CHAPTER 4

DISCUSSION AND CONCLUSIONS

4.1 Discussion

Flow injection analysis (FIA), a simple method to the chemical analysis based on continuous flow measurement without air segmentation, has recently received considerable attention in various fields. FIA are popular due to their excellent reproducibility and reliability, low cost, ease of automation, and rapid turnover time. In this research work, ***normal FIA (nFIA)*** and ***reversed FIA (rFIA)*** modes have been developed using cheap and easily available materials and applied to the determination of aluminium(III) and europium(III) in water samples. In nFIA, the method is based on injection of sample into the carrier stream of reagent. On the other hand, rFIA is based on injection of reagent(s) into a carrier stream of sample, thus saving an important of reagent and improving the overall economy [41,57]. rFIA has proven suited in cases where sample material is abundant while reagents ought to be spared.

In selecting a therapeutic agent for use as ligand for the proposed FI-spectrophotometric method, general requirement need to be taken into consideration. This requirement was discussed in Chapter 2 and Chapter 3 of this thesis. TC and CTC which are widely used in pharmacology as therapeutic agents have been proposed as ligands for the determination of aluminium(III) and europium(III) respectively. In this research project, all parameters namely pH of buffer, reagent concentration, mixing coil length, reagent or sample flow rates and injection volume were optimized by the ***univariate*** (or ***conventional***) method was carried out by varying each parameter individually to obtain the best experimental conditions (Tables 2.19 and 3.12). Each optimum condition was judged from the condition which gave the greatest peak height.

4.1.1 Determination of aluminium(III) with TC

The FI-spectrophotometric method proposed by Thuchpramook [70] was adapted for the development of novel reversed-FIA spectrophotmetric method for determining aluminium(III) in water using TC. After some preliminary studies, it was found to be more satisfactory to inject the TC in buffer medium into the stream of sample or standard solution (see Figure 2.2). The absorption spectra of the aluminium(III) - TC complex and TC solutions were scanned from 350 to 500 nm. The complex has an absorption maximum at 390 nm (Figure 2.4). The absorption spectrum of an TC solution was recorded under the same operating conditions. The spectrum of TC shows a maximum absorption at 360 nm. The molar absorptivity of the aluminium(III) - TC complex at 390 nm was 1.12×10^{4} $l.mol^{-1}.cm^{-1}$. A study of the composition of the aluminium(III) - TC complex using the mole ratio method indicate that the complex has a 1 : 3 composition (Figure 2.5).

Under the topic of optimization of experimental conditions starting with optimum wavelength of aluminium(III)-TC complex, Table 2.3 shows peak heights obtained from the injection of 100 µl of 2.08×10^{-4} M TC solution at different wavelengths from 360 to 430 nm whereas Figure 2.6 shows a relationship between absorbance expressed as peak height (mV) and detector wavelengths. The optimum wavelength chosen was 390 nm due to high sensitivity provided for aluminium(III)-TC complex at this wavelength.

Most complex formation reactions are pH dependent so it is essential to investigate the optimum pH for the formation of Al(III) - TC complex to obtain the best sensitivity. Optimization of pH of tris-buffer was done by varying the pH of tris-buffer over the range 2.0 - 7.0, as shown in Table 2.4 and Figures 2.7 - 2.8. The buffer pH 6.0 gave the highest sensitivity for this investigation. Below pH 6.0 the absorbance decreased and above pH 6.0 the wavelength of the maximum absorption was shifted from 390 to 395 nm, probably because of incomplete formation of the complex or a change in the structure of the complex itself [15].

Concentration of the reagents can more or less affect the complexation reactions. The results on the optimization of TC concentration are shown in Table 2.5 and Figures 2.9 - 2.10. By varying the TC concentrations from 1.04×10^{-5} - 5.20×10^{-5} M different slopes of calibration curves were obtained. Figure 2.10 showed that the sensitivity (expressed as slope (mV/ppm)) increased with increasing TC concentration up to 3.12×10^{-5} M, which was therefore chosen as the optimum concentration for subsequent study.

Since reagents and/or sample rates can affect the dispersion and hence affect the sensitivity and the sample throughput so it is very necessary to investigate the effect of varying flow rate on the analyte peak height. Optimization of flow rate was done by varying the flow rate from 3.0 to 8.0 ml/min, as shown in Tables 2.7 - 2.8 and Figures 2.11 - 2.13. It can be seen that higher flow rate provided higher sample throughput (lower t_{base}), as shown in Table 2.8. The optimum flow rate chosen for this experiment was 4.0 ml/min because it provided more satisfactory sensitivity (slope=86.46 mV/ppm), correlation coefficient (0.9966) and adequate sample throughput (80 hr^{-1}).

