CHAPTER 2

EXPERIMENTAL, RESULTS AND DISCUSSION

2.1 Apparatus and Chemicals.

2.1.1 Apparatus

1. Aminco-Bowman Ratio spectrofluorimeter.

2. Fluorescense detector : Model 420, Water-Associate Company, USA.

3. Flow Injection Analyser : Model 5010, Tecator, Sweden.

4. Injection valve : Model V-100, Tecator, Sweden.

5. Chemifold tray and chemifold type III, Tecator, Sweden.

6. Spectrophotometer : Model 5023, Tecator, Sweden.

7. Detector controller : Model 5032, Tecator, Sweden.

8. Chart recorder : Model KIPP & ZONEN BD 12, Germany.
   : Model Lineseis type L 6512-1, USA.

2.1.2 Chemicals (All chemicals are analytical grade.)

1. Uranyl nitrate ; [$UO_2(NO_3)_2 . 6H_2O$] , BDH, England.

2. Quinine sulphate ; [$(C_{20}H_{24}N_2O_2)_2 . H_2SO_4 . 2H_2O$] , BDH, England.

3. Sodium tetraborate ; [$Na_2B_4O_7 . 10H_2O$] , BDH, England.

4. Sodium hydroxide ; [NaOH] , BDH, England.

5. Sulfuric acid ; [H_2SO_4] , BDH , England.

6. Phosphoric acid ; [H_3PO_4] , BDH, England.

7. Dipotassium hydrogen phosphate ; [K_2HPO_4] , purum , Fluka, Switzerland.

8. Sodium dihydrogen phosphate ; [NaH_2PO_4] , purum , Fluka, Switzerland.

9. Arsenazo III [2,2'-(1,8-dihydroxy-3,6-disulpho-2,7-naphthylene-bis (azo) dibenzene-arsenic acid] ; $C_{22}H_{18}As_2N_4O_{14}S_2$, Aldrich, Germany.

10. Glacial acetic acid ; [CH_3COOH] , BDH, England.

11. Hydrochloric acid ; [HCl] , BDH, England.

12. Sodium acetate trihydrate ; [$CH_3COONa.3H_2O$] , BDH, England.

13. Yttrium oxide ; [Y_2O_3] , Johnson Matthey, England.

14. Amberlite IR-120 (H) , 14-52 mesh size [USS] , BDH, England.

15. Amberlite IRC-50 (H) , 14-52 mesh size [USS] , BDH, England.

16. Dowex 50-WX8 (Na^+) , 20-50 mesh size [USS] , BDH, England.

17. Duolite C-225 (H) , 14-50 mesh size [USS] , Rohm & Hass, USA.

18. Chelex-100 (Na^+), 50-100 mesh size , SIGMA, USA.

2.1.3 Stock Solutions

(1). Uranium standard.

A stock standard solution of uranium (1000 mg/l) was prepared by dissolving uranyl nitrate (2.1098 g) in deionized water and diluting the solution with 1% v/v HNO_3 solution to 1000 ml.

(2). Sodium tetraborate (1.0 M)

Prepared by dissolving sodium tetraborate (38.14 g) in deionized water and diluting the solution to 100 ml.

(3). Dipotassium hydrogen phosphate-phosphoric acid buffer, pH 3.5 (0.1 M)

Dipotassium hydrogen phosphate (13.61 g) was dissolved in water. Phosphoric acid (2.7 ml) was added, followed by 900 ml of water. The pH of the resulting solution was adjusted to 3.5 with dilute sodium hydroxide before fixed dilution with water to 1000 ml.

(4). Yttrium standard

A stock standard solution of yttrium (1000 mg/l) was prepared by dissolving yttrium oxide (0.1270 g) in 5.0 ml of hot hydrochloric acid 1:1 (v/v) and diluting the solution with deionized water to 100 ml.

(5). Arsenazo III reagent (0.1 % w/v)

Prepared by dissolving Arsenazo III (0.10 g) in deionized water 100 ml.

(6). Acetic/acetate buffer, pH 4.0 (1.0 M)

Prepared by dissolving sodium acetate trihydrate (2.08 g) and glacial acetic acid (5.0 ml) in 1 litre of deionized water and adjusting the pH to 4 with dilute hydrochloric acid or sodium hydroxide.

(7). Hydrochloric acid solution (1.0 M)

Prepared by diluting concentrated hydrochloric acid [37% w/v] (83.3 ml) to 1 litre with deionized water.

Other concentrations of solutions were prepared by diluting stock solutions with deionized water.

2.2 Determination of Uranium by Flow Injection Fluorimetry

2.2.1 Preliminary study on fluorescence spectra of uranium in aqueous solution

In solution uranium (VI) compounds [UO_2^{2+}] fluoresce, but uranium (IV) and uranium (V) compounds do not (58). To study the fluorescence spectra of uranium in solution, the uranyl ion as the uranyl nitrate salt [$UO_2(NO_3)_2.6H_2O$] was used. Preliminary investigations were carried out by recording excitation spectra and emission spectra of solutions in deionized water containing 10 mg/l uranyl ion. These solutions were adjusted to pH 8 by using sodium hydroxide solution. The spectra was recorded by a spectrofluorimeter previously calibrated with quinine sulphate solution (1 mg/l in 0.1 N sulfuric acid) with an 1 cm quartz cell. Emission spectra were recorded over the range 200 to 800 nm at a scan speed of 500 nm/min. The excitation and fluorescence spectra of the uranyl ion are shown in Fig. 2.2.1 .

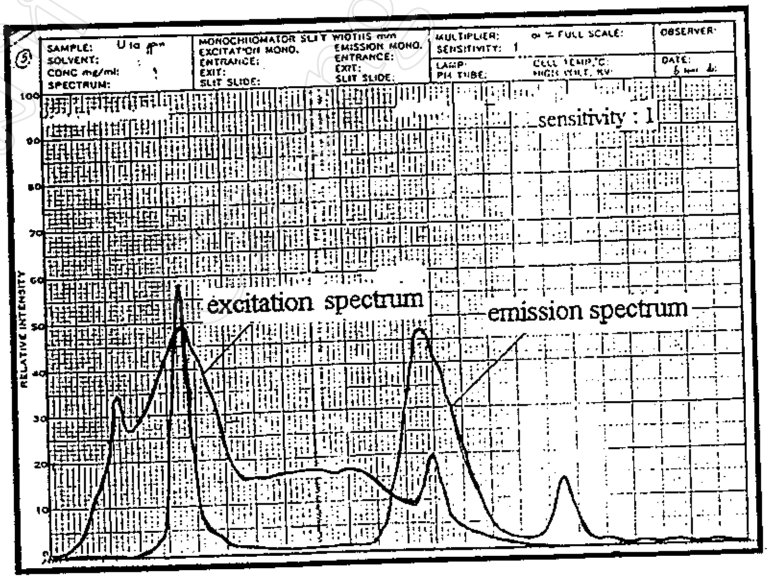

Fig. 2.2.1 Fluorescence spectra of uranyl ion in aqueous solution

The maximum excitation wavelength of uranyl ion in an aqueous medium was found to be 315 nm while the emission wavelength was 515 nm. Subsequent investigations, however, employed an excitation wavelength of 340 nm because of the inconvenience of using a 315 nm excitation filter.

The effect of pH on fluorescence spectra

Uranium standard solutions (10 mg/l) in aqueous medium were adjusted to an appropriate pH (between 2 and 9) with sodium hydroxide solution and were measured for emission intensity with an excitation wavelength of 340 nm using the same conditions as those used in the preliminary study (section 2.2.1). Results, (Fig. 2.2.2), show that the maximum emission wavelength of uranium in aqueous solutions at pH between 4 and 9 is 515 nm. The solution of pH 8 yielded the highest intensity.

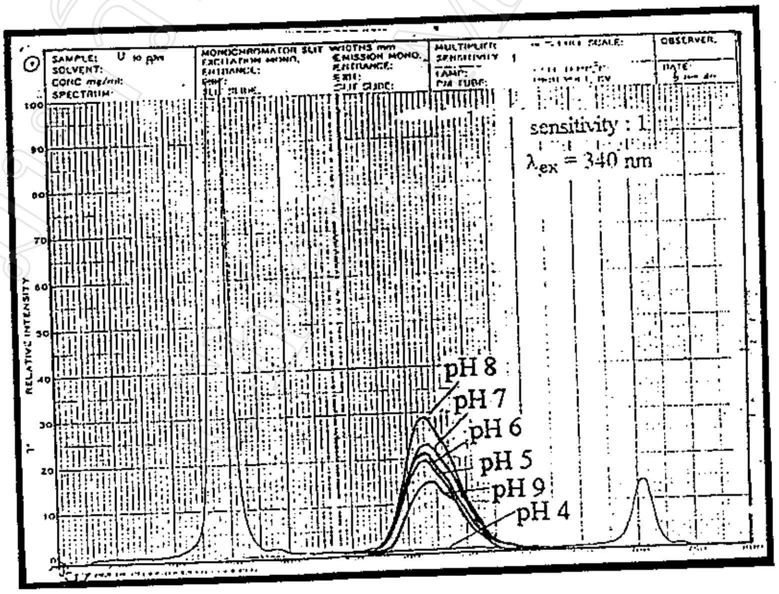

Fig. 2.2.2 Effect of pH on fluorescence spectra of uranyl solutions

A calibration at pH 8 was carried out by measuring fluorescence signals of uranium standard solutions from 5 to 50 mg/l. The results are shown in Figs. 2.2.3 and 2.2.4 and in Table 2.2.1. Linear calibrations over the ranges of 5 to 20 and 20 to 50 mg/l were obtained.

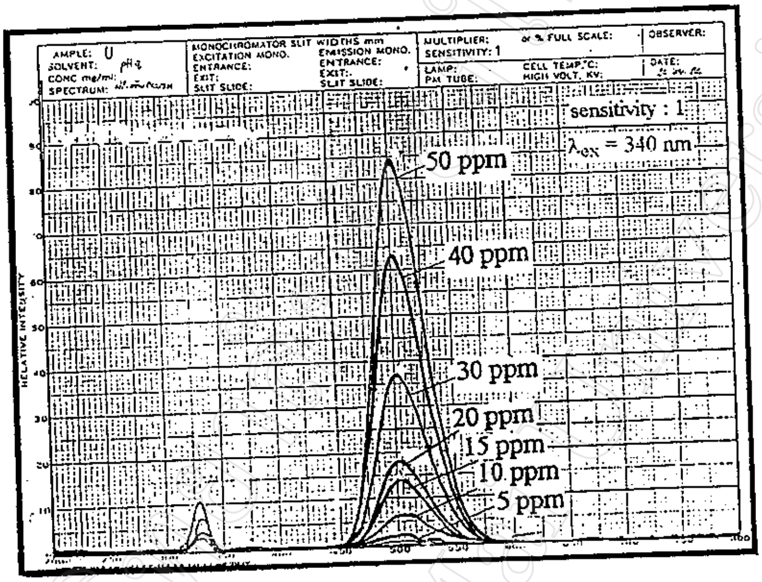

Fig. 2.2.3 Fluorescence spectra of uranium in aqueous media at pH 8

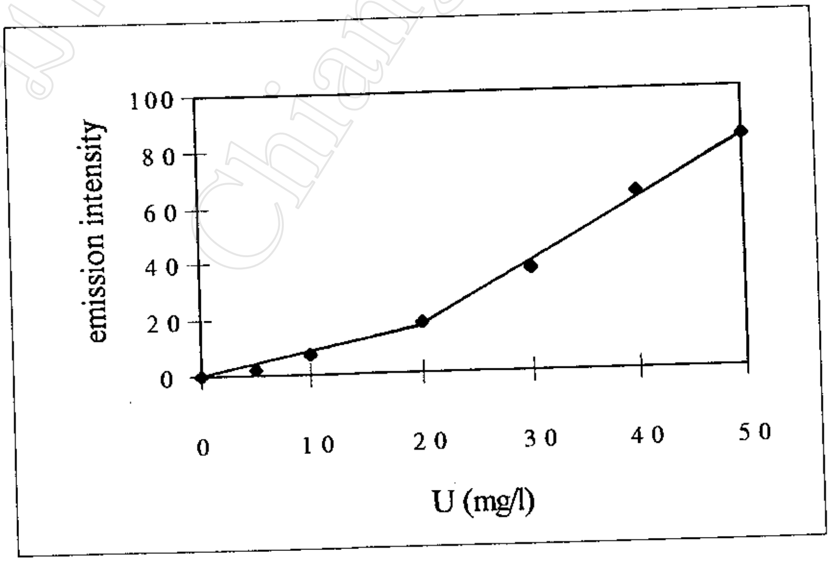

Fig. 2.2.4 Calibration for uranium in aqueous media at pH 8.

Table 2.2.1 Emission intensity of uranium in aqueous media at pH 8

λex = 340 nm, λem = 500 nm, sensitivity : 1.

U (mg/l)	0	5	10	20	30	40	50
emission intensity (arbitary unit)	0	2	7	18	37	63	83

2.2.2 Preliminary study on fluorescence spectra of uranium in buffer solutions of pH 8

The emission intensities of uranium standards in various buffer solutions of pH 8 were measured. The uranium standard solutions (5, 10, 20, 30, 40 and 50 mg/l) were prepared using buffer solutions as diluents.

2.2.2.1 Buffer solutions with phosphate species.

Excitation and fluorescence spectra of an uranium standard solution of 50 mg/l in 0.01 M dipotassium hydrogen phosphate was recorded (Fig 2.2.5).

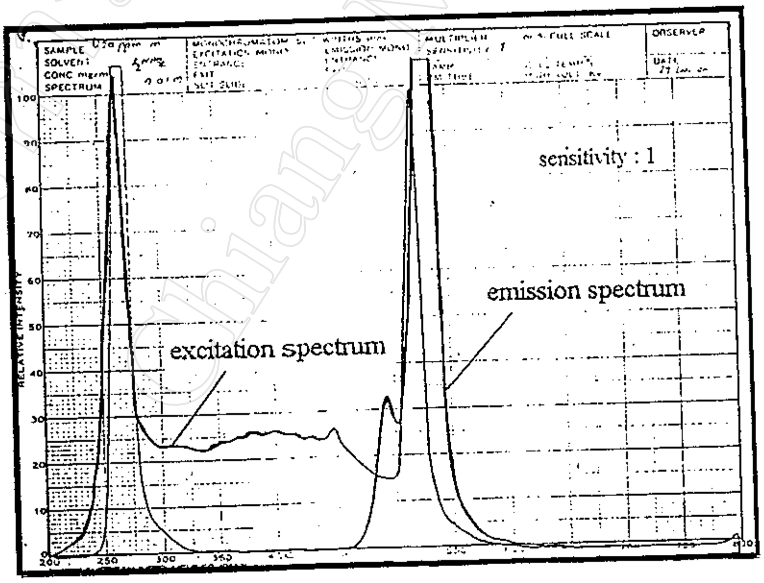

Fig. 2.2.5 Excitation and fluorescence spectra for uranium in phosphate buffer solution. (pH 8)

It was found that in the presence of phosphate species the maximum excitation wavelength [λex] was shifted from 315 nm to 270 nm and maximum emission wavelength [λem] was 530 nm. Consequently, the uranium fluorescence spectra in phosphate buffers should be monitored at an excitation wavelength of 270 nm. Uranium fluorescence spectra and emission intensities in other phosphate buffer solutions are shown in Figs. 2.2.6 - 2.2.11 and in Tables 2.2.2 - 2.2.4.

(a) Citric acid (0.1 M)-Disodium hydrogen phosphate buffer (0.2 M)

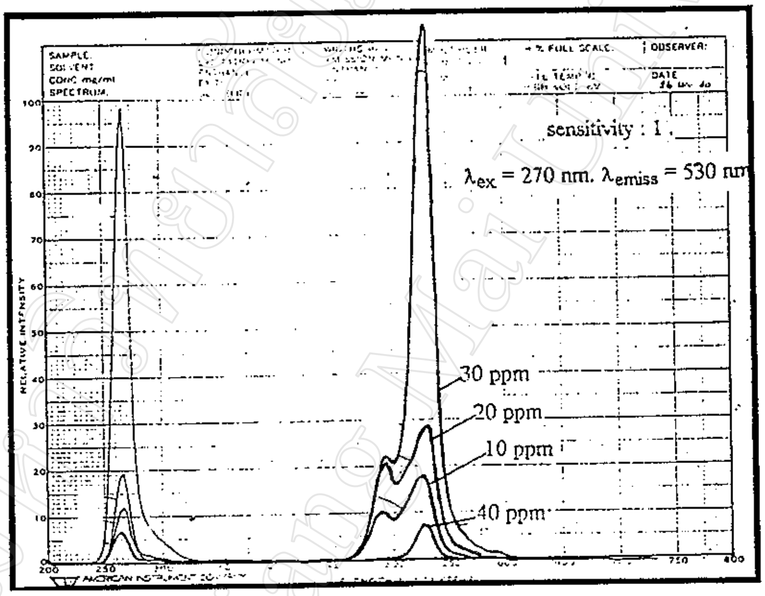

Fig. 2.2.6 Fluorescence spectra of uranium in citric acid (0.1 M) - Na_2HPO_4 (0.2 M) buffer.

Table 2.2.2 Emission intensity of uranium in citric acid (0.1 M) - Na_2HPO_4 (0.2 M) buffer ; λex = 270 nm, λem = 530 nm, sensitivity : 1

U (mg/l)	0	10	20	30	40
emission intensity (arbitary unit)	0	18	29	113	7

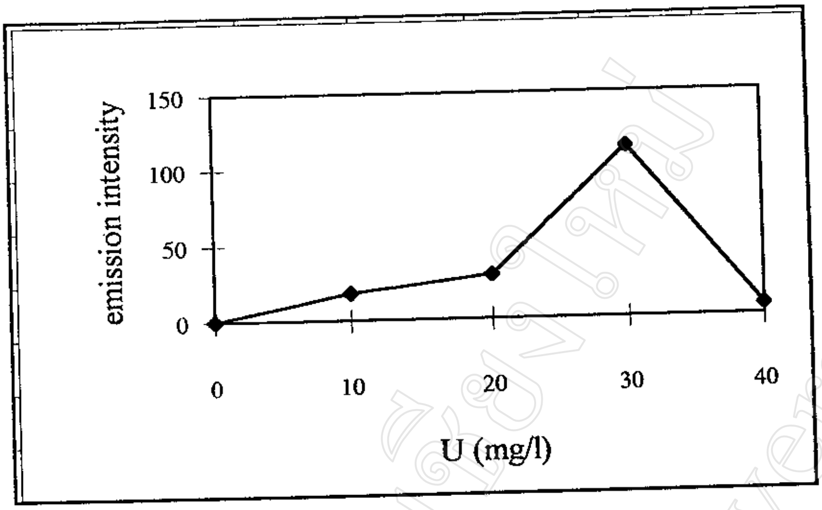

Fig. 2.2.7 Emission intensity of uranium in citric acid (0.1 M) - Na_2HPO_4 (0.2 M) buffer ; λex = 270 nm, λem = 530 nm, sensitivity : 1

(b) Potassium dihydrogen phosphate-sodium hydroxide mixture (0.1M)

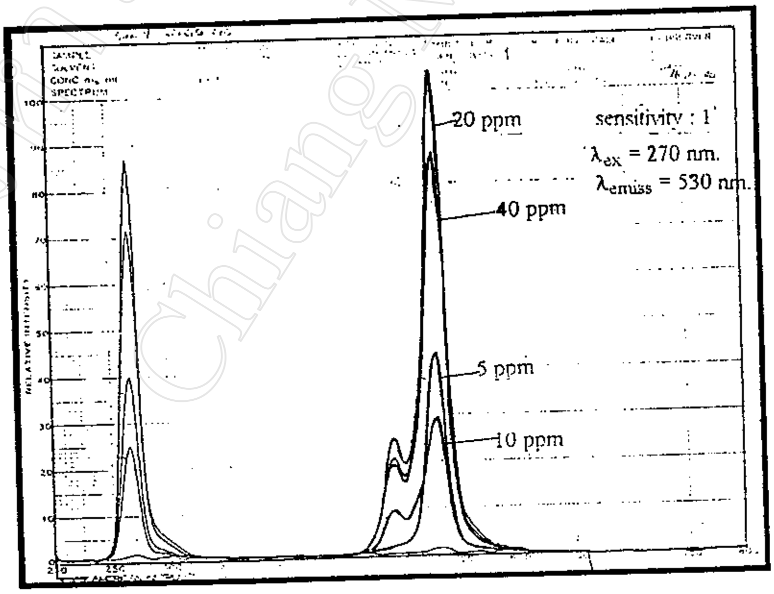

Fig. 2.2.8 Fluorescence spectra of uranium in KH_2PO_4 - NaOH mixture (0.1 M)

