

CHAPTER 2

DETERMINATION OF ALUMINIUM(III) WITH TETRACYCLINE AS A COMPLEXING AGENT

2.1 Introduction

Tetracycline(TC)[4-dimethylamino-1,4,4 α ,5,5 α ,6,11,12 α -octahydro-3,6,10,12,12 α -pentahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide].HCl is an important member of the tetracycline group of antibiotics which are used extensively because of their broad-spectrum antibacterial properties [67]. It is active against a wide range of gram-positive and gram-negative bacteria, spirochetes, mycoplasmas, rickettsiae, and clamydiae. The structure of TC is shown in Figure 2.1. The molecule contains four rings in its structure capable to forming stable complexes with many metal ions such as iron(III) [15,68-69], aluminium(III) [70], magnesium(II) [71], cobalt(III) [72], copper(II) [69], zirconium(IV) [73], etc.

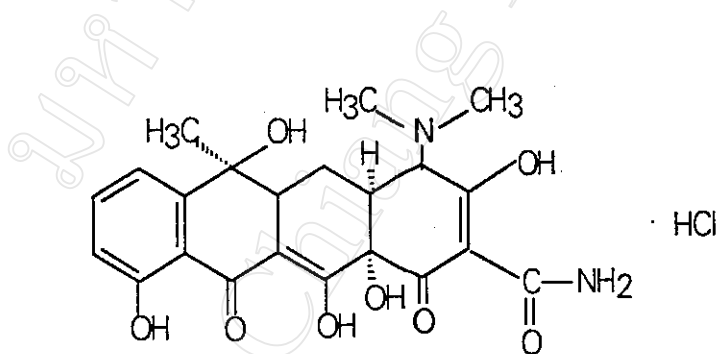


Figure 2.1 : Structure of Tetracycline Hydrochloride [74].

This research project will describe the formation of a 1:3 complex between aluminium(III) and TC, the study of some structural and spectral characteristics, and

the development of a simple and rapid flow injection (FI) spectrophotometric method for the determination of trace amounts of aluminium(III) in some environmental samples.

2.2 Experimental

2.2.1 Apparatus and Instruments

1. UV-VIS spectrophotometer model U-2000 Spectrophotometer, Hitachi, Japan.
2. CECIL 1000 SERIES, Cecil Instruments, Cambridge, England.
3. Spectronic 21, Milton Roy Company, USA.
4. Recorder, Servograph REC 51, Radiometer Copenhagen, Denmark.
5. Atomic absorption/ Flame emission spectrophotometer model AA-680, Shimadzu, Japan.
6. Home-made injection valve [75].
7. Flow-through cell for spectrophotometer, Hellma, Germany.
8. Peristaltic pump EYELA SMP-23S, Tokyo Rikakikai Co., Ltd., Japan.
9. pH/mV meter model 5986-2S, Cole - Parmer, Co., Ltd., USA.
10. Plastic tube (I.D. 0.0173, 0.020, 0.030, 0.0402, 0.0449, and 0.060 in).
11. Teflon tube (I.D. 0.050 in).
12. Test tube (O.D. 1.20, 1.60, 1.80, and 2.40 in).
13. Disposable syringe (1 ml), Nissho Nipro Corporation Ltd., Thailand.

2.2.2 Chemicals

1. Aluminium chloride hexahydrate ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$), A.R. grade, BDH, England.
2. Tris(hydroxymethyl) aminomethane ($\text{C}_4\text{H}_{11}\text{NO}_3$), BDH, England.

3. Hydrochloric acid (HCl), 36.5-38.0%, A.R. grade, J.T.Baker Inc., Phillipsberg, USA.
4. Tetracycline hydrochloride, TC.HCl, $C_{22}H_{24}N_2O_8.HCl$, Fluka, Switzerland.
5. Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$), A.R. grade, E.Merck, Germany.
6. Zinc sulphate heptahydrate ($ZnSO_4.7H_2O$), A.R. grade, E.Merck, Germany.
7. Sodium nitrate ($NaNO_3$), A.R. grade, M&B Ltd., England.
8. Potassium chloride (KCl), A.R. grade, Carlo Erba, Italy.
9. Sodium fluoride (NaF), A.R. grade, E. Merck, Germany.
10. Copper(II) chloride dihydrate ($CuCl_2.2H_2O$), A.R. grade, E. Merck, Germany.

2.2.3 Preparation of Standard Solutions and Reagents

(1) Standard aluminium(III) solution (100 ppm)

Aluminium(III) stock solution was prepared by dissolving 0.8948 g aluminium(III) chloride hexahydrate ($AlCl_3.6H_2O$) in deionized water and diluted in a 1 l volumetric flask. Solution of lower concentration were obtained by accurate dilution of this solution.

(2) Standard TC solution (2.08×10^{-4} M)

This solution was prepared by dissolving 0.01 g of TC in water, adjusted to pH 6.0 with tris-buffer solution, and then diluted to 100 ml with deionized water. The solution was kept cool in the dark place.

(3) Tris - buffer solution pH 6.0

Tris(hydroxymethyl) aminomethane (12 g) was mixed with 600 ml of deionized water, then the pH of the solution was adjusted to 6.0 with 1M HCl

solution and diluted to 1 l with deionized water. Finally, add 31 ml of 1M KCl to the portion of solution (500 ml) before use.

(4) Potassium chloride solution (1 M)

Potassium chloride (7.4551 g) was dissolved in the minimum of water and diluted to 100 ml.

2.3 Theory

The proposed method is based on the reaction of TC with Al(III) in buffer solution to form a yellow complex which can be used for the spectrophotometric determination of Al(III) by measuring its absorbance at 390 nm. The intensity of the coloured product formed is related directly to the amount of Al(III) present and depends very much on the reaction conditions.

In this research work, the reversed FIA mode (rFIA) as shown in Figures 2.2 and 2.3 is the most convenient because it is of great use when very frequent analyzes of abundant and inexpensive samples or increased sensitivity.

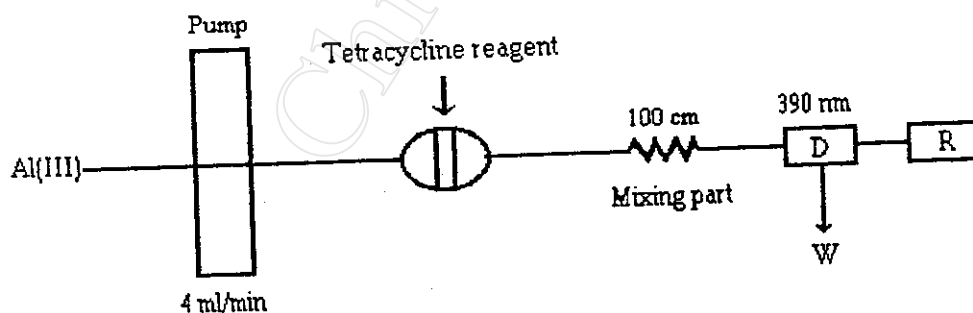


Figure 2.2 : Manifold for the determination of Al(III) by rFIA.



Figure 2.3 : reversed FIA system for the determination of Al(III) with tetracycline as a complexing agent.

- | | |
|-----------------------|----------------------|
| 1 = sample reservoir | 2 = peristaltic pump |
| 3 = injection valve | 4 = mixing part |
| 5 = flow through cell | 6 = detector |
| 7 = recorder | 8 = waste |

2.4 Preliminary Studies of Spectrometric Determination of Aluminium(III) by Using TC as a Complexing Agent*

2.4.1 Absorption Spectra

A 5 ml of 100 ppm aluminium(III) solution was transferred into a 25 ml volumetric flask. A 5 ml aliquot of the 100 ppm TC solution was added and, after mixing well, the pH was adjusted to 6.0 with a pH 6.0 tris - buffer solution. The contents were diluted to final volume with deionized water and mixed thoroughly. Finally, the absorption spectra of TC and Al(III) - TC complex solutions were scanned from 350 - 500 nm.

The absorption spectra of TC and Al(III) - TC complex solutions are shown in Figure 2.4.

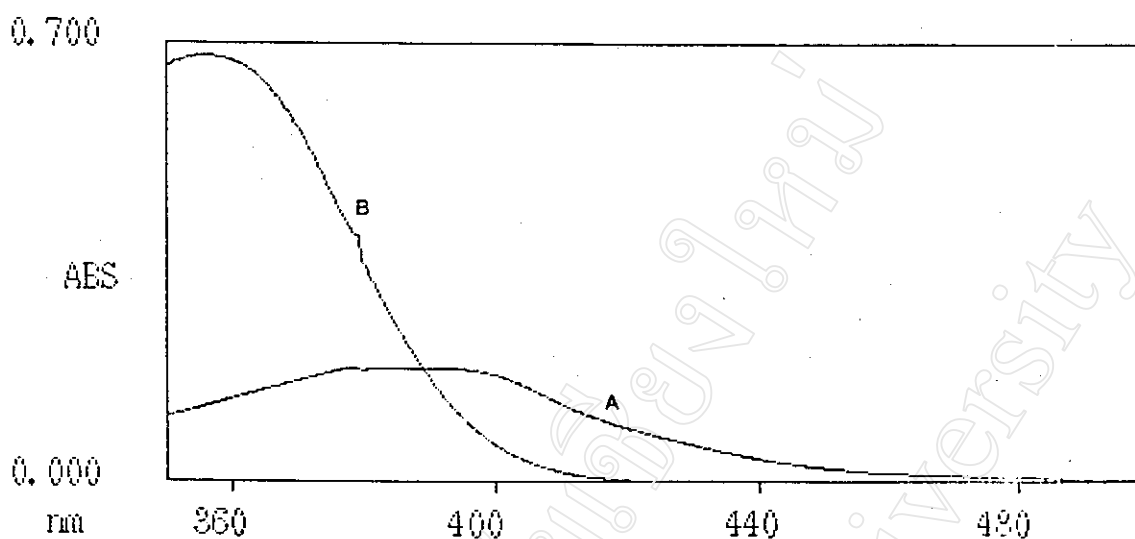


Figure 2.4 : Absorption spectrum of A. Al(III) - TC complex (against reagent blank) and B., TC solution.

The Al(III) - TC complex showed an absorption maximum at 390 nm (against water) while the yellow solution of TC having an absorption maximum at 360 nm (against blank solution) (see Figure 2.4).

2.4.2 Composition of the Aluminium(III) - TC complex

Study of the composition of complex by Mole - ratio Method

The mole-ratio method is defined as a series of solution is prepared in which the analytical concentration of one reactant (usually the cation) is constant while that of the other is varied. A plot of absorbance versus mole ratio of the reactant is then prepared. If the formation constant is reasonably favorable, two straight lines of different slopes that intersect at a mole ratio that corresponds to the combining ratio in the complex are obtained [76].

