CHAPTER 3

Results and discussion

3.1 FIA Spectrophotometric Determination of Copper Using Nitroso-R Salt as a Complexing Agent

A number of flow-injection methods for the determination of copper have been reported using fluorescence, ICP-MS and FAAS as detectors **[73, 124]**. However, these methods are inconvenient for monitoring copper in wastewater because they are usually expensive and too sensitive for the determination of copper(II) in wastewater, especially those from heavy machinery plants and refineries.

Most flow-injection systems have been increasingly used with various detectors **[73-75, 81, 86, 88, 89, 92, 102, 106, 124]** owing to their high sample throughput, cost-effective performance and versatility. However, a colorimetric detection system is often preferred, because it involves less instrumentation and provides better sensitivity when appropriate chromogenic reagents are available.

Although numerous organic reagents, such as PAN **[125]**, zincon **[105]**, PAR **[107]** and DDTC **[126]** have been proposed for the spectrophotometric determination of copper, none of the available reported methods are completely satisfactory.

In this study, Nitroso-R salt was used to react with copper(II), forming a colored, water-soluble anionic complex, the absorbance of which was measured spectrophotometrically at 492 nm. The stoichiometry of Cu(II)-nitroso-R complex was studied, and suggested the formation of $Cu(C_{10}H_5O_8NS_2)_2$ **[112]**. This reagent forms complexes with certain metal ions (*e.g.* Cr^{3+}, Pb^{2+}, Cd^{2+}, Zn^{2+}, *etc.*), but does not interfere with the reaction between copper(II) and Nitroso-R salt. The sensitivity of the reagent has been made selective by controlling the pH of the solution.

Therefore, in this investigation, an effort was made to develop a simple, selective, reproducible and sensitive FIA method for the determination of copper(II) in a wide range of materials. Various factors influencing the sensitivity of this method (such as the pH, concentration of buffer solution, flow rate of the solutions, sample loop length, concentration of nitroso-R and length of mixing coil) were optimized by using the univariate and simplex methods. These methods were developed for the selective determination at μg/ml-levels of copper(II) by FIA with spectrophotometric determination, which can be precise and accurate. This method is suitable for the quantitation of copper in wastewater and copper ore samples for routine analysis.

3.1.1 Absorption Spectra

The solutions with concentration of nitroso-R (0.02% w/v), with and without 10 μg/ml of copper(II) in acetate buffer medium (pH 7), were prepared. The absorption spectra of both $Cu(C_{10}H_5O_8NS_2)_2$ and the reagent blank solutions were scanned over a range from 370-550 nm and recorded, using a Hitachi U-2000 spectrophotometer (Figure 3.1). The complex exhibited two absorption maxima at about 492 and 390 nm, with at the highest absorbance 492 nm. However, in order to obtain the best sensitivity the λ_{max} at 492 nm was chosen for further studies.

3.1.2 Manifold Design

The term "manifold or chemifold", usually considered to consist of all parts of the system that contribute to the dispersion, will be restricted to mean the tubing, connectors, confluences, etc. **[2, 127]**. The real dispersion effects are due to the manifold designed in conjunction with the flow-rate of the carrier stream, and the volume of sample injected. The contribution to the over-all dispersion by the injection process is difficult to quantify and is usually considered to be negligible **[2]**.

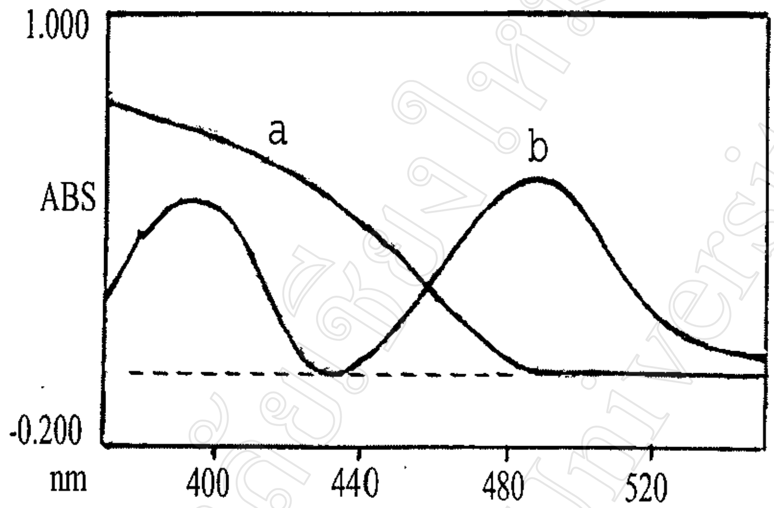
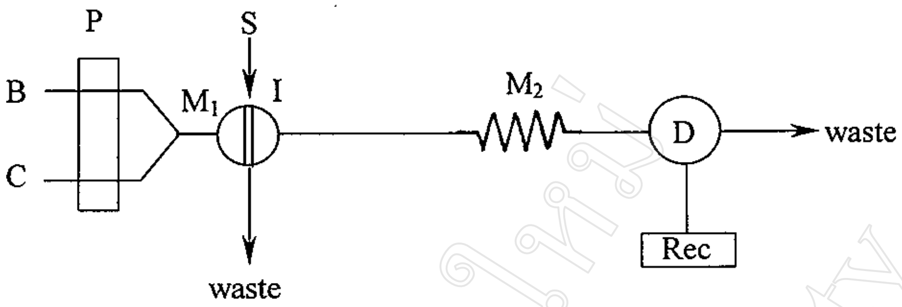
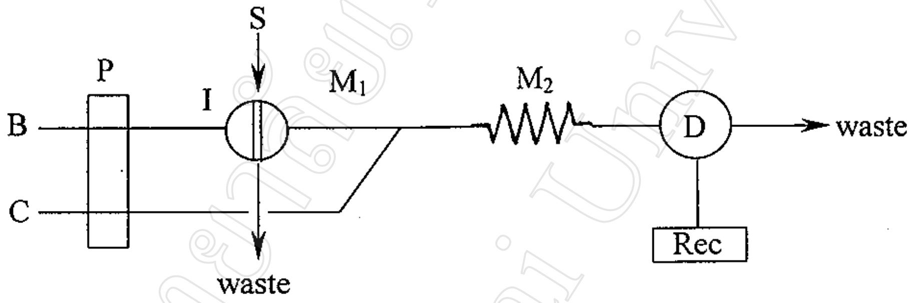


Figure 3.1 The spectra of the reagent solution (a) (against water as blank solution) and the Cu(II)-nitroso-R complex (b) (against reagent as blank solution).

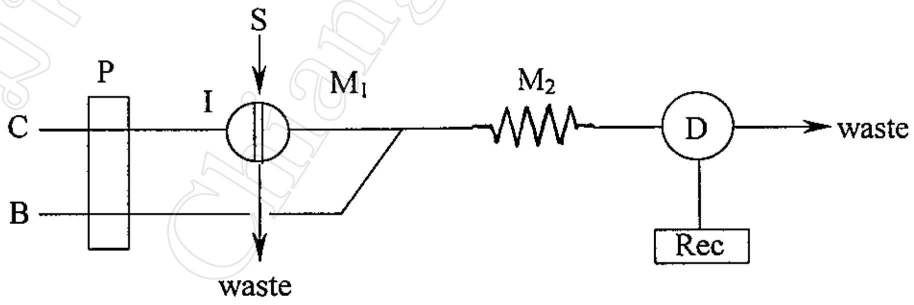
Preliminary studies revealed that, nitroso-R could be used as a coloring agent in FIA for copper(II) determination and that it could also be adopted to the development of a novel flow injection spectrometric procedure for copper(II) determination. The optimum conditions for operation of the FIA system were investigated. A variety FIA manifolds were designed and tested as shown in Figure 3.2. Three types of manifolds were designed and constructed: copper solution was injected via a rotary valve into carrier stream of buffer solution before merging into a stream of the coloring agent (Figure 3.2-b). In Figure 3.2-c copper(II) solution was injected via rotary valve into a stream of coloring agent again before the two streams were merged and the FIA manifold with injection of the sample solution after the merged streams of both reagent streams (Figure 3.2-a). The peak heights were obtained by injecting the copper(II) standard solutions with concentration of 1.0, 3.0, 5.0 and 7.0 mg/l successively. The results are shown in Table 3.1 and Figure 3.3.



Manifold-a



Manifold-b



Manifold-c

Figure 3.2 Various types of the manifolds. (S, sample; B, acetate buffer (pH 7, 0.5 M); C, nitroso-R reagent (0.04% w/v); P, peristaltic pump; I, home-made injection valve; M_1, mixing part; M_2, mixing coil; D, spectrophotometer; and Rec, recorder.

Table 3.1 Effect of various type of manifold on peak height.

Manifold	Peak height* (mV) obtained from the standard Cu (II)(mg/l)				y = a(x) + b	r^2
	1.0	3.0	5.0	7.0		
a	7.5	27.0	42.0	55.3	y = 7.92(x)+1.27	0.9921
b	8.0	23.5	36.8	47.8	y = 6.64(x)+2.48	0.9872
c	0.0	17.3	31.7	43.0	y = 7.71(x)-5.68	0.9943

(* average of triplicate injections)

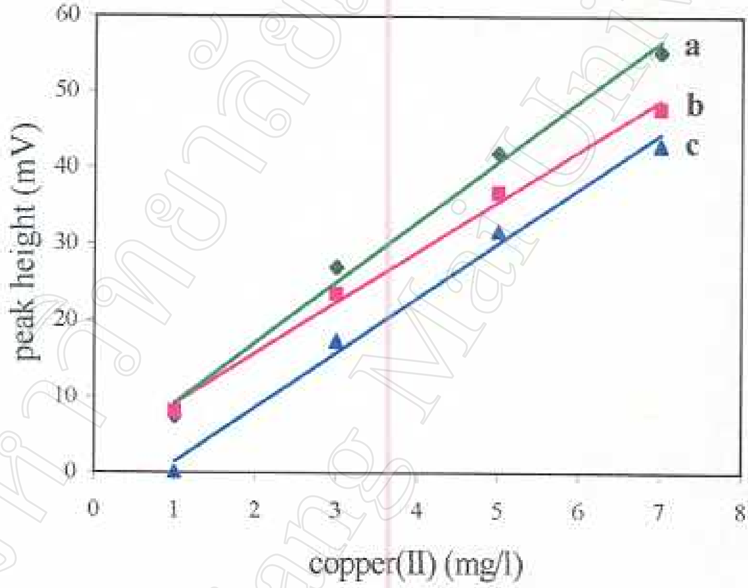


Figure 3.3 Effect of various types of manifold on the calibration curve (a, manifold-a; b, manifold-b; c, manifold-c).

By comparing the results obtained from the three manifolds (Table 3.1 and Figure 3.3), it was found to be more satisfactory to inject the sample into the merged stream (manifold-a) rather than injecting into the buffer stream or the coloring streams before merging; higher peak height and slope were obtained. So, the manifold-a was chosen as a feasible manifold for further investigations for copper(II) determination.

3.1.3 Optimization of the flow system by Univariate Method

An FIA system uses lower concentrations of reagents than corresponding batch method. This is done in order to reduce the effect of refractive index differences between the reagent and sample solution, especially when the colored reagent is used that has a high absorption for base line measurement. Then the variable-by-variable method was applied to select the optimum conditions for the FIA spectrophotometric determination of copper(II). For this reason, a variable was modified while maintaining the other variables at their constant values (chosen by random). Then, by maintaining that variable at its optimum value, another was modified; all variables were optimized via this method [**128**].

To optimize the conditions, the FIA manifold in Figure 3.2-a was used. The mixing coil had the following dimensions M_1: 1.5 mm i.d. 10 cm long, M_2: 1.5 mm i.d., 30 cm long. The injection volume was 103 μl. Concentration of nitroso-R was 0.04% (w/v). The acetate buffer (pH 7), concentration 0.5 M was used. The flow rate of each reagent was 3.2 ml/min. Copper(II) in the range of 1.0 to 7.0 mg/l were injected in the system.

All optimum values were chosen by judging from the highest peak height, stability of the base line, low or no positive blank signals, low analysis time, availability and economy. Preliminary conditions used were as shown in Table 3.2.

Table 3.2 Preliminary conditions before optimization of the FIA systems.

Variable	Fixed Value
Reagent concentration	0.04% (w/v)
Acetate buffer	pH 7.0, 0.5 M
Flow rate of reagent and buffer	2.1 ml/min
M_1 dimension	20 cm, 1.5 mm i.d.
M_2 dimension	70 cm, 1.5 mm i.d.
Sensitivity of recorder	10 mV/cm

3.1.3.1 Wavelength

The results shown in Table 3.3 and Figure 3.4 indicated that the highest peak height was obtained when the absorbance was measured at 492 nm. The analytical wavelength at 492 nm was selected for the further studies.

Table 3.3 Effect of the wavelength on peak height.

Wavelength λ (nm)	**Peak height* (mV) obtained from the standard Cu(II)(mg/l)**			
	1.0	**3.0**	**5.0**	**7.0**
484	0.0	16.8	34.0	47.7
488	3.8	22.0	38.2	50.8
492	8.7	25.8	40.3	53.0
496	8.7	26.2	39.5	51.2
500	8.8	24.0	38.0	48.3
504	8.5	22.5	35.2	45.0
508	7.0	19.0	30.5	39.2
512	6.2	15.7	25.5	33.7

(* average of triplicate injections)

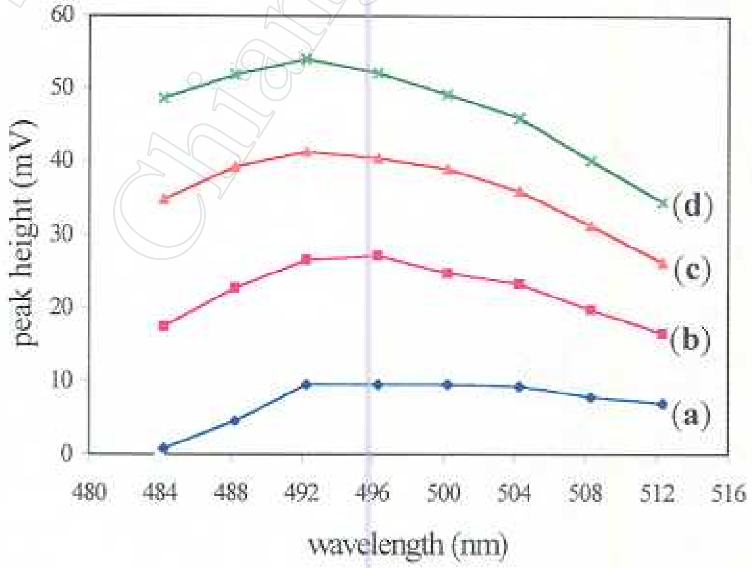


Figure 3.4 Effect of the wavelength on peak height [Cu(II) standard solution (a)1.0, (b) 3.0, (c) 5.0 and (d) 7.0 mg/l].

3.1.3.2 Effect of pH of Buffer Solutions

The effects of acetate and phosphate buffer solutions have been investigated. It was found that phosphate buffer solution provided poor sensitivity for copper(II) determination. Therefore, acetate buffer solution was selected as suitable.

The complexation of copper(II) with nitroso-R was studied at different pH values in the ranges of 4.0-10.0. The pH values of buffer solution were adjusted with acetic acid/sodium acetate. Using the manifold as shown in Figure 3.2-a, a solution of copper(II) 2.0 mg/l and a blank solution (1% (v/v) HNO_3) were injected in the system. The peak heights obtained are shown in Table 3.4 and Figure 3.5 indicated that pH values below 6.0 or above 8.0 the peak height decreased significantly. Hence, pH 7.0 was chosen.