Table 2.2.3 Emission intensity of uranium in KH_2PO_4 - NaOH mixture (0.1 M)

λex = 270 nm, λem = 530 nm, sensitivity : 1

U (mg/l)	0	10	20	30	40
emission intensity (arbitary unit)	1	43	30	104	86

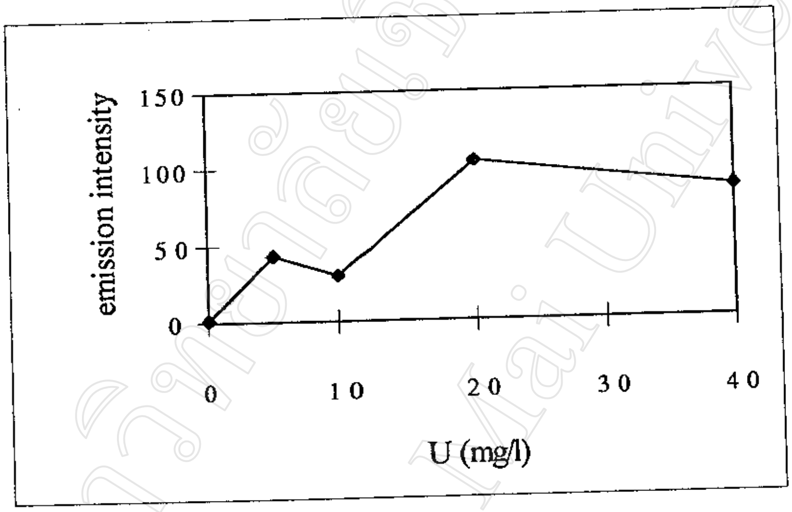

Fig. 2.2.9 Emission intensity of uranium in KH_2PO_4 - NaOH mixture (0.1 M)

λex = 270 nm, λem = 530 nm, sensitivity : 1

(c) Disodium hydrogen phosphate-potassium dihydrogen phosphate buffer (0.1 M)

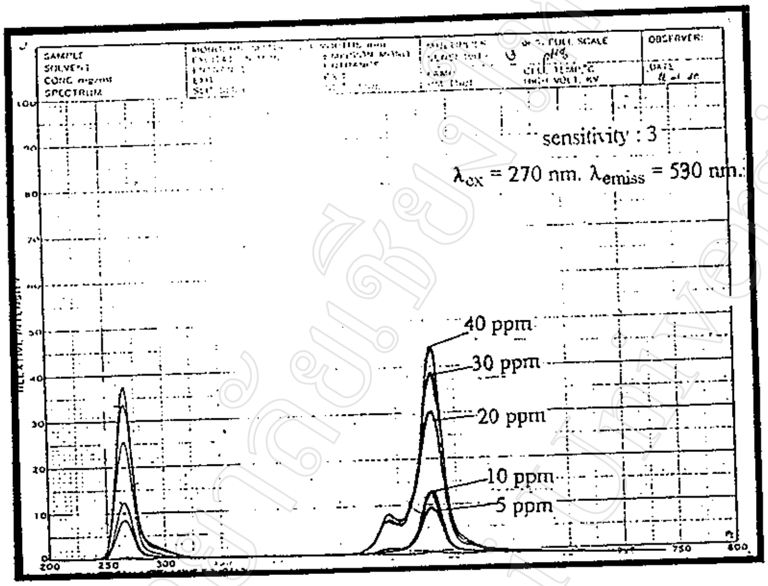

Fig. 2.2.10 Fluorescence spectra of uranium in Na_2HPO_4 - KH_2PO_4 buffer (0.1 M)

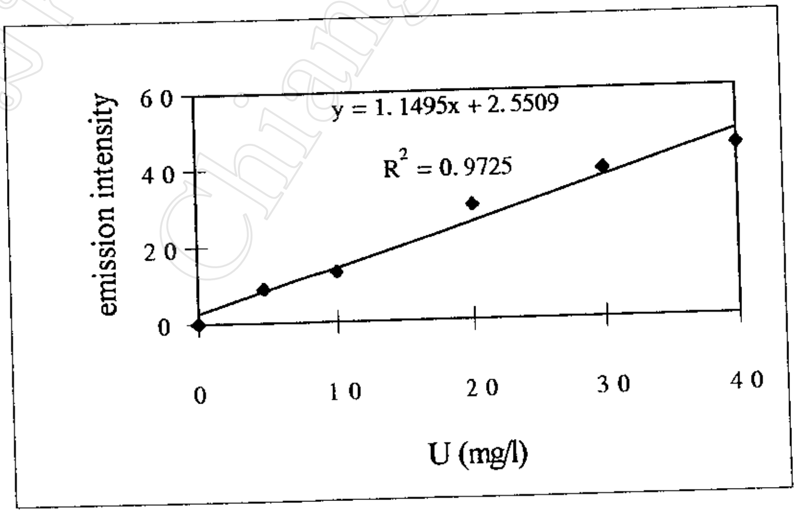

Fig. 2.2.11 Emission intensity of uranium in Na_2HPO_4 - KH_2PO_4 buffer (0.1 M)

λex = 270 nm, λem = 530 nm, sensitivity : 3

Table 2.2.4 Emission intensity of uranium in Na_2HPO_4 - KH_2PO_4 buffer (0.1 M)

$\lambda ex = 270$ nm, $\lambda em = 530$ nm, sensitivity : 3

U (mg/l)	0	5	10	20	30	40
emission intensity (arbitary unit)	0	9	13	30	39	45

2.2.2.2 Other buffers

Buffer solutions without phosphate species were also examined. The spectra obtained were affected by the types of buffer used, as shown in Figs. 2.2.12 - 2.2.18, and Tables 2.2.5 and 2.2.6 .

(a) Ammonium chloride-ammonia buffer (1.0 M)

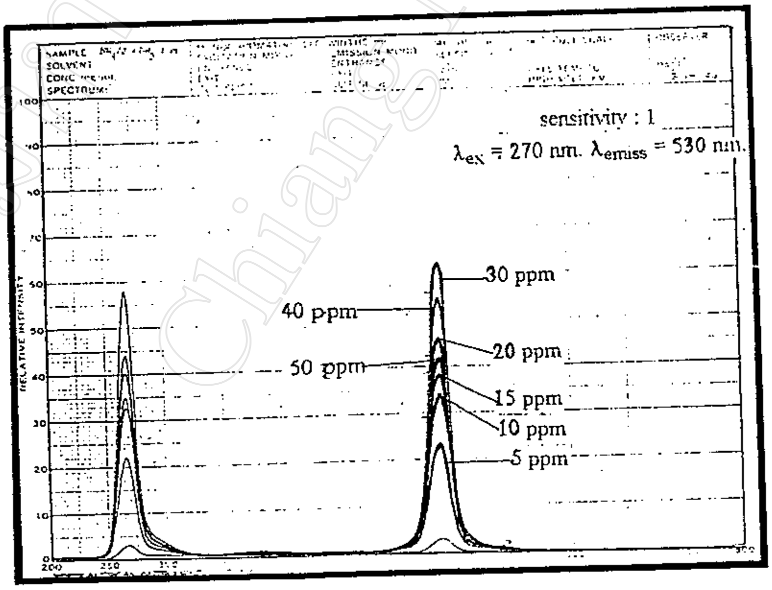

Fig. 2.2.12 Fluorescence spectra of uranium in NH_4Cl - NH_3 buffer (1.0 M)

Table 2.2.5 Emission intensity of uranium in NH_4Cl - NH_3 buffer (1.0 M)

λex = 270 nm, λem = 530 nm, sensitivity : 1

U (mg/l)	0	5	10	15	20	30	40	50
emission intensity (arbitary unit)	0	23	34	38	46	63	55	42

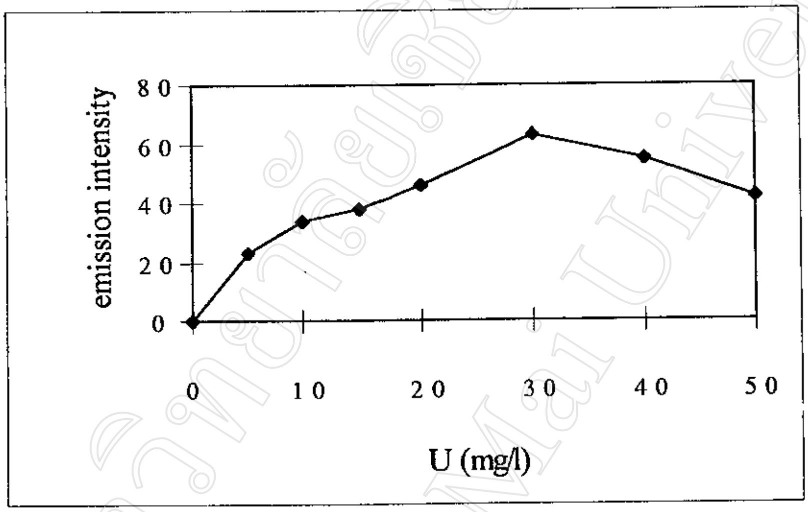

Fig. 2.2.13 Emission intensity of uranium in NH_4Cl - NH_3 buffer (1.0 M)

λex = 270 nm, λem = 530 nm, sensitivity : 1

(b) Ammonium nitrate - ammonia buffer (1.0 M)

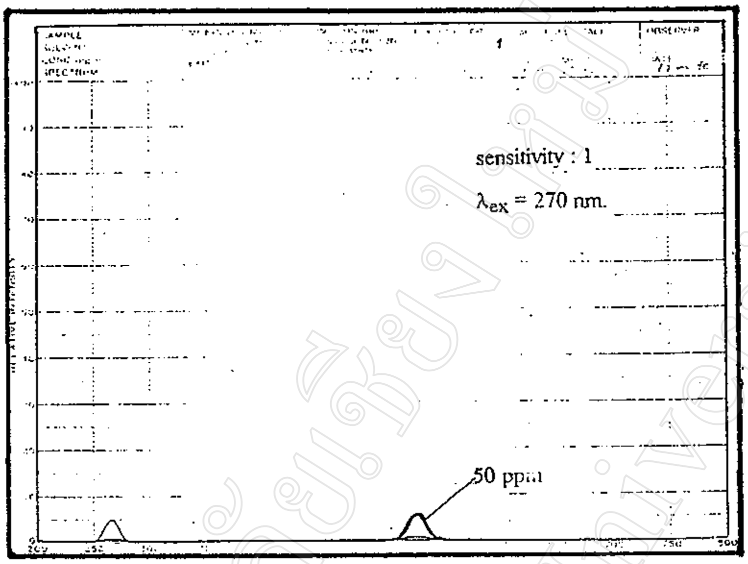

Fig. 2.2.14 Fluorescence spectrum of uranium in NH_4NO_3 - NH_3 buffer (1.0 M)

λex = 270, sensitivity : 1

(c) Triethanolamine buffer (0.1 M)

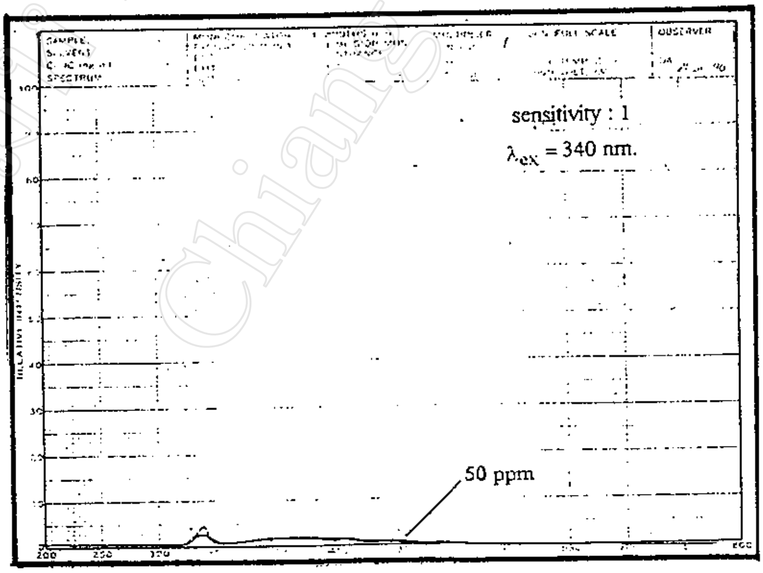

Fig. 2.2.15 Fluorescence spectrum of uranium in triethanolamine buffer (0.1 M)

λex = 340, sensitivity : 1

(d) Glycine - sodium hydroxide buffer (0.1 M)

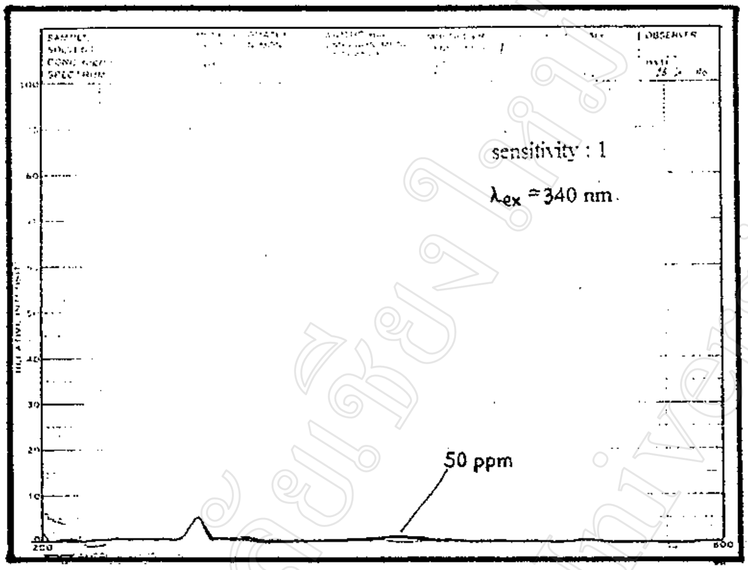

Fig. 2.2.16 Fluorescence spectrum of uranium in glycine - NaOH buffer (0.1 M)

λex = 340, sensitivity : 1

(e) Sodium tetraborate buffer (0.001 M)

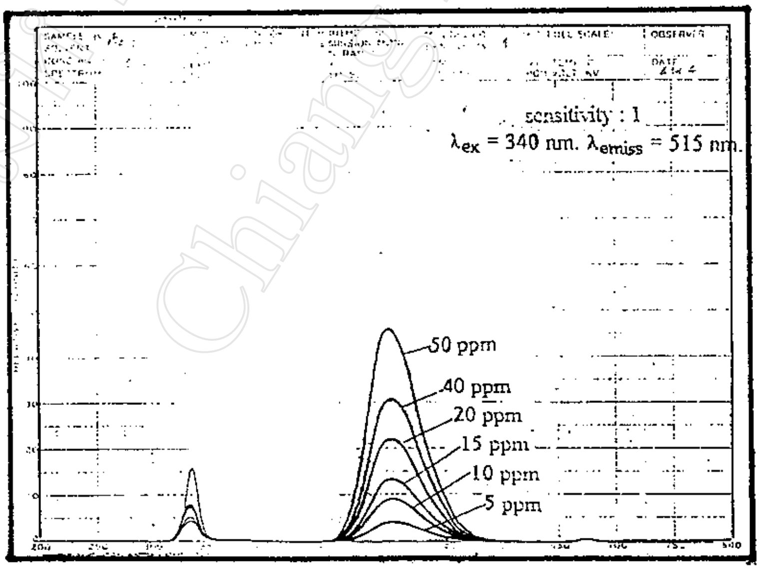

Fig. 2.2.17 Fluorescence spectra of uranium in $Na_2B_4O_7$ buffer (0.001 M)

λex = 340, λem = 515 nm, sensitivity : 1

Table 2.2.6 Emission intensity of uranium in $Na_2B_4O_7$ buffer (0.001 M)

λex = 340 nm, λem = 515 nm, sensitivity : 1

U (mg/l)	0	5	10	15	20	40	50
emision intensity (arbitary unit)	0	4	8	13	22	31	47

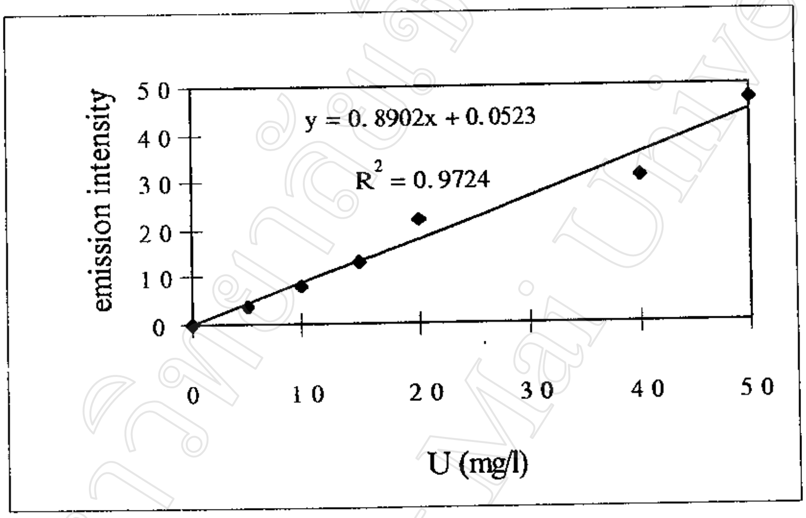

Fig. 2.2.18 Emission intensity of uranium in $Na_2B_4O_7$ buffer (0.001 M)

λex = 340 nm, λem = 515 nm, sensitivity : 1

2.2.2.3 Summary

The wavelengths of maximum excitation and emission found for uranyl ion in the presence of various buffer solutions are shown in Table 2.2.7

Table 2.2.7 Maximum excitation and emission wavelengths of uranyl ion at pH 8

Buffer	**λex**	**λem**	**sens.**
citric acid 0.1 M- Na_2HPO_4 0.2 M	270	530	1
KH_2PO_4-NaOH 0.1 M	270	530	1
KH_2PO_4-Na_2HPO_4 0.1 M	270	530	3
NH_4Cl-NH_3 1.0 M	270	530	1
NH_4NO_3-NH_3 1.0 M	270	-	1
triethanolamine 0.1 M	340	-	1
glycine 0.1 M	340	-	1
$Na_2B_4O_7$ 0.001 M	340	515	1

It was found that all the phosphate buffers can enhance the emission intensity at the excitation wavelength of 270 nm, the maximum intensity occuring in potassium dihydrogen phosphate - disodium hydrogen phosphate solution. Unfortunately, the excitation wavelength in the flow fluorimetric detector was limited at 340 nm. At this wavelength, uranium fluoresces in the presence of sodium tetraborate buffer (0.001 M) solution. Therefore, sodium tetraborate buffer solution at pH 8 was considered to be the appropriate for further study.

2.2.3 Preliminary study on fluorescence spectra of uranium in phosphoric and sulfuric acid solutions.

The presence of phosphoric acid has been reported as enhancing the fluorescence of uranium in solution (25). A similar effect occurs with sulfuric acid. Uranium fluorescence intensity has been found to be considerably enhanced in the presence of a mixture of these acids (65). The effect of those acids was confirmed by recording the fluorescence spectra of uranium standard solutions (5,10, 20, 40 and 50 mg/l) in sulfuric acid (1.3 M), phosphoric acid (1.3 M) and mixtures of the two acids and measuring their fluorescence signals. The spectra obtained are shown in Figs. 2.2.19 - 2.2.22 and the emission intensities are listed in Table 2.2.8. Although, sulfate species or phosphate species alone can enhance the emission intensity. Mixtures containing both acids show the most enhancement.

