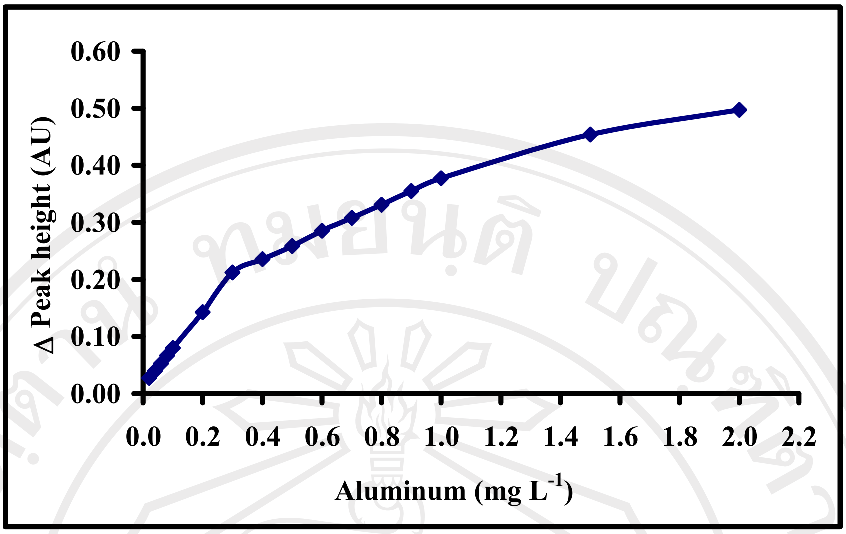


Figure 3.27 Relationship between Δpeak height and concentration of aluminum

3.3.3.2 Precision of the flow injection system

The precision of the proposed method was verified by 11 replicated determination of standard aluminum solution, using the optimum conditions (Table 3.35). Table 3.37 as shown the relative standard deviation was found to be 0.83, 0.56, 0.59 and 0.88 % of aluminum 0.08, 0.20, 0.40 and 1.00 mg L^{-1}, respectively.

Table 3.37 Precision verification using various concentrations of aluminum standard

Experimental number	Peak height (mV) obtained from the standard Al(III) (mg L^{-1})			
	0.08	**0.20**	**0.40**	**1.00**
1	1.368	1.288	1.196	1.023
2	1.384	1.302	1.196	1.043
3	1.389	1.296	1.206	1.03
4	1.381	1.306	1.202	1.04
5	1.366	1.302	1.200	1.027
6	1.366	1.291	1.204	1.041
7	1.361	1.307	1.191	1.029
8	1.373	1.293	1.199	1.032
9	1.378	1.296	1.192	1.024
10	1.369	1.288	1.188	1.045
11	1.354	1.300	1.185	1.049
$\bar{x}$	1.264	1.206	1.130	1.032
S.D.	0.010	0.007	0.007	0.009
% R.S.D.	0.83	0.56	0.59	0.88

3.3.3.3 Calibration curve

As depicted in Figures 2.2 and 2.3, the standard solutions containing 0.02-0.30 and 0.30-1.00 mg L^{-1} were aspirated with appropriate volume into the SI system under the established optimum conditions (Table 3.22). The results are shown in Table 3.38. The calibration curve as shown in Figure 3.28 and 3.29 were established by plotting Δpeak height versus the various aluminum concentrations. A correlation coefficients (r^2) and the regression equation are as follows:

$y = 0.6588x – 0.0134$ ($r^2 = 0.9997$) (Al 0.02-0.30 mg L^{-1})

$y = 0.2367x – 0.1413$ ($r^2 = 0.9997$) (Al 0.30-1.00 mg L^{-1})