Table 3.4 Effect of pH of buffer solution on peak height.

pH of buffer solution	Peak height (mV)*
4	9.2
5	13.2
6	19.9
7	21.2
8	21.0
9	11.2
10	7.2

(* average of triplicate injections)

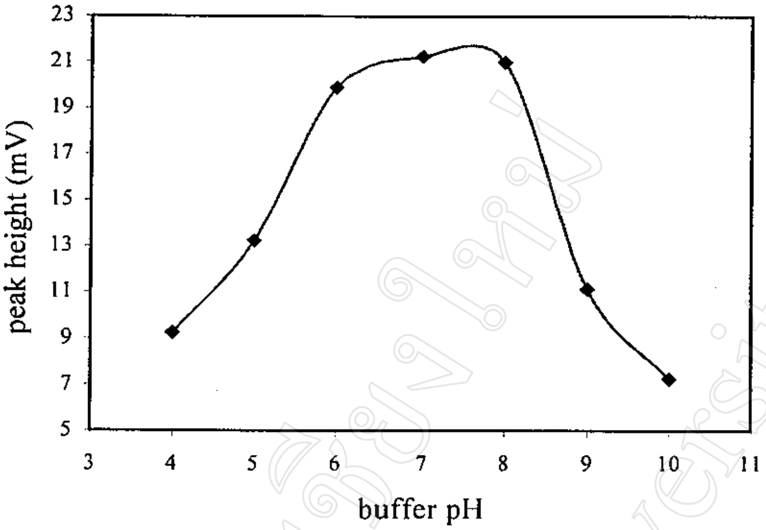


Figure 3.5 Effect of pH of the buffer solution on peak height.

3.1.3.3 Effect of Buffer Concentration

Effect of the concentration of buffer solution (pH 7.0, 0.2-1.4 M) was studied, using the condition in section 3.1.3.2. The copper(II) solution in different concentrations were injected in the system. The results are presented in Table 3.5 and Figure 3.6. It was found that the high concentrations of buffer were a waste of chemicals. A buffer concentration of 0.5 M should be used for the further investigation.

3.1.3.4 Effect of Mixing Part (M_1) Length

The mixing parts are responsible for ensuring complete reagent mixing and, therefore, result in a time delay for development of a reaction between the analyte and reagent. The 1.5 mm i.d. Tygon tubing was examined to use as mixing part M_1. The 0.04% (w/v) nitroso-R salt and 0.5 M acetate buffer pH 7 were used. The flow rate of both reagents was 3.2 ml/min. By injecting 103 μl of a series of standard copper(II) solutions, calibration curves were constructed. The results shown in Table 3.6 and Figure 3.7 indicated that the peak height was not different when the mixing part lengths were used in range 10-25 cm. So, the shortest length should be selected.

Table 3.5 Effect of acetate buffer concentration on peak height.

Acetate buffer concentration (M)	Peak height* (mV) obtained from the standard Cu (II)(mg/l)				y = a(x) + b	r^2
	1.0	3.0	5.0	7.0		
0.2	1.0	11.0	21.0	27.8	y=4.520(x)-2.880	0.9925
0.4	6.0	23.5	37.0	28.2	y=7.005(x)+0.655	0.9898
0.5	8.5	25.5	40.2	53.3	y=7.455(x)+2.055	0.9966
0.6	9.0	26.3	41.7	54.0	y=7.520(x)+2.670	0.9944
0.8	9.0	27.0	43.0	56.0	y=7.850(x)+2.350	0.9949
1.0	9.2	27.3	44.5	56.5	y=7.955(x)+2.555	0.9920
1.2	9.2	27.3	44.0	56.5	y=7.930(x)+2.530	0.9935
1.4	9.3	27.3	44.0	56.6	y=7.930(x)+2.580	0.9939

(* average of triplicate injections)

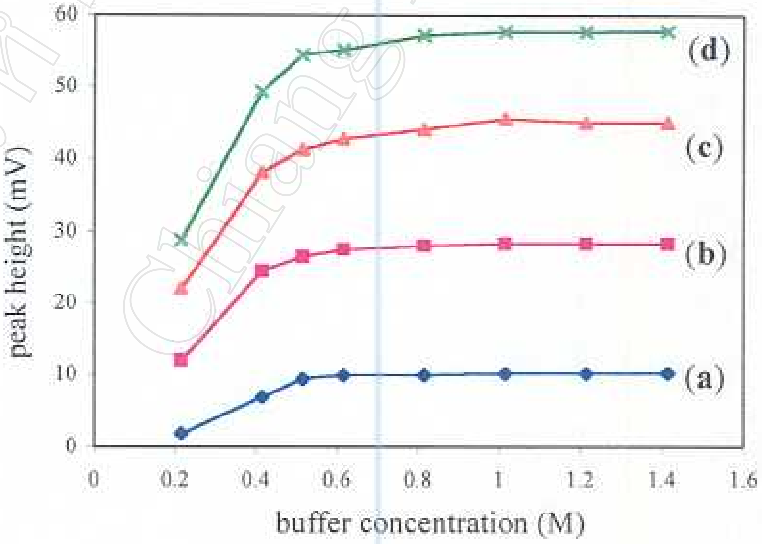


Figure 3.6 Effect of the buffer concentration on peak height [Cu(II) standard solution (a)1.0, (b) 3.0, (c) 5.0 and (d) 7.0 mg/l].

Table 3.6 Effect of the length of the mixing part (M_1) on peak height.

Length (cm)	Peak height* (mV) obtained from the standard Cu (II)(mg/l)				y = a(x) + b	r^2
	1.0	**3.0**	**5.0**	**7.0**		
10	8.0	26.3	41.5	53.3	y=7.555(x)+2.055	0.9908
15	7.8	26.3	41.7	53.5	y=7.625(x)+1.825	0.9904
20	7.8	26.2	41.7	53.5	y=7.630(x)+1.780	0.9907
25	7.8	26.5	42.0	54.0	y=7.705(x)+1.755	0.9906

(* average of triplicate injections)

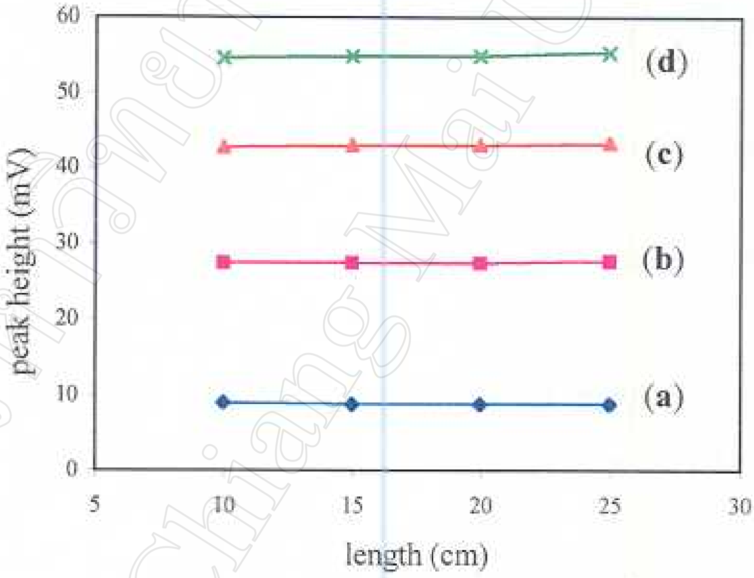


Figure 3.7 Effect of the length of the mixing part (M_1) on peak height [Cu(II) standard solution (a)1.0, (b) 3.0, (c) 5.0 and (d) 7.0 mg/l].

3.1.3.5 Effect of Mixing Coil (M_2) Length

The 1.5 mm i.d. Tygon tubing was examined to use as mixing coil M_2 of 1.5 cm diameter and variable length (10-60 cm long), and using the conditions in section 3.1.3.4. The results were shown in Table 3.7 and Figure 3.8. The results indicated that the M_2 coil length 30 cm gave the highest peak height, therefore, it was chosen for further studies.

Table 3.7 Effect of the length of the mixing coil (M_2) on peak height.

Length (cm)	**Peak height* (mV) obtained from the standard Cu (II)(mg/l)**				**y = a(x) + b**	**r^2**
	1.0	**3.0**	**5.0**	**7.0**		
10	6.0	22.2	36.8	48.8	y=7.150(x)-0.150	0.9957
20	7.5	24.7	38.0	50.5	y=7.115(x)+1.715	0.9941
30	9.0	27.0	40.8	53.7	y=7.395(x)+3.045	0.9936
40	8.0	26.0	40.7	53.7	y=7.590(x)+1.740	0.9945
50	9.0	25.3	39.5	51.3	y=7.055(x)+3.055	0.9949
60	8.5	20.5	38.0	51.2	y=7.280(x)+0.430	0.9952

(* average of triplicate injections)

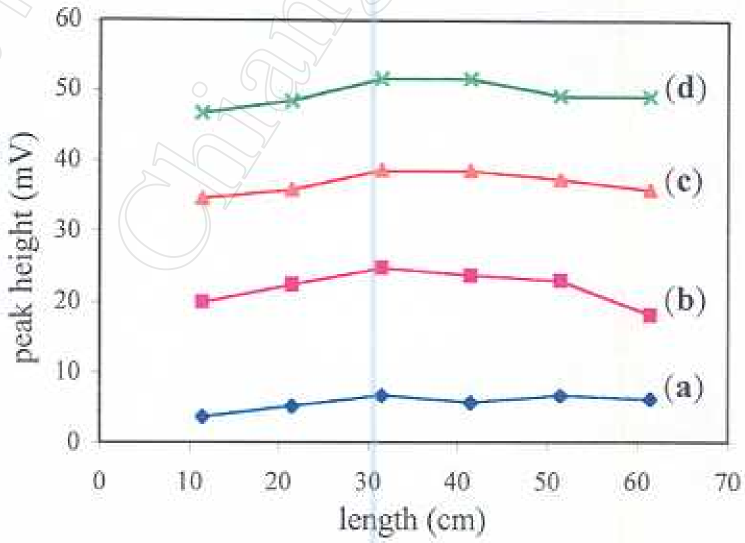


Figure 3.8 Effect of the length of the mixing coil (M_2) on peak height [Cu(II) standard solution (a)1.0, (b) 3.0, (c) 5.0 and (d) 7.0 mg/l].

3.1.3.6 Effect of Reagent Concentration

The concentrations of the coloring reagent would have more or less effects on the Cu(II):nitroso-R complex. Therefore, it is essential to investigate the optimum concentrations of nitroso-R solution in order to obtain the selectivity and sensitivity.

An amount of reagent greater than that required by stiochiometry (Cu(II):nitroso-R = 1:2) **[112]** is needed for complete color development. The results are shown in Table 3.8 and Figure 3.9. The concentration of nitroso-R was sufficient for complete color development; higher concentration resulted in a higher negative peak of the reagent blank. Thus, low sensitivity was obtained. Concentrations of nitroso-R solution in the range 0.02-0.1% (w/v) were studied using the conditions as shown in section 3.1.3.5. By considering the highest peak height, economy and correlation coefficient of the curves, the 0.04 % (w/v) nitroso-R was chosen for further study.

Table 3.8 Effect of the reagent concentration on peak height.

Reagent concentration (% w/v)	Peak height* (mV) obtained from the standard Cu (II)(mg/l)				y = a(x) + b	r^2
	1.0	3.0	5.0	7.0		
0.02	8.0	22.7	35.0	45.7	y=6.270(x)+2.770	0.9949
0.04	8.0	23.7	39.7	51.8	y=7.370(x)+1.320	0.9962
0.06	6.5	24.5	42.2	53.8	y=7.980(x)-0.170	0.9907
0.08	4.8	24.7	42.0	56.0	y=8.545(x)-2.305	0.9941
0.10	2.8	21.8	42.2	57.3	y=9.195(x)-5.755	0.9964

(* average of triplicate injections)

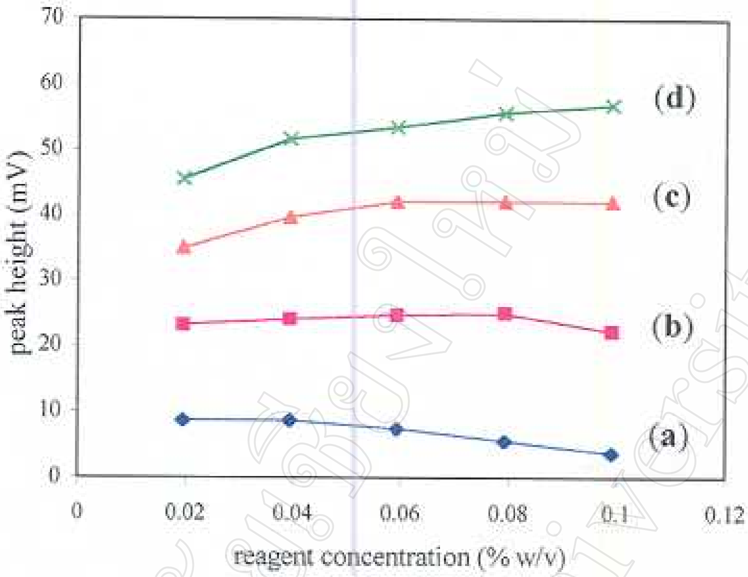


Figure 3.9 Effect of the reagent concentration on peak height [Cu(II) standard solution (a)1.0, (b) 3.0, (c) 5.0 and (d) 7.0 mg/l].

3.1.3.7 Effect of Flow rate

Reagent and carrier flow rate effects were studied, by injecting various concentrations of standard copper(II) solutions (1.0-7.0 mg/l) in to the flow system. The flow rate of each stream is varied in the range 1.6-4.4 ml/min. The effect of flow rate on the peak height was measured as shown in Table 3.9 and Figure 3.10. A high flow rate gives rise to a shorter time required for each sample passing through the system, a low precision of the peak height, and a high rate of reagent consumption and did not significantly affect the sensitivity. With the lower flow rate, the residence time for each sample was longer and the dispersion was large and caused low sensitivity. Therefore, the flow rate of each stream of 3.2 ml/min was selected, because it provides high sensitivity and consumes less reagent volume.

Table 3.9 Effect of flow rate on peak height.