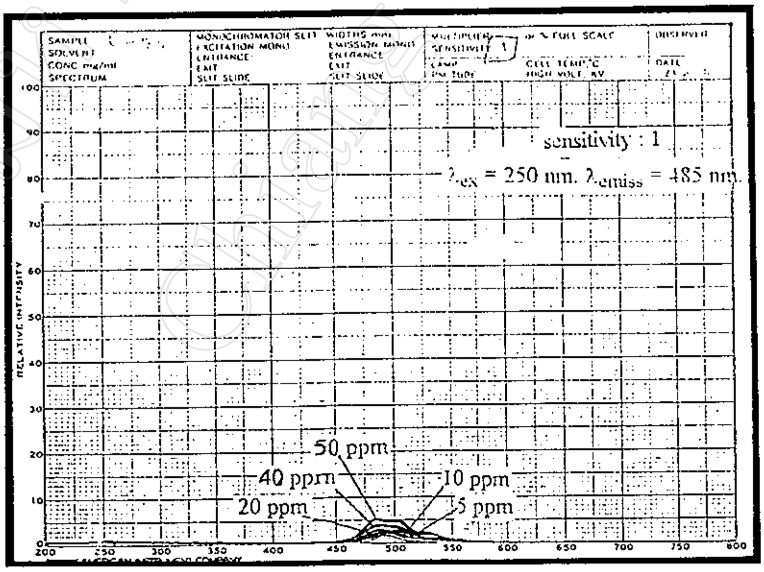

Fig. 2.2.19 Fluorescence spectra of uranium in H_2SO_4 (1.3 M)

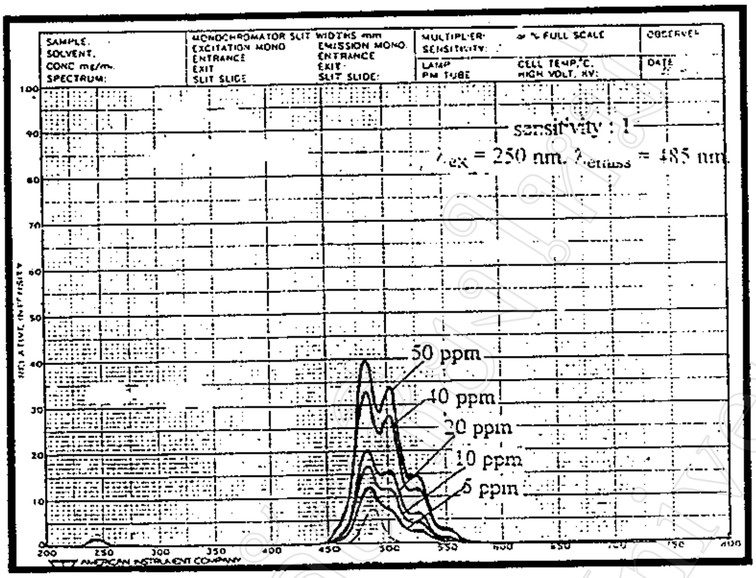

Fig. 2.2.20 Fluorescence spectra of uranium in H_3PO_4 (1.3 M)

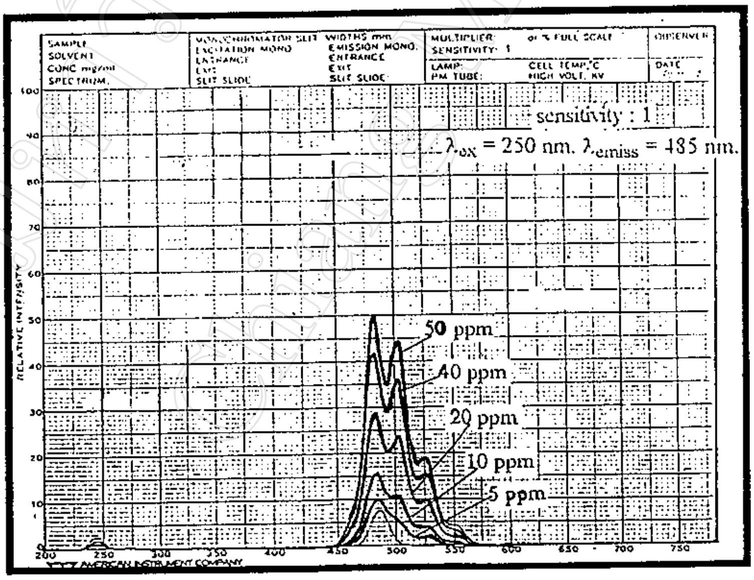

Fig. 2.2.21 Fluorescence spectra of uranium in H_2SO_4-H_3PO_4 (1.3 M)

Table 2.2.8 Fluorescence intensity of uranium in acid solutions

λex = 250, λem = 485, sensitivity : 1

U (mg/l)	emission intensity (arbitary unit)		
	H_2SO_4	H_3PO_4	H_2SO_4/H_3PO_4
0	1	7	7
5	2	12	10
10	2	17	16
20	3	21	29
40	4	33	42
50	5	40	51

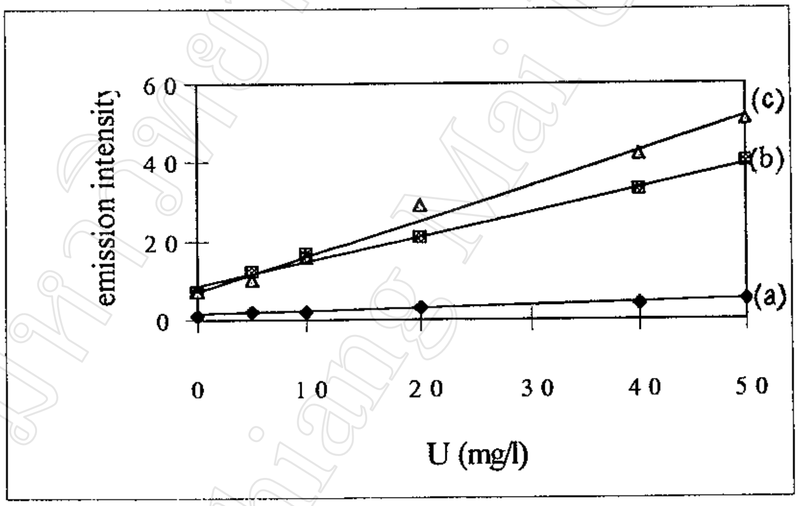

Fig. 2.2.22 Calibration of uranium in acid solutions ; (a) H_2SO_4 (b) H_3PO_4

(c) H_2SO_4/H_3PO_4

2.2.3.1 The effect of pH of acid solutions

Phosphate and sulfate species have been shown to enhance the fluorescence intensity of uranium (section 2.2.3). To study the effect of pH on an emission intensity of phosphate solution uranium standards (30 mg/l) in mixtures of potassium dihydrogen phosphate and disodium hydrogen phosphate (0.001 M) were prepared and adjusted pH values from 2 to 10 using sodium hydroxide, nitric acid, sulfuric acid or phosphoric acid solution. Fluorescence signals were measured under the same conditions as described earlier (section 2.2.1). The results are shown in Table 2.2.9 and Fig. 2.2.23

Table 2.2.9 Fluorescence intensity of uranium in KH_2PO_4-Na_2HPO_4 at various pH values obtained by adjusting to the desire pH values with dil.HNO_3, H_2SO_4 or H_3PO_4.; sensitivity : 1

pH	emission intensity (arbitary unit)		
	HNO_3	H_2SO_4	H_3PO_4
2.0	1	13	2
2.5	-	2	2
3.0	1	150	67
3.5	-	180	161
4.5	-	130	134
5.0	5	89	73
6.5	-	58	65
8.0	6	105	87
9.0	7	5	30

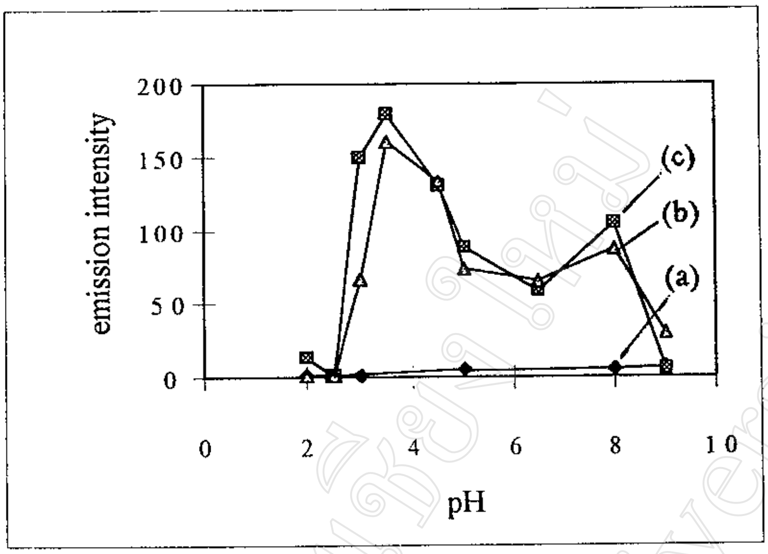

Fig. 2.2.23 Fluorescence intensity of uranium at various pH values ; adjusted with

(a) HNO_3 (b) H_2SO_4 (c) H_3PO_4

At a pH of 3.5 in sulfuric acid or phosphoric acid solution the highest fluorescence intensities were obtained. This result agrees with a previous finding (65). Nitric acid has been reported to be a strong quencher (65). Therefore, phosphate species alone at pH of 3 to 4 were chosen for further investigations.

2.2.3.2 Buffer solutions of pH 3 - 4

Various buffers of pH 3 to 4 were chosen as diluents of uranium standard solutions in order to find an appropriate buffer for the FIA technique. A series of uranium standard solutions was prepared using various buffer solutions at pH 3.5 as diluent. Fluorecence intensities were measured under the conditions described in section 2.2.1. Results are shown in Figs. 2.2.24 - 2.2.28 and Tables 2.2.10 and 2.2.11.

(a) Sodium acetate - acetic acid buffer (0.1 M)

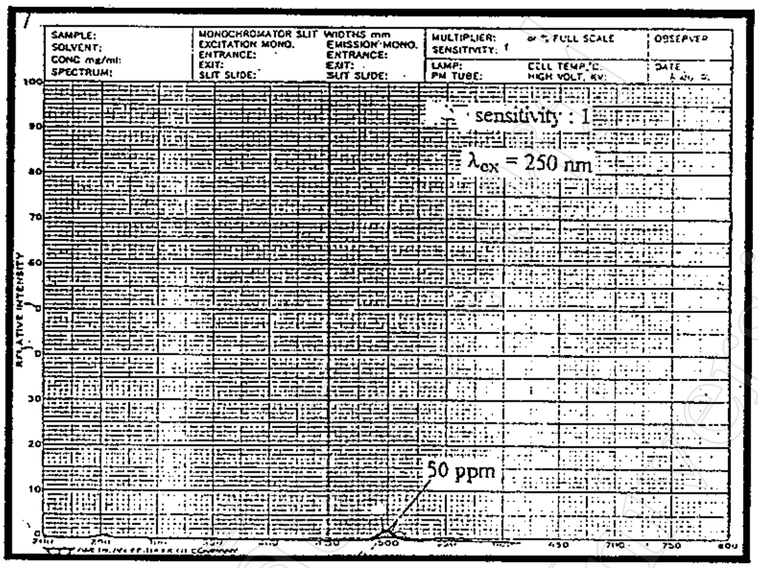

Fig. 2.2.24 Fluorescence spectrum of uranium in sodium acetate-acetic acid buffer (0.1 M)

(b) Potassium hydrogen tartrate buffer (0.03 M)

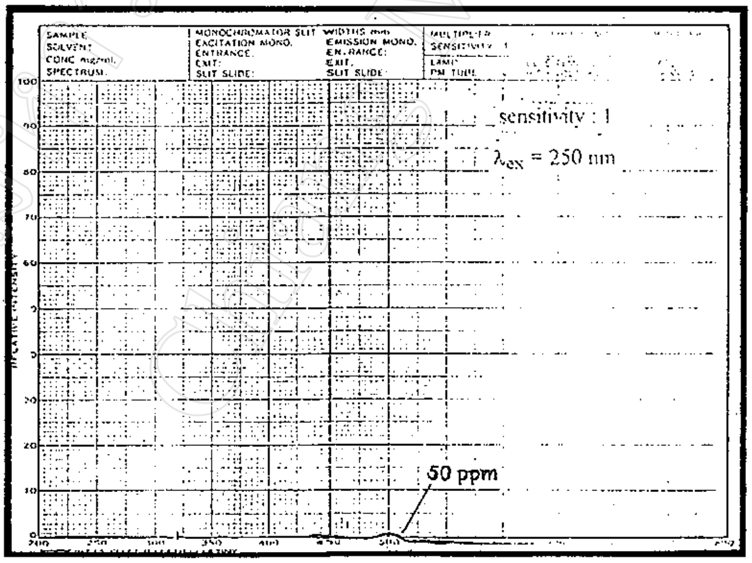

Fig. 2.2.25 Fluorescence spectrum of uranium in potassium hydrogen tartrate buffer (0.03 M)

(c) Sodium formate - formic acid buffer (0.1 M)

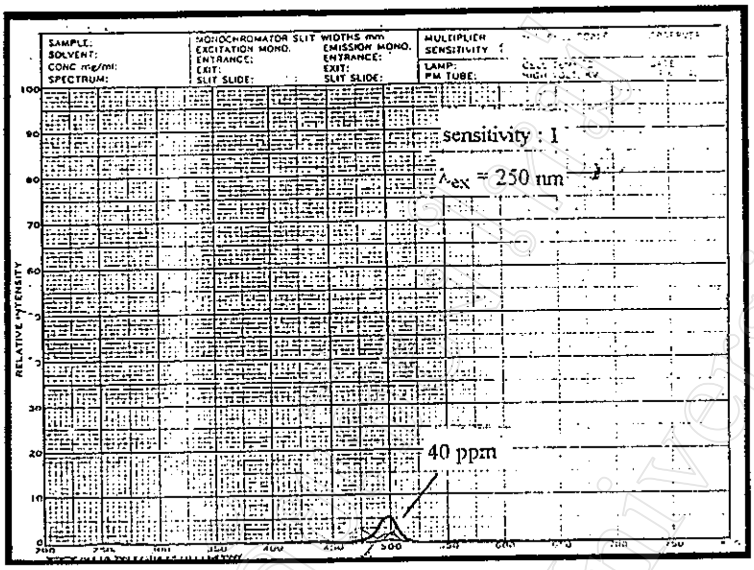

Fig. 2.2.26 Fluorescence spectrum of uranium in sodium formate-formic acid buffer (0.1 M)

(d) Glycine - hydrochloric acid buffer (0.1 M)

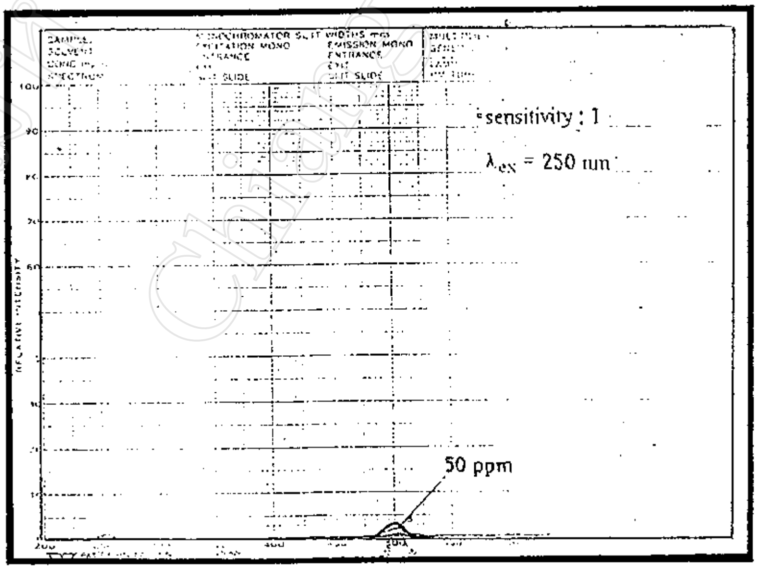

Fig. 2.2.27 Fluorescence spectrum of uranium in glycine-HCl buffer (0.1 M)

(e) Dipotassium hydrogen phosphate - phosphoric acid buffer (0.1 M)

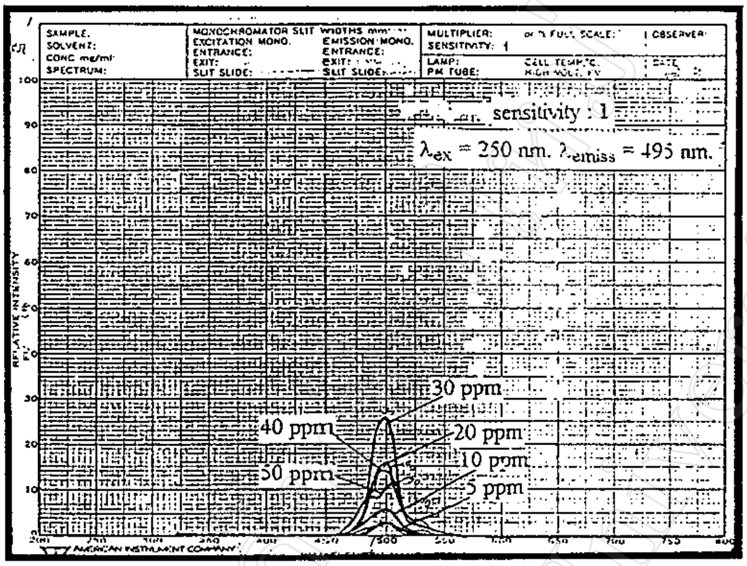

Fig. 2.2.28 Fluorescence spectra of uranium in KH_2PO_4-H_3PO_4 buffer (0.1 M)

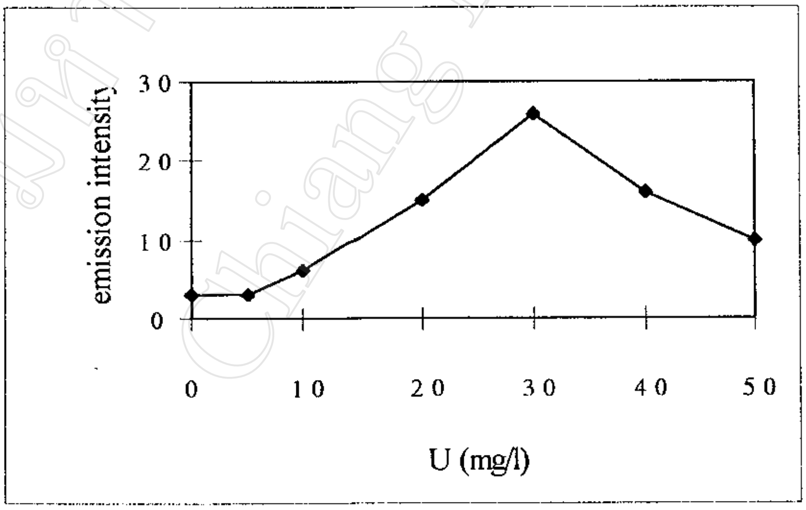

Fig. 2.2.29 Calibration for uranium in K_2HPO_4-H_3PO_4 buffer (0.1 M)

Table 2.2.10 Uranium intensity in K_2HPO_4-H_3PO_4 buffer (0.1 M)

U (mg/l)	0	5	10	20	30	40	50
emission intensity (arbitary unit)	3	3	6	15	26	16	10

The wavelengths of maximum excitation and emission found in the presence of various buffer solutions at pH 3.5 are shown in Table 2.2.11. Of the buffer solutions examined, it was found that uranium can only fluoresce in dipotassium hydrogen phosphate - phosphoric acid buffer solution indicating that phosphate species enhance emission but the other species quench it.

Table 2.2.11 Summary of maximum excitation and emission wavelengths of uranyl ion at pH 3.5

Buffer	**λex**	**λem**	**sens.**
sodium acetate-acetic acid 0.1 M	254	-	1
potassium hydrogen tartrate 0.03 M	254	-	1
sodium formate-formic acid 0.1 M	254	-	1
glycine- HCl 0.1 M	254	-	1
K_2HPO_4-H_3PO_4 0.1 M	254	495	1

2.2.4 Development of flow injection analysis systems

2.2.4.1 Single line manifold

Uranium in sodium tetraborate solution (0.001 M) fluoresces with a maximum emission intensity at 515 nm, when excited at 340 nm (section 2.2.2). The sodium tetraborate buffer was chosen because of the availability of an excitation filter at 340 nm, the optimum excitation wavelength in the presence of this buffer. Uranium standard solutions were injected into the carrier stream of sodium tetraborate solution (0.001 M) which was then passed through a mixing coil to a flow cell of a fluorimetric detector connected to a chart recorder.

(a) The experimental set-up

An HPLC fluorescence detector (Model 420 ; Water-Associates, Inc.) was used. The flow cell of the detector was replaced by a larger quartz cell (see appendix D) in order to diminishing back pressure which causes leakage. The excitation and emission wavelengths were adjusted by changing the filters.

The maximum uranium emission intensity was 515 nm in the green region of the visible spectrum. Unfortunately, there was no emission wavelength filter available at this wavelength, so the emission filter was replaced with GG (a piece of cellophane sheet) or PP (a piece of polypropylene (atactic) sheet). Uranium standard solutions (5, 10, 20, 30, 40 and 50 mg/l) were injected into the single line manifold shown in Fig. 2.2.30 .