In this research project, the composition of the aluminium(III) - TC complex was determined at pH 6.0, the various volumes (2.0 - 10.0 ml) of 1.0×10^{-3} M TC solution were added to solution containing 1.0×10^{-3} M aluminium(III), 10.0 ml buffer solution and diluted with deionized water in 25 ml volumetric flasks. Absorbance of each solution was measured at 390 nm against a reagent blank. The results obtained are shown in Table 2.1 and Figure 2.5.

Table 2.1 : Absorbance of various mole ratio of Al(III) and TC solutions.

[Al] : [TC] (M : M)	Absorbance
1:0	0.012
1:1	0.100
1:2	0.195
1:3	0.280
1:4	0.320
1:5	0.360

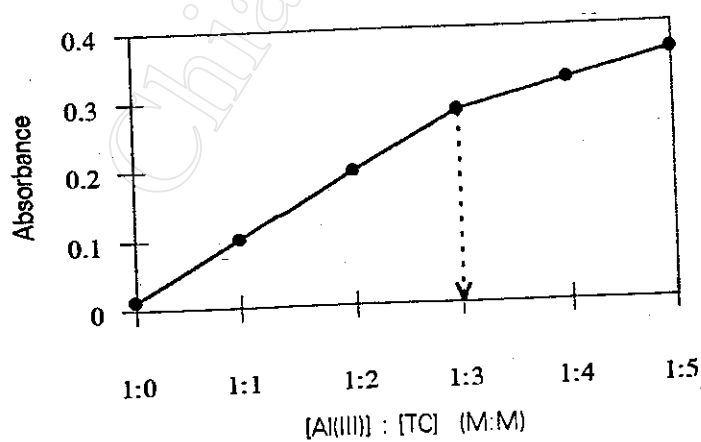


Figure 2.5 : Mole - ratio plots for a 1:3 Al(III) - TC complex.

2.5 Optimization of the flow system

2.5.1 Optimum wavelength

The optimum wavelength for the Al(III) - TC complex was studied over the range 360 - 430 nm by using the experimental condition as shown in Table 2.2. The results obtained are shown in Table 2.3 and Figure 2.6.

Table 2.2 : Experimental conditions for studying optimum wavelength of Al(III) - TC complex.

Analytical Characteristics	Information
conditions of the FI system	
conc. of TC (M)	2.08×10^{-4}
conc. of Al(III) (ppm)	1.0
reagent volume (μ l)	100
pH	6.0
pump flow rate (ml/min)	4.0
inner diameter of mixing tubing (in)	0.0402
length of mixing tubing (m)	1.00
shape of mixing part	coil
conditions of detector	
absorbance range	0 - 2
conditions of recorder	
chart speed (min/cm)	5
sensitivity (mV/cm)	20

Table 2.3 : Peak heights at various wavelengths.

Wavelength (nm)	Peak height *	
	cm	mV
360	1.03	20.60
365	1.38	27.60
370	1.97	39.40
375	2.45	49.00
380	3.27	65.40
385	4.05	81.00
390	4.93	98.60
395	4.92	98.40
400	4.90	95.00
405	4.03	80.60
410	2.83	56.60
415	1.83	36.60
420	0.92	18.40
425	0.40	8.00
430	0.20	4.00

* average of triplicate results.

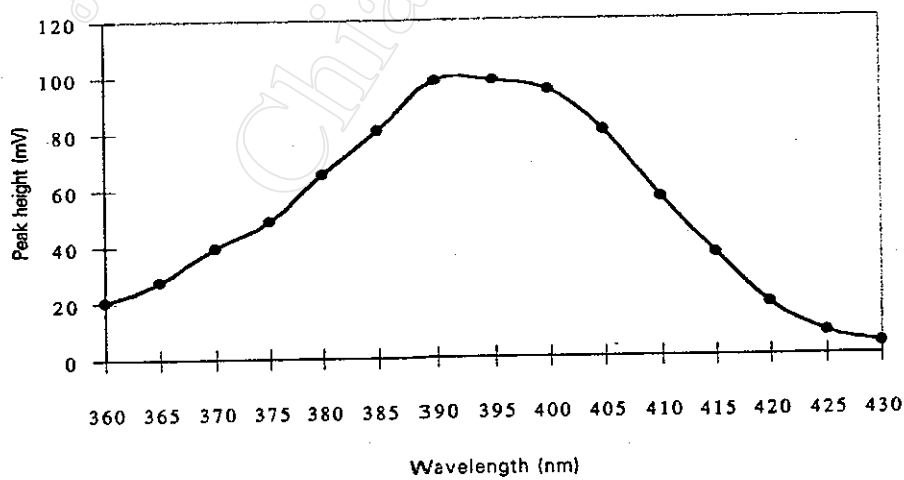


Figure 2.6 : Peak height at various wavelengths.

As the curve in Figure 2.6 show, a maximum peak height was observed at 390 nm. All measurements were, therefore, made at this wavelength.

2.5.2 Effect of pH

The effect of pH on the formation of the Al(III) - TC complex in aqueous solution was studied over the range 2.0 - 7.0. The results obtained are shown in Table 2.4 and Figures 2.7 and 2.8.

Table 2.4 : Effect of the pH of tris - buffer solution on peak heights.

Wavelength (nm)	Peak height (mV) * at pH							
	2.0	3.0	4.0	5.0	5.5	6.0	6.5	7.0
380	46.60	53.00	51.60	54.60	64.60	62.40	64.20	60.60
385	42.40	44.80	50.00	55.00	61.00	64.60	62.40	62.00
390	40.60	47.60	54.60	56.00	64.00	70.40	68.40	67.40
395	34.00	42.00	50.00	55.40	58.00	60.20	68.40	70.00
400	36.60	37.60	43.60	46.40	53.00	57.40	53.40	58.00
405	34.00	41.40	40.00	41.00	52.60	50.60	52.60	55.60
410	34.00	44.60	44.00	45.00	43.40	44.00	42.40	47.40

* average of triplicate results.

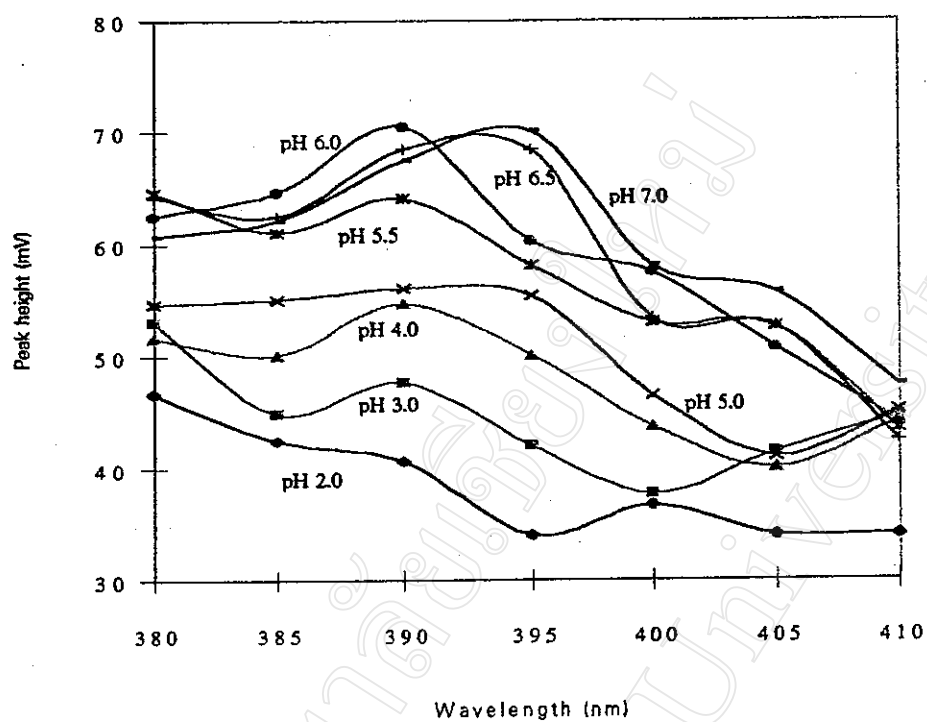


Figure 2.7 : Absorption spectra of Al(III) - TC complex at various pH.

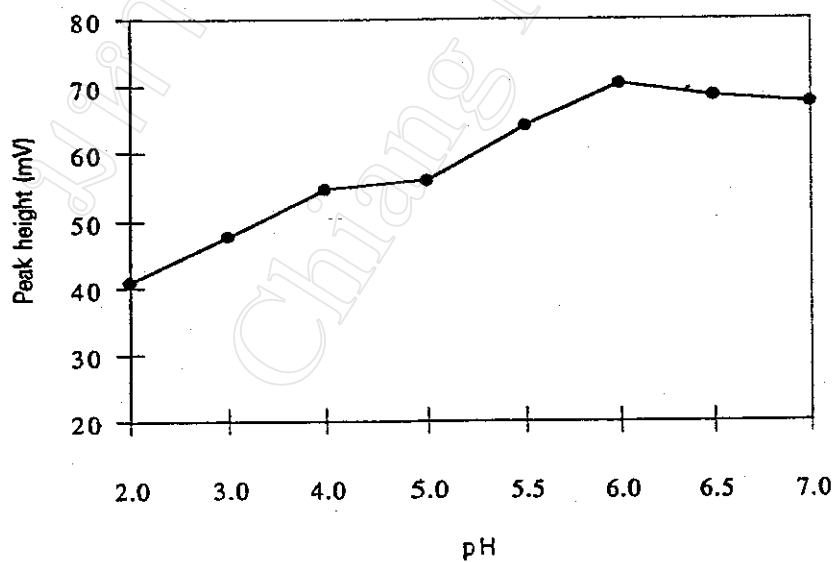


Figure 2.8 : Effect of pH on peak height at wavelength 390 nm.

The optimum pH for a constant maximum absorbance at 390 nm was found to be 6.0. By increasing the acidity or the alkalinity of the absorbance decreased and λ_{\max} was shifted.

2.5.3 Effect of TC concentration

The effect of various TC concentrations on peak heights was studied by varying the concentration of TC solutions in the range of 1.04×10^{-5} - 5.20×10^{-5} M. The results obtained are shown in Table 2.5 and Figures 2.9 - 2.10.

Table 2.5 : Effect of the TC and Al(III) concentration on peak heights.