Flow rate (ml/min)	Peak height* (mV) obtained from the standard Cu (mg/l) (II)				y = a(x) + b	r^2
	1.0	**3.0**	**5.0**	**7.0**		
1.6	4.5	23.5	38.0	50.5	y=7.625(x)-1.375	0.9907
2.1	7.0	26.2	41.0	53.0	y=7.640(x)+1.240	0.9889
2.6	7.0	26.8	43.0	54.8	y=7.980(x)+0.980	0.9876
3.2	8.5	26.7	43.0	54.8	y=7.760(x)+2.210	0.9913
3.8	8.5	26.2	41.0	53.2	y=7.445(x)+2.445	0.9932
4.4	8.5	26.0	41.5	52.5	y=7.375(x)+2.625	0.9901

(* average of triplicate injections)

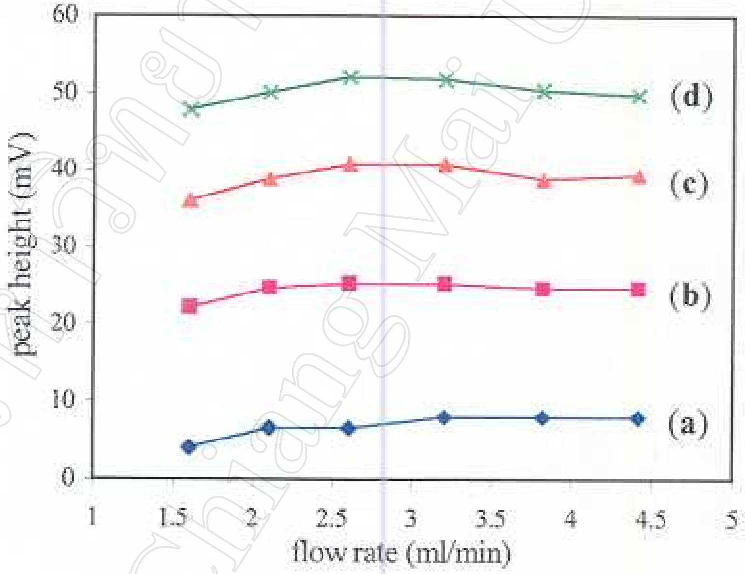


Figure 3.10 Effect of flow rate on peak height [Cu(II) standard solution (a)1.0, (b) 3.0, (c) 5.0 and (d) 7.0 mg/l].

3.1.3.8 Effect of Sample Volume

Using the mixing coil 30 cm long, the 0.04% (w/v) nitroso-R, 0.5 M acetate buffer (pH 7) and the flow rate of both lines of 3.2 ml/min the sample volume was varied by changing the length of sample loop. The 0.86 mm i.d.

Teflon tubing was examined to use as sample loop. The effects of a sample loop length studied are shown in Table 3.10 and Figure 3.11. The results indicate that the sample volume of 103 µl injected provides a good linearity and correlation in the calibration range of 1.0-7.0 mgCu(II)/l. The sample loop length of 20 cm was chosen for further studies.

Table 3.10 Effect of sample volume on peak height.

The length of injection loop (cm)	Sample volume (µl)	Peak height* (mV) obtained from the standard Cu (II)(mg/l)				y = a(x) + b	r^2
		1.0	3.0	5.0	7.0		
10	52	7.5	20.8	33.7	45.5	y=6.34(x)+1.49	0.9993
15	77	8.0	24.5	38.7	51.0	y=7.16(x)+1.91	0.9957
20	103	8.0	26.3	54.3	54.3	y=7.72(x)+1.72	0.9930
25	129	8.7	27.0	41.8	53.3	y=7.43(x)+2.98	0.9896
30	155	8.3	25.8	42.0	52.7	y=7.47(x)+2.32	0.9890

(* average of triplicate injections)

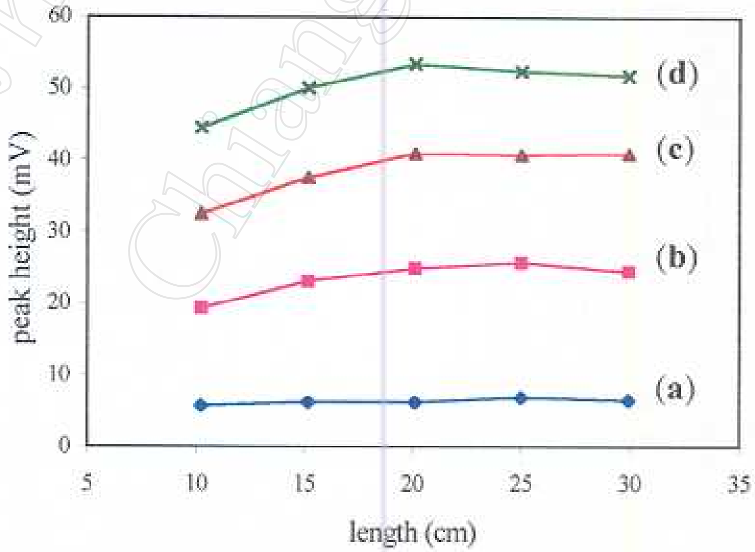


Figure 3.11 Effect of sample loop length on peak height [Cu(II) standard solution (a)1.0, (b) 3.0, (c) 5.0 and (d) 7.0 mg/l].

3.1.3.9 Summary of the Studied Range and Optimum Conditions

A diagram of the recommended FIA manifold is displayed in Figure 2.1. Table 3.11 shows the ranges over which the variables involved in the FIA system were studied and their optimum values.

Table 3.11 Univariate optimization of chemical and FIA variables.

Variable	Studied range	Optimum value
Wavelength (λ)	484-512 nm	492 nm
pH of buffer	4.0-10.0	7.0
Buffer concentration	0.2-1.4 M	0.5 M
Mixing coil length	10-60 cm	30 cm
Reagent concentration	0.02-0.10% (w/v)	0.04% (w/v)
Flow rate	1.6-4.4 ml/min	3.2 ml/min
Sample loop length	10-30 cm	20 cm (103 μl)

3.1.4 Simplex Optimization

A simplex is a geometric figure in which there are $n + 1$ vertices, where n represents the number of variables **[129]**. The parameters to be varied are chosen by initial experiments. Although optimization means maximization of the response, it could be applied equally well to the process of finding a minimum. The simplex method is highly efficient, capable of optimizing several factors at one time, and the variable-size simplex (rule and worksheet for the variable-size simplex, see Appendix A) is not stranded by ridge systems. The interaction with the experimenter is not necessary, and because the simplex technique uses only empirical feedback, it is of special interest to conduct investigations involving totally automated optimization.

In this work, the simplex method was used to confirm the optimum conditions, which were obtained by the univariate procedure. Four major parameters (flow rate, reaction coil length, acetate buffer concentration, and reagent concentration variables) were optimized by the simplex procedure, while the other minor parameters were optimized by the univariate method. The initial parameters to be optimized by the simplex method were chosen from the optimum conditions obtained by the univariate method; others are those appearing next to the optimum values. Table 3.12, Figure 3.12 and Appendix B show the results of the four-variable optimization. Points (or vertexes) 1-5 represent the first cycle, and the first point is the optimum condition of the univariate technique. The best point attained was point 1 with a peak height of 11.5 mm; the worst was point 5 with a peak height of 9.5 mm. Therefore, point 5 was reflected through the centroid of other points to obtain point 6. An experiment was then performed utilizing the variable setting as the reflected point; a peak height of 9.7 mm was obtained. Because this value was better than that at point 5, the latter was rejected and replaced by point 6. A contraction was then performed, because this point was not better than the next-to-the-worst point, point 4. Then, by using the experimental setting of variables generated by contraction, a peak height of 11.2 mm was obtained, which was not better than the best point; hence, this completed the cycle. However, although attempts were made to make further improvements towards maximization, this was found to necessitate additional experiments and was deemed not to be worthwhile; the procedure was therefore halted. It was found that the optimum conditions by the simplex optimization are similar to those obtained by the univariate method.

Table 3.12 Simplex optimization of chemical and FIA variables.

Expt.No*	% Nitro-R (w/v)	Mixing coil length (cm)	Flow rate (ml/min)	Sample loop length (cm)	Peak height (mV)
1	0.04	30.0	5	20	11.5
2	0.03	30.0	4	20	11.0
3	0.03	20.0	5	20	10.0
4	0.04	40.0	6	25	10.5
5	0.05	40.0	6	25	9.5
6 R	0.02	20.0	4	18	9.7
7 C_r	0.03	25.0	5	19	11.2
8 R	0.04	43.0	5	22	10.3
9 C_r	0.04	37.0	5	22	10.7
10 R	0.03	21.0	4	16	10.5

* R = reflection ; C_r = contraction

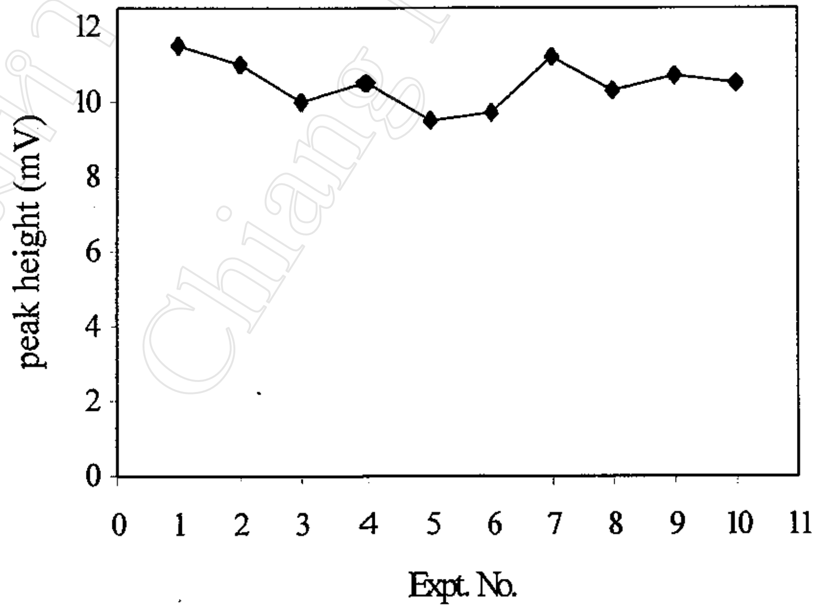


Figure 3.12 Peak height vs. experiment number for the simplex self-test results.

3.1.5 Calibration Curve

Using the FIA manifold (Figure 3.2-a) and the optimum conditions in Table 3.11, the calibration curve was constructed by injecting a series of standard copper(II) solutions. Results are shown in Table 3.13, Figure 3.13 and Figure 3.14. With the proposed flow injection system for copper(II) determination, the linearity was over the concentration range that caused peak height deviation not exceeding 5% from the linear regression line. It was found to be a linear plot for the concentration range studied 1.0-7.0 mgCu(II)/l, which can be expressed by the regression equation as shown below:

$$Y = 7.0714\ (X) + 2.2857, \quad (r^2 = 0.9989),$$

Where Y is the peak height (mV) and X the copper(II) concentration in mg/l. Procedural blanks were determined and subtracted from the sample peak height.

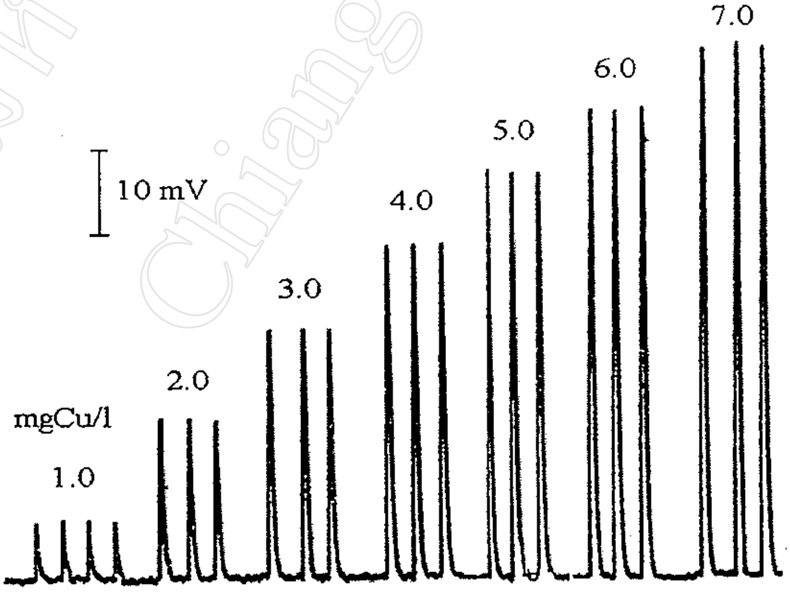


Figure 3.13 The FIA-gram of standard copper(II) solution.

Table 3.13 Peak height for calibration curve.

Cu(II) (mg/l)	Peak height (mV)*
1.0	9.0
2.0	16.0
3.0	24.0
4.0	31.0
5.0	38.0
6.0	45.0
7.0	51.0

(* average of triplicate injections)

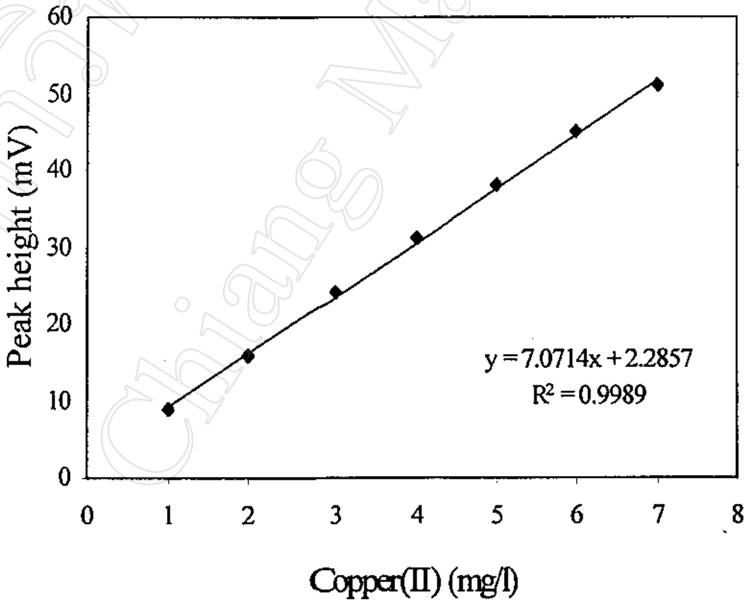


Figure 3.14 The calibration curve of FIA spectrophotometric determination of copper(II).

3.1.6 Precision and Detection Limit

The precision of the recommended method was evaluated by injecting 11 replicates of standard copper(II) 4.0 mg/l (Figure 3.15), using the optimum conditions in section 3.1.3.9. The results are summarized in Table 3.14. The relative standard deviation was estimated to be 0.47%. The percentage recoveries were studied by spiking various amounts of copper(II) standard solutions into copper ore solution and wastewater samples. The results indicated that the percentage recoveries of copper(II) found in copper ore solution and wastewater samples ranged from 94 to 102 and 93 to 102%, respectively. The sample throughput was 150 samples/h.

The detection limit was determined from the regression equation with the calculated parameters of the intercept of the straight line and three-times the standard deviation of the regression time **[130]** LOD = 0.68 mgCu(II)/l.

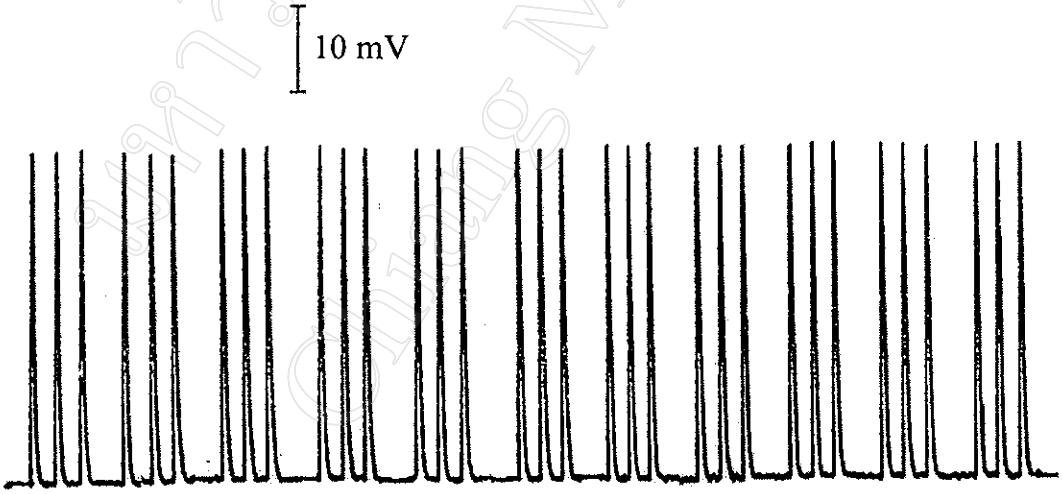


Figure 3.15 FIA peaks obtained from 11 replicate determinations of standard copper(II) 4.0 mg/l.