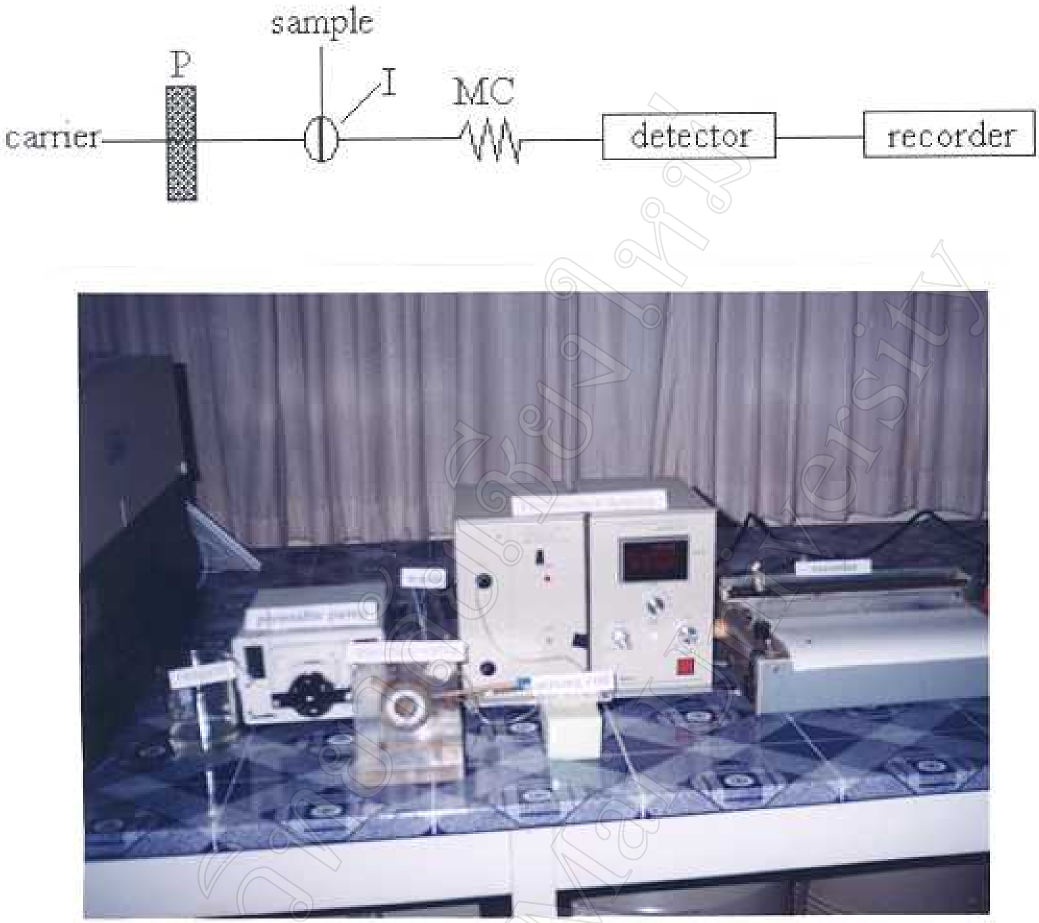

Fig. 2.2.30 Single line manifold for the fluorimetric determination of uranium ;

P= peristaltic pump, I = injection valve, MC = mixing coil.

Preliminary conditions were as follows :

carrier stream = 0.001 M $Na_2B_4O_7$

carrier flow rate = 2 ml/min

sample volume = 200 µl

mixing coil = 70 cm

excitation wavelength = 340 nm

emission wavelength = 515 nm

detector Gain Factor = 2

detector Span Factor = minimum

recorder sensitivity = 0.001 V.

The results obtained, shown in Figs. 2.2.31-2.2.32 and Tables 2.2.12-2.2.13, indicated that responses obtained using PP are greater than those obtained using GG. With 2 PP filters, the blank solution showed a high intensity which was out of range unless the recorder was set at low sensitivity, resulting in low sample peak heights. With 3 PP filters the baseline was not stable : therefore, 4 PP filters were chosen as a compromise to replace the emission filter.

Table 2.2.12 Emission intensity of uranium when using GG as an emission filter

U(mg/l)	peak height (mV)				
	no. of filters each of thickness 0.01 mm				
	1	2	3	4	5
0	overrange	0	0	0	0
3	overrange	0.	0	0	0
5	overrange	0	0	0	0
10	overrange	0.12	0.06	0.04	0.02
20	overrange	0.20	0.13	0.08	0.06
30	overrange	0.27	0.17	0.11	0.08
40	overrange	0.30	0.22	0.13	0.09
50	overrange	0.38	0.26	0.15	0.12

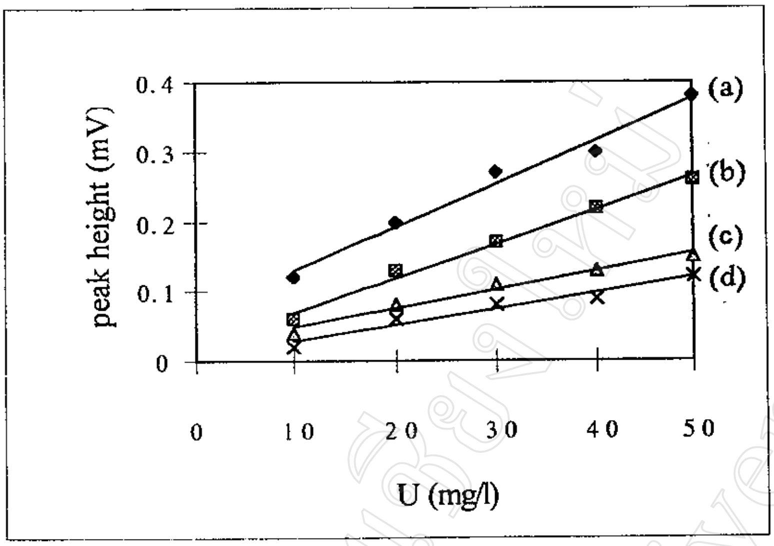

Fig. 2.2.31 Calibration with GG emission filters ; (a) 2 (b) 3 (c) 4 (d) 5 filters

Table 2.2.13 Emission intensity of uranium when using PP as an emission filters (a) 2 (b) 3 (c) 4 (d) 5 filters

U (mg/l)	peak height (mV)				
	no. of filters each of thickness 0.01 mm				
	1	2	3	4	5
0	overrange	0	0	0	0
3	overrange	0	0.02	0.02	0.01
5	overrange	0.07	0.06	0.05	0.04
10	overrange	0.20	0.16	0.14	0.11
20	overrange	0.43	0.37	0.32	0.24
30	overrange	0.64	0.54	0.48	0.37
40	overrange	0.82	0.73	0.66	0.52
50	overrange	0.92	0.81	0.72	0.58

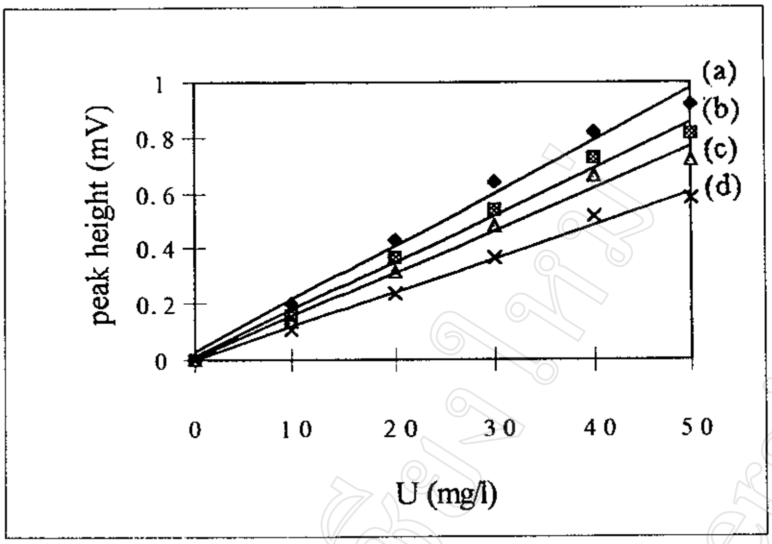

Fig. 2.2.32 Calibration with PP emission filters ; (a) 2 (b) 3 (c) 4 (d) 5 filters

(b) Optimisation

Carrier flow rate

Using the single line manifold (Fig. 2.2.30) and preliminary conditions [section (a)] the effect of flow rate was monitored at an excitation wavelength of 340 nm with 4 PP filters for emission. Flow rates were varied from 1 to 4 ml/min. Results, show in Table 2.2.14 and Fig 2.2.33, indicate that an appropriate flow rate is 3 ml/min.

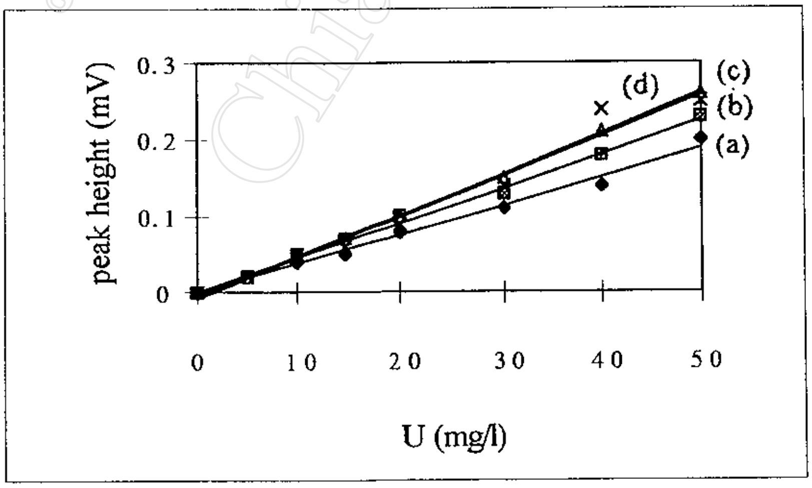

Fig. 2.2.33 Effect of carrier flow rates on peak height ; (a) 1 (b) 2 (c) 3 (d) 4 ml/min.

Table 2.2.14 Effect of carrier flow rates on peak height

U (mg/l)	peak height (mV)			
	carrier flow rate (ml/min)			
	1	2	3	4
0	0	0	0	0
5	0.02	0.02	0.02	0.02
10	0.04	0.05	0.05	0.05
15	0.05	0.07	0.07	0.06
20	0.08	0.10	0.10	0.09
30	0.11	0.13	0.15	0.14
40	0.14	0.18	0.21	0.24
50	0.20	0.23	0.26	0.25

Sodium tetraborate concentration

Uranium standard solutions were injected into carrier streams of various sodium tetraborate concentrations. Results obtained are shown in Fig. 2.2.34 and Table 2.2.15.

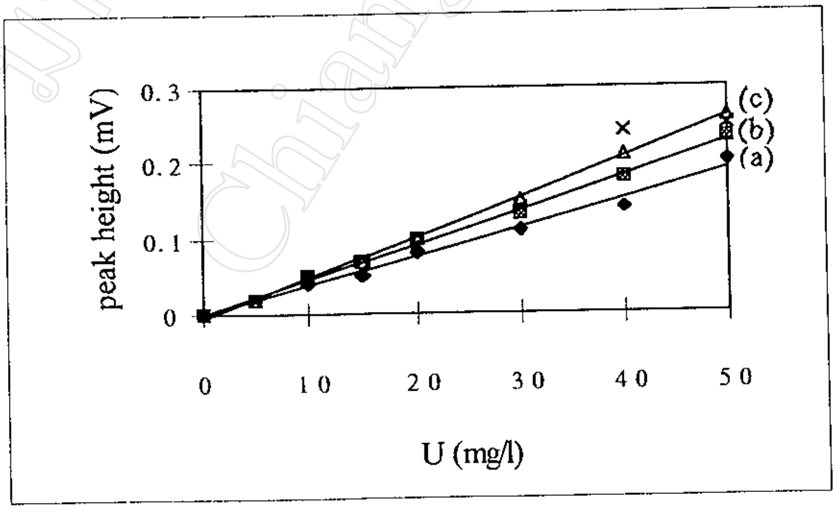

Fig. 2.2.34 Effect of $Na_2B_4O_7$ concentrations on peak height ; (a) 0.1 (b) 0.01 (c) 0.001 M

Table 2.2.15 Effect of $Na_2B_4O_7$ concentrations on peak height

U (mg/l)	peak height (mV)		
	$Na_2B_4O_7$ (M)		
	0.1	0.01	0.001
0	0	0	0
5	0	0.02	0.02
10	0.01	0.05	0.08
15	0.02	0.08	0.10
20	0.06	0.15	0.17
30	0.10	0.23	0.26
40	0.14	0.29	0.34
50	0.18	0.34	0.41

It was found that 0.001 M sodium tetraborate solution was an appropriate carrier stream concentration. Higher concentrations quenched the fluorescence signal. Also, if the concentration was too low the peak height was not enhanced.

Mixing coil length

Mixing coil lengths of 0 and 70 cm were examined. Results, show in Table 2.2.16 and Fig. 2.2.35, indicate that the peak height decreased when the mixing coil length was increased due to increased dispersion if a mixing coil was used. For optimum conditions a mixing coil was not therefore needed.

Table 2.2.16 Effect of mixing coil lengths on peak height.

U (mg/l)	peak height (mV)	
	length of mixing coil (cm)	
	0	70
0	0	0
5	0.03	0.02
10	0.07	0.05
15	0.09	0.07
20	0.13	0.10
30	0.19	0.13
40	0.25	0.18
50	0.32	0.23

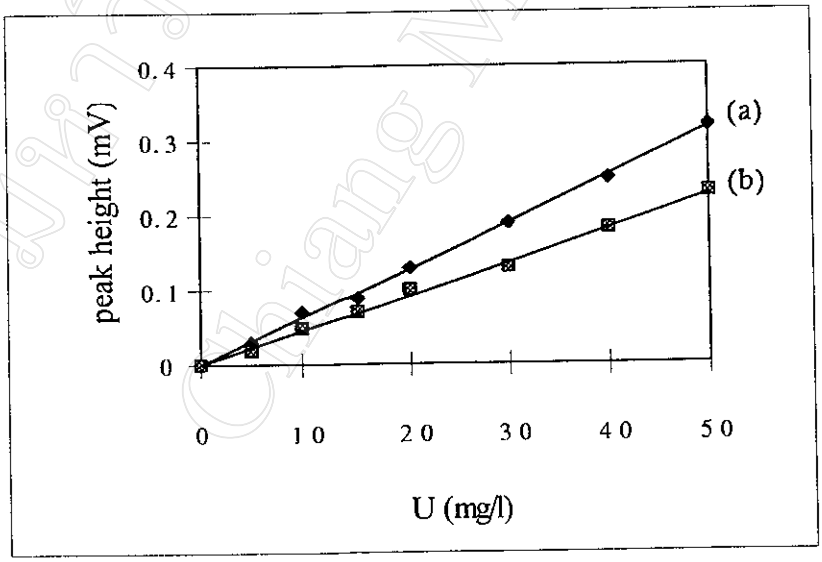

Fig. 2.2.35 Effect of mixing coil lengths on peak height ; (a) 0 (b) 70 cm

Sample volume

The effect of sample volume was investigated by varying the lengths of the sample loop. Results, show in Table 2.2.17 and Fig 2.2.36, indicate that the highest response was obtained with a sample volume of 300 μl .

Table 2.2.17 Effect of sample volumes on peak height

U (mg/l)	peak height (mV)		
	sample volume (μl)		
	200	300	400
0	0	0	0
5	0.02	0.02	0.02
10	0.08	0.08	0.08
15	0.10	0.12	0.12
20	0.17	0.20	0.19
30	0.26	0.29	0..28
40	0.34	0.39	0.34
50	0.41	0.52	0.48

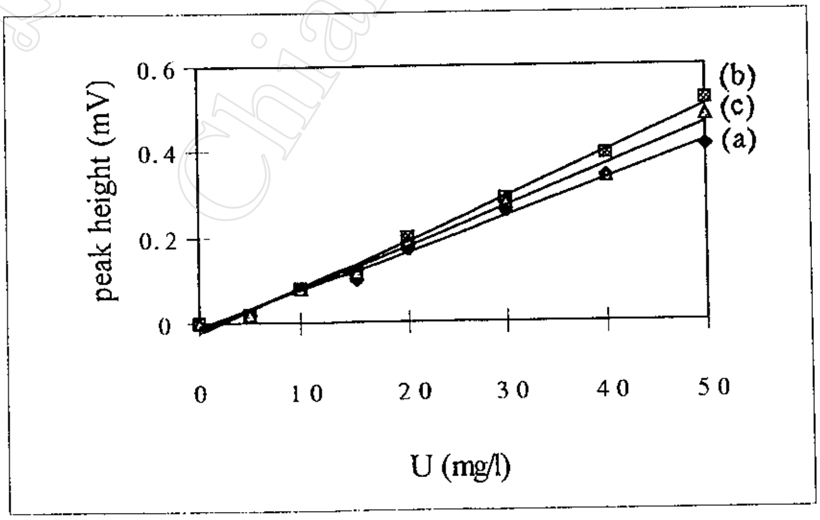

Fig. 2.2.36 Effect of sample volumes on peak height : (a) 200 (b) 300 (c) 400 μl

Summary of optimum conditions

Using the single line FIA manifold shown in Fig. 2.2.30, the optimum conditions obtained are as follows :

carrier concentration = $Na_2B_4O_7$ 0.001 M

carrier flow rate = 3 ml/min

mixing coil length = 0 cm

sample volume = 300 μl

excitation wavelength = 340 nm

emission wavelength = 515 nm

number of PP emission filters of thickness 0.01 mm each = 4

(c) Buffering capacity of sodium tetraborate

Uranium standard solutions of 10 mg/l were prepared by dilution of 100 mg/l uranium standards with 0.001 M sodium tetraborate. The 100 mg/l uranium standards were each adjusted to pH values of from pH 2 to 10.5 prior to dilution to 10 mg/l, the resulting pH values of each were measured. Results, show in Table 2.2.18 and Fig. 2.2.37 indicate that when the 100 mg/l uranium standard solution has a pH between 6 and 9.5, the pH of final solution is 8 which is the optimum pH. So the 0.001 M sodium tetraborate can be considered as the suitable buffer solution in this system if the pH of the sample lies within these limits.

Table 2.2.18 Effect of initial pH on that of the final solution (0.001 M $Na_2B_4O_7$ as diluent)

pH of U 100 mg/l	2	3	4	5	5.5	6	6.5	7	8	9	9.5	10.5
pH of U 10 mg/l	3.6	7.2	7.5	7.7	7.8	8	8	8	8	8	8	8.1

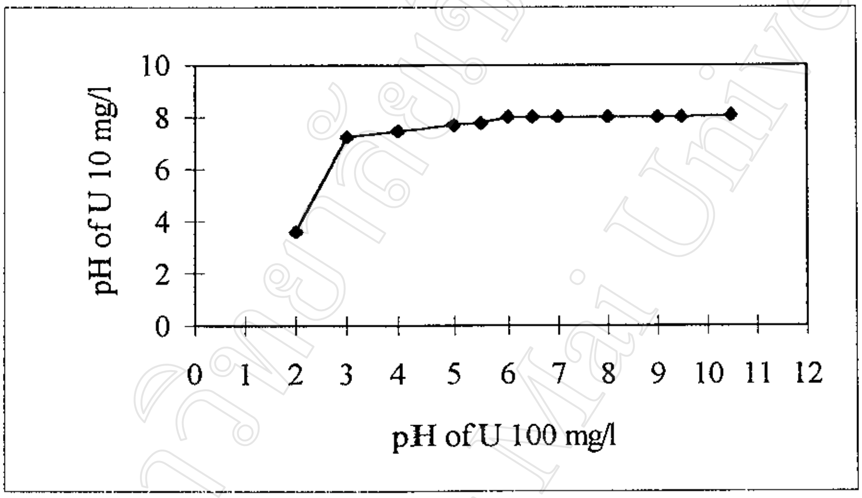

Fig. 2.2.37 Effect of initial pH on peak height (0.001 M $Na_2B_4O_7$ as diluent)

(d) Comparision of conventional filter with plastic filters

Uranium in 0.001 M sodium tetraborate can fluoresce with a maximum emission intensity at 515 nm (green light). It has been shown [section (a)] that 4 PP filters can be used in place of a normal emission filter. The efficiency of 4 PP filters was compared with that of the normal emission filter. The results are shown in Table 2.2.19 and Fig. 2.2.38.