[Al(III)]		Peak height (mV)* obtained for the TC concentration (M)				
ppm	M	1.04×10^{-5}	2.08×10^{-5}	3.12×10^{-5}	4.16×10^{-5}	5.20×10^{-5}
0.10	3.7×10^{-6}	2.00	4.40	7.40	9.00	5.00
0.20	7.4×10^{-6}	5.00	10.40	13.40	14.00	9.60
0.30	1.1×10^{-5}	6.40	16.00	19.20	22.00	14.60
0.40	1.5×10^{-5}	8.00	18.00	25.00	26.00	16.00
0.50	1.8×10^{-5}	10.00	22.00	31.00	30.60	20.40
Slope (mV/ppm)		19.83	44.69	61.03	60.57	40.00
Correlation coefficient		0.9940	0.9906	0.9991	0.9916	0.9907

* average of triplicate results.

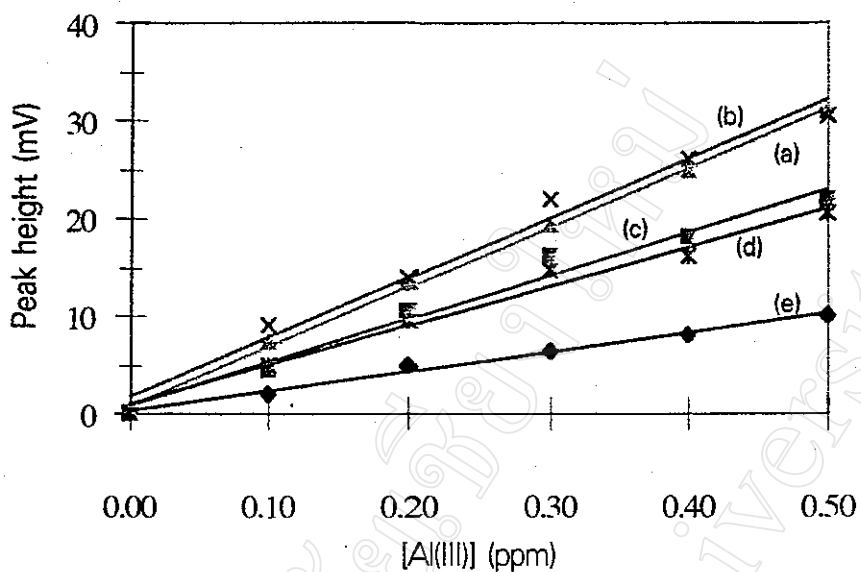


Figure 2.9 : Effect of TC concentration on peak height; (a) 4.16×10^{-5} ,
 (b) 3.12×10^{-5} , (c) 2.08×10^{-5} , (d) 5.20×10^{-5} , and (e) 1.04×10^{-5} M.

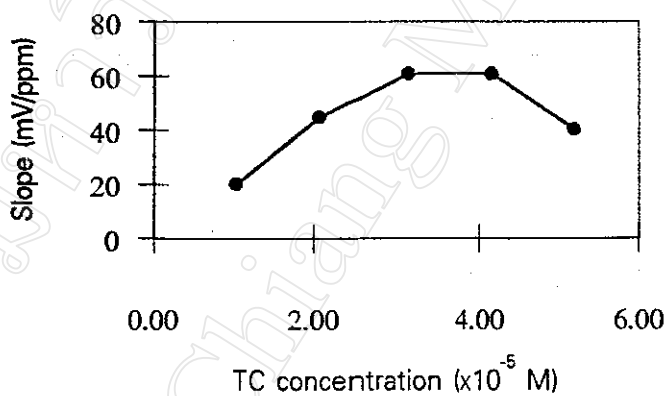


Figure 2.10 : Relationship between slope (mV/ppm) and TC concentration (M).

The results indicate that the highest peak height and optimum sensitivity are obtained when the concentration of TC in buffer solution is between 3.12×10^{-5} - 4.16×10^{-4} M but the appropriate concentration is 3.12×10^{-5} since the linearity of calibration curve is better.

2.5.4 Effect of flow rate

The flow rate of standard aluminium(III) and/or sample solution plays an important role in the FI system. Therefore, it is necessary to investigate the optimum flow rate in order to obtain the maximum sensitivity. The flow rate was studied by keeping all other conditions constant as shown in Table 2.6. The results obtained are summarized in Tables 2.7 - 2.8 and Figures 2.11 - 2.13, which show that the better sensitivity can be attained at the flow rate 4.0 ml/min. A flow rate of 4.0 ml/min was chosen in order to obtain a reasonable sample throughput which is the essence of using a FI method.

Table 2.6 : Experimental condition for the study of the pump flow rate on peak heights.

Analytical Characteristics	Information
conditions of the FI system	
conc. of TC (M)	3.12×10^{-5}
conc. of Al(III) (ppm)	1.0
reagent volume (μ l)	100
pH	6.0
pump flow rate (ml/min)	3.0 - 8.0
inner diameter of mixing tubing (in)	0.0402
length of mixing tubing (m)	1.00
shape of mixing part	coil
conditions of detector	
absorbance range	0 - 2
conditions of recorder	
chart speed (min/cm)	5
sensitivity (mV/cm)	20

Table 2.7 : Effect of the pump flow rate on peak height.

[Al(III)] (ppm)	Peak height (mV)* obtained from the pump flow rate (ml/min)					
	3.0	4.0	5.0	6.0	7.0	8.0
0.10	4.40	6.60	4.40	5.60	5.40	4.90
0.20	9.60	15.00	12.60	13.00	13.00	10.00
0.30	12.00	22.40	20.60	21.00	20.00	13.00
0.40	13.60	34.00	28.00	29.00	28.00	17.40
0.50	16.00	42.60	35.00	37.00	36.00	20.00
Slope (mV/ppm)	31.43	86.46	72.51	84.15	72.80	40.14
Correlation coefficient	0.9766	0.9966	0.9975	0.9752	0.9984	0.9946

* average of triplicate results.

Table 2.8 : Analytical characteristics for the determination of standard aluminium(III) solution 0.50 ppm at various pump flow rate.

Pump flow rate (ml/min)	t_{base} (sec)	Sample / hr
3.0	75.0	48
4.0	45.0	80
5.0	42.0	85
6.0	36.0	100
7.0	30.0	120
8.0	30.0	120

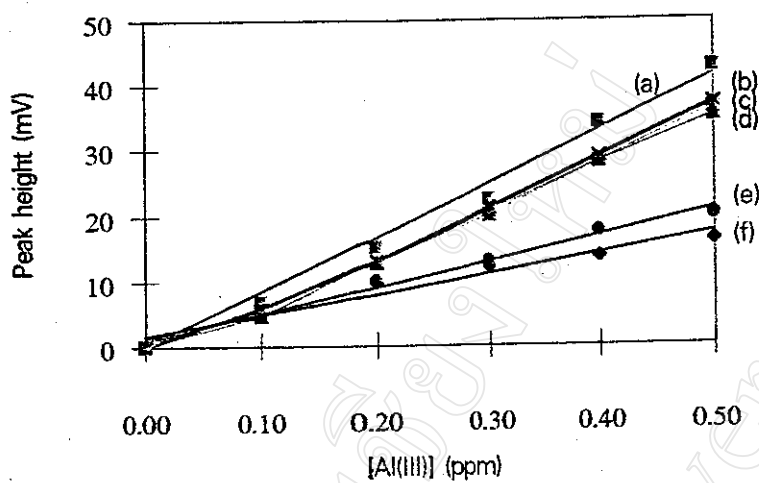


Figure 2.11 : Effect of the pump flow rate; (a) 4.0, (b) 6.0, (c) 7.0, (d) 5.0, (e) 8.0, and (f) 3.0 ml/min.

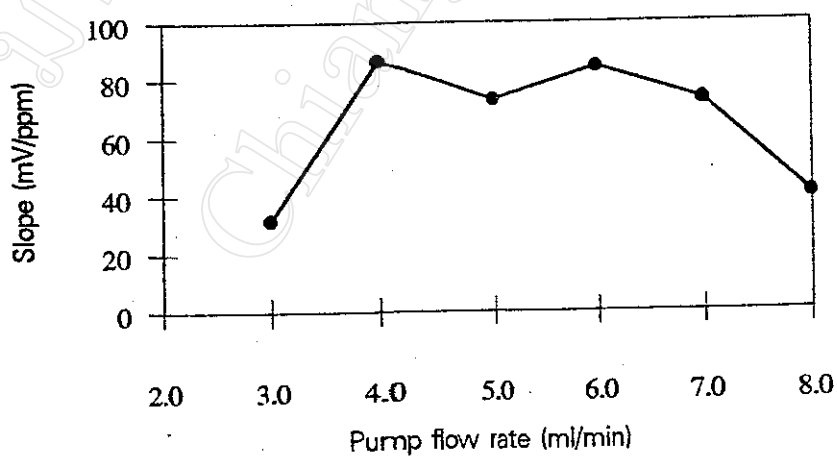


Figure 2.12 : Relationship between slope (mV/ppm) and pump flow rate (ml/min).

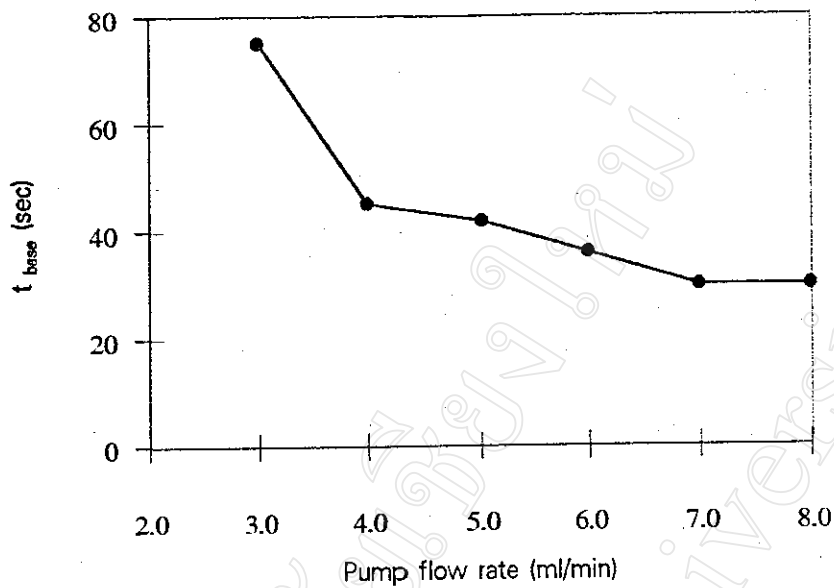


Figure 2.13 : Relationship between t_{base} (sec) and pump flow rate (ml/min).

2.5.5 Effect of the inner diameter of mixing tubing

The diameter of mixing tubing has a significant effect on the flow parameters, influencing the maximum achievable sampling rate. To investigate this, the whole line length was made of tubing ranging from 0.0173" to 0.060" I.D. The results obtained from the experiment are given in Tables 2.9-2.10 and Figures 2.14 - 2.16.