Where y is Δpeak height in AU

x is concentration of aluminum in mg L^{-1}

Table 3.38 ΔPeak height for calibration curve

Aluminum (mg L^{-1})	Peak height (AU)						ΔP.H.* (mV)
	1	2	3	4	5	$\bar{x}$	
0.00	1.431	1.437	1.439	1.405	1.427	1.428	0.000
0.02	1.404	1.396	1.397	1.393	1.413	1.401	0.027
0.04	1.395	1.395	1.387	1.382	1.382	1.388	0.040
0.06	1.373	1.381	1.371	1.377	1.375	1.375	0.052
0.08	1.357	1.368	1.356	1.362	1.363	1.361	0.067
0.10	1.347	1.351	1.345	1.348	1.347	1.348	0.080
0.20	1.282	1.284	1.287	1.289	1.283	1.285	0.143
0.30	1.210	1.226	1.212	1.220	1.209	1.215	0.212
0.40	1.190	1.192	1.192	1.193	1.194	1.192	0.236
0.50	1.166	1.174	1.170	1.168	1.169	1.169	0.258
0.60	1.148	1.149	1.136	1.151	1.129	1.143	0.285
0.70	1.125	1.117	1.117	1.120	1.122	1.120	0.308
0.80	1.101	1.100	1.097	1.094	1.094	1.097	0.331
0.90	1.079	1.071	1.073	1.080	1.062	1.073	0.355
1.00	1.052	1.051	1.055	1.051	1.044	1.051	0.377

*average of pentaplicate results

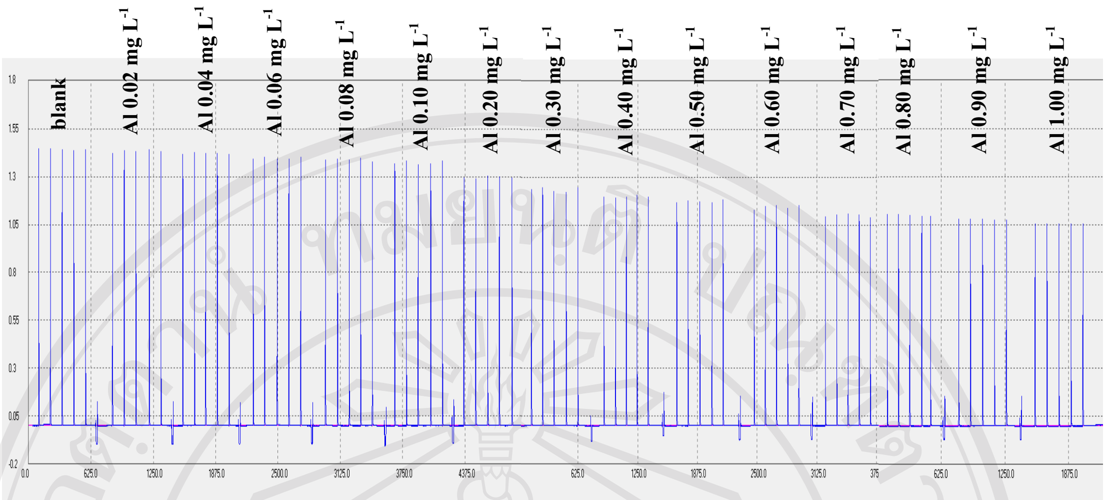
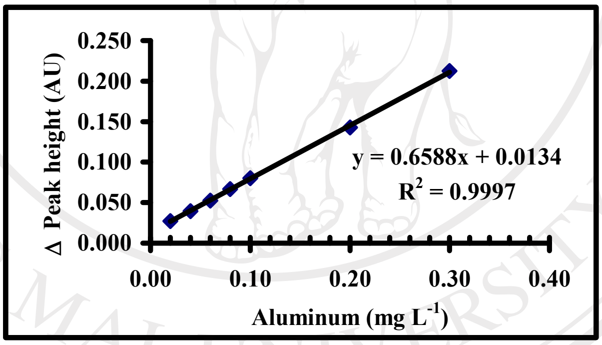
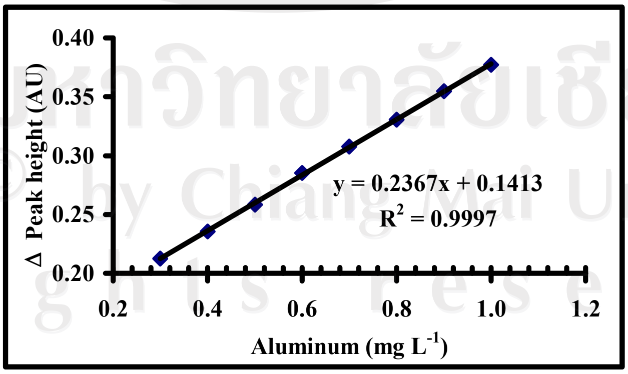


Figure 3.28 Calibration signal of SIA spectrophotometric determination of aluminum 0.02-0.30 and 0.30-1.00 mg L^{-1}



(a)



(b)

Figure 3.29 The Calibration curve of SIA spectrophotometric determination of aluminum: (a) aluminum 0.02-0.30 mg L^{-1}; (b) aluminum 0.30-1.00 mg L^{-1}

3.3.3.4 Detection limit

The detection limit was determined by the method reported by Miller and Miller [61], which was calculated from the linear regression line of twice calibration curves. The results are given in Tables 3.39 and 3.40. The detection limit of the proposed method was found to be 0.007 and 0.209 mg L^{-1} from aluminum 0.02-0.30 and 0.30-1.00 mg L^{-1}. The concentration at limit of detection (C_L) can be calculated from equation 2.2-2.3.