Table 2.2.19 The emission efficiency of a normal glass filter compared with that of 4 PP filters

U (mg/l)	peak height (mV)		
	4 pieces of PP	filter 495 nm.	filter 530 nm.
0	0	0	0
5	0.02	0.09	0.06
10	0.08	0.22	0.15
15	0.10	0.39	0.24
20	0.17	0.48	0.32
30	0.26	0.78	0.54
40	0.34	1.07	0.74
50	0.41	1.15	0.67

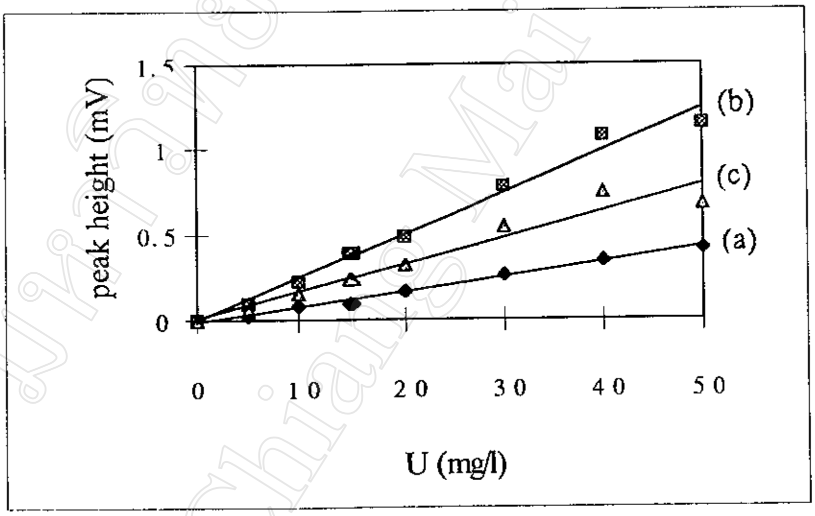

Fig. 2.2.38 Calibrations using a normal glass filter compared with that of 4 PP filters : (a) 4 PP filters (b) glass filter, 495 nm (c) glass filter, 530 filter

Results indicate that glass emission filters gave better sensitivities than those obtained from plastic filters. Also glass filters cut-off more scattered light. However, the sensitivity obtained from PP filters was considered for use.

(e) Calibration

Following the optimised conditions with a 495 nm emission filter, calibrations were found to be linear in a range from 5 to 50 mg/l. In a further study, the uranium standard solutions ranging from 1 to 5 mg/l were injected into the proposed FIA system. Results, show in Table 2.2.20 and Fig. 2.2.39, indicate that uranium concentrations of lower than 4 mg/l cannot be detect even when using maximum Gain Factor and Span Factor. The working linear range that was obtained from this FIA system was from 5 to 50 mg/l. The excitation and emission filters used in the study did not correspond to the optimum wavelengths, this yielded the poor sensitivity found at the lower uranium concentrations.

Table 2.2.20 Peak heights obtained with uranium standard solutions from 1 to 5 mg/l; Gain Factor = 32, Span Factor = maximum.

U (mg/l)	0	1	2	3	4	5
peak height (mV)	0	0.02	0.11	0.17	1.43	2.18

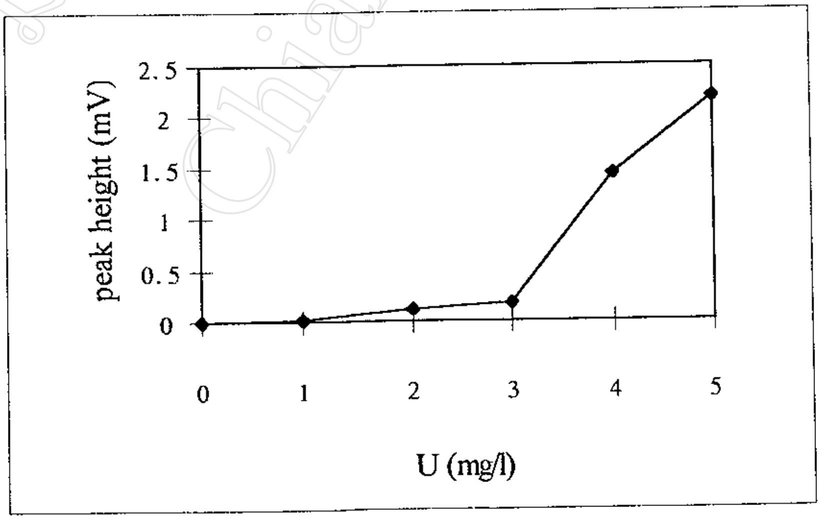

Fig. 2.2.39 Response of uranium standard solutions from 1 to 5 mg/l

2.2.4.2 Two line manifold

In section 2.2.3, it was found that uranium in potassium dihydrogen phosphate - phosphoric acid buffer solution can fluoresce. A flow injection analysis based upon this fluorescence was developed. The single line manifold design is the same as that shown in Fig. 2.2.30 .

A series of uranium standard solutions was injected into a carrier stream of potassium dihydrogen phosphate - phosphoric acid buffer at pH 3.5. The fluorescence of the solution was measured in the flow cell of the fluorimetric detector under the same conditions as those described in section 2.2.4.1. The excitation wavelength was 250 nm and the emission wavelength was 495 nm. The results show that the fluorescence signal was very low : this led to the development of a two line manifold (Fig 2.2.40).

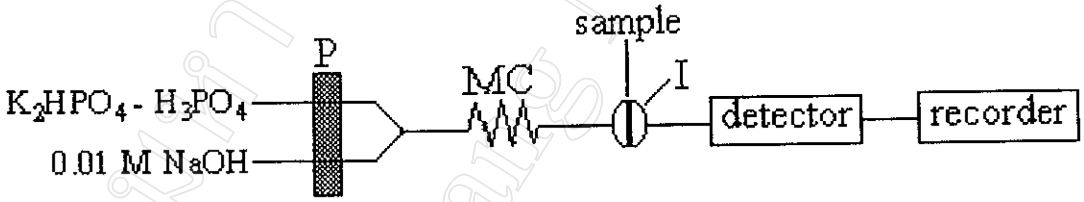

Fig. 2.2.40 Two line manifold for the fluorimetric determination of uranium ;

P = peristaltic pump, I = injection valve, MC = mixing coil.

In the two line manifold, the buffer solution of potassium dihydrogen phosphate - phosphoric acid was prepared at pH 3 and a stream of 0.01 M sodium hydroxide was merged in order to adjust the pH. The sample was injected into the merged stream which was then transported to the flow cell where the emission peak height was recorded. Results obtained from the single line and the two line manifolds are compared in Table 2.2.21 and Fig. 2.2.41. The two line manifold gave the better results than the single line manifold. The stream of the sodium hydroxide solution in the two line manifold is used to adjust the merged solution to a pH closes to the optimum value.

Table 2.2.21 Peak heights obtained from single and two line manifolds
; Gain factor = 8, Span Factor = maximum.

U (mg/l)	peak height (mV)	
	single line	two line
0	8	8
1	6	5
2	9	5
3	10	5
4	16	6
5	19	8
10	21	12
20	10	21
30	13	56

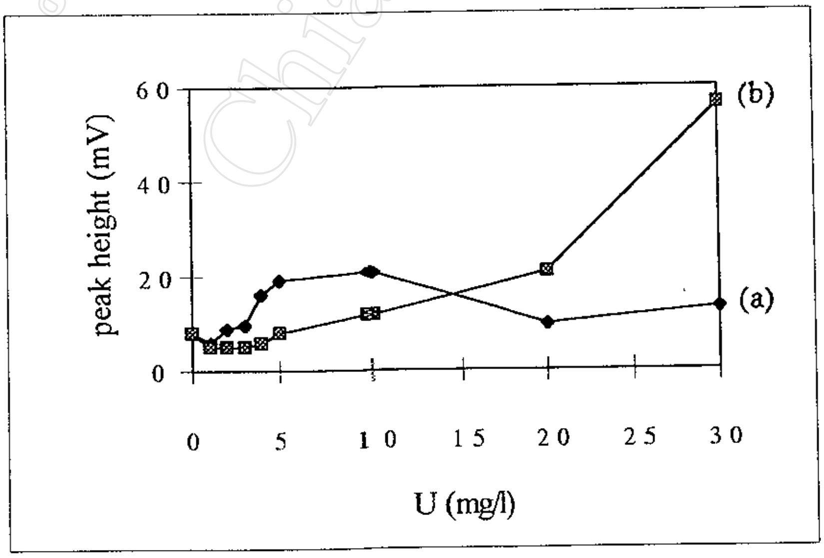

Fig. 2.2.41 Calibrations from single and two line manifolds : (a) single line (b) two line

(a) Sodium hydroxide concentration

A series of uranium standard solutions was injected into FIA system using conditions illustrated in Fig. 2.2.40 but with different sodium hydroxide concentrations. Results, show in Table 2.2.22 and Fig. 2.2.42 indicate that the highest peak heights were obtained using 1.0 M sodium hydroxide although the blank signal at this concentration was high. The 0.1 M sodium hydroxide concentration was considered to give an acceptable peak height and low blank signal and was therefore adapted.

Table 2.2.22 Effect of NaOH concentrations on peak height

U (mg/l)	peak height (mV)		
	NaOH (M)		
	1.0	0.1	0.01
0	0.08	0.02	0.01
5	0.11	0.03	0.03
10	0.13	0.05	0.04
20	0.15	0.07	0.05
30	0.17	0.09	0.07
40	0.19	0.13	0.09

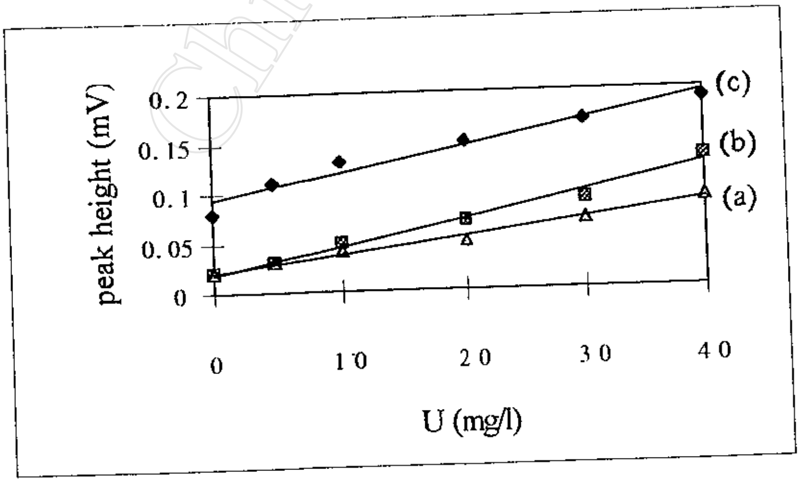

Fig. 2.2.42 Effect of NaOH concentrations on peak height : (a) 0.01 (b) 0.1 (c) 1.0 M

(b) Dipotassium hydrogen phosphate concentration

The concentration of phosphoric acid in the buffer system dipotassium hydrogen phosphate - phosphoric acid was fixed at $1\text{x}10^{-5}$ M and the concentration of dipotassium hydrogen phosphate was varied. Uranium standard solutions were injected into the system with various concentrations of dipotassium hydrogen phosphate by using the conditions illustrated in Fig. 2.2.40. Results, show in Table 2.2.23 and Fig. 2.2.43, indicate that the highest peak is obtained with 0.1 M dipotassium hydrogen phosphate.

Table 2.2.23 Effect of K_2HPO_4 concentrations on peak height.

U (mg/l)	peak height (mV)			
	KH_2PO_4 (M)			
	1×10^{-1}	1×10^{-2}	1×10^{-3}	1×10^{-4}
0	0.03	0.02	0.02	0
5	0.05	0.03	0.03	0.02
10	0.07	0.04	0.04	0.03
20	0.07	0.05	0.05	0.05
30	0.09	0.07	0.06	0.06
40	0.11	0.09	0.09	0.08
50	0.10	0.09	0.06	0.07

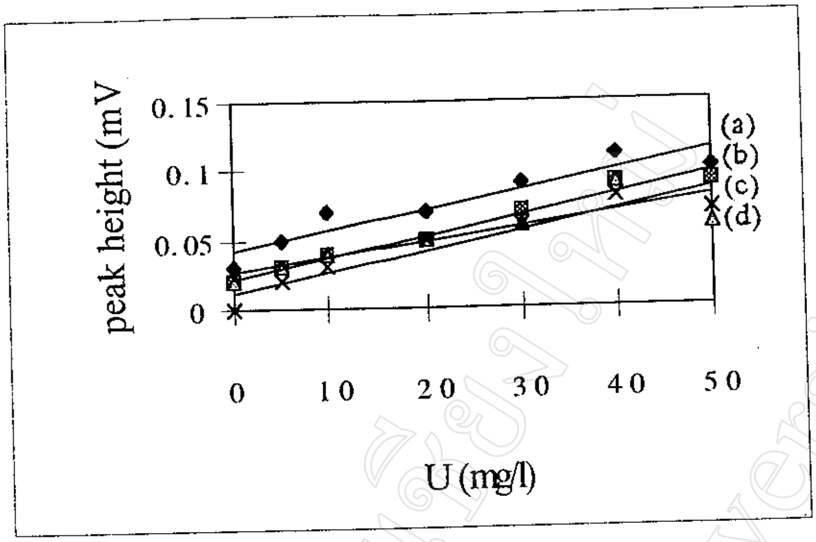

Fig. 2.2.43 Effect of K_2HPO_4 concentrations on peak height ; (a) 1×10^{-1} (b) 1×10^{-2} (c) 1×10^{-3} (d) 1×10^{-4} M

In the present work, a number of parameters have been optimised for the fluorimetric determination of uranium by FIA. However, there are other effects such as flow rate of sodium hydroxide and dipotassium hydrogen phosphate - phosphoric acid buffer which need to be studied in order to fully optimise the conditions for this method.

2.3 Determination of Yttrium by Flow Injection Analysis

2.3.1 Molar absorptivity study

The molar absorptivity of yttrium-Arsenazo III complex was evaluated as described in previous work (55). Absorption spectra of a solution containing yttrium (4.5×10^{-6} M) with colouring reagent (0.001% Arsenazo III (w/v) + acetic/acetate buffer 0.1M) and the reagent blank solution were recorded from 400 to 800 nm (Fig. 2.3.1).

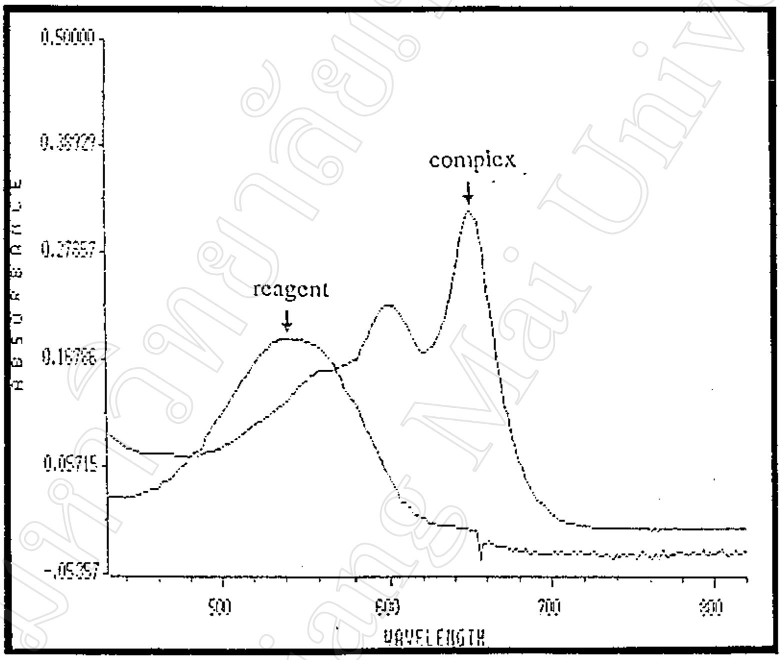

Fig. 2.3.1 Absorption spectra of yttrium-Arsenazo III complex and reagent blank

The spectrum of yttrium-Arsenazo III complex exhibited a maximum absorption at 650 nm. The observed absorbance [A] was 0.322. The molar absorptivity [ε] was estimated to be 71,000 $l.cm^{-1}.M^{-1}$ using Beer' s law [$A = \varepsilon\, b\, c$; $b = 1$ cm and $c = 4.5 \times 10^{-6}$ M]

2.3.2. FIA manifold design

Arsenazo III is a sensitive reagent for yttrium determination, and has therefore been adapted to a simple FIA procedure. Further development has been carried out (55). Yttrium standard solutions (0.01, 0.02, 0.04, 0.06, 0.08 and 0.10 mg/l) were injected into FIA manifolds with either three or two line (Figs. 2.3.2 and 2.3.4 respectively) under the following preliminary conditions.

carrier stream = HCl solution (0.01 M or 0.001 M)

buffer solution = acetic/acetate buffer pH 4.0 (0.1 M or 1.0 M)

colouring reagent = Arsenazo III reagent (0.001 % (w/v))

flow rate (each line) = 2 ml/min

mixing coil length = 100 cm

sample volume = 200 μl

wavelength = 650 nm

2.3.2.1 Three line manifold

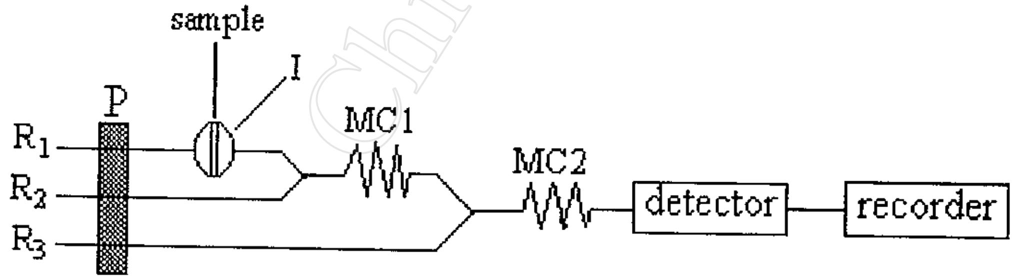

Fig. 2.3.2 Three line FIA manifold : R_1 = carrier solution, R_2 = buffer solution, R_3 = colouring reagent, P = pump, MC = mixing coil, detector = spectrophotometer and I = injection valve.

The yttrium standard solutions were injected into a carrier stream of hydrochloric acid solution [R_1] and merged with a buffer stream [R_2] in mixing coil 1 before merging with a stream of colouring reagent [R_3] in mixing coil 2, and passing to the spectrophotometric detector which was connected to a chart recorder. The results are represented in Table 2.3.1 and Fig. 2.3.3.

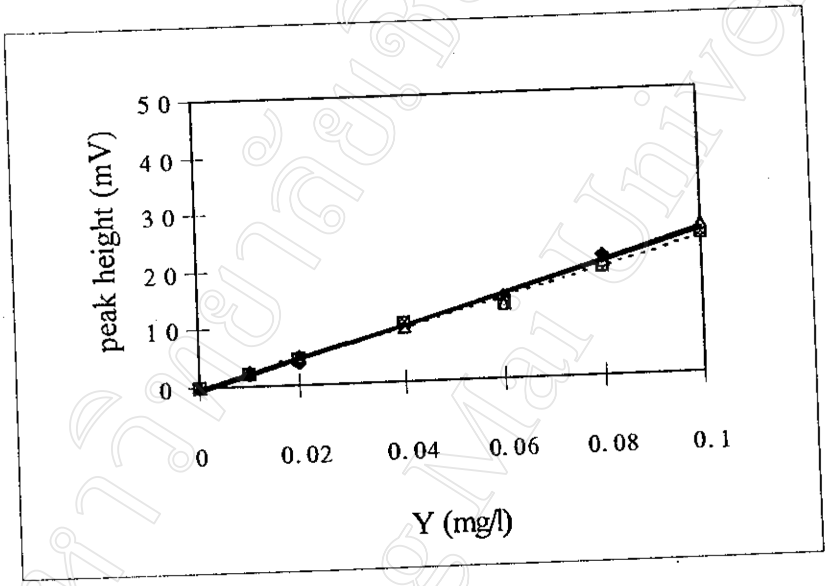

Fig. 2.3.3 Calibrations for yttrium standard solutions with a three line manifold where :

- - - -◆- - - = HCl 0.01 M , acetic/acetate buffer solution 0.1 M

-.-.-.-■-.-.-.- = HCl 0.001 M , acetic/acetate buffer solution 0.1 M

.........▲.......... = HCl 0.01 M , acetic/acetate buffer solution 1.0 M

——×—— = HCl 0.001 M , acetic/acetate buffer solution 1.0 M

Table 2.3.1 Peak heights obtained for yttrium standard solutions using a three line manifold : Line 1; HCl (0.01 M or 0.001 M), Line 2; buffer solution (0.1 M or 1.0 M), Line 3 ; Arsenazo III solution (0.001%)

Yttrium standard (mg/l)	Peak height (mV)			
	buffer solution 0.1 M		buffer solution 1.0 M	
	HCl 0.01 M	HCl 0.001 M	HCl 0.01 M	HCl 0.001 M
0.01	2.0	2.0	2.5	2.4
0.02	4.0	4.7	5.0	5.2
0.04	9.5	10.0	9.3	8.6
0.06	14.2	13.3	13.0	14.5
0.08	21.0	19.0	20.0	21.4
0.10	25.0	24.0	26.0	25.3

2.3.2.2 Two line manifold

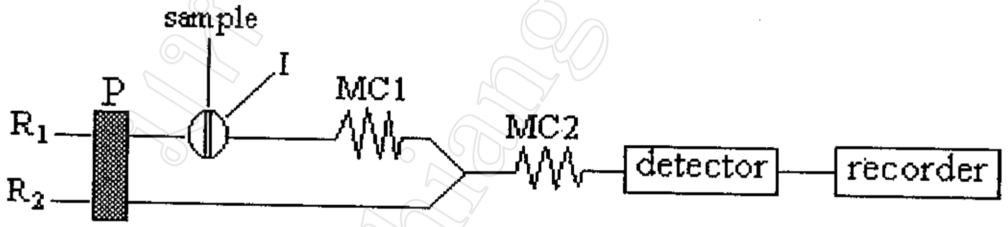

Fig. 2.3.4 Two line FIA manifold : R_1 = carrier solution, R_2 = premixed solution of buffer with colouring reagent, P = pump, I = injection valve, MC = mixing coil, detector = spectrophotometer.