Table 3.14 The precision using standard 4.0 mgCu(II)/l .

Expt. No.	Peak height (mV)*
1	39.7
2	39.3
3	39.5
4	39.7
5	39.3
6	39.5
7	39.8
8	39.7
9	39.8
10	39.8
11	39.8
	$\bar{x}$ = 39.6
	SD = 0.19
	% RSD = 0.47

(* average of triplicate injections)

3.1.7 Interference Studies

The inference effects of some possible foreign ions in FIA system for copper(II) was studied by the proposed FIA procedure using the optimum conditions obtained. A systematic study to check for the effects of some possible interference of foreign ions on the determination of copper(II) was undertaken for the maximum w/w ratio of foreign ions to copper(II) ion up to 100:1. A solution of a 4.0 mg/l copper(II) standard containing varying concentration of diverse ions were injected into the FIA system. The tolerance is defined as the foreign-ion concentration causing an error smaller than ±10% for determining the analyte of interest. The tolerance values for the ions studied are summarized in Table 3.15 and Table 3.16. The most serious interferences are caused by Fe^{3+}, Ni^{2+} and Co^{2+} ions, probably due to the formation of complexes with nitroso-R which absorb at the same or very near to the working wavelength.

Table 3.15 Effect of interference study for 4.0 mg/l standard copper(II).

Ions	Chemical form added	Concentration added (mg/l)	Peak height (mV)*	% Relative error
none	-	-	34.0	-
Pb^{2+}	$Pb(NO_3)_2$	40	33.3	-2.2
		80	31.3	-7.8
		200	30.7	-9.7
		400	29.2	-14.1
Cr^{3+}	$Cr(NO_3)_3$	40	34.2	-0.6
		200	33.8	-0.6
		360	32.8	-3.8
		400	31.4	-7.6
Mn^{2+}	$Mn(NO_3)_2$	40	34.2	-0.6
		80	33.0	-2.9
		200	31.5	-7.4
		400	28.9	-15.0
Mg^{2+}	$Mg(NO_3)_2$	40	34.2	+0.2
		80	34.0	0.0
		200	32.8	-0.4
		400	29.2	-14.1
Cd^{2+}	$Cd(NO_3)_2$	40	34.2	+0.2
		80	33.4	-1.8
		200	31.9	-6.2
		400	27.9	-17.9

Table 3.15 (Continued).

Ions	**Chemical form added**	**Concentration added (mg/l)**	**Peak height (mV)***	**% Relative error**
Zn^{2+}	$Zn(NO_3)_2$	40	34.0	0.0
		80	33.1	-3.8
		200	32.6	-7.9
		400	29.3	-13.8
Na^{+}	$NaNO_3$	40	33.5	-1.5
		120	31.3	-7.9
		160	31.0	-8.8
		200	30.4	-10.6
Ca^{2+}	$Ca(NO_3)_2$	40	33.0	-2.9
		160	30.0	-11.8
		200	29.0	-14.7
Fe^{3+}	$Fe(NO_3)_3$	4	36.0	+5.9
		8	37.6	+10.6
		10	38.5	+13.2
Ni^{2+}	$Ni(NO_3)_2$	2	36.8	+8.2
		4	38.6	+13.5
		8	45.2	+32.9
Co^{2+}	$Co(NO_3)_2$	4	56.1	+65.0
NO_2^{-}	$NaNO_2$	40	34.3	+0.9
		120	32.1	-5.6
		160	30.7	-9.7
		200	29.2	-14.1
Cl^{-}	KCl	40	33.9	-0.3
		120	31.7	-6.8
		160	30.8	-9.4
		200	28.6	-15.9

Table 3.15 (Continued).

Ions	Chemical form added	Concentration added (mg/l)	Peak height (mV)*	% Relative error
SO_4^{2-}	Na_2SO_4	40	33.7	-0.9
		120	32.2	-5.3
		160	30.2	-11.2
		200	30.1	-11.5
PO_4^{3-}	NaH_2PO_4	40	33.2	-2.4
		120	30.6	-10.0
		160	29.6	-12.9
		200	28.2	-17.1

(* average of triplicate injections)

Table 3.16 Summary of the interference effects of some ions on the response obtained from 4.0 mgCu(II)/l .

Interference ions	Tolerable concentration ratio*(mg/l) of ion/Cu(II)
Cr^{3+}	> 100
Pb^{2+}, Mn^{2+}, Mg^{2+}, Cd^{2+}, Zn^{2+}	50
Na^+ , NO_2^-, Cl^-	40
SO_4^{2-}, PO_4^{3-}	30
Ca^{2+}	10
Fe^{3+}	1
Ni^{2+}, Co^{2+}	< 1

* The concentration of an ion is considered to be interfering when causing a relative error of more than ±10 % with respect to the signal of copper(II) alone.

3.1.8 Determination of Copper in Wastewater Samples and Copper Ores Samples

The carrier and the reagent streams were pumped into the proposed flow system. After the base line had stabilized on the chart recorder, 103 μl of the pretreated sample [procedures in section 2.5.1-(1) and (2)] solution was injected into the system and the absorbance was continuously monitored at 492 nm. The data were collected as peak height by a chart recorder. The peak heights are rectilinearly related to the copper(II) concentrations. The results were compared with those obtained by FAAS, both of conventional and standard addition method, as shown in Table 3.17 and Table 3.19. The accuracy was found to be high, because the student t-values were calculated to be less than the theoretical values at a confident level of 95%, as shown in Table 3.18 and Table 3.20, indicating that the results obtained by both FIA and FAAS are in excellent agreement.

Table 3.17 Concentration of copper in wastewater samples.

Sample No.	**Concentration of copper (mg/l)**			
	a	**b**	**c**	**d**
A 1	1440	1420	1520	1460
A 2	1580	1580	1660	1620
A 3	1960	2000	2020	1980
A 4	700	720	760	720
A 5	720	730	830	760
A 6	510	510	560	510
A 7	1130	1150	1270	1180

(a = recommended method FIA, b = standard addition FIA, c = FAAS, d = standard addition FAAS)

Table 3.18 Calculation for t-Test.

Comparison	t-value
a – b	0.04
a – c	0.49
a – d	0.06

(a = recommended method FIA, b = standard addition FIA, c = FAAS, d = standard addition FAAS)

Table 3.19 The weight percentage of copper contents in samples (Copper Ores).

Sample No.	Cu (% w/w)			
	a	b	c	d
S 1	17.68	17.80	17.60	16.88
S 2	35.00	35.24	36.00	34.48
S 3	8.24	8.16	8.44	8.04
S 4	32.20	32.40	33.60	32.20
S 5	20.96	22.84	21.44	21.56
S 6	8.24	8.32	8.28	8.16
S 7	21.28	21.28	20.72	21.00
S 8	8.60	8.48	9.00	8.60
S 9	27.44	26.84	26.16	25.56
S 10	6.96	6.96	7.68	7.36
S 11	8.64	8.64	9.24	9.16
S 12	16.32	15.92	16.80	16.72

(a = recommended method FIA, b = standard addition FIA, c = FAAS, d = standard addition FAAS)

Table 3.20 Calculation for t-Test.

Comparison	t-value
a – b	0.03
a – c	0.19
a – d	0.04

(a = recommended method FIA, b = standard addition FIA, c = FAAS, d = standard addition FAAS)

3.1.9 Summary of Optimum Conditions of FIA Method for Copper(II) Determination

The optimum conditions and the features of the proposed method are summarized in Table 3.21.

Table 3.21 Optimum conditions.

Variable	Optimum conditions
Manifold	a two channel FI manifold (Figure 2.1)
Reagent	0.04% (w/v) nitroso-R
Mixing coil length	30 cm
Reagent flow rate	3.2 ml/min
Analytical wavelength	492 nm
Linear calibration range	1.0-7.0 mg/l
Calibration equation	$Y = 7.0714x + 2.2857$, $r^2 = 0.9989$
Detection limit	0.68 mg/l
Precision	RSD = 0.47% (for 4.0 mgCu(II)/l, n = 11)
Samples rate	150 h^{-1}

3.2 Study of Copper Adsorption on Perlites and Synthetic Zeolites from Perlites as Packing Material for FIA On-line Preconcentration Column

Recently, several methods, including precipitation **[131]**, coprecipitation **[132]**, and ion exchange **[133]** have been used for the removal of copper(II) from real samples. The adsorption phenomenon has still been found economically appealing for the removal of copper(II) from wastewater by choosing some adsorbents under optimum operating conditions. The behaviours of some adsorbents like sawdust **[134]**, soil and clay **[135]**, raw chacoal **[136]**, modified silica gel **[137]**, agricultural solid wastes **[138]**, fly ash **[139]**, seafood processing sludge **[140]**, peanut hull carbon **[141]**, apple residues **[142]**, white-rot fungus **[143]**, algae **[144]**, sugar beet pulp **[145]**, chitosan **[146]**, natural zeolite **[123]** have been studied for the adsorption of copper(II) ions.

The natural and expanded perlite samples were obtained from Thai Tridymite Co. Ltd. (Thailand). The starting material was ground to approximately 100 mesh, then sieved by a 100-mesh sieve. The perlite fines (less than 100 mesh) was then washed with hydrochloric acid solution and deionized water until the pH of the effluent was about pH 7. The solid thus obtained was filtered and dried at temperature of 100-110°C for 2 hours.

In this study, the adsorption behaviour of copper(II) on the Lopburi perlite has been investigated. The natural and expanded perlites have been converted to zeolite. Subsequently the natural and expanded perlites together with the synthetic zeolites were investigated as sorbents for the removal of copper(II) from aqueous solution containing copper(II). If, the adsorption of copper(II) ions on both the perlites and zeolites was proved to be favorable then each sorbent could be proposed as a novel packing material for FIA on-line preconcentration column.

3.2.1 Effect of pH

As can be seen in Table 3.22 and Figure 3.16.The pH of a solution at which adsorption occurs may influence the extent of adsorption of copper(II) on the sorbents studied. The influence of solution pH value on copper(II) uptake was investigated under the following conditions: the test solution containing 5 mg of copper(II); a shaking time 30 min; v:m ratio of 50 ml: 0.2000 g of the sorbent, and pH values (referred to as initial pH) of 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0. It is shown that highest efficiency removal of copper(II) in the test solution due to uptake ability of zeolite (most probably by ions exchange adsorption) is observed at pH values more than 3.0. An increase in the copper(II) removal efficiency of copper(II) for all adsorbent was observed initially at initial pH of 6.0. It could be assigned to precipitation of copper(II) **[142]**. Initially the removal efficiency of copper(II) due to precipitation was found to be 36.6-98.8% in the adsorbents. These values are in the range which corresponds to the values of copper(II) uptake by natural perlite and synthetic zeolite from expanded perlite from aqueous solutions at pH 3-5. Hence, the pH of the aqueous solution is one of the important parameters in the copper(II) adsorption process.

Table 3.22 Effect of pH on % adsorption. (N, natural perlite; E, expanded perlite; ZN, synthetic zeolite from natural perlite; ZE, synthetic zeolite from expanded perlite)

pH	**% Adsorption***			
	N	**E**	**ZN**	**ZE**
2	0	0	0	5.9
3	4.9	7.0	18.7	99.2
4	24.9	34.0	77.9	99.0
5	31.2	44.9	77.7	99.0
6	36.6	49.8	81.9	98.8
7	43.1	52.2	80.4	93.4

(* average of duplicate results)

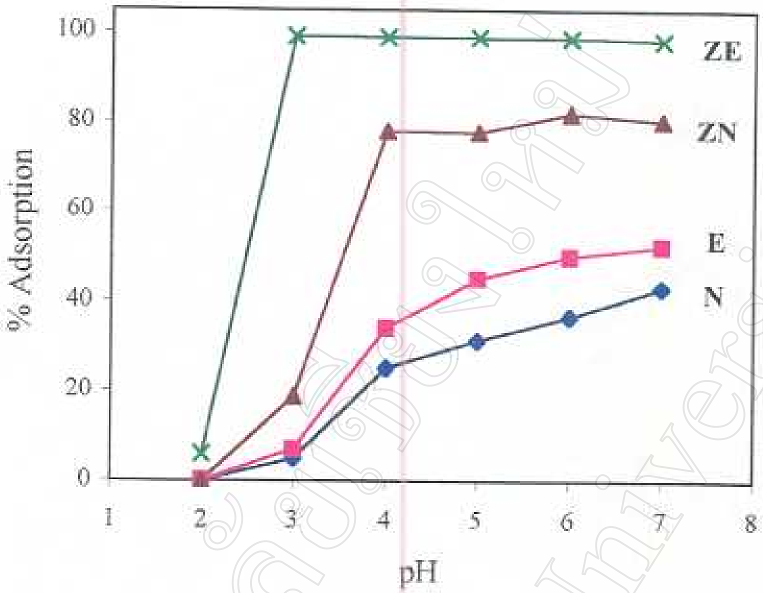


Figure 3.16 The effect of pH on adsorption of copper(II) on various sorbents (N, natural perlite; E, expanded perlite; ZN, synthetic zeolite from natural perlite; ZE, synthetic zeolite from expanded perlite).

3.2.2 Effect of Shaking Time

The effect of shaking time on the retention behavior of copper(II) was studied over the range of 10-80 min at a copper(II) concentration of 5 mg/l, pH 5 and temperature of 30°C. Batch experiments were carried out by shaking 0.2000 g of adsorbents with 50 ml of copper(II) 5 mg/l solution. The adsorbent was removed by filtration at definite copper(II) concentration was measured by using a Shimudzu AA-680 atomic absorption spectrophotometer. Table 3.23 and Figure 3.17 show the adsorption of copper(II) on synthetic zeolite from natural perlite and synthetic zeolite from expanded perlite reached equilibration very rapidly but for natural perlite and expanded perlite it indicated that the equilibrium was attained after shaking for 10 min.

The rate of copper(II) uptake on natural and expanded perlite were rapid initially and unchanged markedly after 10 min, while both of the synthetic zeolites remain almost unchanged regardless of any change in the shaking time. Similar results for natural zeolite has been reported by Panayotova [**123**]. This is an advantage for wide applications in the treatment of wastewater, trace copper(II) preconcentration or recovery of trace copper(II) by using perlites and zeolites formed.