The geometry of the mixing tubing (inner diameter and length) could affect the flow rate and hence, the sensitivity and sample throughput. It is again very important to find out an appropriate geometry of the mixing tube. The results of investigation for the optimum diameter of mixing tubing are shown in Tables 2.9 -

2.10 and Figures 2.14 - 2.16. The mixing tubing with the inner diameter of 0.030" was chosen as optimum.

The effect of the length of mixing tubing was investigated and the results are shown in Tables 2.11 - 2.12 and Figures 2.17 - 2.19. The optimum length of the mixing tubing chosen for this experiment was 1.00 m because it provided the highest sensitivity (93.49 mV/ppm) and sample throughput (85 hr^{-1}). This optimum length of mixing tubing allowed sufficient time for colour development and/or effective reaction taking place.

The mixing part of the system was made of the narrow bore tubing by winding round a glass test tube and securing it at the ends with tape. Optimization of the size of coiled mixing part to obtain satisfactory sensitivity was done by varying the size of test tube over the range 0.75 - 2.40 cm. The optimum size of the coiled mixing part was restrictly chosen to be 0.75 cm, because it was the smallest test tube available in the laboratory. However, it would be useful to compare the results obtained from the use of the 0.75 cm O.D. test tube with any other materials with the smaller sizes as cores on which the mixing tubings were wound.

Apart from the coiled mixing part, other types of mixing parts namely single bead string (s.b.s.r.), knitted, etc., mixing part or reactors were tested. Optimum length of s.b.s.r. was investigated in order to obtain adequate sensitivity. The s.b.s.r. 7.00 cm was chosen as optimum of this type of reactor. The effect of various shapes of mixing part were also studied. The results (Table 2.16) show that the knitted mixing part is the optimum shape.

The effect of injection volume was investigated by varying a loop length of the injection valve. The results are shown in Table 2.18 and Figures 2.26 - 2.27. A volume of 100 μl was chosen as optimum injection volume with highest sensitivity.

Optimized experimental conditions for aluminium(III) analysis are summarized in Table 4.1. These optimum conditions were used throughout the research work.

Table 4.1 : Analytical characteristics for trace aluminium(III) determination with TC.

Analytical characteristics	Information
1. Wavelength (nm)	390
2. pH of tris - buffer solution	6.0
3. Concentration of TC reagent (M)	3.12×10^{-5}
4. Pump flow rate (ml/min)	4.0
5. Length of mixing tubing (cm)	100
6. Shape of mixing part	knitted
7. Inner diameter of mixing tubing (in)	0.030
8. Reagent volume (µl)	100
9. Linear range of calibration graph (ppm)	0 - 1.40 and 2.2 - 4.0
10.Slope of calibration graph (mV/ppm)	66.29
11.Reproducibility (%)	2.65
12.Repeatability (%)	0.91
13.Detection limit (ppm)	0.02
14.Percentage recovery (%)	96.32
15.Sample throughput (hr^{-1})	80

A calibration curve which was a plot of peak heights against various concentrations of aluminium(III) was linear over the range of 0.0 - 1.0 ppm aluminium(III), with the correlation coefficient of 0.9993. The relative standard deviation 0.91% (for 1.0 ppm) of aluminium(III), n = 12), a detection limit of 0.02

ppm aluminium(III) and the percentage recovery of the added aluminium(III) of 96.32% were obtained and shown in Tables 2.23 and 2.28 respectively.

In any analytical procedure, it is necessary to determine some possible interferences before any quantitative analysis is carried out. Table 2.26 summarized the results obtained in the study of interfering species for the determination of 1 ppm of aluminium(III). Species generally present in waters including potassium, sodium, magnesium(II), copper(II), zinc(II), iron(III), chloride, sulphate, nitrate and fluoride were tested. It was found that sodium, copper(II), or zinc(II) was present at the concentration ratio up to 10 folds that of the aluminium, the relative errors were less than 10%. While the presence of potassium at a concentration of 2 folds that of aluminium, less than 10% relative error was obtained. The presence of magnesium(II) or iron(III) was found to interfere by increasing the FIA signals in all ratio. Most of the interferences might be due to the formation of coloured complex and competitive complexation of the aluminium. The tetracycline structure contains numerous sites at which chelation with metal ions might occur, and thus affect the absorption for the determination of aluminium(III) [78].