In this manifold design, a mixture of Arsenazo III reagent and acetic/acetate buffer was used as reagent stream [R_2]. A yttrium standard solution was injected into a carrier stream of hydrochloric acid solution [R_1]. The results are shown in Table 2.3.2 and Fig. 2.3.5.

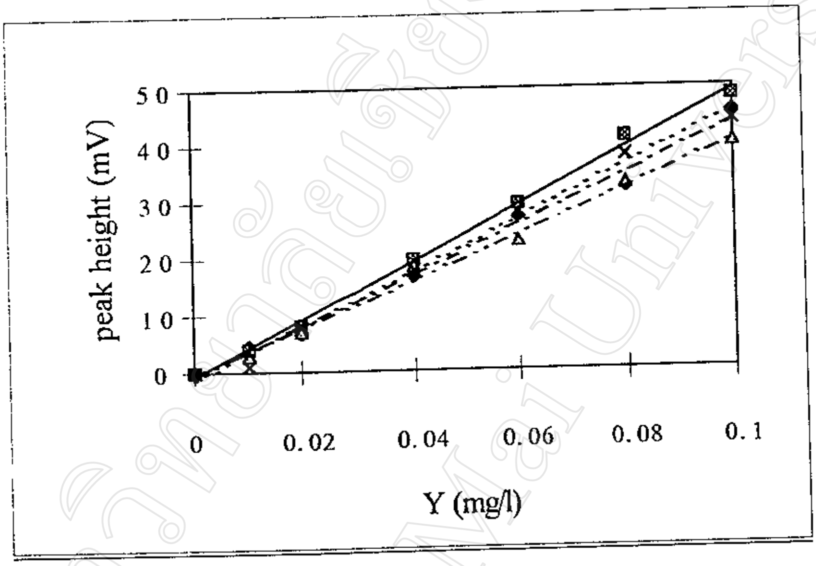

Fig. 2.3.5 Calibrations for yttrium standard solutions with a two line manifold where :

---◆--- = HCl 0.01 M , acetic/acetate buffer solution 0.1 M

——■—— = HCl 0.001 M , acetic/acetate buffer solution 0.1 M

---▲--- = HCl 0.01 M , acetic/acetate buffer solution 1.0 M

·······×······· = HCl 0.001 M , acetic/acetate buffer solution 1.0 M

Table 2.3.2 Peak heights obtained for yttrium standard solutions using a two line manifold : Line 1; HCl (0.01 M or 0.001 M), Line 2; premixed solution of Arsenazo III reagent (0.001%) with buffer solution (0.1 M or 1.0 M).

Yttrium standard (mg/l)	Peak height (mV)			
	buffer solution 0.1 M		buffer solution 1.0 M	
	HCl 0.01 M	HCl 0.001 M	HCl 0.01 M	HCl 0.001 M
0.01	4.7	4.0	3.0	1.0
0.02	6.7	8.3	7.0	8.3
0.04	16.7	19.7	18.7	17.7
0.06	27.3	29.3	22.7	28.0
0.08	32.3	41.0	32.7	38.0
0.10	45.3	48.3	40.0	44.0

Using a two line manifold higher peaks were obtained, at all the concentrations of buffer and carrier stream examined. Using a three line system, the linear equation of the most sensitive calibration of linear equation was $y = 257x - 0.610$ (HCl 0.01 M, acetic/acetate buffer 0.1 M), with a coefficient of linear regression (r^2) of 0.992. Using a two line system, the linear equation of the most sensitive calibration was $y = 500x - 0.665$, $r^2 = 0.997$ (HCl 0.001 M, acetic/acetate buffer solution 0.1 M). The slope of the equation is higher for the two line system indicating its higher sensitivity. The sample was not diluted so much in the two line system as in the three line system. A two line manifold was chosen for further preconcentration studies.

2.3.3 Resins for yttrium preconcentration

Low concentrations of yttrium must be preconcentrated prior to determination. Ion exchange resins were used for this purpose. In-valve preconcentration was used in this study : the resin column being placed in the sample loop of the injection valve. Five types of resin ; Amberlite IRC-50, Dowex-50 WX8, Amberlite-IR 120, Duolite C-225 and Chelex-100, were examined. The resins were packed into glass columns (I.D. 2 mm, length 2 cm), plugged at both ends with teflon wool. Each type of resin was pretreated with 6 M HCl (20) to convert it to the H^+-form. Standard yttrium solutions (0.01 and 0.05 mg/l) were passed through the resin column at a flow rate of 2 ml/min, for 3 or 5 minutes ; the column was then washed with deionized water for 1 minute. After preconcentration, an acid solution was used to elute the yttrium from the column, which was then determined as described earlier. The manifold design is shown in Fig. 2.3.6.

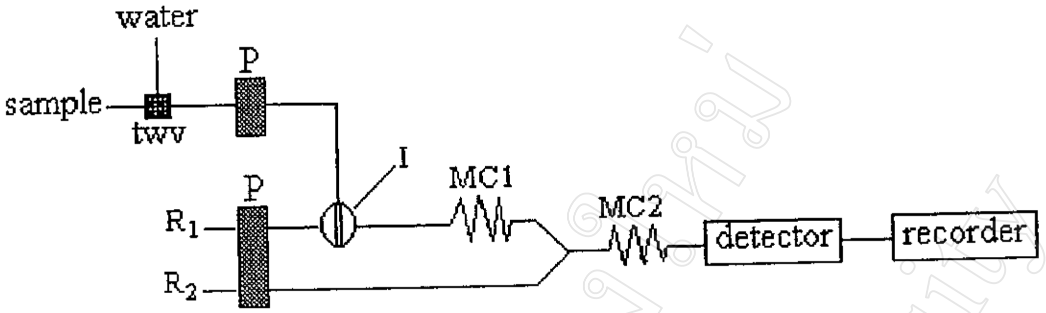

Fig. 2.3.6 FIA manifold for preconcentration study : R_1 = eluent carrier stream, R_2 = premixed solution of buffer with colouring reagent, P = pump. twv = three ways valve, I = injection valve, MC = mixing coil, detector = spectrophotometer.

Preliminary conditions were the same as those used in the FIA manifold design (section 2.3.2) where HCl was the carrier stream (0.01 M or 0.001 M) and reagent stream was a mixture of acetic/acetate buffer pH 4.0 (0.1 M or 1.0 M) and Arsenazo III reagent (0.001%). The results of this study are shown in Tables 2.3.3, 2.3.4 and Figs. 2.3.7, 2.3.8.

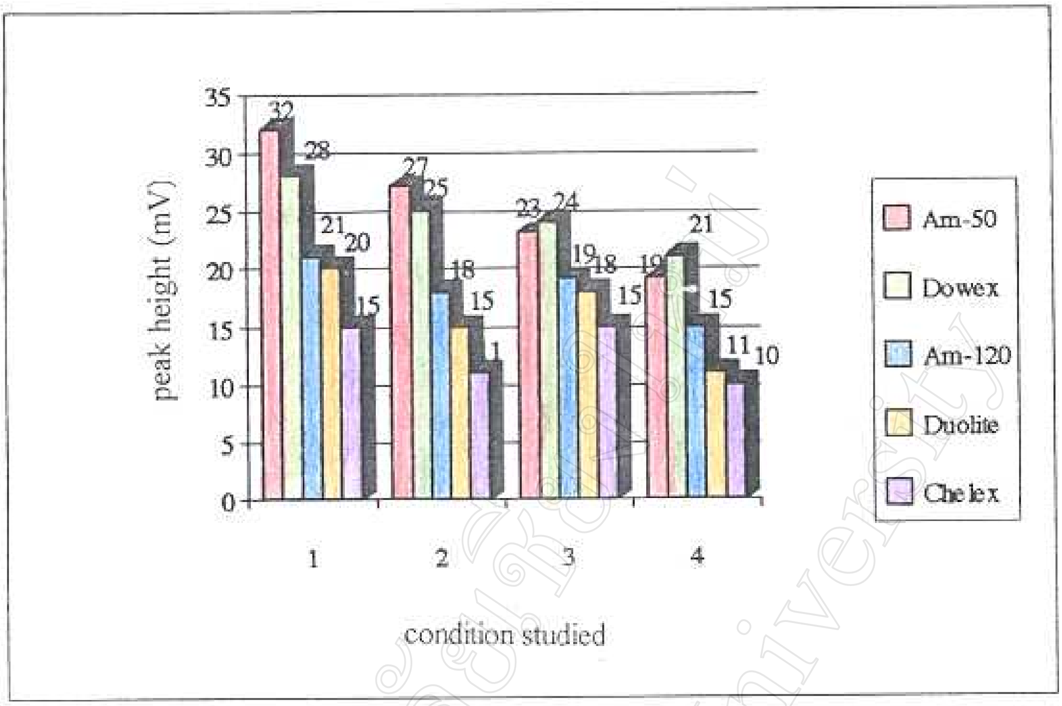

Fig. 2.3.7 Peak heights obtained from various types of resin, using yttrium standard solution 0.01 mg/l with preconcentration time 5 minutes at a loading flow rate of 2 ml/min

condition 1 = buffer solution 0.1 M , HCl 0.01 M

condition 2 = buffer solution 0.1 M , HCl 0.001 M

condition 3 = buffer solution 1.0 M , HCl 0.01 M

condition 4 = buffer solution 1.0 M , HCl 0.001 M

Table 2.3.3 Peak heights of yttrium standard solution (0.01 mg/l) with pre-concentration time of 5 minutes at loading flow rate of 2 ml/min by various types of resin.

Types of resin	Peak height (mV)			
	buffer solution 0.1 M		buffer solution 1.0 M	
	HCl 0.01 M	HCl 0.001 M	HCl 0.01 M	HCl 0.001 M
Amberlite-IRC 50	32.0	27.0	23.0	19.0
Dowex-50 WX8	28.0	25.0	24.0	21.0
Amberlite-IR 120	21.0	18.0	19.0	15.0
Duolite C-225	20.0	15.0	18.0	11.0
Chelex-100	15.0	11.0	15.0	10.0

Table 2.3.4 Peak heights of yttrium standard solution (0.05 mg/l) with a pre-concentration time of 3 minutes at a loading flow rate of 2 ml/min by various types of resin.

Types of resin	Peak height (mV)			
	buffer solution 0.1 M		buffer solution 1.0 M	
	HCl 0.01 M	HCl 0.001 M	HCl 0.01 M	HCl 0.001 M
Amberlite-IRC 50	95.0	84.0	91.0	78.0
Dowex-50 WX8	57.0	69.0	53.0	43.0
Amberlite-IR 120	53.0	47.0	45.0	36.0
Duolite C-225	77.0	68.0	60.0	50.0
Chelex-100	36.0	25.0	28.0	19.0

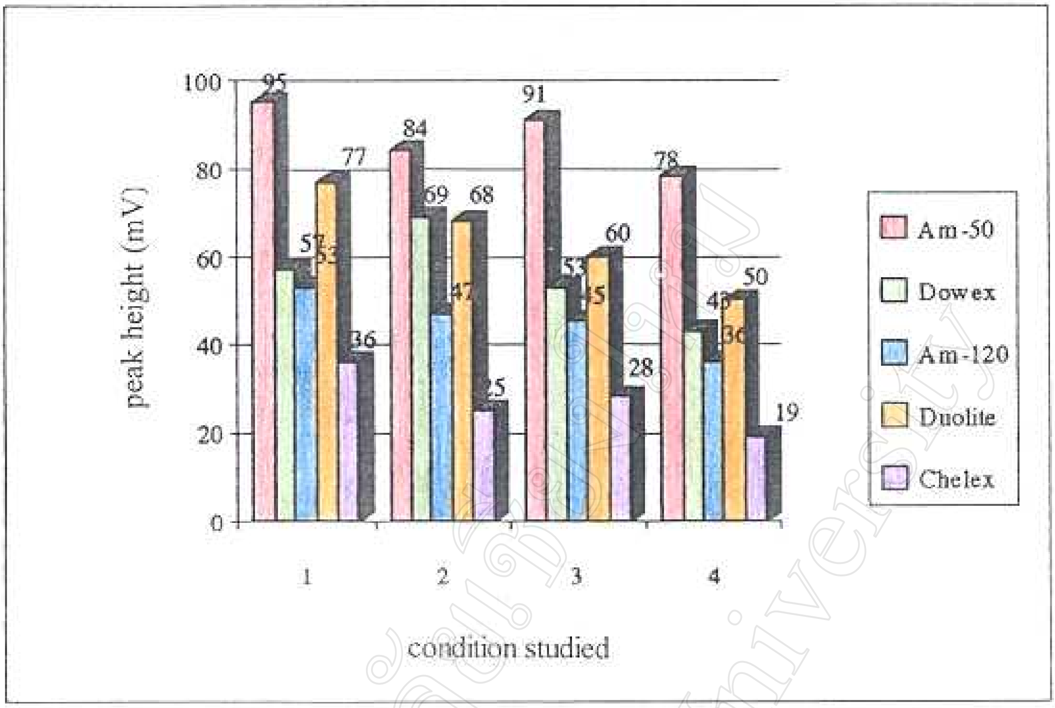

Fig. 2.3.8 Peak heights obtained from various types of resin, using yttrium standard solution (0.05 mg/l) with a preconcentration time of 3 minutes at a loading flow rate of 2 ml/min

condition 1 = buffer solution 0.1 M , HCl 0.01 M

condition 2 = buffer solution 0.1 M , HCl 0.001 M

condition 3 = buffer solution 1.0 M , HCl 0.01 M

condition 4 = buffer solution 1.0 M , HCl 0.001 M

It can be seen that when various conditions were used to find the best resin by varying the concentrations of HCl and acetic/acetate buffer solutions in the FIA system, Amberlite-IRC 50 gave the highest peak heights. Peak heights obtained with the other resins under similar conditions always followed the order Duolite C-225 > Dowex - 50 WX8 > Amberlite-IR 120 > Chelex-100. Amberlite-IRC 50 is superior because it is a weakly acid cation exchanger ; so the yttrium ion bound to the ionogenic groups of the resin to be easily eluted with HCl acid [H^+ ion]. The other resins, are all strongly acid cation exchangers and therefore require the more concentrated acid to elute the yttrium. Also the shortest residence time in the column, indicated by the sharpest response peak, obtained from Amberlite-IRC 50. Therefore, Amberlite-IRC 50 was used for all further investigations.

2.3.4 Optimisation

Optimisation of the system was carried out either by varying each parameter individually to obtain the best conditions [conventional method], or by a method of simplex optimisation, a multiparameter technique.

2.3.4.1 Conventional method

(a) Eluent concentration

Using the manifold shown in Fig. 2.3.6, the concentration of the HCl eluent was varied under the preliminary conditions already described (section 2.3.2). An yttrium standard solution of 0.05 mg/l was passed through the glass column (I.D. 2 mm, length 2 cm) containing Amberlite-IRC 50, for a time of 3 minutes at flow rate of 2 ml/min. Results, show in Table 2.3.5 and Fig. 2.3.9, indicate that 0.01 M HCl solution gave the highest peak. This concentration was chosen for further study.

Table 2.3.5 Effect of HCl concentrations on peak height.

HCl (M)	Peak height (mV)
0.001	239
0.01	305
0.1	28

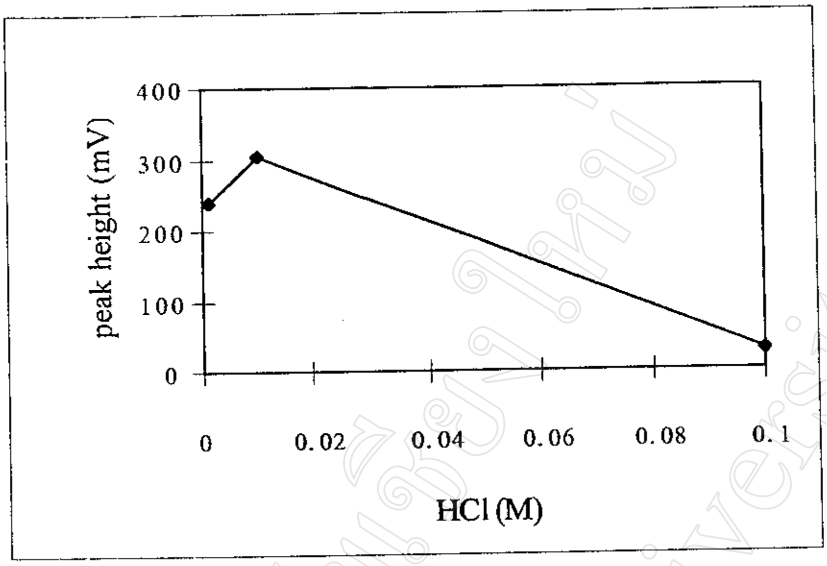

Fig. 2.3.9 Effect of HCl concentrations on peak height

(b) Buffer concentration

At pH 3-4 yttrium forms a complex with Arsenazo III with the highest sensitivity (55, 59). Acetic/acetate buffer pH 4.0 of various concentrations was used with Arsenazo III under the preliminary conditions already described (section 2.3.2). Results, show in Table 2.3.6 and Fig. 2.3.10, indicate that using a 0.1 M buffer solution yielded the highest peak. This concentration of buffer had enough capacity to maintain the reagent in the range over which yttrium-Arsenazo III complex exhibits its highest sensitivity and was used in subsequent experiments.

Table 2.3.6 Effect of buffer concentrations on peak height

buffer solution (M)	Peak height (mV)
0.01	234.0
0.1	305.0
1.0	239.0

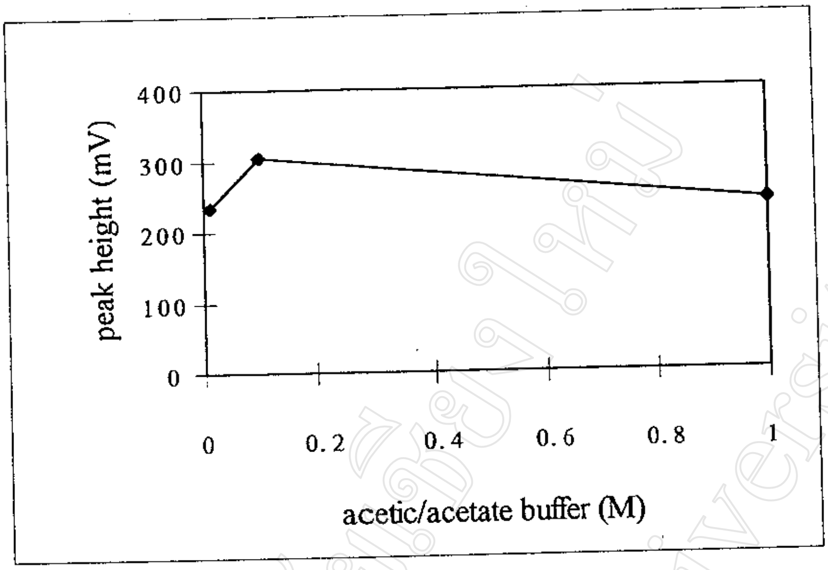

Fig. 2.3.10 Effect of buffer concentrations on peak height.

(c) Arsenazo III reagent concentration

The concentration of reagent was studied using various premixtures with 0.1 M acetic/acetate buffer. Results, show in Table 2.3.7 and Fig. 2.3.11, indicate that Arsenazo III concentrations in the range from 0.001 to 0.005% give a maximum peak height. Higher concentrations cause decreased responses and high blanks. 0.001% Arsenazo III was chosen for further investigations for economic reasons.