Table 3.23 Effect of shaking time on % adsorption. (N, natural perlite; E, expanded perlite; ZN, synthetic zeolite from natural perlite; ZE, synthetic zeolite from expanded perlite)

Shaking time (min)	**% Adsorption***			
	N	**E**	**ZN**	**ZE**
0	8.7	15.4	77.5	97.0
5	26.4	28.8	77.4	98.5
10	29.7	41.4	76.7	97.1
20	27.2	43.5	75.9	98.4
30	27.6	41.9	79.6	99.0
40	24.4	40.3	81.8	98.6
60	26.4	40.5	76.6	98.5
80	29.2	44.9	81.0	98.9

(* average of duplicate results)

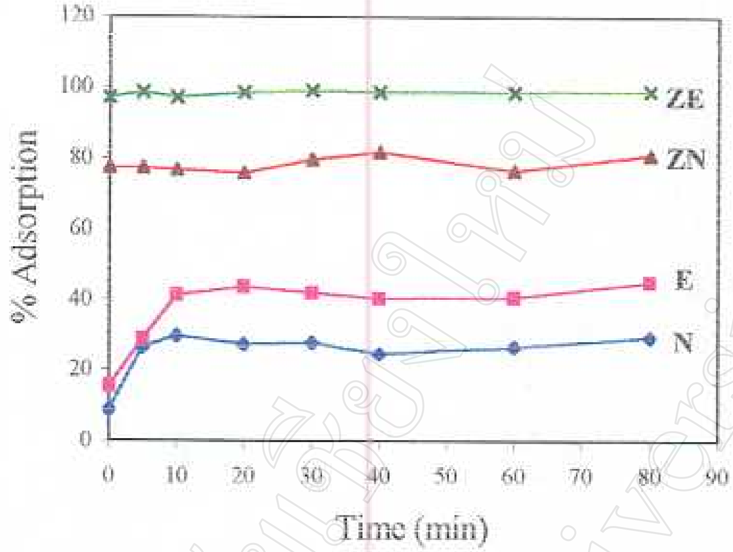


Figure 3.17 The effect of shaking time on adsorption of copper(II) on various sorbents (N, natural perlite; E, expanded perlite; ZN, synthetic zeolite from natural perlite; ZE, synthetic zeolite from expanded perlite).

3.2.3 Effect of Temperature

By varying the temperature of a mechanical shaker bath at 22, 30, and 40°C, no change in the percentage adsorption of copper(II) was observed. The results are shown in Table 3.24 and Figure 3.18. It was indicated that these adsorbents could be used over the temperature range 22-40°C. The temperature of 30°C was chosen as suitable.

Table 3.24 Effect of temperature on % adsorption. (N, natural perlite; E, expanded perlite; ZN, synthetic zeolite from natural perlite; ZE, synthetic zeolite from expanded perlite)

Temperature (°C)	% Adsorption*			
	N	E	ZN	ZE
22	28.9	42.7	71.3	99.2
30	32.9	43.2	76.4	98.8
40	33.9	47.6	78.6	98.5

(* average of duplicate results)

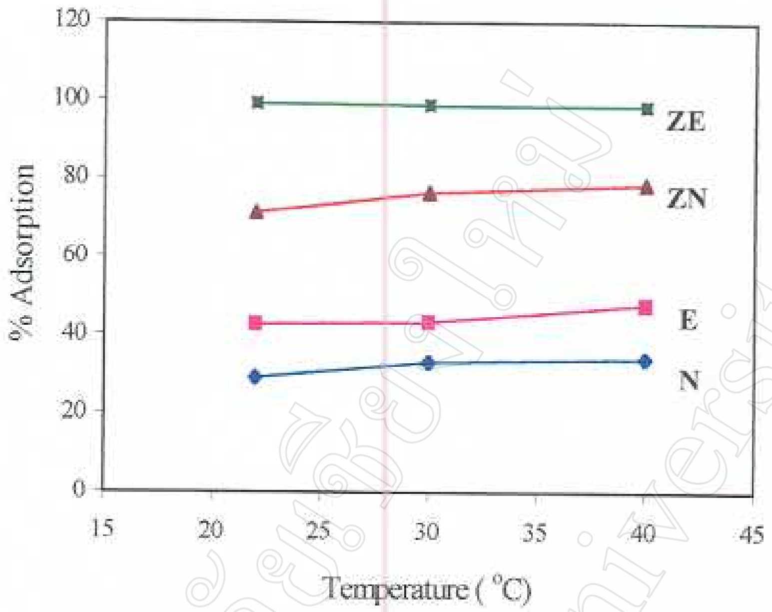


Figure 3.18 The effect of temperatures on adsorption of copper(II) on various sorbents (N, natural perlite; E, expanded perlite; ZN, synthetic zeolite from natural perlite; ZE, synthetic zeolite from expanded perlite).

3.2.4 Adsorption Isotherms

In order to model the adsorption behavior of copper(II) on the sorbents studied, the adsorption isotherms were studied. To quantify the adsorption capacity of perlites and zeolites from perlites for the removal of copper(II) from aqueous solution, the Langmuir and Freundlich models were used. A computer simulation technique has been applied to fit the Langmuir and the Freundlich equations for the adsorption data. The coefficients of least square fitting to a straight line (R) were computed for these two models.

3.2.4.1 Langmuir Model [134]

Langmuir model. are according to the equation as shown below:

$$C_e/A_m = 1/K \cdot 1/b + (1/b) \cdot C_e$$

Where C_e is the equilibrium concentration (mol/l) and A_m is the amount adsorbed per specified amount of adsorbent (mol/g), K is the equilibrium constant and b is the amount of adsorbate required to form a monolayer. Hence, a plot of C_e/A_m vs C_e should be a straight line with a slope $1/b$ and intercept as $1/Kb$ (Table 3.25-3.28 and Figure 3.19-3.22).

An adsorption isotherm is characterized by certain constants the values of which express the surface properties and affinity of the sorbent and can also be used to compare adsorptive capacity of perlites and synthetic zeolites from perlites for copper(II) ions.

3.2.4.2 Freundlich Model [134]

Freundlich model. Freundlich (1907) proposed the following model.

$$A_m = K \cdot C_e^{1/n}$$

or

$$\ln A_m = \ln K + 1/n \cdot \ln C_e$$

Where all the terms have the usual significance and n is an empirical constant. Thus a plot of ln A_m vs ln C_e should be a straight line with a slope $1/n$ and intercept ln K. The intercept, ln K, is an indicator of adsorption ability and the slope, $1/n$, is an indicator of concentration dependence of adsorption. This model deals with the multi layer adsorption of K the substance on the adsorbent. The points in Table 3.29-3.32 and Figure 3.23-3.26 indicate the observed data and the line corresponds to the fitted data.

The linearized Langmuir and Freundlich adsorption isotherms of each adsorbent for copper(II) obtained at 30±2 °C and pH 5 were shown in Table 3.25-3.32 and Figure 3.19-3.26. The Langmuir and Freundlich adsorption constants evaluated from the isotherms with the regression coefficients also given in Table 3.33. The adsorption of copper(II) on perlites and synthetic zeolites are followed by both Freundlich as well as Langmuir type adsorption isotherms. However, the Langmuir equation is better obeyed by the system than the Freundlich one as is evident from the values of regression coefficients.

The Freundlich type adsorption isotherm is an indication of surface heterogeneity of the adsorbent while Langmuir type isotherm hints towards surface homogeneity of the adsorbent. This leads to the conclusion that the surface of natural perlite, expanded perlite and both of the synthetic zeolites from perlites are made up of small homogeneous adsorption patches which are very much similar to each other with respect to adsorption phenomenon.

Table 3.25 The parameters for Langmuir model of copper(II) adsorption on natural perlite.

C_e (mg/l)	**C_e (mol/l)**	**Amount of adsorbent (g)**	**C_a (mg/l)**	**A_m (mg/g)**	**C_e/A_m**
0.818	12.9×10^{-6}	0.2038	0.799	0.196	65.8×10^{-6}
1.460	23.0×10^{-6}	0.2030	1.033	0.254	90.6×10^{-6}
2.336	36.8×10^{-6}	0.2041	1.068	0.262	140.5×10^{-6}
3.692	58.1×10^{-6}	0.2040	1.587	0.389	149.4×10^{-6}
5.798	91.2×10^{-6}	0.2032	1.342	0.330	276.4×10^{-6}
7.494	117.9×10^{-6}	0.2030	1.362	0.336	350.9×10^{-6}

(C_e = the equilibrium concentration, C_a = adsorbed concentration, A_m = the amount adsorbed per specified amount of adsorbent)

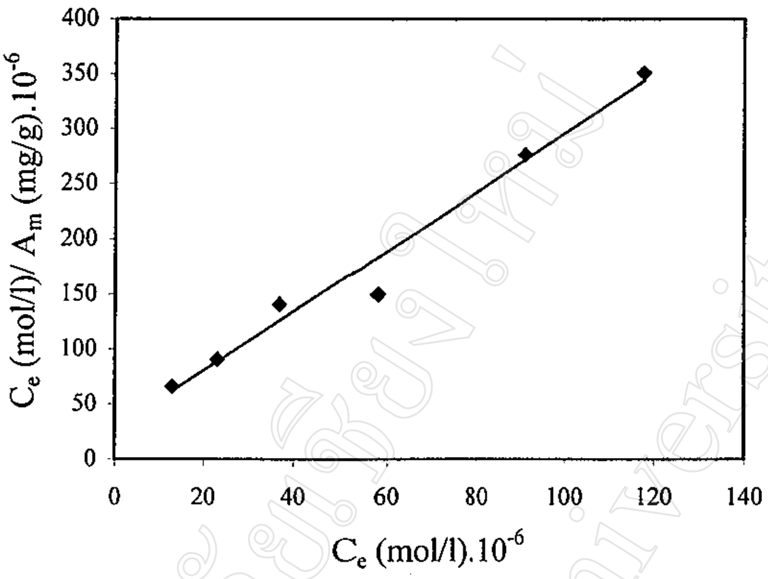


Figure 3.19 Langmuir plots for the adsorption of copper(II) by natural perlite.

Table 3.26 The parameters of copper(II) adsorption for Langmuir model on expanded perlite.

C_e (mg/l)	C_e (mol/l)	Amount of adsorbent (g)	C_a (mg/l)	A_m (mg/g)	C_e/A_m
0.516	8.1×10^{-6}	0.2018	1.102	0.273	29.7×10^{-6}
1.120	17.6×10^{-6}	0.2020	1.374	0.340	51.8×10^{-6}
1.979	31.1×10^{-6}	0.2020	1.425	0.353	88.1×10^{-6}
3.578	56.3×10^{-6}	0.2015	1.701	0.422	133.4×10^{-6}
5.222	82.2×10^{-6}	0.2026	1.918	0.474	173.4×10^{-6}
6.633	104.4×10^{-6}	0.2018	2.224	0.551	189.5×10^{-6}

(C_e = the equilibrium concentration, C_a = adsorbed concentration, A_m = the amount adsorbed per specified amount of adsorbent)

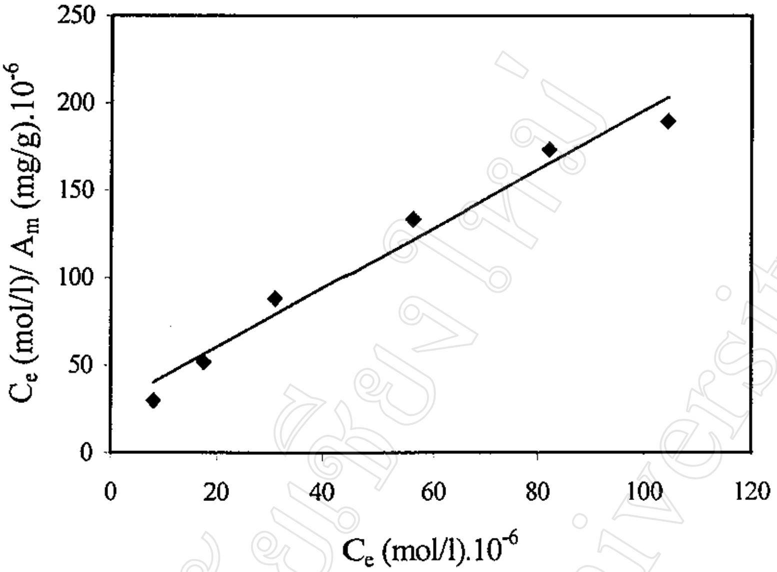


Figure 3.20 Langmuir plots for the adsorption of copper(II) by expanded perlite.

Table 3.27 The parameters of copper(II) adsorption for Langmuir model on synthetic zeolite from natural perlite.

C_e (mg/l)	**C_e (mol/l)**	**Amount of adsorbent (g)**	**C_a (mg/l)**	**A_m (mg/g)**	**C_e/A_m**
0.352	5.5×10^{-6}	0.2016	1.266	0.314	17.5×10^{-6}
0.457	7.2×10^{-6}	0.2004	2.036	0.508	14.2×10^{-6}
0.846	13.3×10^{-6}	0.2006	2.558	0.637	20.9×10^{-6}
1.395	22.0×10^{-6}	0.2005	3.884	0.968	22.7×10^{-6}
2.612	41.1×10^{-6}	0.2018	4.528	1.122	36.6×10^{-6}
4.110	64.7×10^{-6}	0.2012	4.746	1.180	54.8×10^{-6}

(C_e = the equilibrium concentration, C_a = adsorbed concentration, A_m = the amount adsorbed per specified amount of adsorbent)

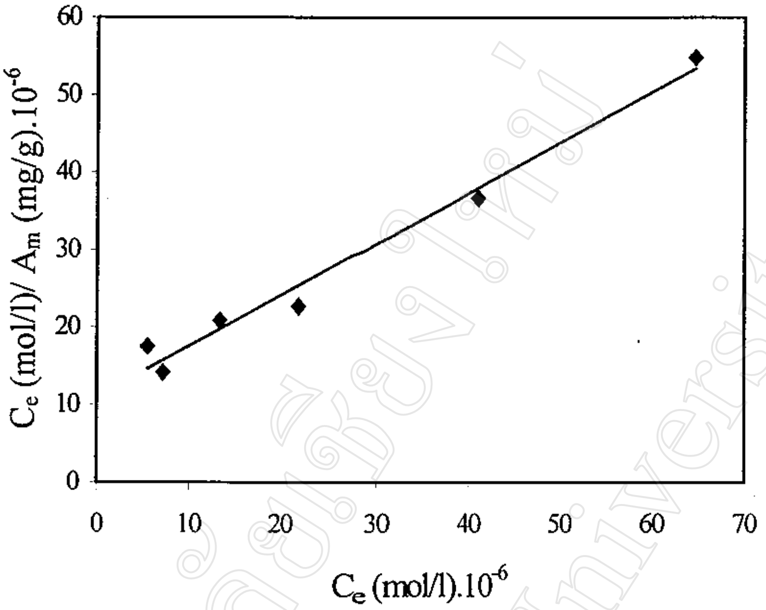


Figure 3.21 Langmuir plots for the adsorption of copper(II) by synthetic zeolite from natural perlite.

Table 3.28 The parameters of copper(II) adsorption for Langmuir model on synthetic zeolite from expanded perlite.