Table 2.9 : Effect of the I.D. of mixing tubing on peak heights.

[Al(III)] (ppm)	Peak height (mV)* obtained from the I.D. of mixing tubing (in)					
	0.0173	0.020	0.030	0.0402	0.0449	0.060
0.10	9.40	5.40	12.60	7.00	4.40	9.00
0.20	11.00	10.60	18.00	13.00	8.00	14.00
0.30	14.60	13.60	32.40	22.60	19.40	18.60
0.40	27.60	14.60	41.60	33.40	31.60	28.00
0.50	28.00	14.00	48.00	41.40	48.60	31.40
Slope (mV/ppm)	56.63	28.74	97.54	84.51	96.00	62.46
Correlation coefficient	0.9669	0.9192	0.9939	0.9956	0.9656	0.9926

* average of triplicate results.

Table 2.10 : Analytical characteristics for the determination of standard aluminium(III) solution 0.50 ppm.

I.D. of mixing tubing (in)	t_{base} (sec)	Sample / hr
0.0173	75.0	48
0.020	75.0	48
0.030	45.0	80
0.0402	60.0	60
0.0449	45.0	80
0.060	75.0	48

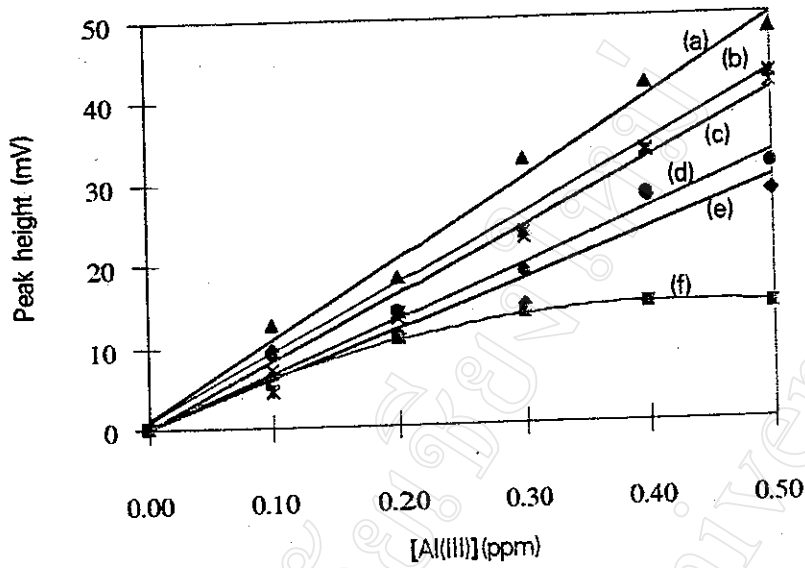


Figure 2.14 : Effect of the I.D. of mixing tubing on peak heights; (a) 0.030", (b) 0.0449", (c) 0.0402", (d) 0.060", (e) 0.0173", and (f) 0.020".

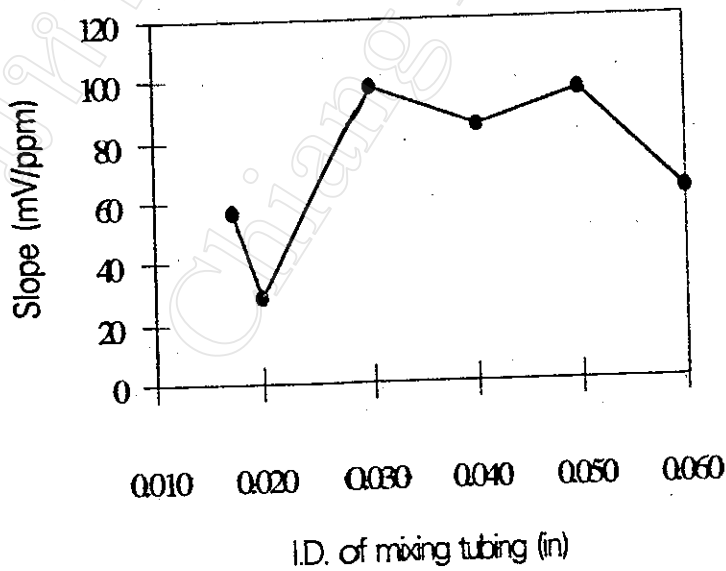


Figure 2.15 : Relationship between slope (mV/ppm) and I.D. of mixing tubing (in).

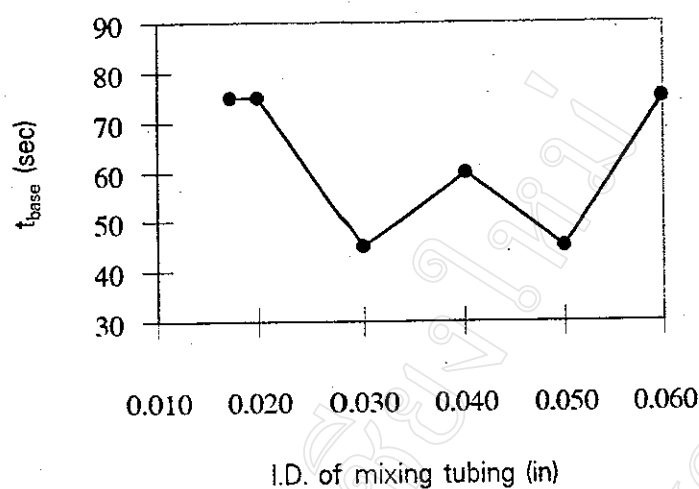


Figure 2.16 : Relationship between t_{base} (sec) and I.D. of mixing tubing (in).

2.5.6 Effect of the length of mixing tubing

The length of mixing tubing is defined as the portion or part of the tubing, where the sample and reagent are mixed and reacted. The colour develops and the absorbance of which is measured. The distance between the point of injection and the flow through cell should be kept as short as possible, yet should still be long to allow sufficient time for colour development and/or effective reaction taking place. In this work, the effect of the mixing tubing length was studied by varying the tubing length from 0.25 to 1.25 m. The results are shown in Tables 2.11 - 2.12 and Figures 2.17 - 2.19.

Table 2.11 : Effect of the length of mixing tubing on peak heights.

[Al(III)] (ppm)	Peak height (mV)* obtained from the length of mixing tubing (m)				
	0.25	0.50	0.75	1.00	1.25
0.10	5.60	6.20	10.00	6.00	8.00
0.20	10.60	13.80	16.40	13.00	21.00
0.30	16.60	21.40	22.80	26.00	32.00
0.40	20.60	29.00	29.20	37.40	36.00
0.50	28.40	36.60	35.60	44.00	45.00
Slope (mV/ppm)	55.14	74.00	69.14	93.49	91.43
Correlation coefficient	0.9973	0.9995	0.9963	0.9924	0.9911

* average of triplicate results.

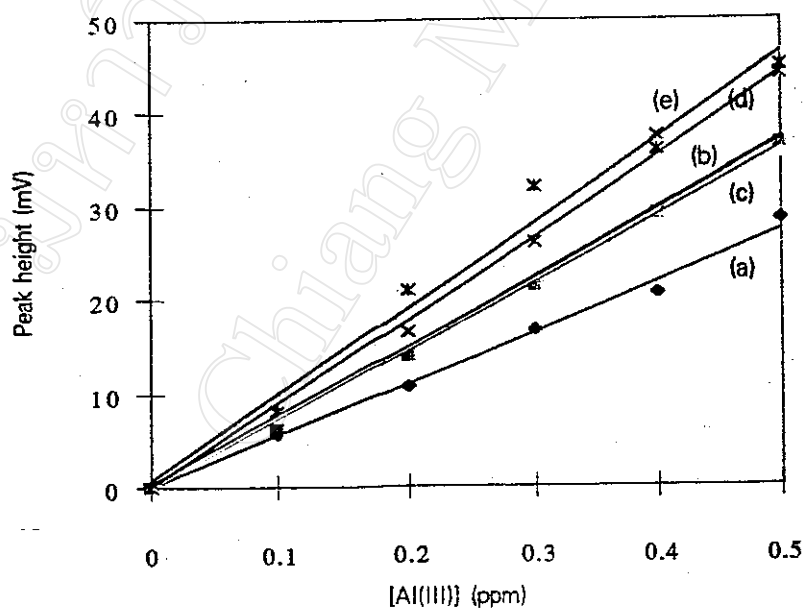


Figure 2.17 : Effect of the length of mixing tubing on the calibration curve;

(a) 0.25, (b) 0.50, (c) 0.75, (d) 1.00 and (e) 1.25 m.

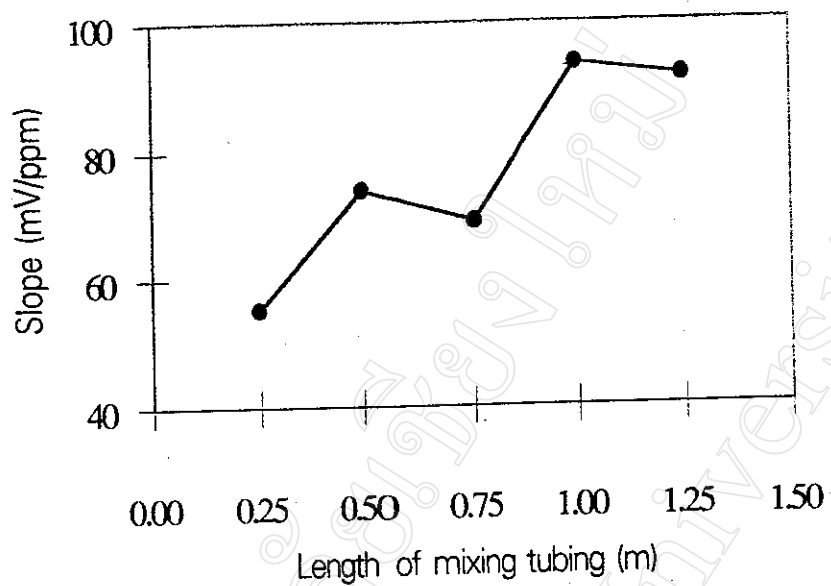


Figure 2.18 : Relationship between slope (mV/ppm) and length of mixing tubing (m).

Table 2.12 : Analytical characteristics for the determination of standard aluminium(III) solution 0.50 ppm.