Table 2.3.7 Effect of Arsenazo III concentrations on peak height

Arsenazo III (%)	Peak height (mV)
0.001	305.0
0.003	308.0
0.005	303.0
0.007	286.0
0.01	278.0

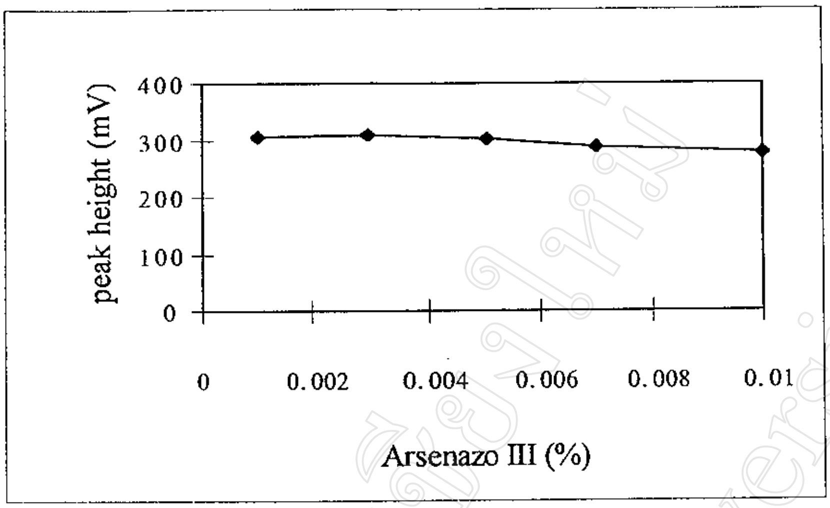

Fig. 2.3.11 Effect of Arsenazo III concentrations on peak height.

(d) Flow rate of eluent

The speed of elution was investigated by passing 0.01 M hydrochloric acid through the resin column at different flow rates. Results, show in Table 2.3.8 and Fig. 2.3.12, indicate that an appropriate flow rate to elute yttrium from the resin column is 2 ml/min.

Table 2.3.8 Effect of flow rates of eluent on peak height

Flow rate of HCl (ml/min)	Peak height (mV)
1.2	165.0
2.0	305.0
2.8	263.0

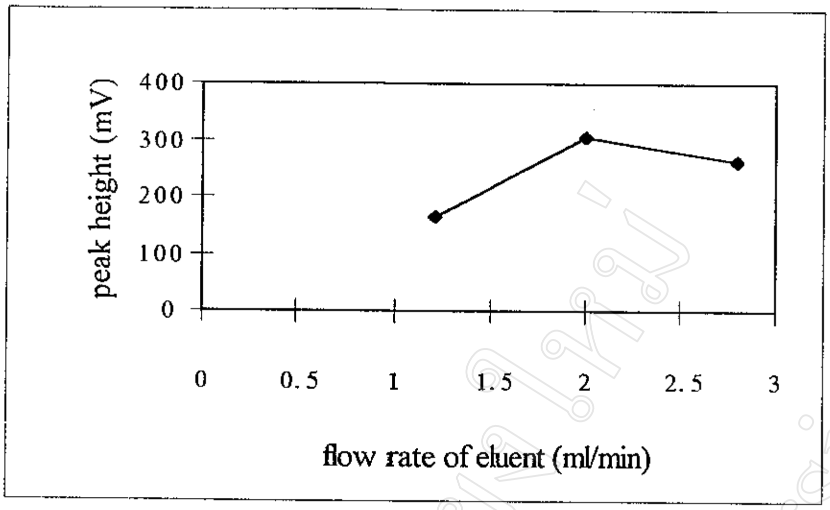

Fig. 2.3.12 Effect of flow rates of eluent on peak height.

(e) Flow rate of reagent

The reagent stream was a mixture of Arsenazo III reagent (0.001%) and acetic/acetate buffer (0.1 M). The effect of changing the flow rate of this reagent stream is shown in Table 2.3.9 and Fig. 2.3.13, from which it can be seen that a flow rate of 2 ml/min is appropriate. The flow rate of reagent influences the extent of formation of the complex. With an appropriate flow rate, yttrium forms complex quantitatively with Arsenazo III, in the presence acetic/acetate buffer controlling the pH range to give the highest sensitivity.

Table 2.3.9 Effect of flow rates of reagent on peak height

Flow rate of reagent (ml/min)	Peak height (mV)
1.2	254.0
2.0	305.0
2.8	233.0

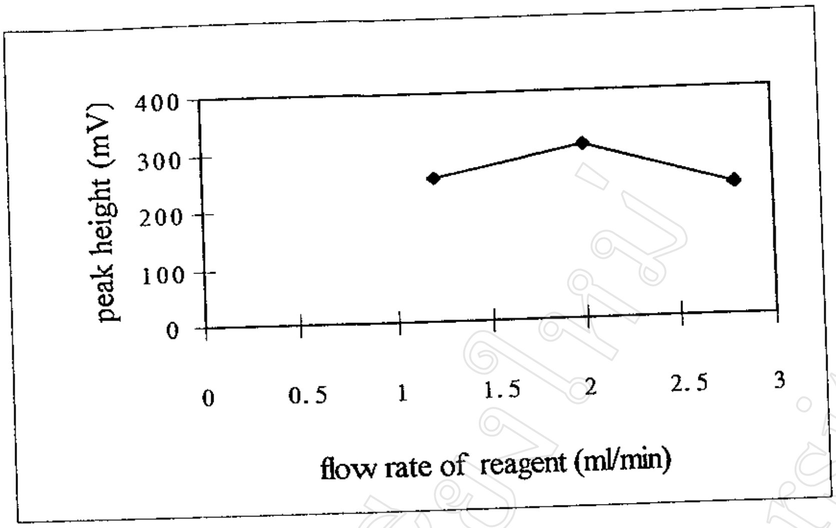

Fig. 2.3.13 Effect of flow rates of reagent on peak height

(f) Mixing coil lengths

In FIA system, the sample zones can disperse and react with the components of the carrier stream whilst passing through a mixing device such as a mixing coil. The effect of the eluent mixing coil (mixing coil 1) where it was connected with the carrier stream line is shown by the results in Table 2.3.10 and Fig. 2.3.14 which indicate that the greatest peak height is achieved without using a mixing coil and the dispersion is low.

Table 2.3.10 Effect of mixing coil 1 lengths on peak height

mixing coil 1 length (cm)	Peak height (mV)
0	305.0
25	246.0
50	232.0

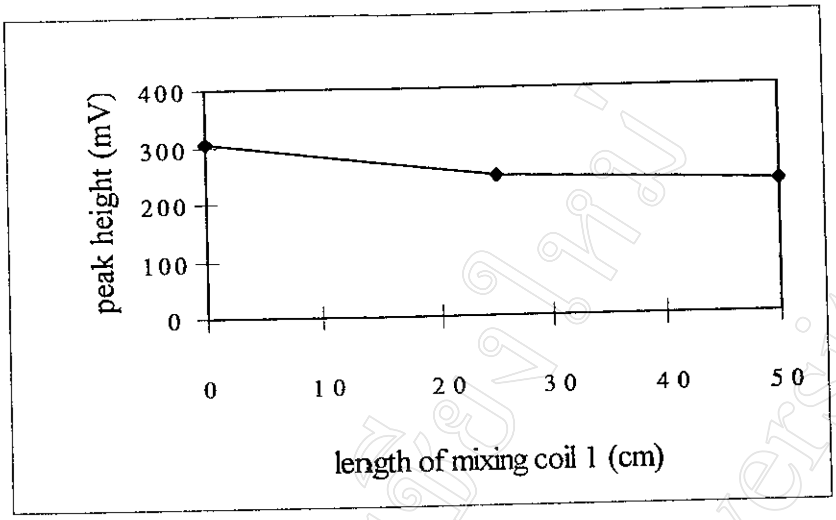

Fig. 2.2.14 Effect of mixing coil 1 lengths on peak height.

The mixing coil after the merging of sample and reagent streams (mixing coil 2) was used in order to permit the yttrium sample zone to disperse and react more effectively with the Arsenazo III reagent. The effect of mixing coil 2 was studied using various mixing coil lengths from 25 to 100 cm. Results, show in Table 2.3.11 and Fig. 2.3.15, indicate that a mixing coil length of 50 cm gave the highest peak. Peak height decreases when longer mixing coils are used because of the higher dispersions.

Table 2.3.11 Effect of mixing coil 2 lengths on peak height

mixing coil 2 length (cm)	Peak height (mV)
25	270.0
50	305.0
75	255.0
100	160.0

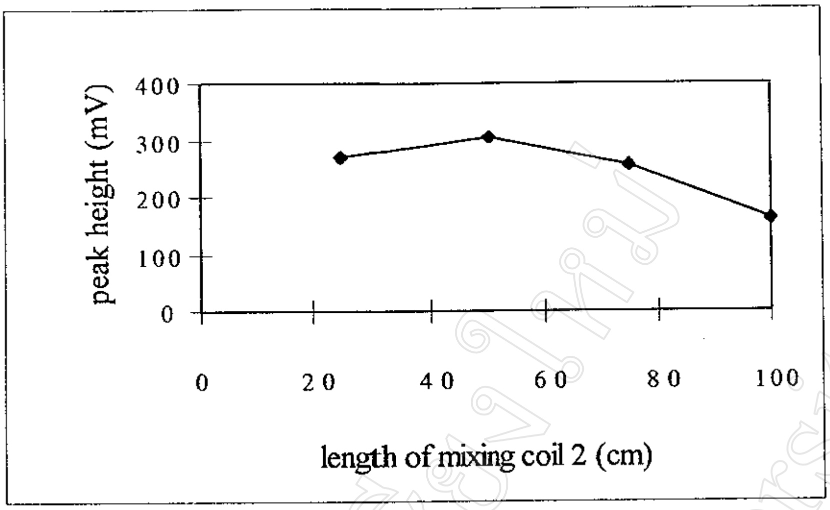

Fig. 2.3.15 Effect of mixing coil 2 lengths on peak height.

(g) Loading flow rate

The exchange rate of ions is affected by the flow rate of solution as it flows through the ion exchange column : therefore, it was essential to study the effect of loading flow rate on the preconcentration. The flow rate for preconcentrating yttrium on the ion exchange column was investigated by passing a yttrium standard solution (0.01 mg/l) through the resin column at different rates for 2 minutes. The results (Table 2.3.12 and Fig. 2.3.16) show that the optimum flow rate is 5.5 ml/min.

Table 2.2.12 Effect of loading flow rates on peak height

Loading flow rate (ml/min)	μg of yttrium passed column	Peak height (mV)
1.2	0.02	17.0
2.5	0.05	37.0
4.0	0.08	59.0
5.3	0.10	77.0
5.5	0.11	80.0
6.5	0.13	65.0

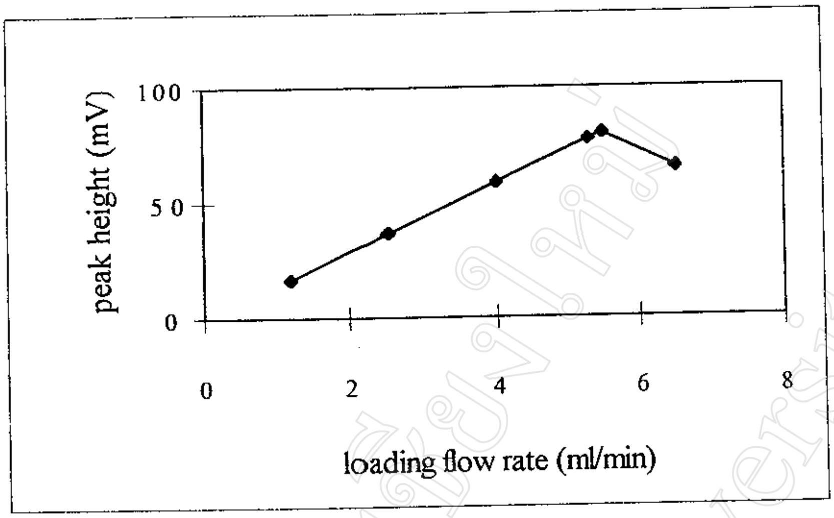

Fig. 2.3.16 Effect of loading flow rates on peak height

(h) Resin column lengths

This was studied by using an FIA system optimised from the previous studies. An yttrium standard solution (0.05 mg/l) was passed through resin column at constant loading flow rate of 2 ml/min, for 3 minutes. Glass columns of varying lengths between 2 and 5 cm were examined.

Table 2.3.12 Effect of resin column lengths on peak height.

column length (cm)	Peak height (mV)
2.0	271.5
3.0	295.0
4.0	355.0
5.0	66.0

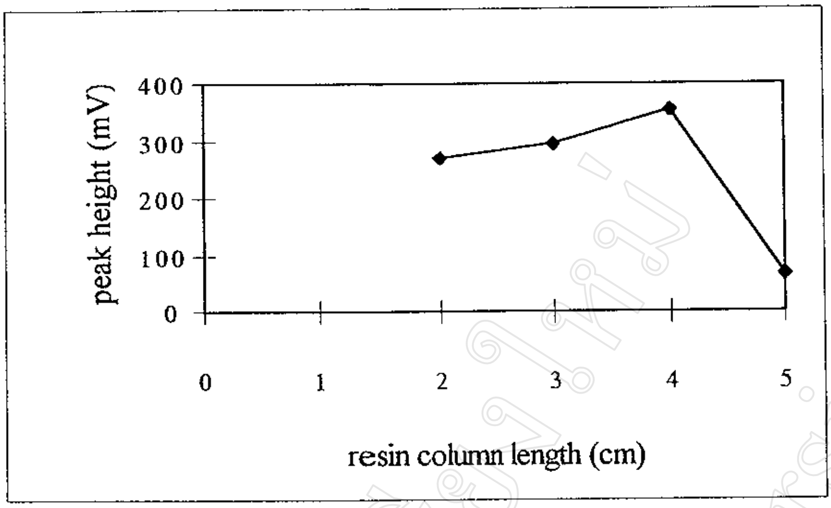

Fig. 2.3.17 Effect of resin column lengths on peak height.

The results (Table 2.3.13 and Fig. 2.3.17) show that a resin column length of 4 cm yields the highest peak, whilst columns greater than 4 cm in length caused decreases peak height due to peak broadening. This is because the yttrium ion retained on long columns and cannot be quantitatively eluted with H^+-ion in a short time.

(i) Summary of FIA optimum conditions obtained by conventional methods

A summary of optimised conditions for an FIA manifold shown in Fig. 2.3.6 , are as follows :

eluent solution = HCl 0.01 M

buffer solution = acetic/acetate buffer (pH 4.0) 0.1 M

colouring reagent = Arsenazo III solution 0.001 %

flow rate of eluent = 2 ml/min

flow rate of reagent = 2 ml/min

mixing coil 1 length = 0 cm

mixing coil 2 length = 50 cm

loading flow rate = up to 5.5 ml/min

resin column length = 4.0 cm

2.3.4.2 Simplex optimisation

Simplex optimisation is a mathematical method which can be applied to optimise factors in a multicomponent system. It may be applied when all the factors are continuous variables, such as the many factors that must be optimised in this study.

By this method, the vertices of the simplex come from experiments where values are determined randomly. The initial vertices for a simplex must have at least ***n+1*** vertices when ***n*** are a number of factors that are required to be optimised. For example, in this study there are 9 factors therefore at least 10 experiments must be carried out to determine in the initial vertices. From the initial values the vertex that gives the lowest response is rejected and a new vertex calculated as follows :

1) Sum values of each factor except the one in the replaced vertex
2) Divide by the number of factors (*n*) excluding the replaced vertex.
3) Calculate the displacement of the new point by using the value in 2) minus the value in the replaced vertex.
4) Calculate the new vertex by using the value in 2) plus the value obtained in 3)

The calculated vertex indicates the new experimental conditions under which a response must be determined. Similarly, the vertex in the new vertices that give the lowest response will be replaced with new calculated parameters until no further improvement can be obtained, then the vertex that give the best response represents the best conditions. The number of experiment required in the simplex method does not increase rapidly with the number of factors as it does in the conventional method.

(a) Experiments by simplex method

For the determination of yttrium in the present study there are 9 factors to be optimised levels (see section 2.3.4.1) so at least 10 vertices must be obtained with initial random vertices. The experiments are carried out using the FIA manifold shown in Fig. 2.3.6. The initial vertices of this study are shown in Table 2.3.14. Also shows is the method used to calculate the new vertex.

Table 2.3.14 The initial vertices of simplex method for yttrium determination

VERTEX	FACTOR**									Peak height (mV)	Noted
	A	B	C	D	E	F	G	H	I		
Vertex 1	0.001	0.001	0.1	100	100	2.0	2.0	2.0	2.0	94.0	
Vertex 2	0.01	0.001	0.1	100	100	2.0	2.0	2.0	2.0	109.0	
Vertex 3	0.01	0.005	0.1	100	100	2.0	2.0	2.0	2.0	125.0	
Vertex 4	0.01	0.001	1.0	100	100	2.0	2.0	2.0	2.0	91.0	
Vertex 5	0.01	0.001	0.1	0	100	2.0	2.0	2.0	2.0	258.0	
Vertex 6	0.01	0.001	0.1	0	200	2.0	2.0	2.0	2.0	139.0	
Vertex 7	0.01	0.001	0.1	0	200	1.2	2.0	2.0	2.0	146.0	
Vertex 8	0.01	0.001	0.1	0	200	1.2	2.8	2.0	2.0	145.0	
Vertex 9	0.01	0.001	0.1	0	200	1.2	2.8	2.0	5.0	40.0	
Vertex 10	0.01	0.001	0.1	0	200	2.0	2.8	1.2	2.0	86.0	
1) Sum [excluding vertex 9]	0.081	0.013	1.8	400	1300	16.4	19.6	19.2	18.0		
2)Sum/n [excluding vertex 9]	0.009	0.001	0.2	44	144	1.8	2.2	2.2	2.0		
3) Rejected vertex [vertex 9]	0.01	0.001	0.1	0	200	1.2	2.8	2.0	5.0		
4) Displacement = 2) - 3)	-0.001	0	0.1	44	-56	0.6	-0.6	0.2	-3.0		
5) New vertex = 2) + 4)	0.008	0.001	0.3	88	88	2.4	1.6	2.4	-1.0		
Vertex 11*	0.01	0.001	0.3	100	100	2.0	2.0	2.0	2.0		

* new vertex can use approximate values

** FACTOR A = HCl concentration (M)
B = Arsenazo III concentration (%)
C = acetic/acetate buffer concentration (M)
D = mixing coil 1 length (cm)
E = mixing coil 2 length (cm)
F = flow rate of eluent (ml/min)
G = flow rate of reagent (ml/min)
H = loading flow rate (ml/min)
I = resin column length (cm)

From Table 2.3.14 it can be seen that vertex 9 gives the lowest response therefore it will be replaced by the new vertex. Vertex 11 is the new vertex in which the value of each factor is an approximate one close to that obtained from calculations. Table 2.3.15 shows all of vertices which were obtained in the yttrium determination.