Table 3.39 Calculation of detection limit of SIA spectrophotometric determination of aluminum 0.02-0.30 mg L^{-1}

Aluminum (mg L^{-1})	**Y_i^***	**$\hat{Y}_i$**	**$\lvert Y_i-\hat{Y}_i\rvert$**	**$\lvert Y_i-\hat{Y}_i\rvert^2$**
0.02	0.027	0.027	0.001	0.000000
0.04	0.040	0.040	0.000	0.000000
0.06	0.052	0.053	-0.001	0.000000
0.08	0.067	0.066	0.000	0.000000
0.10	0.080	0.079	0.001	0.000001
0.20	0.143	0.145	-0.002	0.000006
0.30	0.212	0.211	0.001	0.000002
$\Sigma\lvert Yi-\hat{Y}i\rvert^2$				9×10^{-6}
S_y/x				0.002
C_L , LOD				0.007
LOQ				0.023

*average of triplicate results

The linear regression equation is Y = 0.6588x + 0.0134

$$S_y/x = [9 \times 10^{-6}/(7-2)]^{1/2}$$
$$= 0.002$$
$$C_L\text{ , LOD} = (3 \times 0.002)/0.6588$$
$$= 0.007 \text{ mg L}^{-1}\text{ Al(III)}$$
$$\text{LOQ} = (10 \times 0.002)/0.6588$$
$$= 0.023 \text{ mg L}^{-1}\text{ Al(III)}$$

Table 3.40 Calculation of detection limit of SIA spectrophotometric determination of aluminum 0.30-1.00 mg L^{-1}

Aluminum (mg L^{-1})	**Y_i^***	**$\hat{Y}_i$**	**$\lvert Y_i-\hat{Y}_i \rvert$**	**$\lvert Y_i-\hat{Y}_i \rvert^2$**
0.3	0.212	0.212	0.000	0.0000
0.4	0.236	0.236	0.000	0.0000
0.5	0.258	0.260	0.001	0.0000
0.6	0.285	0.283	0.002	0.0000
0.7	0.308	0.307	0.001	0.0000
0.8	0.308	0.331	0.023	0.0005
0.9	0.331	0.354	0.024	0.0006
1.0	0.355	0.378	0.023	0.0005
$\Sigma\lvert Yi-\hat{Y}i \rvert^2$				0.002
S_y/x				0.017
C_L , LOD				0.209
LOQ				0.698

*average of triplicate results

The linear regression equation is Y = 0.2367x + 0.1413

$$S_y/x = [0.002/(8-2)]^{1/2}$$

$$= 0.017$$

$$C_L\text{ , LOD} = (3 \times 0.017)/0.2367$$

$$= 0.209 \text{ mg L}^{-1} \text{ Al(III)}$$

$$\text{LOQ} = (10 \times 0.017)/0.2367$$

$$= 0.698 \text{ mg L}^{-1} \text{ Al(III)}$$

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 3.22). A systematic study to check for the effects of some possible foreign ions on the determination of aluminum 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} aluminum 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 3.41.

Table 3.41 Interference studies for 0.20 mg L^{-1} standard aluminum by SIA method

Interference	Aluminum : Interference	Peak height* (AU)	% Recovery
Cu^{2+}	1 : 0	0.143	-
	1 : 0.5	0.145	101
	1 : 1.0	0.158	110
	1 : 1.5	0.168	117
Fe^{2+}	1 : 0	0.143	-
	1 : 0.1	0.145	101
	1 : 0.3	0.145	101
	1 : 0.5	0.177	124
Fe^{3+}	1 : 0	0.143	-
	1 : 0.1	0.156	109
	1 : 0.3	0.156	109
	1 : 0.5	0.181	127
Ni^{2+}	1 : 0	0.143	-
	1 : 2.5	0.147	103
	1 : 3.0	0.158	110
	1 : 3.5	0.173	121
Co^{2+}	1 : 0	0.143	-
	1 : 10	0.150	105
	1 : 20	0.158	110
	1 : 30	0.165	115
Cr^{3+}	1 : 0	0.143	-
	1 : 10	0.136	95
	1 : 15	0.132	92
	1 : 20	0.127	89

*average of triplicate results

Table 3.41 (Continued).