Length of mixing tubing (m)	t_{base} (sec)	Sample / hr
0.25	72	50
0.50	60	60
0.75	42	85
1.00	42	85
1.25	36	100

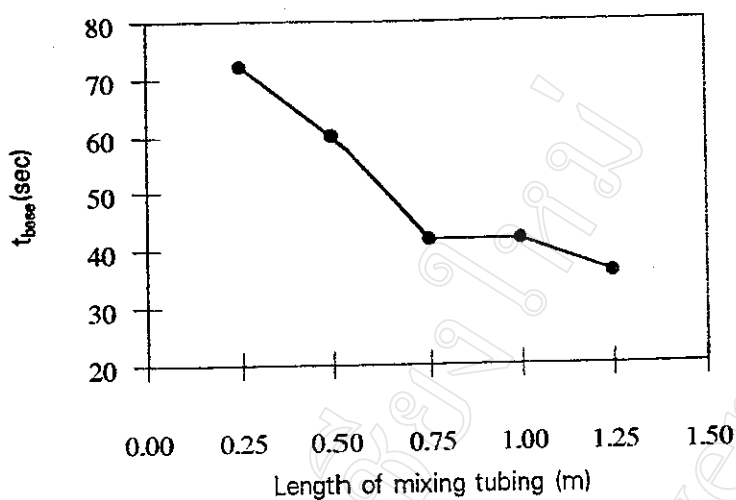


Figure 2.19 : Relationship between t_{base} (sec) and length of mixing tubing (m).

The results showed that the optimum length of mixing tubing is 1.00 m which giving the best sensitivity and appropriate sample throughput for using a FI method.

2.5.7 Effect of the size of coiled mixing part

The effect of O.D. of coiled mixing part was tested by using various size of test tube over range 0.75 - 2.40 cm. The results obtained are shown in Table 2.13 and Figure 2.20. The results showed that the optimum size of coiled mixing part is 0.75 cm.

Table 2.13 : Effect of the O.D. of test tube of coiled mixing part on peak heights.

O.D. of test tube (cm) [Al(III)] (ppm)		Peak height *					
		0.75		1.00		1.20	
		cm	mV	cm	mV	cm	mV
0.10	0.33	6.60	0.37	7.40	0.40	8.00	
0.20	0.77	15.40	0.72	14.40	0.63	12.60	
0.30	0.98	19.60	1.00	20.00	0.90	18.00	
0.40	1.36	27.20	1.35	27.00	1.00	20.00	
0.50	1.70	34.00	1.70	34.00	1.10	22.00	
Slope (mV/ppm)		67.43		66.97		43.26	
Correlation coefficient		0.9976		0.9994		0.9700	

O.D. of test tube (cm) [Al(III)] (ppm)		Peak height *					
		1.60		1.80		2.40	
		cm	mV	cm	mV	cm	mV
0.10	0.20	4.00	0.17	3.40	0.52	10.40	
0.20	0.65	13.00	0.62	12.40	0.83	16.60	
0.30	0.80	16.00	0.95	19.00	1.07	21.40	
0.40	0.93	18.60	1.18	23.60	1.30	26.00	
0.50	1.20	24.00	1.35	27.00	1.45	29.00	
Slope (mV/ppm)		45.46		57.77		56.09	
Correlation coefficient		0.9752		0.9893		0.9661	

* average of triplicate results.

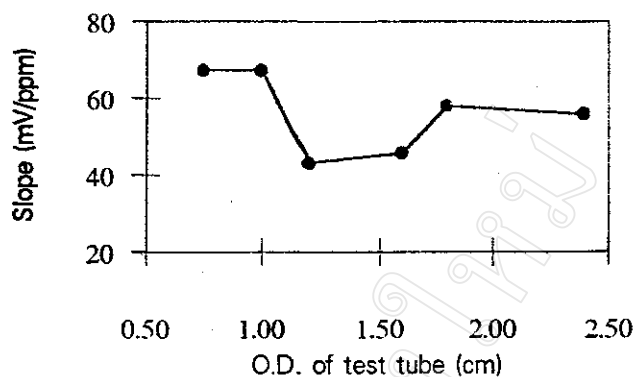


Figure 2.20 : Relationship between slope (mV/ppm) and size of coiled mixing part.

2.5.8 Effect of the length of s.b.s.r.

The effect of the length of single bead string reactor (s.b.s.r.) on peak height was studied over the range 3.00 - 15.00 cm. The results are shown in Tables 2.14 - 2.15 and Figures 2.21 - 2.22. The results indicated that the s.b.s.r. length of 7.00 cm should be the most appropriate as giving the best sensitivity.

Table 2.14 : Effect of the length of s.b.s.r. on peak heights.

[Al(III)] (ppm)	Length of s.b.s.r. (cm)	Peak height *					
		3.00		5.00		7.00	
		cm	mV	cm	mV	cm	mV
0.10	0.15	3.00	0.48	9.60	0.40	8.00	
0.20	0.50	10.00	0.63	12.60	0.80	16.00	
0.30	0.70	14.00	1.20	24.00	1.17	23.40	
0.40	0.85	17.00	1.50	30.00	1.50	30.00	
0.50	1.25	25.00	1.73	34.60	1.87	37.40	
Slope (mV/ppm)		48.86		70.17		74.4	
Correlation coefficient		0.9908		0.9907		0.9994	

* average of triplicate reselts.

Table 2.14 : (continued)

[Al(III)] (ppm)	Length of s.b.s.r. (cm)	Peak height *					
		9.00		13.00		15.00	
		cm	mV	cm	mV	cm	mV
0.10	0.50	10.00	0.30	6.00	0.43	8.60	
0.20	0.88	17.60	0.60	12.00	0.93	18.60	
0.30	1.20	24.00	1.00	20.00	1.37	27.40	
0.40	1.30	26.00	1.13	22.60	1.50	30.00	
0.50	1.70	34.00	1.23	24.60	1.80	36.00	
Slope (mV/ppm)		64.11		51.66		72.28	
Correlation coefficient		0.9846		0.9802		0.9849	

* average of triplicate results.

Table 2.15 : Analytical characteristics for the determination of aluminium(III)
standard solution 0.50 ppm at various length of s.b.s.r. mixing part.

Length of s.b.s.r. (cm)	t_{base}		Sample/hr
	cm	sec	
3.00	1.00	60.0	60
5.00	1.10	66.0	54
7.00	1.10	66.0	54
9.00	1.10	66.0	54
13.00	1.10	66.0	54
15.00	1.20	72.0	50

(chart speed 1 min/cm)

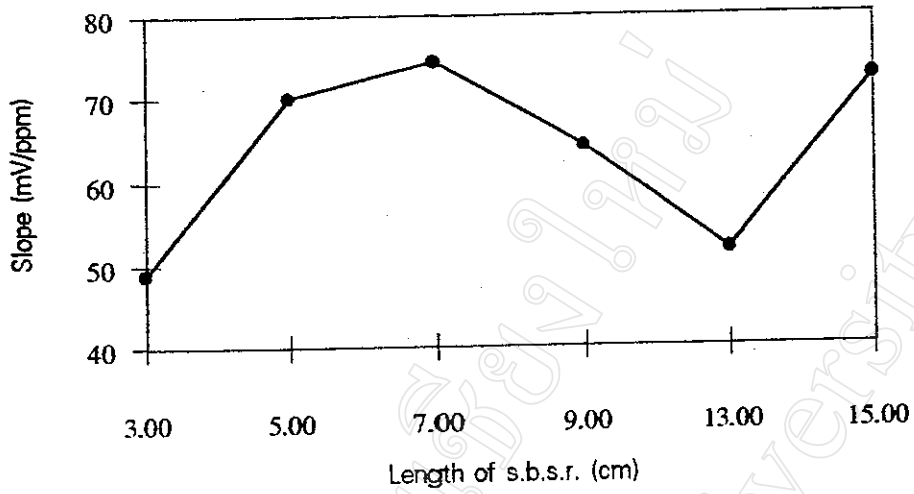


Figure 2.21 : Relationship between slope (mV/ppm) and length of s.b.s.r. (cm).

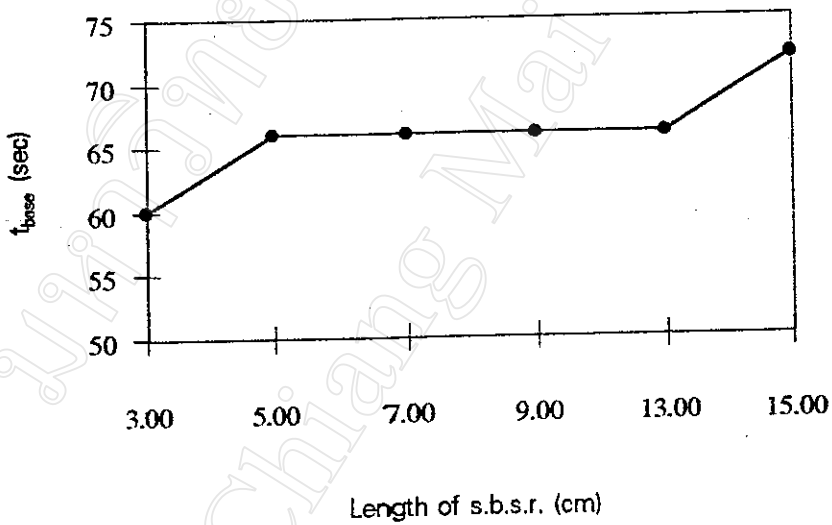


Figure 2.22 : Relationship between t_{base} (sec) and length of s.b.s.r. (cm).

2.5.9 Effect of various shapes of mixing part

This effect was studied by comparing the peak heights obtained from the use of various shapes of the mixing parts namely coil, zig zag, knitting, straight, and

s.b.s.r. The results obtained are shown in Tables 2.16 - 2.17 and Figures 2.23 - 2.25. It was found that the knitted mixing part is the optimum shape, since it exhibits the best sensitivity.

Table 2.16 : Effect of various shapes of mixing part on peak heights.

Shape of mixing [Al(III)] part (ppm)	Peak height *					
	Coil		Zig zag 1 x 4 cm		Zig zag 3 x 4 cm	
	cm	mV	cm	mV	cm	mV
0.10	0.33	6.60	0.20	4.00	0.33	6.60
0.20	0.65	13.00	0.32	6.40	0.50	10.00
0.30	1.00	20.00	0.70	14.00	0.87	17.40
0.40	1.30	26.00	0.88	17.60	1.00	20.00
0.50	1.53	30.60	1.40	28.00	1.50	30.00
Slope (mV/ppm)	62.34		53.83		55.14	
Correlation coefficient	0.9983		0.9781		0.9762	

Shape of mixing [Al(III)] part (ppm)	Peak height *					
	Knitted		Straight		s.b.s.r.	
	cm	mV	cm	mV	cm	mV
0.10	0.70	14.00	0.32	6.40	0.30	6.00
0.20	1.13	22.60	0.60	12.00	0.47	9.40
0.30	1.55	31.00	0.90	18.00	0.77	15.40
0.40	1.98	39.60	1.10	22.00	0.90	18.00
0.50	2.42	48.40	1.50	30.00	1.27	25.40
Slope (mV/ppm)	93.48		57.94		48.28	
Correlation coefficient	0.9955		0.9975		0.9938	

* average of triplicate results.