The proposed method was applied to the determination of aluminium(III) in tap water samples collected from student dormitories in Chiang Mai University. There was good agreement between the proposed and flame - AAS methods (Table 2.29). The aluminium(III) contents in the tap water sample were ranging from 0.043 to 0.556 ppm and 0.035 to 0.548 ppm Al(III) by the proposed and flame-AAS methods respectively. The proposed method permits the determination of aluminium(III) in water samples. Also, this proposed method was applied to the determination of tetracycline in proprietary drugs by using single standard calibration curve. The results obtained are shown in Table 2.31.

4.1.2 Determination of europium(III) with CTC

Preliminary studies showed that europium(III) reacts with CTC resulting in a yellow complex in tris-buffer medium. The complex presents absorption maximum at 400 nm. The CTC has an absorbance at 368 nm and low absorbance at 400 nm in the same experimental conditions. It was found that the composition of complex examined by mole ratio and continuous variation methods was found to be 1:1 (Figure 3.5) which was in good agreement with the previously reported [77]. The apparent molar absorptivity at 400 nm was 1.00×10^{4} $l.mol^{-1}.cm^{-1}$ for europium(III)-CTC complex in tris-buffer medium. In the present work, a flow injection colorimetric procedure was proposed for determining europium(III) using CTC as colorimetric reagent. The tris-buffer was used because it is one of the few buffer materials that does not interfere with the europium(III) - CTC complexation.

The optimum conditions for europium(III) determination were investigated by injecting a 100 µl of the standard or sample solutions containing europium(III) into the merged streams of CTC and the tris-buffer solution with flow rates of 4.5 ml/min in each channel. It was found to be more satisfactory to inject the standard or sample solutions into the merged streams rather than injecting into the buffer stream before merging. Since the FIA peaks obtained by the former manifold provides reproducible results with the high sensitivity than those obtained by the latter manifold. The proposed design of the manifold was shown in Figure 3.2.

The peak height of the europium(III) - CTC complex was measured over the pH range 7.0 - 9.0, by using tris - buffer together with a sufficient volume of 1 M HCl to give the required pH. pH 8.0 was chosen as optimum, because at this pH the peaks were relatively high with a good reproducibility and the linearity of the calibration curve was better (r=0.9998) as shown in Table 3.4 and Figure 3.7.

The effect of various concentrations of CTC solutions (1.0×10^{-5} - 1.0×10^{-4} M) on the absorption of the europium(III) - CTC complex (as peak height) was examined. At higher CTC concentrations, the slope (mV/ppm) of standard calibration curve increases. The CTC concentration which exhibited the largest slope increment with reasonable sample throughput was found to be 4.0×10^{-5} M and was therefore chosen as optimum CTC concentration.

The flow rate of the solutions is very important and should be regulated. High flow rates shorten the reaction time, hence the reaction is not allowed to reach completion as indicated by the decrease in absorbance of the complex formed [15]. On the other hand, low flow rates will decrease the sample throughput. A flow rate of 3.0 ml/min was chosen in order to obtain a reasonable sample throughput, the highest sensitivity and the better linearity of standard calibration curve.

The effect of inner diameter of mixing tubing was investigated. The results were tabulated in Table 3.7. An inner diameter of 0.0449" was selected as a compromise between sensitivity (6.58 mV/ppm Eu(III)) and sample throughput (60 hr^{-1}).

The effect of the mixing tubing length was investigated by varying the tubing length from 25 to 100 cm. A 75 cm mixing tubing was chosen as optimum length. With the shorter mixing tubing the baseline is unstable and the measurements of absorbance are not reproducible.

The effect of using glass bead column as a mixing reactor was examined. the results obtained are summarized in Table 3.9 and Figure 3.11, which show that good sensitivity and reasonable sampling rate could be obtained when the glass bead column length was 5.0 cm.

Various mixing reactor types (zig zag 1x1 cm, zig zag 1.5x1.5 cm, coil, knitted, straight line, s.b.s.r.) were tested, as shown in Table 3.10. The s.b.s.r. was selected as optimum reactor for the system, because it provided the better sensitivity (6.12 mV/ppm), detection limit [defined as 3σ] = 0.01 ppm Eu(III), and sample throughput than those obtained by any other reactors. By comparing the results obtained from the use of straight line and s.b.s.r. as a mixing reactors (Table 3.10), it can be seen that both types of reactors gave identical analytical characteristics for Eu(III) but the use of straight line as a mixing reactor in commercial FIA system should be satisfactory owning to the lower cost combined with a relatively suitable sensitivity, correlation coefficient, and sample throughput.