Interference	Aluminum : Interference	Peak height* (AU)	% Recovery
Cd^{2+}	1 : 0	0.143	-
	1 : 10	0.145	101
	1 : 40	0.153	107
	1 : 100	0.160	112
Zn^{2+}	1 : 0	0.143	-
	1 : 20	0.147	103
	1 : 30	0.155	108
	1 : 40	0.164	115
Mn^{2+}	1 : 0	0.143	-
	1 : 100	0.144	101
	1 : 300	0.145	101
	1 : 500	0.147	103
Mg^{2+}	1 : 0	0.143	-
	1 : 100	0.147	103
	1 : 300	0.150	105
	1 : 500	0.153	107
Na^{+}	1 : 0	0.143	-
	1 : 500	0.143	100
	1 : 1500	0.138	97
	1 : 2500	0.137	96
Ca^{2+}	1 : 0	0.143	-
	1 : 500	0.143	100
	1 : 1500	0.142	99
	1 : 2500	0.139	97
NO_2^{-}	1 : 0	0.143	-
	1 : 100	0.142	99
	1 : 300	0.139	97
	1 : 500	0.138	97

*average of triplicate results

Table 3.41 (Continued).

Interference	Aluminum : Interference	Peak height* (AU)	% Recovery
SO_4^{2-}	1 : 0	0.143	-
	1 : 100	0.143	100
	1 : 300	0.139	97
	1 : 500	0.139	97
HCO_3^-	1 : 0	0.143	-
	1 : 100	0.145	101
	1 : 300	0.150	105
	1 : 500	0.151	106
Br^-	1 : 0	0.143	-
	1 : 100	0.145	102
	1 : 300	0.148	103
	1 : 500	0.150	105
I^-	1 : 0	0.143	-
	1 : 100	0.144	101
	1 : 300	0.145	101
	1 : 500	0.148	103
Cl^-	1 : 0	0.143	-
	1 : 500	0.143	100
	1 : 1500	0.142	99
	1 : 2500	0.141	99

*average of triplicate results

The interference effects of some possible foreign ions in the SIA system for aluminum were summarized in Table 3.42. It was found that Fe^{2+}, Fe^{3+} and Cu^{2+} interfered determination aluminum in tap water, which refered standard quality of tap water defined by metropolitan waterworks authority (Appendix A).

Table 3.42 Summary of interference effects of some ions on the response obtained from aluminum 0.2 mg L^{-1} by SIA method

Interference ion	**Tolerable concentration ratio***
Na^{+}, Ca^{2+}, Cl^{-}	2500
Mn^{2+}, Mg^{2+}, NO_2^{-}, HCO_3^{-}, SO_4^{2-}, Br^{-}, I^{-}	500
Cd^{2+}	100
Zn^{2+}	30
Cr^{3+}, Co^{2+}	20
Ni^{2+}	3
Cu^{2+}	1
Fe^{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 3.43. It was found that Fe^{2+} was masked with 0.008 mol L^{-1} of 1,10-phenanthroline [62] and Fe^{3+} was reduced to Fe^{2+} with 1% v/v of hydroxylammonium chloride and was masked in the same manner as Fe^{2+} [62]. Cu^{2+} was masked with 0.5% v/v of thiourea [63].

Table 3.43 Effect of masking agent for mask Fe^{2+}, Fe^{3+} and Cu^{2+} the response obtained from aluminum 0.2 mg L^{-1} by SIA method

Interference	Concentration of masking agent	Aluminum :Interference	Peak height* (mV)	% Recovery
Fe^{2+}	0.004 mol L^{-1} PHT	1 : 0	0.143	-
		1 : 10	0.156	109
		1 : 15	0.167	117
		1 : 20	0.170	119
	0.008 mol L^{-1} PHT	1 : 0	0.143	-
		1 : 10	0.155	108
		1 : 15	0.157	110
		1 : 20	0.170	119
	0.012 mol L^{-1} PHT	1 : 0	0.143	-
		1 : 5	0.155	108
		1 : 10	0.156	109
		1 : 15	0.165	115

*average of triplicate results

PHT was 1,10-phenanthroliene

Table 3.43 (continued).