C_e (mg/l)	C_e (mol/l)	Amount of adsorbent (g)	C_a (mg/l)	A_m (mg/g)	C_e/A_m
0.016	0.3×10^{-6}	0.2008	1.602	0.399	0.8×10^{-6}
0.040	0.6×10^{-6}	0.2004	2.454	0.612	1.0×10^{-6}
0.048	0.8×10^{-6}	0.2007	3.356	0.836	1.0×10^{-6}
0.067	1.0×10^{-6}	0.2008	5.212	1.297	0.8×10^{-6}
0.084	1.3×10^{-6}	0.2011	7.056	1.754	0.7×10^{-6}
0.431	6.8×10^{-6}	0.2005	8.426	2.101	3.2×10^{-6}

(C_e = the equilibrium concentration, C_a = adsorbed concentration, A_m = the amount adsorbed per specified amount of adsorbent)

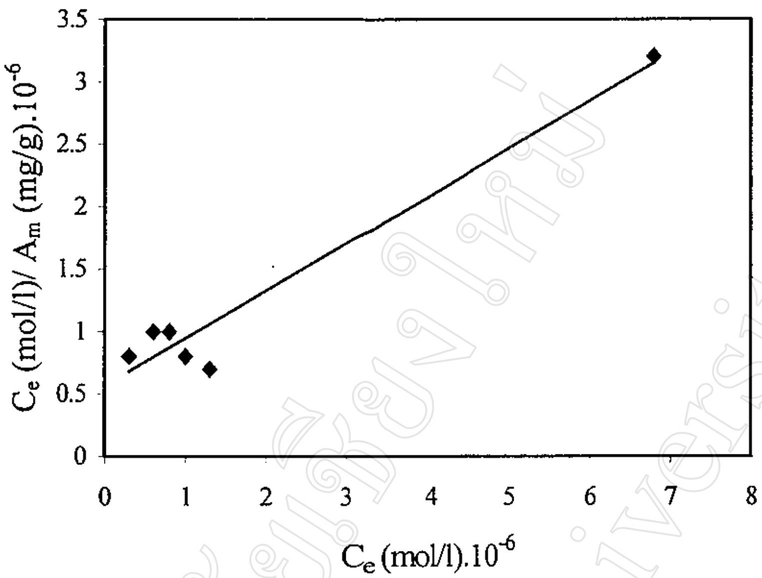


Figure 3.22 Langmuir plots for the adsorption of copper(II) by synthetic zeolite from expanded perlite.

Table 3.29 The parameters of copper(II) adsorption on natural perlite for Freundlich model.

A_m (mg/g)	ln A_m	C_e (mol/l)	ln C_e
0.196	-1.6	12.9×10^{-6}	-11.3
0.254	-1.4	23.0×10^{-6}	-10.7
0.262	-1.3	36.8×10^{-6}	-10.2
0.389	-0.9	58.1×10^{-6}	-9.8
0.330	-1.1	91.2×10^{-6}	-9.3
0.336	-1.1	117.9×10^{-6}	-9.0

(A_m = the amount adsorbed per specified amount of adsorbent, C_e = the equilibrium concentration)

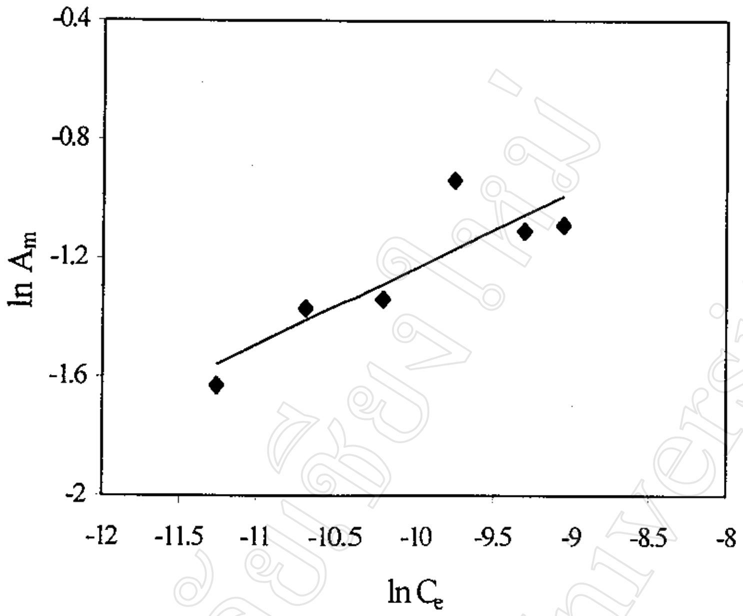


Figure 3.23 Freundlich plots for the adsorption of copper(II) by natural perlite.

Table 3.30 The parameters of copper(II) adsorption on expanded perlite for Freundlich model.

A_m (mg/g)	**$\ln A_m$**	**C_e (mol/l)**	**$\ln C_e$**
0.273	-1.30	8.1×10^{-6}	-11.7
0.340	-1.08	17.6×10^{-6}	-10.9
0.353	-1.04	31.1×10^{-6}	-10.4
0.422	-0.86	56.3×10^{-6}	-9.8
0.474	-0.75	82.2×10^{-6}	-9.4
0.551	-0.60	104.4×10^{-6}	-9.2

(A_m = the amount adsorbed per specified amount of adsorbent, C_e = the equilibrium concentration)

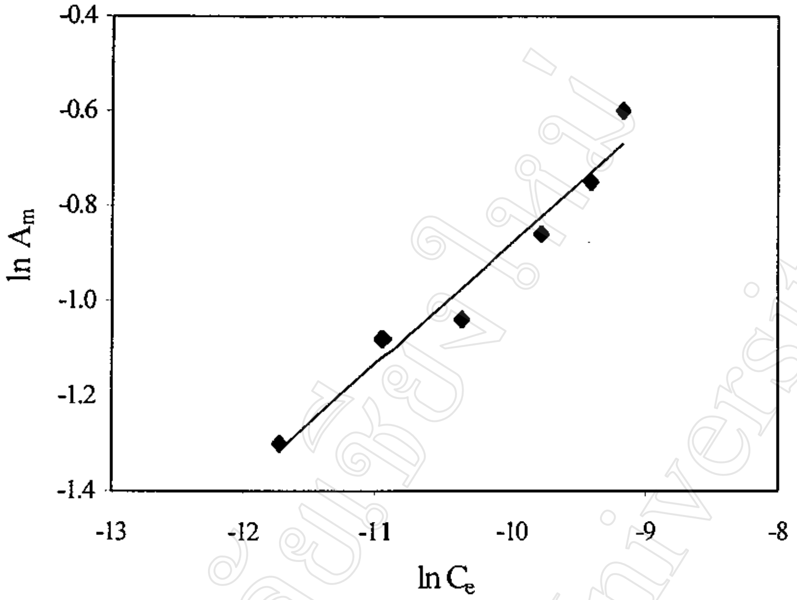


Figure 3.24 Freundlich plots for the adsorption of copper(II) by expanded perlite.

Table 3.31 The parameters of copper(II) adsorption on synthetic zeolite from natural perlite for Freundlich model.

A_m (mg/g)	ln A_m	C_m (mol/l)	ln C_e
0.314	-1.16	5.5×10^{-6}	-12.1
0.508	-0.68	7.2×10^{-6}	-11.8
0.637	-0.45	13.3×10^{-6}	-11.2
0.968	-0.03	22.0×10^{-6}	-10.7
1.122	0.12	41.1×10^{-6}	-10.1
1.180	0.17	64.7×10^{-6}	-9.6

(A_m = the amount adsorbed per specified amount of adsorbent, C_e = the equilibrium concentration)

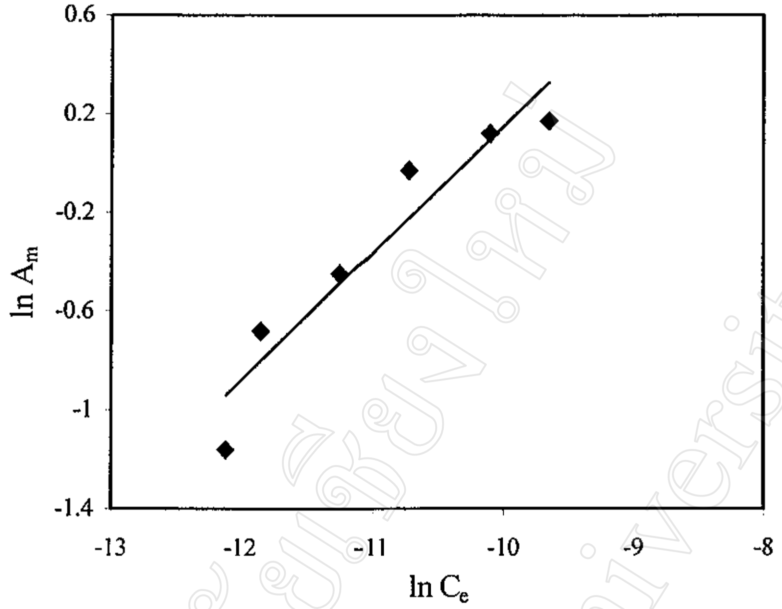


Figure 3.25 Freundlich plots for the adsorption of copper(II) by synthetic zeolite from natural perlite.

Table 3.32 The parameters of copper(II) adsorption on synthetic zeolite from expanded perlite for Freundlich model.

A_m (mg/g)	ln A_m	C_e (mol/l)	ln C_e
0.399	-0.91	0.6×10^{-6}	-15.2
0.612	-0.49	1.0×10^{-6}	-14.3
0.836	-0.18	0.9×10^{-6}	-14.1
1.297	0.26	0.8×10^{-6}	-13.8
1.754	0.56	0.8×10^{-6}	-13.5
2.101	0.74	3.2×10^{-6}	-11.9

(A_m = the amount adsorbed per specified amount of adsorbent, C_e = the equilibrium concentration)

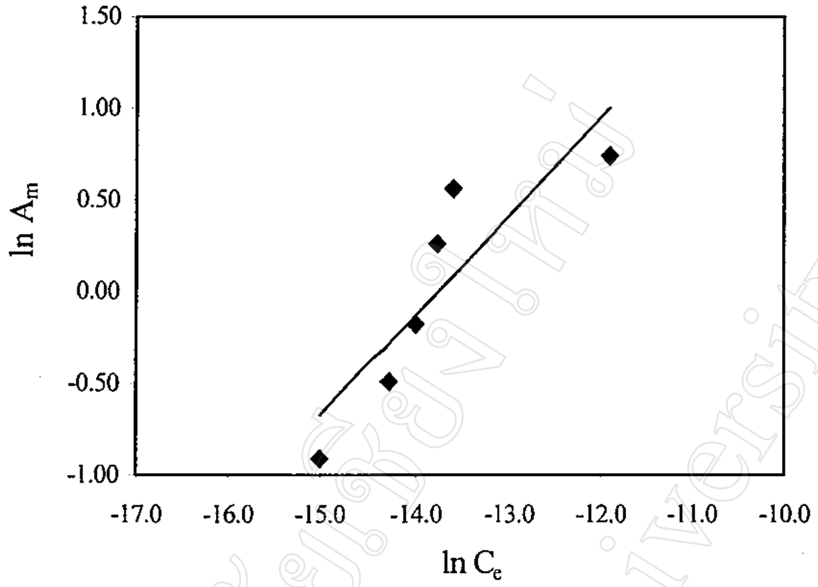


Figure 3.26 Freundlich plots for the adsorption of copper(II) by synthetic zeolite from expanded perlite.

Table 3.33 The related parameters for the adsorption of copper(II) on perlites and synthetic zeolites from perlites.

Material	Langmuir constants			Freundlich constants		
	R	K	b	R	K	1/n
natural perlite	0.9883	0.1007	0.3721	0.8692	0.2844	0.2565
expanded perlite	0.9858	0.0639	0.5910	0.9802	0.5019	0.2531
zeolite from natural perlite	0.9900	0.0597	1.5257	0.9523	1.6666	0.5148
zeolite from expanded perlite	0.9822	0.7806	2.5246	0.8999	1.9710	0.5202

3.2.5 Adsorption capacity

The adsorption capacity of the sorbents have been calculated from equation [147]:

$$\text{Adsorption capacity} = \frac{[\text{initial amount of Cu(II)} - \text{final amount of Cu(II)}]}{\text{amount of sorbent}}$$

The numberical data for the maximum adsorption capacity of the sorbents used are summerized in Table 3.34. The results clearly demonstrate that synthetic zeolite from expanded perlite has a greater adsorption capacity than those of the other sorbents used.

Table 3.34 Limiting Adsorption of copper(II) by perlites and synthetic zeolites from perlites.

Adsorbent	**Capacity (μgCu/g adsorbent)**
natural perlite	336
expanded perlite	551
zeolite from natural perlite	1180
zeolite from expanded perlite	2101

3.3 FIA On-line Column Preconcentration for Trace Copper Determination

A principle drawback of conventional separation and preconcentration procedures is the fact, that they are often time-consuming and frequently require large sample volumes. With the implementation of on-line flow injection techniques for the separation and preconcentration, such drawback are, however, no longer valid, while the beneficial effects are further enhanced. The virtues of flow injection on-line column preconcentration techniques compared to their conventional batch operation counterparts may be summarized as follows [**5**]:

(a) One to two orders of magnitude higher efficiency in terms of sampling frequency.

(b) One to two orders of magnitude lower consumption of sample and reagent.

(c) Better precision. Typically 1-2% RSD.

(d) Reduced risks of contamination in a closed system produced from inert material and isolated from the environment.

(e) Readily automated operation.

There has been an interest in on-line preconcentration since 1980 [**148, 149**]. Many approaches for modification have been proposed to improve the performance.

The first contribution in FIA on-line column preconcentration was the one which used a spectrophotometer as a detector and involved its broad applications to both inorganic and organic analyte species [**2, 127**], but the development has been too slow. There are two important reasons, the first is the interferences in the signal readout arising from variation in the refractive index between sample (or carrier) and eluent. The second is the requirement for coordinating the chemical conditions of the eluent and the ensuring chromogenic reactions, which is not always easy or even possible [3]. However, these obstacles can be overcome. The in-valve microcolumn allows rapid analysis of very small volumes and rapid regeneration of the volume for subsequent analyses.

In this work, synthetic zeolite from natural perlite was used as adsorbent packed in a microcolumn for preconcentration of copper(II). From the previous studies (section 3.2), copper(II) could be adsorbed on the synthetic zeolite from expanded perlite better than the synthetic zeolite from natural perlite. However, the particle size of the synthetic zeolite from the expanded perlite is smaller than 100 mesh, which is too small to pack in a microcolumn. So the synthetic zeolite from natural perlite is more suitable in this work.

3.3.1 Column Design

The column design strongly influences the performance of preconcentration systems. The capacities of the column used in the FIA preconcentration range from 15 µl or more, depending on the specific purpose concerned. In previous reports, the column diameter may be as small as 1.5 mm or as large as 7.5 mm [3]. The optimum column design for achieving high efficiency and low consumption index depends on several factors such as specificity, particle size and type of packing materials.

In this proposed preconcentration method, the column design is shown in Figure 3.27. A portion of synthetic zeolite from natural perlite (0.15 g, 45-80 mesh) was packed into a borosilicate glass tubing (3 mm i.d., 4 cm long) using the so-called “wet packing technique”. The procedure involved a batch-wise addition of small amounts of material in deionized water, by a dropper, into a vertically mounted column. Two ends of the microcolumn were then closed then Teflon wool plugs with covered with Tygon tubings (1.5 mm i.d., 1 cm long) and connected to Teflon tubing (0.86 mm i.d.) for inserting into the injection valve rotor. One of this was placed instead of a sample loop of the six-port injection valve.

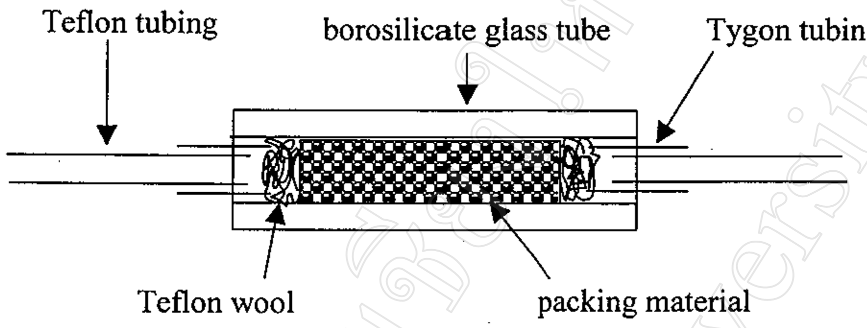


Figure 3.27 Microcolumn design for on-line preconcentration of copper(II) determination.