Table 2.3.15 All of vertices of simplex method for yttrium determination

VERTEX	FACTOR**									Peak height (mV)	Noted
	A	B	C	D	E	F	G	H	I		
Vertex 1 [5th rejected]	0.001	0.001	0.1	100	100	2.0	2.0	2.0	2.0	94.0	
Vertex 2	0.01	0.001	0.1	100	100	2.0	2.0	2.0	2.0	109.0	
Vertex 3	0.01	0.005	0.1	100	100	2.0	2.0	2.0	2.0	125.0	
Vertex 4 [4th rejected]	0.01	0.001	1.0	100	100	2.0	2.0	2.0	2.0	91.0	
Vertex 5	0.01	0.001	0.1	0	100	2.0	2.0	2.0	2.0	258.0	
Vertex 6	0.01	0.001	0.1	0	200	2.0	2.0	2.0	2.0	139.0	
Vertex 7	0.01	0.001	0.1	0	200	1.2	2.0	2.0	2.0	146.0	
Vertex 8	0.01	0.001	0.1	0	200	1.2	2.8	2.0	2.0	145.0	
Vertex 9 [1st rejected]	0.01	0.001	0.1	0	200	1.2	2.8	2.0	5.0	40.0	
Vertex 10 [3rd rejected]	0.01	0.001	0.1	0	200	2.0	2.8	1.2	2.0	86.0	
Vertex 11 [2nd rejected]	0.01	0.002	0.3	100	100	2.0	1.5	2.0	1.0	70.0	
Vertex 12	0.01	0.001	0.1	0	200	1.5	0.8	2.0	3.0	168.0	
Vertex 13	0.01	0.002	0.3	100	100	1.5	1.2	3.0	2.0	169.0	
Vertex 14	0.01	0.002	0.01	0	200	1.5	1.5	2.0	2.0	98.0	
Vertex 15	0.02	0.001	0.1	0	200	1.2	1.5	2.0	2.0	106.0	stopped

[** see Table 2.3.14]

After the fifth rejected vertex, a further experiment do not improve on the peak height obtained for vertex 5. Because there is no further improvement, it is concluded that vertex 5 represents the optimum experimental conditions

(b) Summary of FIA conditions obtained by simplex method

By using FIA manifold as shown in Fig. 2.3.6, the summary of FIA optimised conditions obtained by the simplex method are as follows :

eluent solution [A] = HCl 0.01 M
coloring reagent [B] = Arsenazo III solution 0.001%
buffer solution [C] = Acetic/acetate buffer (pH 4.0) 0.1 M
mixing coil 1 length [D] = 0 cm
mixing coil 2 length [E] = 100 cm
flow rate of eluent [F] = 2 ml/min
flow rate of reagent [G] = 2 ml/min
loading flow rate [H] = 2 ml/min
resin column length [I] = 2.0 cm

(c) Comparison of optimum conditions obtained by conventional and simplex methods

The comparison of FIA optimum conditions obtained from the conventional method and the simplex method are shown in Table 2.3.16. It was found that almost all factors were in agreement, except the mixing coil 2 lengths, resin column lengths and loading flow rate. For the initial simplex matrix, the factor values were chosen at random but in the actual study, the mixing coil 2 lengths and the resin column lengths were not chosen as variables. Nevertheless, a mixing coil 2 length of 100 cm and resin column length of 2 cm were used because they produced the highest peak. The calculated value of loading flow rate in the simplex method was a single one while in the conventional method a range of variables were used to find the appropriate loading flow rate. Hence, for the conventional optimisation method the factor was a continuous variable. The sample flow rate of 2 ml/min was predicted by the simplex method and also conventionally determined. By the simplex method, only 15 experiments were required for optimisation whilst in the conventional method at least 27 experiments were needed.

Table 2.3.16 The comparison of FIA optimum conditions obtained between conventional and simplex methods

FACTORS	**conventional method**	**simplex method**
eluent concentration (M)	0.01	0.01
buffer concentration (M)	0.1	0.1
Arsenazo III concentration (%)	0.001	0.001
flow rate of eluent (ml/min)	2.0	2.0
flow rate of premixing reagent (ml/min)	2.0	2.0
mixing coil 1 length (cm)	0	0
mixing coil 2 length (cm)	50	100
sample flow rate (ml/min)	up to 5.5	2.0
resin column length (cm)	4.0	2.0

2.3.5 pH of sample

Yttrium standard solutions (0.05 mg/l) of varying pH from 3 to 9 were passed through a resin column, for 2 minutes under FIA optimised conditions obtained by the conventional method (section 2.3.4.1). Results (Table 2.3.17 and Fig. 2.3.18) indicate that, maximum peak height is obtained over a pH range from 4 to 7. At pH lower than 4 binding of the yttrium ion to the ionogenic group of the resin can be inhibited by H^+-ion so yttrium ion is not bound completely and thus the peak height decreases. At pH higher than 7 yttrium ion is hydrolysed to hydroxy complexes [$Y(OH)_2^+$ and $Y_2(OH)_2^{4+}$] (28) which affects the exchange equilibrium. Also, if the pH of sample solution is too high yttrium ion can precipitate and block the FIA system. Therefore the pH of sample solutions should lie between 4 to 7.

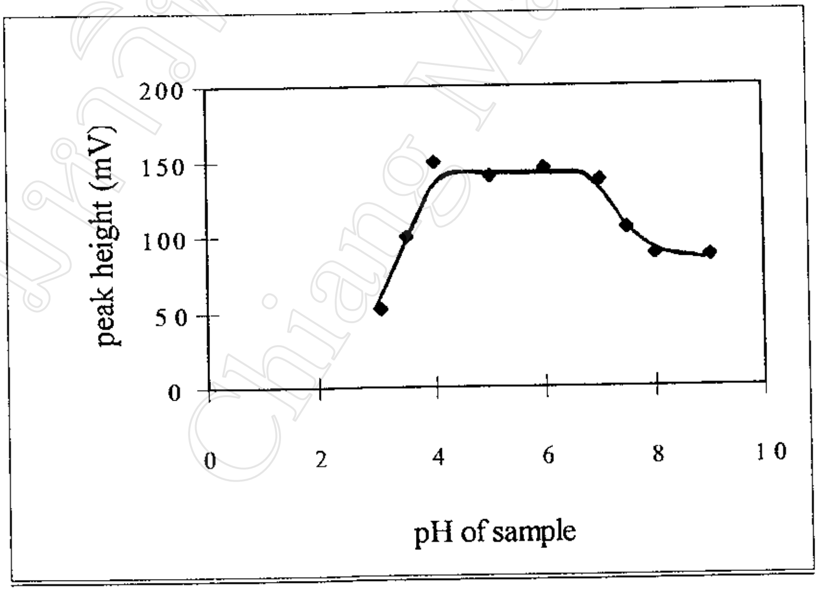

Fig. 2.3.18 Effect of pH of sample solutions on peak height

Table 2.3.17 Effect of pH of sample solution on peak height

pH	3	3.5	4	5	6	7	7.5	8	9
peak height (mV)	52.0	100.0	150.0	140.0	146.0	138.0	105.0	88.0	87.0

2.3.6 Effect of water washing time

Peak heights and reproducibilities were compared after 1 and 2 minutes of water washing after loading. Results (Table 2.3.18) indicate that a suitable time for washing with water was 2 minutes. Water washing after loading may eliminate some matrix components that might otherwise interfere. But long periods of water washing might remove some yttrium ion before elution. Washing after elution is also required in order to get rid off remaining HCl eluent [H^+-ion] before reloading, otherwise the remaining H^+-ion may change the pH of the reloaded sample.

Table 2.3.18 Effect of water washing time on peak height : preconcentration time 3 minutes, loading flow rate 1.1 ml/min.

Yttrium standard solution (mg/l)	Washing time	
	1 minute	2 minutes
0.01	0.061, 0.062, 0.063	0.080, 0.078, 0.077
0.05	0.378, 0.372, 0.391, 0.391, 0.389	0.392, 0.395, 0.401

2.3.7 Calibration curve

A calibration for yttrium preconcentration was achieved by varying the concentration of yttrium standard or the preconcentration time at a loading flow rate of 1.5 ml/min. When various loading times were varied, the amounts of yttrium loaded onto the column versus absorbance were plotted. The results are shown in Table 2.3.19 and Figs. 2.3.19 - 2.3.21.

Table 2.3.19 The absorbances obtained from varying either yttrium standard solutions or preconcentration time at a loading flow rate of 1.5 ml/min.

Y standard solution (mg/l)	Preconcentration time (min)	Y loaded onto column (μg)	Absorbance
0.005	6	0.05	0.047
0.01	3	0.05	0.050
	6	0.09	0.102
0.02	1	0.03	0.041
	3	0.09	0.120
	6	0.18	0.254
0.03	1	0.05	0.061
	3	0.14	0.180
	6	0.27	0.359
	12	0.54	0.722
	15	0.68	0.646
	20	0.90	0.899
0.05	1	0.08	0.094
	3	0.23	0.289
	6	0.45	0.608
	12	0.90	0.898

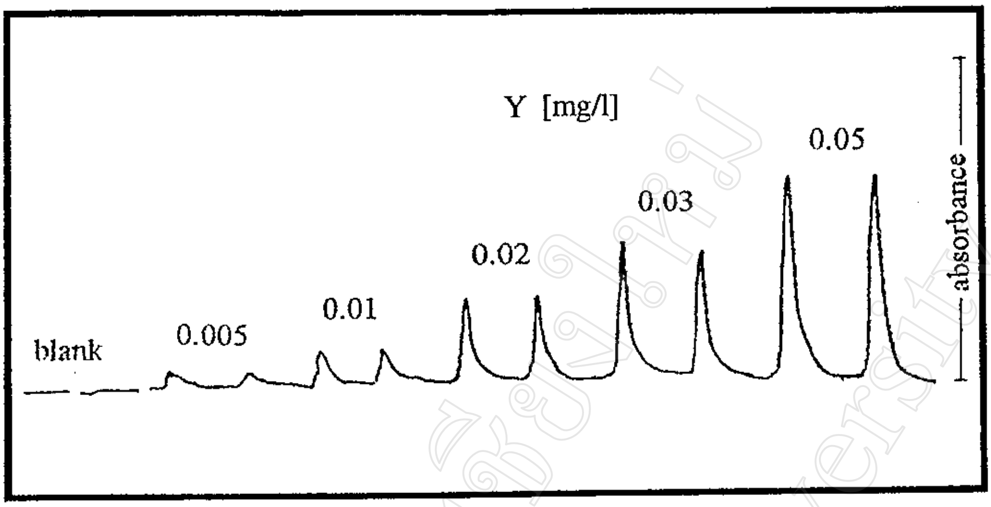

Fig. 2.3.19 Signals from yttrium standard solutions loaded for a preconcentrations time of 6 minutes at a flow rate of 1.5 ml/min.

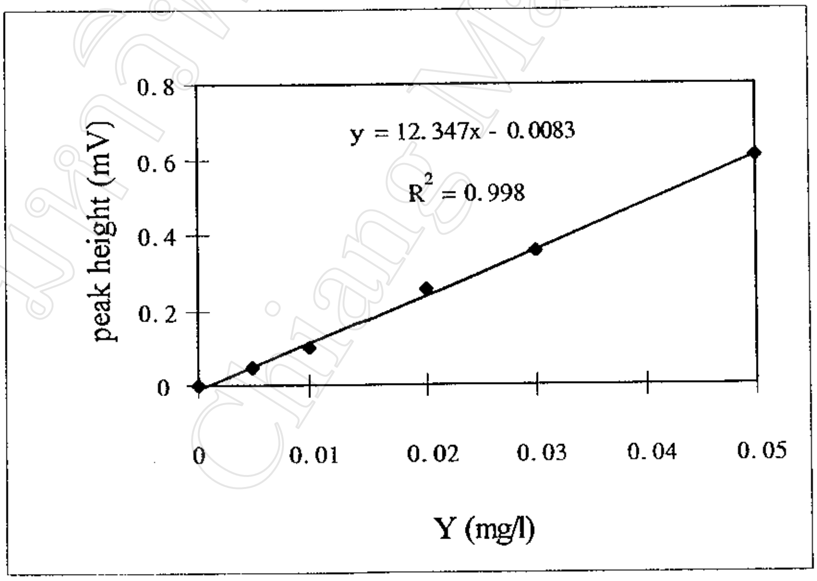

Fig. 2.3.20 Calibration for yttrium standard solutions loaded for a preconcentration time of 6 minutes at a flow rate of 1.5 ml/min

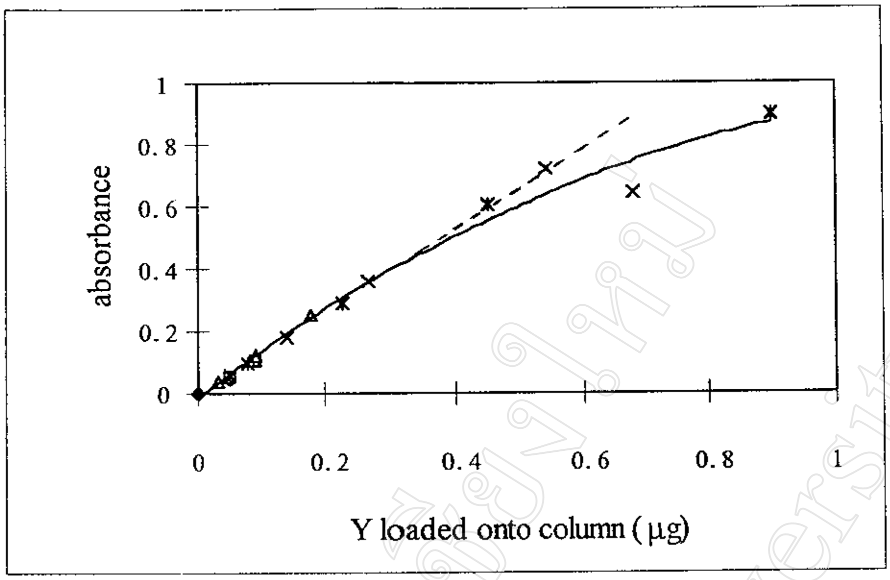

Fig. 2.3.21 Calibration for yttrium standard solutions loaded for various preconcentration times ; (♦) 0.005, (■) 0.01, (▲) 0.02, (×) 0.03 and (✱) 0.05 mg Y/l

It is evident that the calibration linear is up to 0.45 μg of Y, the equation of the straight line being : $y = 1.331x - 0.014$ ($r^2 = 0.996$). When the quantity of yttrium was greater than 0.45 μg negative deviation observed. Below 0.45 μg of Y, the linearity is independent of the concentration of yttrium standard passing through the column.

2.3.8 Effect of preconcentration time

A calibration curve was constructed as already described (section 2.3.7) but extending the preconcentration time of yttrium standard solutions (0.005 mg/l) up to 45 minutes at loading flow rate of 1.3 ml/min, then compared the calibration curve. The results, (Table 2.3.20 and Fig. 2.3.22) indicate that the calibration curve from the yttrium standard of 0.005 mg/l began to deviate from the linear calibration derived from the data for yttrium standards of higher concentrations at a preconcentration time of 7 minutes. Yttrium at concentration of 0.005 mg/l can only be determined quantitatively if the preconcentration time is less than 7 minutes. After times longer than 7 minutes, yttrium ion bound to the resin might be released owing to kinetic features of the exchange process leading to the breakthrough. Also for a trace concentration which is needed preconcentration time of longer than 7 minutes cannot be achieved by this condition.

Table 2.3.20 Effect of preconcentration times on peak height

Y standard solution (mg/l)	Preconcentration time (min)	Y loaded onto column (µg)	Absorbance
0.005	5	0.03	0.046
	15	0.10	0.088
	30	0.20	0.154
	45	0.29	0.207
0.01	2	0.03	0.045
	5	0.07	0.092
0.02	1	0.03	0.060
	2	0.05	0.085
	5	0.13	0.177
0.03	2	0.08	0.112
	5	0.20	0.239
0.05	1	0.07	0.100
	2	0.13	0.227
	5	0.33	0.417

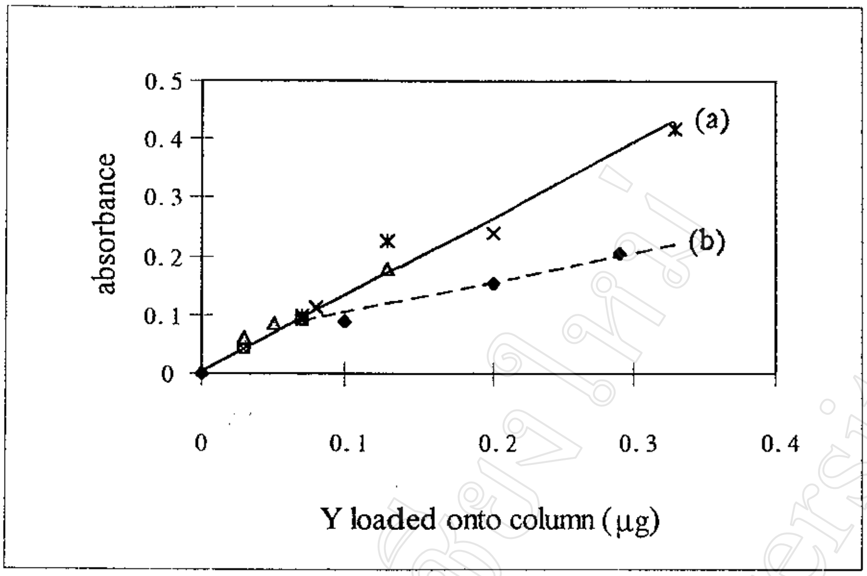

Fig. 2.3.22 Effect of preconcetration time on peak height ;

(◆) 0.005, (■) 0.01, (▲) 0.02, (×) 0.03 and (✱) 0.05 mg Y/l

; loading flow rate 1.3 ml/min ; data with preconcentration time

(a) less than 7 minutes and (b) exceeding 7 minutes.

2.3.9 Adsorption onto glass

Calibration were made using yttrium standard solutions (0.005, 0.01, 0.02 and 0.03 mg/l) that had been stored in plasticware or glassware with a preconcentration time of 5 or 10 minutes at a loading flow rate of 1.3 ml/min under the optimised conditions. Results (Table 2.3.21 and Fig. 2.3.23) indicate that when yttrium standard solutions are stored in glassware, some yttrium ion might be adsorbed onto the glass surface. Therefore, this causes errors in the calibration. Using glasswares, yttrium solutions of 0.005-0.02 mg/l yielded low absorbances. Repeated experiments gave consistent results. A solution of higher concentration resulted in higher absorbance. This could be due to that saturation of sorption of yttrium onto the glass-wall would be achieved for the concentration of higher than 0.02 mg/l. Using plasticwares to store the solutions, the phenomena were not observed ; a linear relationship (0 - 0.03 mg/l) was found. Yttrium standards should be stored in plasticware to prevent such adsorption.

Table 2.3.21 Effect of adsorbed ions onto glass surface.

Yttrium standard solutions (mg/l)	Absorbance			
	Preconcentration time			
	5 minutes		10 minutes	
	glassware	plasticware	glassware	plasticware
0	0.022	0.026	0.027	0.020
0.005	0.068	0.034	0.103	0.073
0.01	0.115	0.136	0.276	0.251
0.02	0.131	0.243	0.167	0.811
0.03	0.477	0.377	1.022	1.223

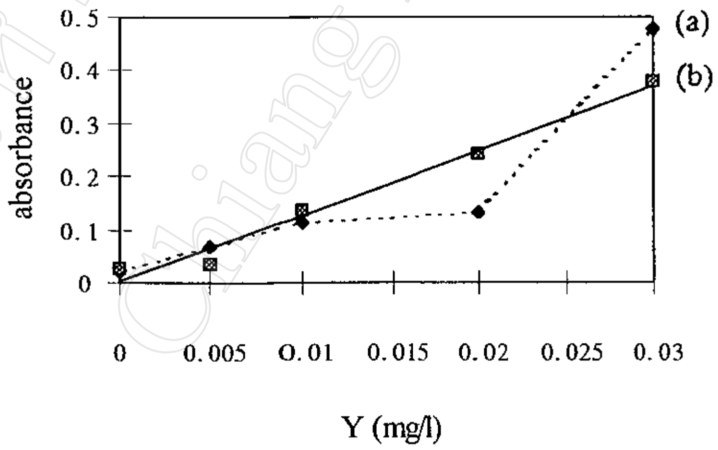

Fig. 2.3.23 Effect of adsorbed ions onto glass surface ; preconcentration time of 5 minutes at a loading flow rate of 1.3 ml/min ; store in (a) glassware (b) plasticware

2.3.10 Interferences

Yttrium standards containing various quantities of foreign ions were injected into FIA system under the optimum conditions already described. The summarised results are shown in Table 2.3.22 and the detailed results are shown in appendix B.

Table 2.3.22 Summary of interferences : maximum mass ratio which causes relative errors of less than 10%

Ion added	Ion added/Y (III) mass ratio
Na^+, K^+, NO_3^-	3000
Co^{2+}, Mg^{2+}, Te^{2+}, Cd^{2+}, Ca^{2+}, SO_4^{2-}	100
Ni^{2+}, CO_3^{2-}	50
Zn^{2+}, V^{5+}, Pb^{2+}, Ge^{4+}	10
La^{3+}, Al^{3+}	1
Ti^{2+}, Fe^{3+}, Cu^{2+}	<0.5

Sodium ion, potassium ion and nitrate ion did not interfere seriously. Interferences from cobalt (II), magnesium, tellurium, cadmium, calcium and sulphate ions were negligible up to mass ratio of 100. Aluminium, titanium, iron (III) and copper (II) ions should be removed from the sample before determination. Lantanum (III), a rare earth ion, interferes by enhancing the peak height of yttrium. Chloride and phosphate ions have been reported not to interfere in the determination of rare earth elements with Arsenazo III (27).

2.3.11 Determination of yttrium in tin tailing leachates

The proposed FIA procedure was applied to determine yttrium in leachates of tin tailing samples, obtained by extracting with a hydroxylammonium chloride solution (0.1 M) (60). The results obtained (Table 2.3.23) agree with the ones obtained from ICP-AES.

Table 2.3.23 Determination of yttrium in tin tailing leachates (with 0.1 M hydroxylammonium chloride)

Sample	Yttrium (μg/g)	
	FIA (this work)	ICP-AES (60)
S 1	3.27	2.90
S 2	2.76	2.72
S 3	2.49	2.36
S 4	3.00	2.73
S 5	3.67	3.42