Table 2.17 : Analytical characteristics for the determination of standard aluminium(III) solution 0.50 ppm at various shapes of mixing parts.

Type of mixing part	t_{base}		Sample/hr
	cm	sec	
coil	0.75	45.0	80
zig zag 1x4	0.80	48.0	75
zig zag 3x4	0.60	36.0	100
knitted	0.60	36.0	100
straight	1.00	60.0	60
s.b.s.r.	0.70	42.0	85

(chart speed 1 min/cm).

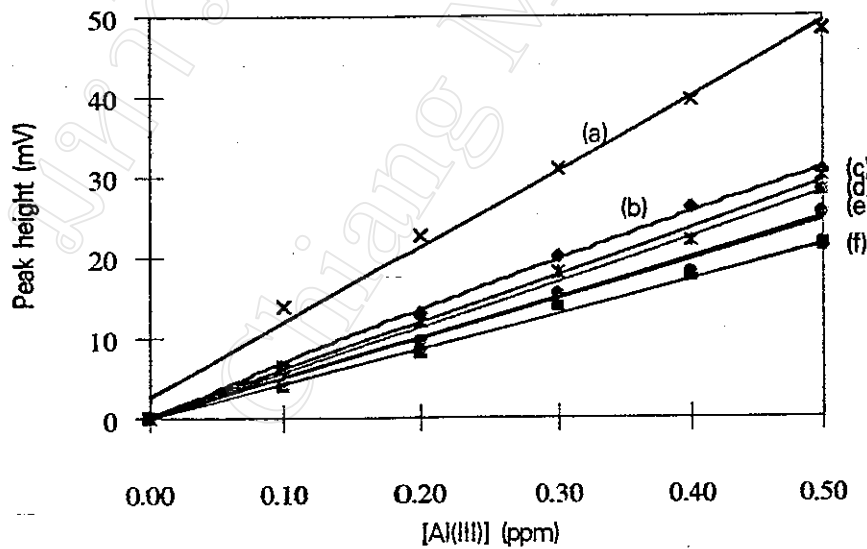


Figure 2.23 : Effect of various shapes of mixing parts on peak height;

(a) knitted, (b) coil, (c) straight, (d) zig zag 3x4 cm, (e) zig zag 1x4 cm and (f) s.b.s.r.

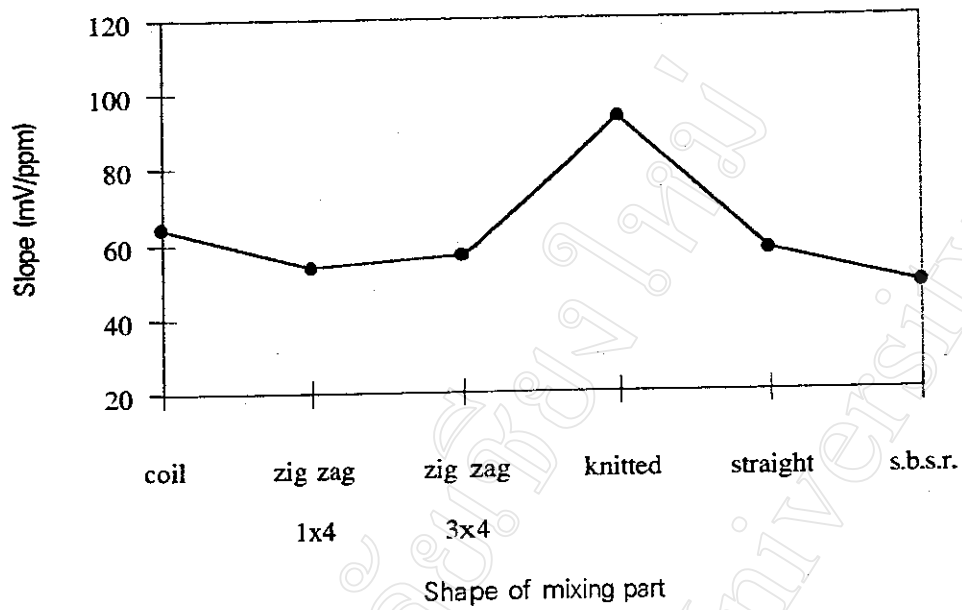


Figure 2.24 : Relationship between slope (mV/ppm) and various shapes of mixing part.

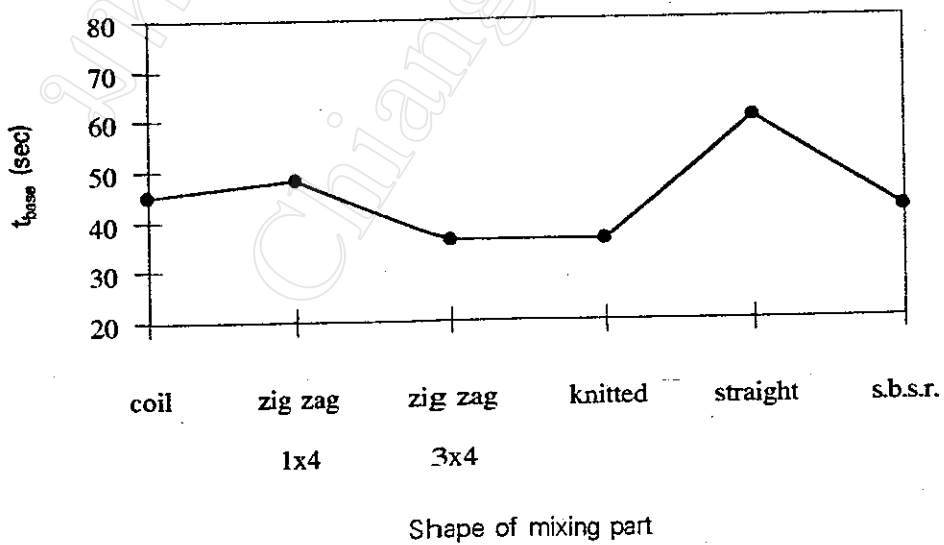


Figure 2.25 : Relationship between t_{base} (sec) and various shapes of mixing part.

2.5.10 Effect of the reagent injection volume

The influence of the reagent injection volume was studied by changing the loop, *i.e.*, to give a reagent volume in the range 50 - 250 μl in order to find out the optimal reagent injection volume. The results obtained are shown in Table 2.18 and Figures 2.26 - 2.27. It is found that the optimum reagent injection volume is 100 μl .

Table 2.18 : Effect of the reagent injection volume on peak heights.

Injection volume [Al(III)] (μl) (ppm)		Peak height *									
		50		100		150		200		250	
		cm	mV	cm	mV	cm	mV	cm	mV	cm	mV
0.10	0.58	11.60	0.58	11.60	0.70	14.00	0.52	10.40	0.72	14.40	
0.20	0.95	19.00	1.23	24.60	1.20	24.00	0.93	18.60	1.16	23.20	
0.30	1.32	26.40	1.88	37.60	1.68	33.60	1.36	27.20	1.59	31.80	
0.40	1.68	33.60	2.50	50.00	2.17	43.40	1.78	35.60	2.03	40.60	
0.50	2.06	41.20	3.18	63.60	2.66	53.20	2.20	44.00	2.46	49.20	
Slope (mV/ppm)	79.83		127.49		103.94		86.91		95.20		
Correlation coefficient	0.9962		0.9998		0.9977		0.9993		0.9951		

* average of triplicate results.

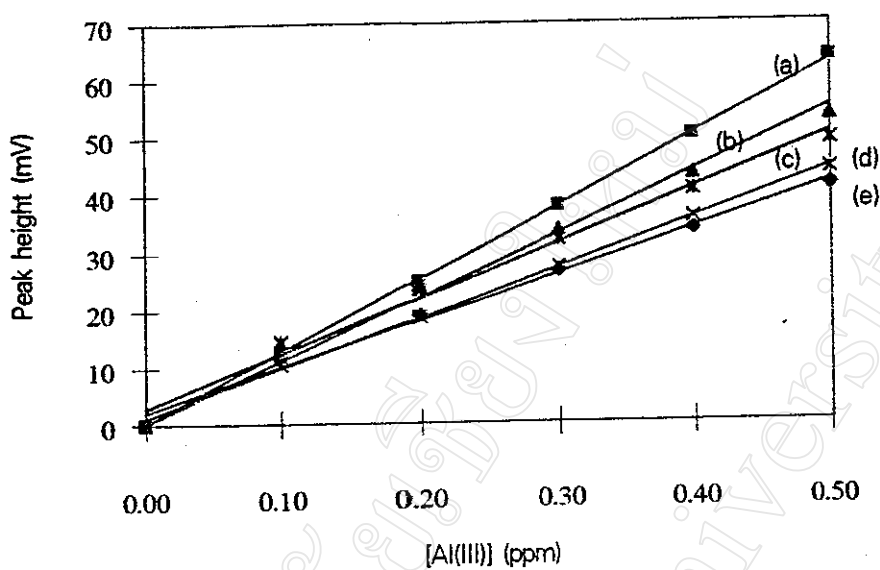


Figure 2.26 : Effect of the reagent injection volume on peak height of Al(III) solution, (a) 100, (b) 150, (c) 200, (d) 250 and (e) 50 μ l.

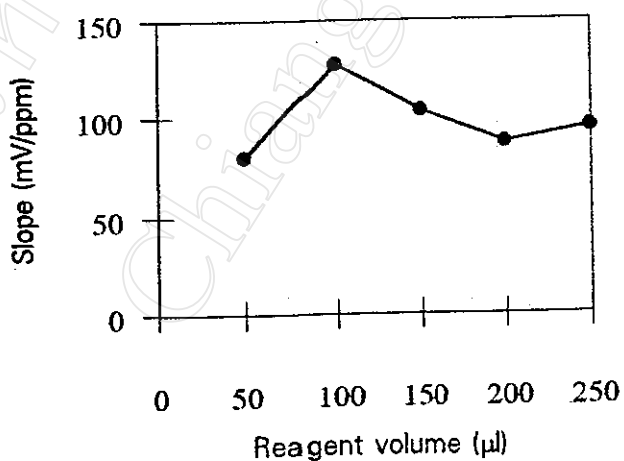


Figure 2.27 : Relationship between slope (mV/ppm) and reagent injection volume (μ l).