3.3.2 The Manifold Design

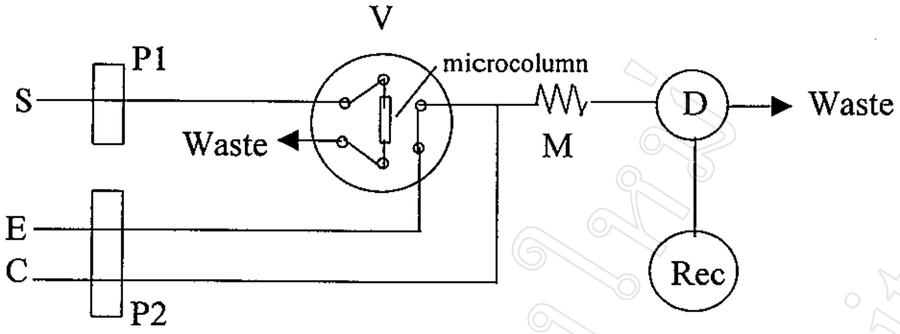
Two manifolds for this study were designed as shown in Figure 3.28. In manifold b the sample, eluent and reagent were propelled via peristaltic pumps 1 and 2 respectively. The flow rate of each line can be controlled independently by adjustment of the pump speeds. An in-valve microcolumn packed with synthetic zeolite from natural perlite was incorporated within the injection valve, replacing one of sample loops (by-passed). When the valve was in the "inject" position, the eluent solution passed through the column to elute the sample into the flow system. When the injection valve was turned to the "load" position, the sample solution passed through the column. After a certain loading time, the valve was turned back to inject position and accumulated copper(II) was eluted to the flow system which was then reacted with the reagent solution at the micro reactor (M) and the colored product was monitored spectrophotometrically at 492 nm.

The preliminary conditions used before optimization conditions by means of univariate method were given in Table 3.35.

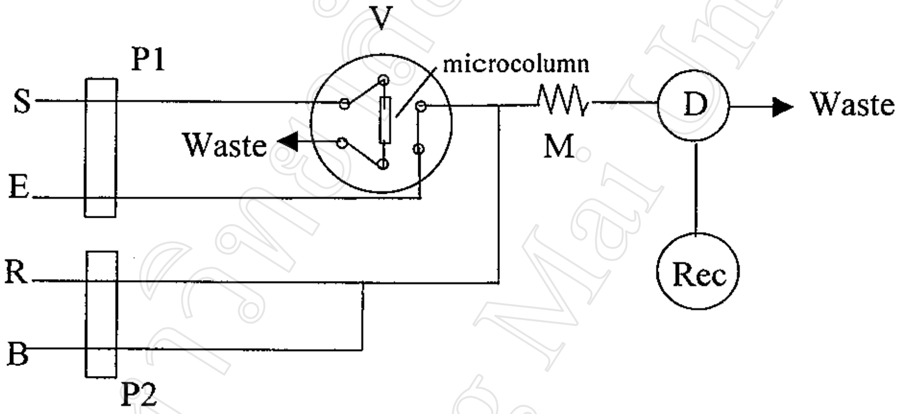
Table 3.35 Preliminary conditions.

Variable	**Fixed value**
Eluent (E)	$NaNO_3$ 4% w/v
Reagent (R)	nitroso-R salt 0.02% (w/v)
Reagent (C)	nitroso-R salt 0.02% (w/v) in acetate buffer pH 7 (0.5 M)
Reagent (B)	acetate buffer pH 7 (0.5 M)
Flow rate of E stream	3.0 ml/min
Flow rate of C stream	2.4 ml/min
Flow rate of R stream	2.4 ml/min
Flow rate of B stream	2.4 ml/min
Flow rate of sample stream (S)	3.0 ml/min
λ_{anal}	492 nm
Mixing coil length	30 cm

The optimization of each manifold was carried out, in which a series of the standard copper(II) solutions containing 0.04, 0.06, 0.08 and 0.10 mg/l were preconcentrated in the microcolumn with the preconcentration times for 2 min at a flow rate of 3.1 ml/min. The injection valve was switched in the eluent stream position as described previously (section 2.5.3). The results are given in Table 3.36 and Figure 3.29. The results indicate that manifold-a should be appropriate since the highest peak height and the good linearity was obtained.



manifold-a



manifold-b

Figure 3.28 Two types of the FIA manifolds used in the on-line microcolumn preconcentration for the determination of copper(II) with loading configurations shown. S, sample; C, nitroso-R reagent (0.02% w/v) in acetate buffer pH 6-7 (0.5 M); B, acetate buffer pH 6-7 (0.5 M); R, nitroso-R reagent (0.02% w/v); E, eluent; P1 and P2, peristaltic pumps; V, six-ports injection valve; M, mixing coil; D, spectrophotometer; Rec, recorder.

Table 3.36 Effect of two types of the manifold on peak height.

Manifold	Peak height*(mV) obtained from the standard Cu(II) (mg/l)				y = a(x)+b	r^2
	0.04	0.06	0.08	0.10		
a	13.5	21.3	25.5	30.0	y=268.5(x)+3.78	0.9764
b	7.0	12.3	17.3	19.3	y=209.5(x)-0.69	0.9660

(*average of triplicate injections)

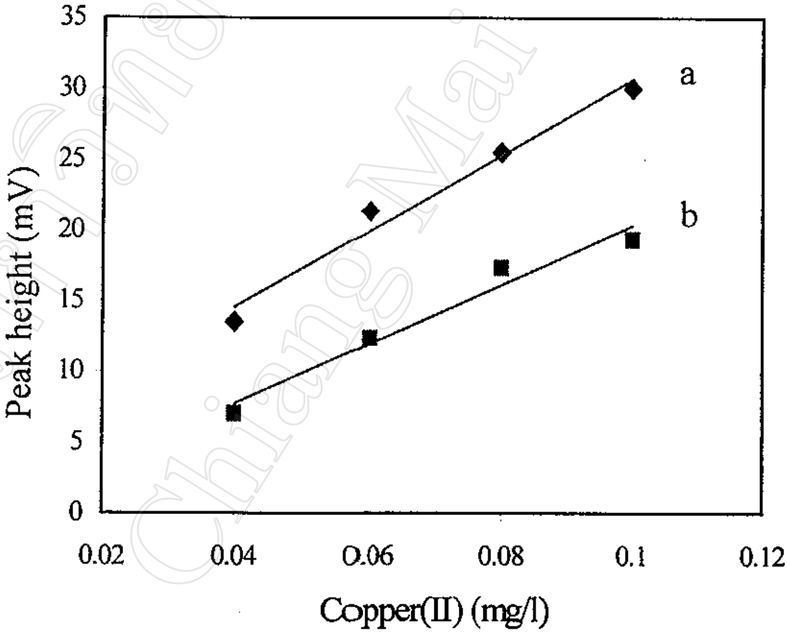


Figure 3.29 Effect of two types of the manifold on peak height (a, manifold-a; b, manifold-b).

3.3.3 Optimization of the FIA System

Generally, the important parameters to be optimized in FIA system are flow rate, concentration of reagents and length and size of mixing part. But in an on-line preconcentration FIA system incorporated with the microcolumn effects from the eluent such as flow rate and concentration of the eluent should be taken into consideration. The effects of these parameters upon dispersion and peak height were studied by injecting appropriate volume of a standard copper(II) solution into the proposed on-line preconcentration system. The preconcentration process was repeated to investigate such effect by using the same FIA manifold under the experimental conditions chosen.

The on-line continuous system was optimized by using the univariate method in order to determine the best chemical and flow conditions for copper(II) determination with good sensitivity and selectivity.

3.3.3.1 Effect of pH of Sample Solution

Using the manifold-a as shown in Figure 3.28, the influence of sample pH was studied for varying pH values ranging from 1.3-6.0 with the standard copper(II) solutions containing 0.04 and 0.08 mg/l and the preconcentration time of 2 min at the flow rate 3.1 ml/min. The results obtained are summarized in Table 3.37 and Figure 3.30. The results indicate that the pH range of the sample solution is 2.5-3.0 the most appropriate as it exhibits the highest peak height.

Table 3.37 Effect of pH of sample on peak height.

pH of sample	**Peak height*(mV) obtained from the standard Cu(II) (mg/l)**	
	0.04	**0.08**
1.3	0.0	0.3
2.8	15.3	28.7
4.0	12.7	22.8
5.0	9.3	20.8
6.0	3.0	12.3

(*average of triplicate injections)

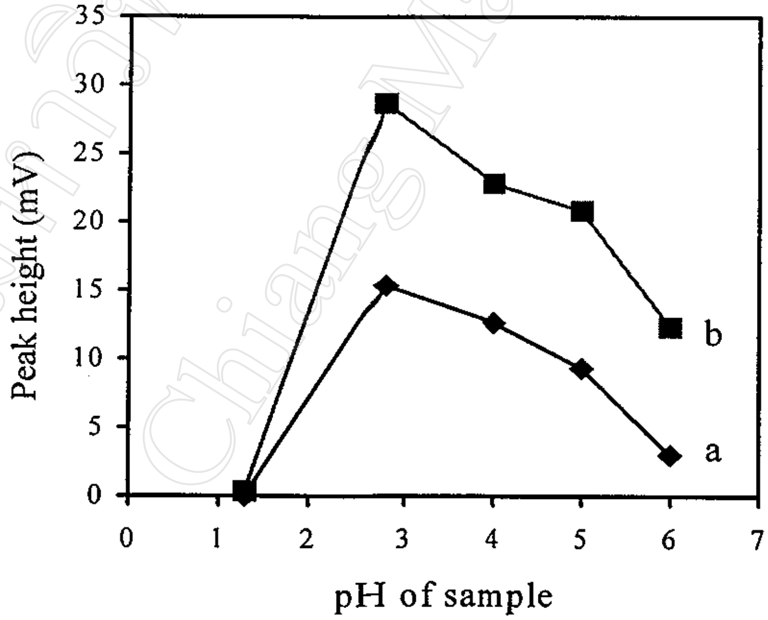


Figure 3.30 Effect of pH of sample on peak height [Cu(II) standard solution (a) 0.04 and (b) 0.08 mg/l].

3.3.3.2 Effect of pH of Buffer Solution

The pH of the solution at different points of a flow system will be dependent on pH of the relevant solution. The effect of pH of buffer solution on peak height was investigated by using acetate buffer (0.5 M) over the pH range of 5-9, using the manifold-a in section 3.3.2. The results obtained are presented in Table 3.38 and Figure 3.31. The maximum peak height occurred between pH 6-7, so pH 6-7 was chosen as optimum in order to achieve the best sensitivity.

Table 3.38 Effect of pH of buffer solution on peak height.

pH of buffer solution	**Peak height*(mV) obtained from the standard Cu(II) (mg/l)**	
	0.04	**0.08**
5	5.2	13.0
6	9.0	19.5
7	9.0	19.7
8	3.0	5.5
9	0.7	3.3

(*average of triplicate injections)

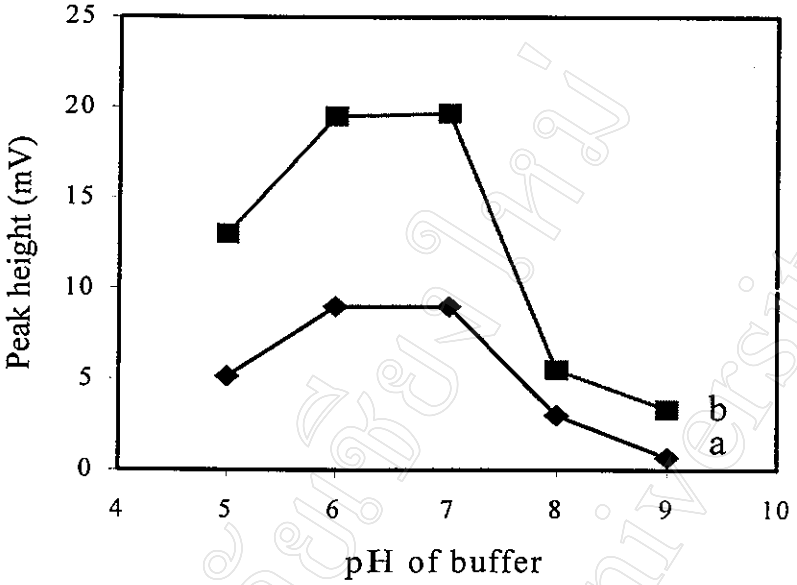


Figure 3.31 Effect of pH of buffer solution on peak height [Cu(II) standard solutions (a) 0.04 and (b) 0.08 mg/l].

3.3.3.3 Effect of Buffer Concentration

Buffer concentration can more or less affect the buffer capacity and hence, the FIA signal as well. To reduce the effect of the buffer concentration the buffer capacity must be increased or the suitable pH of solution of different lines must be adjusted to ensure a suitable pH range of 6-7. The concentration of the buffer solution was also studied in the range of 0.2-0.8 M by using acetate buffer pH 6-7. The results were shown in Table 3.39 and Figure 3.32. It was found that high concentrations of buffer solution were not only a source of waste chemical, but also caused a high blank signal. Therefore a 0.5 M of acetate buffer solution pH 7 was used as a buffer solution.

Table 3.39 Effect of buffer concentration on peak height.

Buffer concentration (M)	Peak height*(mV) obtained from the standard Cu(II) (mg/l)	
	0.04	**0.08**
0.2	8.7	18.3
0.4	9.0	19.8
0.5	11.5	23.2
0.6	8.7	21.3
0.8	8.7	19.0

(*average of triplicate injections)

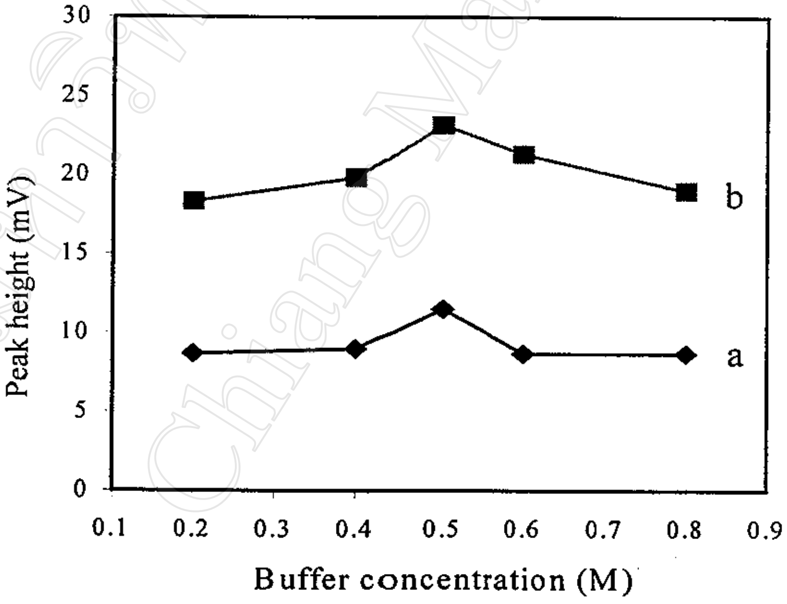


Figure 3.32 Effect of buffer concentration on peak height [Cu(II) standard solution (a) 0.04 and (b) 0.08 mg/l].