Interference	Concentration of masking agent	Aluminum :Interference	Peak height* (mV)	% Recovery
Fe^{3+}	0.5% HAC + 0.008 mol L^{-1} PHT	1 : 0	0.143	-
		1 : 10	0.149	104
		1 : 15	0.157	110
		1 : 20	0.169	118
	1.0% HAC + 0.008 mol L^{-1} PHT	1 : 0	0.143	-
		1 : 10	0.149	104
		1 : 15	0.155	108
		1 : 20	0.163	114
	1.5% HAC + 0.008 mol L^{-1} PHT	1 : 0	0.143	-
		1 : 10	0.149	104
		1 : 15	0.155	108
		1 : 20	0.159	111
Cu^{2+}	0.25% thiourea	1 : 0	0.143	-
		1 : 30	0.155	108
		1 : 50	0.161	113
		1 : 70	0.171	120
	0.50% thiourea	1 : 0	0.143	-
		1 : 30	0.153	107
		1 : 50	0.156	109
		1 : 70	0.162	113
	0.75% thiourea	1 : 0	0.143	-
		1 : 30	0.154	108
		1 : 50	0.156	109
		1 : 70	0.161	113

*average of triplicate results

PHT was 1,10-phenanthroliene

HAC was hydroxylammonium chloride

3.3.3.7 Determination of aluminum in waters

The proposed SI spectrophotometric method was applied to the simultaneous determination of aluminum in tap water samples were collected from several Aumphur in Chiang Mai and boiling water from aluminum ware. The peak heights of each sample were compared with standard calibration curve. The results were given in Table 3.44.

Table 3.44 Determination of aluminum in water sample by SIA method

Water samples	**Peak heights**				**SD**	**Aluminum concentration*** **(mg L^{-1})**	**% recovery***
	1	**2**	**3**	**$\bar{x}$**			
Hangdong	ND**	ND**	ND**	-	-	ND**	-
Sangpatong	0.120	0.121	0.123	0.121	0.002	0.121 ± 0.002	99.78
Muang	0.075	0.073	0.071	0.072	0.001	0.072 ± 0.001	100.33
Mae Jo	0.125	0.127	0.124	0.125	0.002	0.125 ± 0.002	102.96
Sansai	ND**	ND**	ND**	-	-	ND**	-
Sankumpang	ND**	ND**	ND**	-	-	ND**	-
Mae Rim	0.358	0.354	0.360	0.357	0.003	0.357 ± 0.003	99.24
Chiang Mai University	0.247	0.247	0.245	0.246	0.001	0.246 ± 0.001	99.06
Boil water 3 h	0.113	0.109	0.112	0.111	0.002	0.111 ± 0.002	100.89
Boil water 6 h	0.025	0.027	0.03	0.027	0.003	0.027 ± 0.003	98.93
Boil water 9 h	ND**	ND**	ND**	-	-	ND**	-

*average of triplicate results

**not detected

The aluminum contents in the water samples were in the range of 0.027-0.357 mg L^{-1} and 0.011-0.350 mg L^{-1} using the proposed method and ICP-OES respectively. The results obtained by the proposed SI spectrohotometric method compared with those obtained by ICP-OES using the student t-test (Table 3.45 and Appendix B in Table B.2). It was evident that the t-value for Al(III) contents in water samples determined by comparison the results obtained by SI spectrohotometric with those obtained by ICP-OES was 0.893. It was seen that the experimental t-value for Al(III) assay(0.893) which was smaller than the theoretical t-value with a confidence interval of 95% (2.45) indicating that results obtained by both methods were in excellent agreement.

Table 3.45 Comparative determination of aluminum in water sample by proposed SIA method and ICP-OES

Water samples	**Concentrations (mg L^{-1})**		x_d
	SIA*	**ICP-OES***	
Hangdong	ND**	0.011	-
Sangpatong	0.121	0.127	-0.012
Muang	0.072	0.072	0.001
Mae Jo	0.125	0.128	0.000
Sansai	ND	0.011	-
Sankumpang	ND**	0.014	-
Mae Rim	0.357	0.350	0.013
Chiang Mai University	0.246	0.252	-0.006
Boil water 3 hour	0.111	0.110	0.002
Boil water 6 hour	0.027	0.031	-0.008
Boil water 9 hour	ND**	0.014	-
Σ			-0.010
S_d			0.00465
t			0.893

*average of triplicate results

**not detected