2.6 Study of the analytical parameters for aluminium(III) determination

2.6.1 Linear range

The linear range of the proposed method was studied by injecting Al(III) standard solution (0 - 5.00 ppm) into the FI system. The experimental conditions are given in Table 2.19. The results obtained are shown in Table 2.20 and Figure 2.28. The results indicated that linear range was in range 0 - 1.40 and 2.20 - 4.00 ppm Al(III) solution.

Table 2.19 : Experimental conditions for the study of linear range of the determination of Al(III).

Analytical Characteristics	Information
conditions of the FI system	
conc. of TC (M)	3.12×10^{-5}
conc. of Al(III) (ppm)	0 - 5.00
reagent volume (μ l)	100
pH	6.0
pump flow rate (ml/min)	4.0
inner diameter of mixing tubing (in)	0.030
length of mixing tubing (m)	1.00
shape of mixing part	knitted
conditions of detector	
absorbance range	0 - 2
conditions of recorder	
chart speed (min/cm)	5
sensitivity (mV/cm)	20

Table 2.20 : Study of linear range.

[Al(III)] (ppm)	Peak height (cm)				Peak height (mV)	Net signal (mV)
	1	2	3	Mean		
0.00	5.30	5.20	5.30	5.27	105.40	-
0.20	5.65	5.65	5.70	5.67	113.40	8.00
0.40	5.95	6.00	6.08	6.01	120.20	14.80
0.60	6.35	6.40	6.33	6.36	127.20	21.80
0.80	6.70	6.68	6.72	6.70	134.00	28.60
1.00	7.00	7.00	7.12	7.04	148.80	35.40
1.40	7.75	7.75	7.78	7.76	155.20	49.80
1.80	8.00	7.95	7.90	7.95	159.00	53.60
2.20	8.10	8.00	8.00	8.03	160.60	55.20
2.60	8.25	8.25	8.20	8.23	164.60	59.20
3.00	8.45	8.40	8.45	8.43	169.60	63.20
4.00	9.00	8.90	8.90	8.93	178.60	73.20
5.00	9.10	9.12	9.12	9.12	182.40	77.00

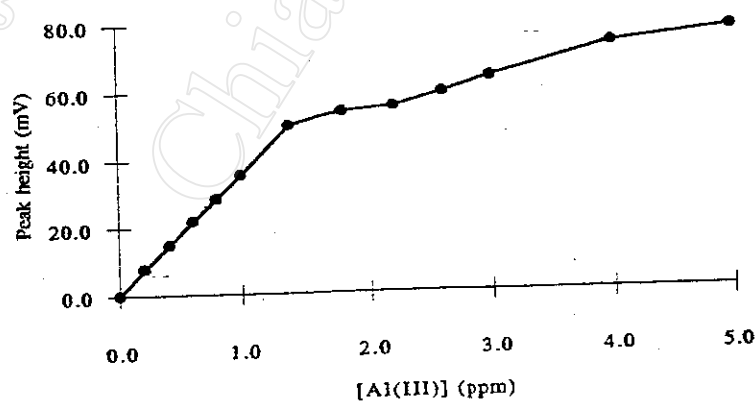


Figure 2.28 : Relationship between net signal (mV) and various concentrations of Al(III) (ppm).

2.6.2 Precision

2.6.2.1 Precision of the flow injection system

The precision of the flow injection (FI) system was determined by repeating injection of a standard solution (1 ppm, 100 μ l) into the FI system for 12 replicates. The results are summarized in Table 2.21.

Table 2.21 : Study of the precision of the flow injection system.

Expt. no.	Peak height in	
	cm	mV
1	7.60	152.00
2	7.70	154.00
3	7.65	153.00
4	7.70	154.00
5	7.75	155.00
6	7.75	155.00
7	7.65	153.00
8	7.85	157.00
9	7.70	154.00
10	7.65	153.00
11	7.70	154.00
12	7.80	156.00
\bar{X}	154.17 mV	
S.D.	1.40 mV	
% R.S.D	0.91 %	

2.6.2.2 Precision of the proposed method

The precision of the proposed method was determined by preparing 12 spiked samples of 1.00 ppm Al(III) which were then, injected into the FI system under the experimental conditions as shown in Table 2.19. The results obtained are shown in Table 2.22.

Table 2.22 : Study of the precision of the proposed method.

Expt. no.	Peak height (cm)				Peak height (mV)
	1	2	3	Mean	
1	8.35	8.45	8.40	8.40	168.00
2	8.30	8.35	8.20	8.28	165.60
3	8.20	8.35	8.20	8.25	165.00
4	8.30	8.20	8.30	8.27	165.40
5	7.95	7.95	8.05	7.98	159.60
6	7.85	7.75	7.85	7.82	156.40
7	8.50	8.50	8.30	8.43	168.60
8	7.90	8.00	8.15	8.03	160.60
9	7.95	7.85	7.80	7.87	157.40
10	8.15	8.05	8.15	8.12	162.40
11	7.75	7.85	7.90	7.83	156.60
12	8.05	8.15	7.95	8.05	161.00
\bar{X}				162.22 mV	
S.D.				4.30 mV	
%R.S.D.				2.65	

2.6.3 Detection limit

The detection limit is defined as the concentration of the analyte which gives the signal equivalent to three times of the standard deviation (3σ) of the blank signals. The results are given in Tables 2.23 and 2.24. The detection limit of the proposed method was found to be 0.020 ppm of aluminium(III) solution.

Table 2.23 : Peak height obtained for blank solutions.

Expt. no	Peak height in	
	cm	mV
1	5.55	111.00
2	5.65	113.00
3	5.60	112.00
4	5.55	111.00
5	5.60	112.00
6	5.55	111.00
7	5.50	110.00
8	5.50	110.00
9	5.55	111.00
10	5.55	111.00
11	5.45	109.00
12	5.40	108.00
\bar{X}	110.75 mV	
S.D.	1.37	
%R.S.D	1.23	

Table 2.24 : Peak heights of various aluminium(III) concentrations near the detection limit.

[Al(III)] (ppm)	Peak height (cm)				Peak height
	1	2	3	Mean	(mV)
0.015	5.68	5.68	5.63	5.66	113.20
0.020	5.73	5.73	5.73	5.73	114.60
0.030	5.75	5.75	5.78	5.76	115.20
0.040	5.70	5.78	5.78	5.78	115.60
0.060	5.83	5.80	5.85	5.83	116.60
0.080	5.93	5.93	5.93	5.93	118.60

2.6.4 Calibration curve and sensitivity

An aliquot of TC solution was injected into standard aluminium(III) solution (0.2 - 1.0 ppm) stream under the recommended optimum conditions. The results were shown in Table 2.25 and Figure 2.29. The calibration curve as shown in Figure 2.30 was established by plotting peak heights versus the various aluminium (III) concentrations. It was found to be linear over the range 0.0 - 1.0 ppm with the following calibration equation:

$$Y = 66.29 X + 0.72$$

where Y is peak height in mV and

X is concentration of Al(III) solution in ppm with a correlation coefficient (r) of 0.9993.

Table 2.25 : Peak heights for calibration curve.

[Al(III)] (ppm)	Peak height (cm)				Peak height (mV)	Net signal (mV)
	1	2	3	avg		
blank	4.90	4.90	4.90	4.90	98.00	-
0.20	5.60	5.60	5.60	5.60	112.00	14.00
0.40	6.30	6.36	6.30	6.32	126.40	28.40
0.60	6.80	6.95	6.95	6.90	138.00	40.00
0.80	7.60	7.60	7.72	7.64	152.80	54.80
1.00	8.30	8.20	8.10	8.20	164.00	66.00

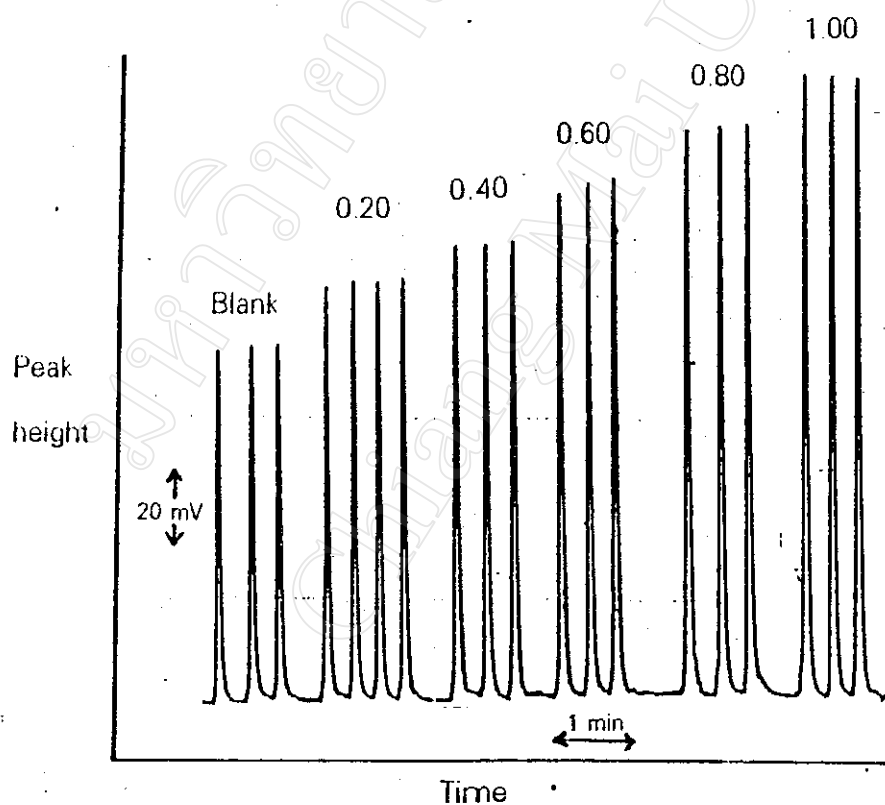


Figure 2.29 : Calibration signals for the determination of Al(III) with TC as a complexing agent (ppm, shown above each set of peaks).

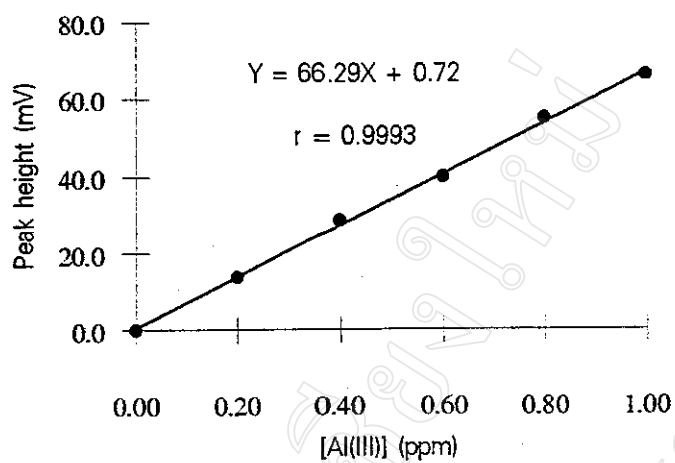


Figure 2.30 : The calibration curve for determining Al(III).