The effect of injection volume was studied by changing the loop, i.e., to give an injection volume in the range 50 - 200 μl in order to find out the optimum injection volume. The results obtained (Table 3.11) show that a 100 μl was found to be the optimum injection volume.

Optimized experimental conditions for europium(III) analysis are summarized in Table 4.2. These optimum conditions were used throughout this research work.

The calibration curve for the determination of europium(III) using CTC was linear over the range up to 0.6 ppm europium(III). Linear regression analysis gave correlation coefficient of 0.9994. The calibration curve showed good correlation coefficient values which indicated excellent agreement. The lower detection limit of europium(III) was also investigated. The detection limit (defined as three times of the standard deviation, 3σ) was determined, calculated and found to be 0.010 ppm of europium(III). The accuracy of the proposed method was verified by analysing spiked water samples containing europium(III). Results were shown in Table 3.19. The mean percentage recovery of the added europium(III) of 92.07% was obtained. The relative standard deviation for replicate injections was found to be 4.32% of europium(III) (n = 12) as shown in Table 3.14.

Table 4.2 : Analytical characteristics for europium(III) determination with CTC.

Analytical characteristics	Information
1. Wavelength (nm)	400
2. pH of tris - buffer solution	8.0
3. Concentration of CTC reagent (M)	4.0×10^{-5}
4. Pump flow rate (ml/min)	3.0
5. Length of mixing tubing (cm)	75
6. Shape of mixing part	s.b.s.r.
7. Inner diameter of mixing tubing (in)	0.0449
8. Injection volume (µl)	100
9. Linear range of calibration graph (ppm)	0.0 - 0.6 and 2.0 - 3.0
10.Slope of calibration graph (mV/ppm)	34.93
11.Reproducibility (%)	4.88
12.Repeatability (%)	4.32
13.Detection limit (ppm)	0.01
14.Percentage recovery (%)	92.07
15.Sample throughput (hr^{-1})	67

The effect of some possible interfering cations and anions was investigated. The results (Table 3.20) indicate that the presence of some interfering ions namely Na, SO_4^{2-}, and NO_3^- at concentration of 100 folds that of europium causes relative errors of less than 5%. The presence of Al(III) and Cl^- ion concentrations upto 100 folds that of europium, resulted in the relative error of less than 10%. While in the presence of Zn(II), Mg(II) and K ions at a concentration of 100 folds that of europium, less than 20% relative error was obtained.

The proposed method was applied to the analysis of europium(III) in spiked water samples. A series of recovery experiments were carried out by adding a standard europium(III) solution to aliquots of deionized water. The results obtained

(3.21) indicate that the proposed method is suitable for the determination of europium(III) in samples.

4.2 Conclusions

Two flow injection spectrophotometric procedures have been developed for determining Al(III) and Eu(III) using TC and CTC as chromogenic reagents respectively. In the former method, a single - line FI manifold incorporating with a knitted reactor was developed for Al(III) determination in water. A rectilinear calibration curve over the range of 0 - 1.0 ppm Al(III) could be established as little as 0.02 ppm Al(III) could be determined with the coefficient of variation of less than 0.91%. Cl^-, SO_4^{2-}, NO_3^-, F^- were not interfere. Na, Cu(II), Zn(II), K give slightly effect but Mg(II), Fe(III) interfere seriously. With respect to the latter FI method, a 2-line manifold equipped with a single bead string reactor instead of a knitted one was found to be appropriate FI manifold for Eu(III) determination. A calibration curve was linear over the range up to 0.6 ppm Eu(III). A detection limit of 0.01 ppm Eu(III), with a precision of 92.07% could be obtained. The presence of Na, Zn(II), SO_4^{2-} and NO_3^- cause slightly effect. Al(III), Cl^-, Mg(II) and K were not interfere only if the analyte to interfering ion ratios were less than 1:100 but these ratios were as high as 1000 fold interference effects would be observed.

Both FI-spectrophotometric methods proposed for the determination of aluminium(III) and europium(III) were found to be simple, sensitive and rapid. They offer several advantages such as low cost, good accuracy (recovery>90%) and reproducibility (R.S.D.<5%), and high sample throughput (80 hr^{-1} and 67 hr^{-1} for Al(III) and Eu(III) respectively).