2.6.5 Effect of foreign ions

The effect of foreign ions was investigated by adding known amounts of each foreign ion to a solution containing 1.0 ppm of Al(III) and then determining the latter using the recommended procedure. The results are presented in Table 2.26.

Table 2.26 : Effect of interfering ions.

Ion	Al(III) ions	Peak height (mV)	%Relative error
K	1 : 0	88.0	-
	1 : 1	89.0	+1.14
	1 : 2	93.6	+6.36
	1 : 5	99.6	+13.2
	1 : 10	100.0	+13.6
	1 : 20	100.6	+14.3
	1 : 50	120.0	+36.4
Na	1 : 0	88.0	-
	1 : 1	88.0	0
	1 : 2	86.6	-1.59
	1 : 5	87.0	-1.14
	1 : 10	80.0	-9.09
	1 : 20	70.4	-20.0
	1 : 50	70.4	-20.0
Mg(II)	1 : 0	88.0	-
	1 : 1	79.0	-10.2
	1 : 2	72.0	-18.2
	1 : 5	65.0	-26.1
	1 : 10	61.6	-30.0
	1 : 20	58.0	-34.1
	1 : 50	49.6	-43.6
Cu(II)	1 : 0	88.0	-
	1 : 1	88.0	0
	1 : 2	88.6	+0.68
	1 : 5	90.4	+2.73
	1 : 10	92.6	+5.23
	1 : 20	99.6	+13.2
	1 : 50	105.0	+19.3

Table 2.26 : (continued)

Ion	A(III) ions	Peak height (mV)	% Relative error
Zn(II)	1 : 0	88.0	-
	1 : 1	88.0	0
	1 : 2	88.0	0
	1 : 5	85.0	-3.41
	1 : 10	84.6	-3.86
	1 : 20	75.0	-14.8
	1 : 50	70.6	-19.8
Fe(III)	1 : 0	88.0	-
	1 : 1	75.4	-14.3
	1 : 2	65.8	-25.2
	1 : 5	61.6	-30.0
	1 : 10	56.6	-35.7
	1 : 20	53.0	-39.8
	1 : 50	48.4	-45.0
Cl ⁻	1 : 0	88.0	-
	1 : 1	88.0	0
	1 : 2	88.0	0
	1 : 5	86.0	-2.27
	1 : 10	85.4	-2.95
	1 : 20	85.6	-2.73
	1 : 50	80.4	-8.64
SO ₄ ²⁻	1 : 0	88.0	-
	1 : 1	88.0	0
	1 : 2	88.0	0
	1 : 5	88.0	0
	1 : 10	89.6	+1.82
	1 : 20	89.6	+1.82
	1 : 50	89.4	+1.59

Table 2.26 : (continued)

Ion	Al(III) : Ions	Peak height (mV)	%Relative error
NO ₃ ⁻	1 : 0	88.0	-
	1 : 1	88.0	0
	1 : 2	89.0	+1.14
	1 : 5	89.6	+1.82
	1 : 10	93.4	+6.14
	1 : 20	97.6	+10.9
	1 : 50	98.0	+11.4
F ⁻	1 : 0	92.0	-
	1 : 1	92.0	0
	1 : 2	91.6	-0.43
	1 : 5	91.6	-0.43
	1 : 10	90.0	-2.17
	1 : 20	86.4	-6.09
	1 : 50	83.0	-9.78

2.6.6 Accuracy

The accuracy of the proposed method was studied by determining the recoveries of the added Al(III) with varying concentrations in the sample solutions. The results are presented in Tables 2.27 and 2.28.

Table 2.27 : Peak height obtained by using standard addition FIA.

[Al(III)] (ppm)	Peak height (mV)	
	standard aluminium	standard + sample
0.00	-	10.50
0.20	14.00	22.60
0.40	28.40	36.40
0.60	40.00	48.80
0.80	54.80	62.00
1.00	66.00	76.00

Table 2.28 : Assessment of the accuracy of the proposed method.

[Al(III)], ppm			%recovery
Added	Initially present	Recovered	
0.00	0.147	-	-
0.20	0.330	0.183	91.50
0.40	0.538	0.391	97.75
0.60	0.725	0.578	96.33
0.80	0.924	0.777	97.12
1.00	1.136	0.989	98.90
\bar{X}			96.32
S.D.			2.85
% R.S.D			2.96

2.7 Application of the proposed FIA method

2.7.1 Determination of aluminium(III) in tap water samples using TC

The proposed FI-spectrophotometric method was applied to the determination of aluminium(III) in tap water samples. Different tap water samples collected from student's dormitories in Chiang Mai University were analyzed by the proposed and flame atomic absorption spectrophotometric (flame-AAS) methods. The results are summarized in Table 2.29 which are showed that the aluminium(III) contents in tap water samples are ranging from 0.043 - 0.556 ppm Al(III) and 0.035 - 0.548 ppm Al(III) by the proposed and the flame-AAS respectively. The results obtained by both methods were compared by applying the t-test at the 95% confidence level. The calculated t values did not exceed the theoretical value ($t=2.12$) indicating that there was no significant difference between the two

methods with respect to accuracy. The proposed method permits the determination of aluminium(III) in tap water samples.

Table 2.29 : Comparative determinations of Al(III) in tap water using the proposed procedure and the standard method (AAS).

Sample*	conc. of Al(III) (ppm)**		Di
	FIA	AAS	
MD1	0.321	0.336	0.015
MD2	0.315	0.322	0.007
MD3	0.110	0.092	- 0.018
MD4	0.179	0.213	0.034
MD5	0.411	0.425	0.014
MD6	0.400	0.476	0.076
MD7	0.556	0.548	- 0.008
FD1	0.110	0.072	- 0.038
FD2	0.170	0.178	0.008
FD3	0.043	0.035	- 0.008
FD4	0.261	0.245	- 0.016
FD5	0.191	0.190	0.001
FD6	0.158	0.147	- 0.011
FD7	0.215	0.118	- 0.097
FD8	0.396	0.443	0.047
FD9	0.261	0.299	0.038
\bar{D}	2.625×10^{-3}		
S.D.	3.766×10^{-2}		
t	0.28		

* MD and FD represent tap water samples collected from male's and female's dormitories respectively.

** average of triplicate results.

2.7.2 Determination of tetracycline in drug formulations by using single standard calibration curve

The proposed method was also applied to the determination of tetracycline in proprietary drugs (TC mycin and TC.HCl capsules) under optimum experimental conditions as shown in Table 2.19. A single standard calibration curve over the range 0 - 10.0 ppm TC was shown in Table 2.30 and Figures 2.31 - 2.32. Table 2.31 summarized the results obtained with percentage labelled amounts 116.6 and 109.7% for TC mycin and TC.HCl capsules respectively.

Table 2.30 : Peak height (mV) for 0.0 - 10.0 ppm TC (100 μ l) by single standard method

[TC] (ppm)	Peak height (mV)
0.5	7.00
1.0	16.80
2.0	28.80
3.0	48.20
4.0	68.40
5.0	86.40
6.0	104.00
8.0	139.00
10.0	176.66
t_{base} (sec)	36
Sample/hr	100

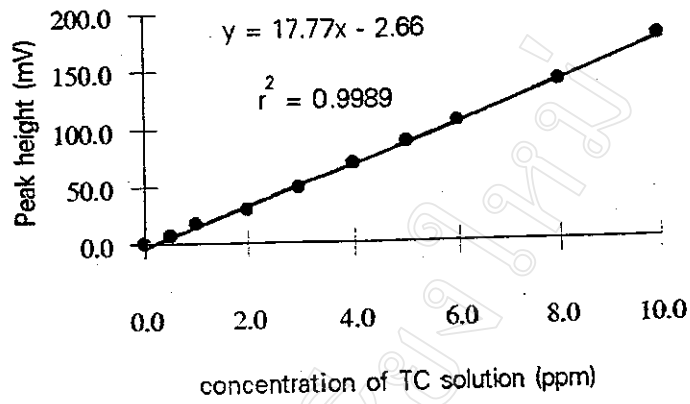


Figure 2.31 : Single standard calibration curve for determination of TC in proprietary drugs.

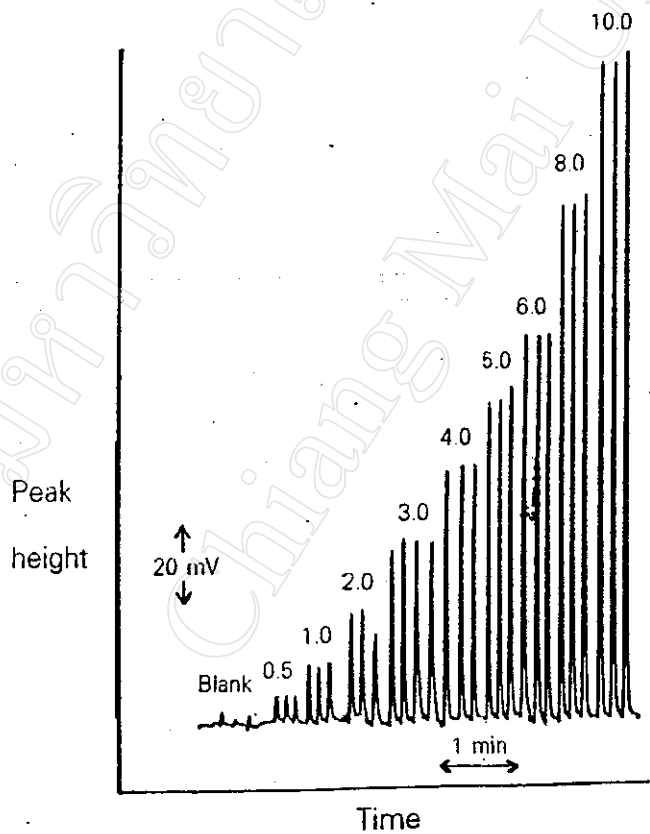


Figure 2.32 : FI signal for various concentrations of TC obtained by using single standard method.

Table 2.31 : Analysis of some proprietary drugs by using single standard calibration curve.

Sample	Amount of TC (mg/capsule)		%labelled amounts
	Certified	Found*	
TC mycin capsule	250	291.4	116.6
TC.HCl capsule	250	274.2	109.7

* average of triplicate results.