3.3.3.4 Effect of Reagent Concentration

The concentration of nitroso-R solution was optimized, in which the concentration of the nitroso-R solution was varied from 0.005-0.04% (w/v). Using the manifold-a in Figure 3.28, a solution of 0.04 and 0.08 mgCu(II)/l and a blank solution were loaded into an in-valve microcolumn for 2 min with loading flow rate 3.1 ml/min. The sorbed copper(II) was then eluted by $NaNO_3$ solution , and the eluent flow rate was 2.4 ml/min. The eluted copper(II) was mixed with nitroso-R (various concentrations) in the buffer solution 0.5 M, pH 7 (flow rate 2.6 ml/min). The mixture was passed through the mixing coil of 20 cm long and through the flow cell, in which the absorbance was continuously monitored. The results are shown in Table 3.40 and Figure 3.33. It was found that increasing the reagent concentration from 0.005-0.02% (w/v) resulted in an increase in peak heights. The peak heights at higher concentrations remained constant, so the concentration of 0.02% (w/v) should be economically used in further investigation.

Table 3.40 Effect of reagent concentration on peak height.

Reagent concentration (% w/v)	Peak height*(mV) obtained from the standard Cu(II) (mg/l)	
	0.04	0.08
0.005	10.0	18.8
0.01	10.0	21.7
0.02	11.5	23.0
0.03	11.3	22.8
0.04	11.2	22.7

(*average of triplicate injections)

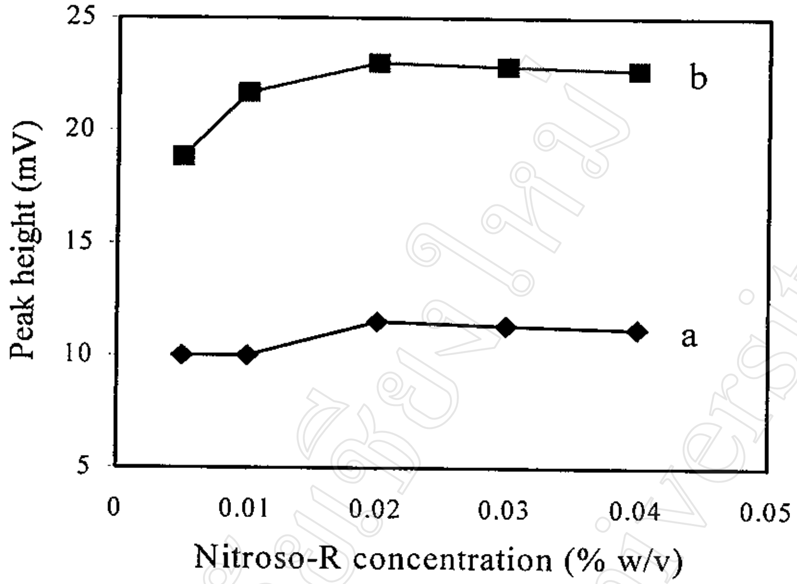


Figure 3.33 Effect of reagent concentration on peak height [Cu(II) standard solution (a) 0.04 and (b) 0.08 mg/l].

3.3.3.5 Effect of Mixing Coil Length

By using the FI manifold as shown in Figure 2.2, the effect of mixing coil length on the peak height of 0.04 mg/l and 0.08 mg/l standard copper(II) solutions were evaluated. Various coils with increasing lengths (10-50 cm) were placed between the T-junction piece and the detector. The results obtained (Table 3.41 and Figure 3.34) shows that the maximum colored complex formation was achieved with the coil length of 20 cm. This can be explained as followed: as the reaction coil length increases more colored complex is produced, up to 20 cm, above which a nearly constant peak height is obtained, owing to the balance between the increase in the colored complex production and the dispersion effect. At the longer coil lengths, dispersion decreases the peak height. A 20-cm coil length was, therefore, chosen as the optimum.

Table 3.41 Effect of mixing coil length on peak height.

Mixing coil length (cm)	Peak height*(mV) obtained from the standard Cu(II) (mg/l)	
	0.04	0.08
10	6.7	20.8
20	12.2	26.8
30	10.8	22.8
40	10.7	21.2
50	10.5	20.8

(*average of triplicate injections)

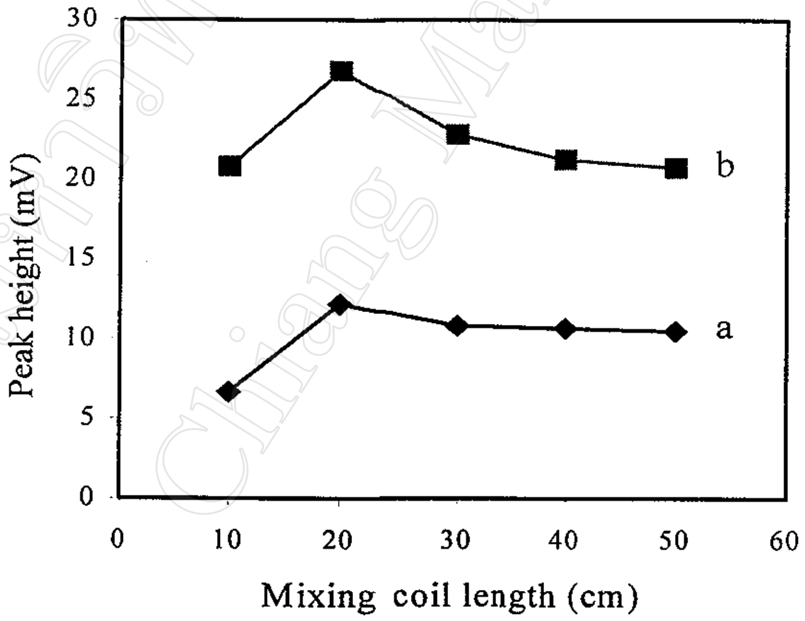


Figure 3.34 Effect of mixing coil length on peak height [Cu(II) standard solution (a) 0.04 and (b) 0.08 mg/l].

3.3.3.6 Investigation of Eluents

Elution of copper(II) which sorbed on the synthetic zeolite is easily done by using methyl isobutyl ketone [79]. For analytical purpose, copper(II) should be rapidly eluted. Various eluents that provided optimum elution of copper(II) from the microcolumn was carried out. The eluent examined were sodium chloride, nitric acid, sodium nitrate and sodium sulphate. The experiments performed to evaluate the best eluent for this method were performed using a loading time of 2 min and elution time of 0.5 min. The flow rates were 3.1 ml/min for the sample, 2.6 ml/min for the nitroso-R reagent and 2.4 ml/min for the eluent. After copper(II) was sorbed from aqueous medium and then the eluent under investigation was passed through the column. The eluent ability of various solutions was classified by comparing the peak height. Table 3.42 and Figure 3.35 shows the results obtained with different types of eluent in a fixed concentration of 4% (w/v). It was found that sodium nitrate provided the highest peak height and a good linearity.

Table 3.42 Effect of various types of eluent on peak height.

Type of eluent	Peak height*(mV) obtained from the standard Cu(II) (mg/l)			y = a(x) + b	r^2
	0.02	0.04	0.06		
NaCl	6.5	12.2	17.0	y=262.5(x)+1.39	0.9979
HNO_3	1.5	6.2	11.5	y=250.0(x)+3.61	0.9985
$NaNO_3$	8.3	15.6	22.2	y=345.8(x)+1.52	0.9994
Na_2SO_4	6.5	11.7	16.3	y=245.8(x)+1.67	0.9992

(*average of triplicate injections)

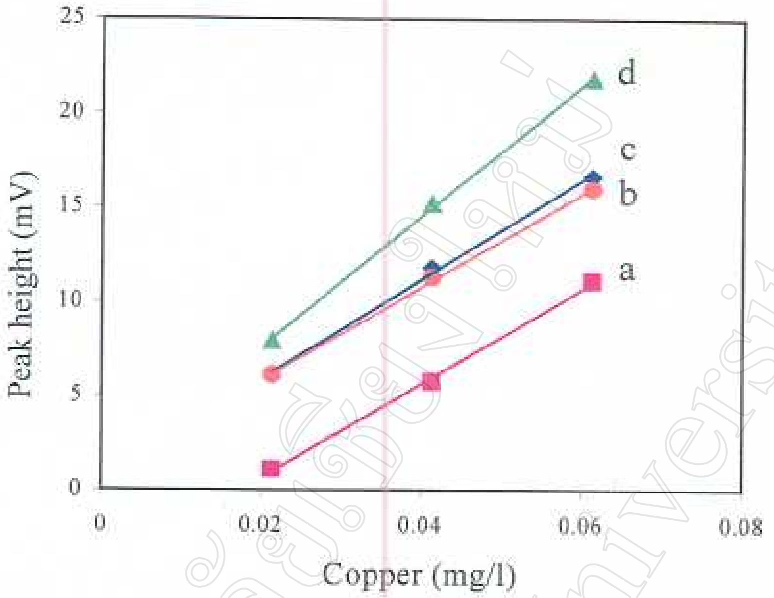


Figure 3.35 Effect of various types of eluent on peak height (a, HNO_3; b, Na_2SO_4; c, NaCl; d, $NaNO_3$).

3.3.3.7 Effect of Eluent Concentration

In the on-line elution, the kinetic features of the process are much more important than the off-line batch procedures. Low concentration eluent requires long equilibrium periods and may significantly degrade the enrichment factor and concentration efficiencies. High concentration may create some problems in color developing step of the FIA [3]. This effect was studied by varying the concentration of sodium nitrate over the range of 1-6% (w/v). The FIA conditions are the same as described earlier. The eluent ability of various concentrations was classified by comparing in the peak height. The results are shown in Table 3.43 and Figure 3.36. It can be seen that the peak height decreased with increasing the eluent concentration more than 2% (w/v). A concentration of 2% (w/v) was the most suitable eluent concentration because it provides the maximum peak height.

Table 3.43 Effect of eluent concentration on peak height.

Concentration of eluent (% w/v)	Peak height*(mV) obtained from the standard Cu(II) (0.04 mg/l)
1.0	14.5
2.0	14.5
3.0	13.7
4.0	13.5
5.0	11.8
6.0	11.5

(*average of triplicate injections)

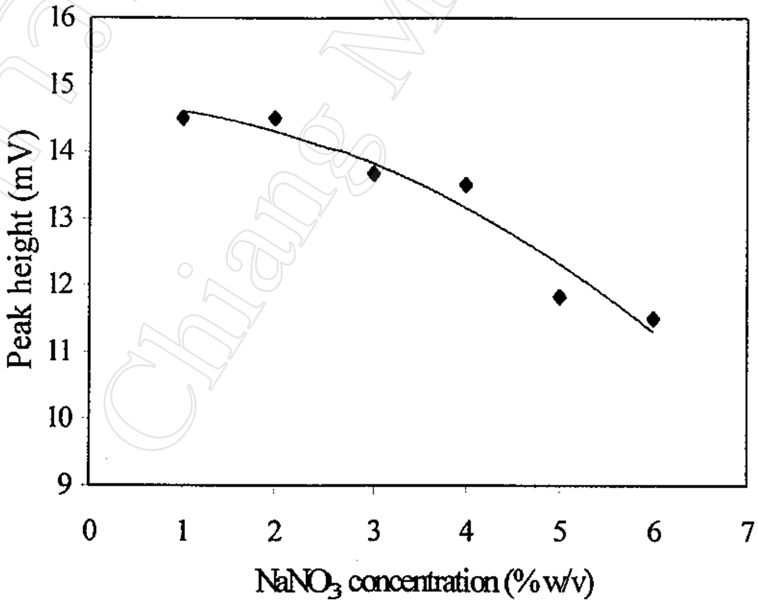


Figure 3.36 Effect of eluent concentration on peak height.

3.3.3.8 Effect of the Length of Microcolumn

The loading capacity of the column depends on the quantity of adsorbent packed in a column. In this work, a borosilicate microcolumn of 3 mm inner diameter was used as column with different lengths. The microcolumns of different lengths of 3, 4 and 5 cm packed with 0.10, 0.15 and 0.20 g of synthetic zeolite from natural perlite with 45-80 mesh size respectively were tested. The effect of column was studied by loading a series of copper(II) standard solution using the condition in section 3.3.3.7. The results obtained are presented in Table 3.44 and Figure 3.37. It can be seen that a column length of 5 cm increases the dispersion coefficient lowering the height of the absorbance signal. The column length of 4 cm provided the highest sensitivity. A short column was due to the band broadening of the extraction zone of copper(II) to the column. Therefore, the 4 cm columns (i.d. 3 mm and packed with 0.15 g, 45-80 mesh of the synthetic zeolite from natural perlite) were chosen for copper(II) preconcentration.

Table 3.44 Effect of column length on peak height.

Column length (cm)	Peak height*(mV) obtained from the standard Cu(II) (mg/l)			y = a(x)+b	r^2
	0.02	0.04	0.06		
3.0	7.8	13.8	19.8	y=250.00(x)-3.61	0.9985
4.0	9.9	18.5	26.3	y=345.75(x)+1.52	0.9994
5.0	9.5	16.8	24.3	y=262.50(x)+1.39	0.9979

(*average of triplicate injections)

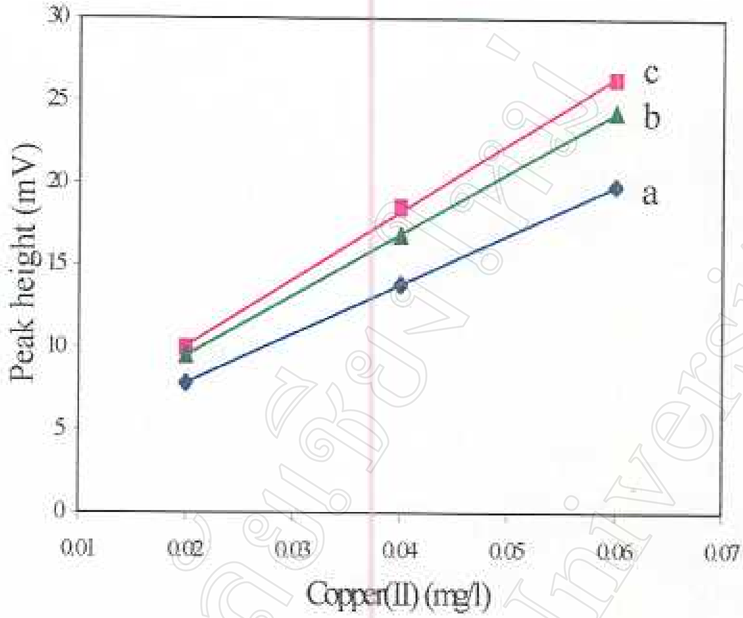


Figure 3.37 Effect of microcolumn length on peak height (a, 3cm; b, 5 cm; c, 4 cm).

3.3.3.9 Effect of Loading Flow Rate

The efficiency of adsorption of copper(II) from a solution onto the solid adsorbent depends on several factors, such as contact time, concentration of ion etc. For continuous loading of a copper(II) solution onto a column, the loading solution flow rate was studied to obtain an adsorption efficiency of copper(II). Under the conditions in section 3.3.3.8 appropriate volume of standard copper(II) solutions were loaded for 2 min with different loading flow rate of 1.9-4.0 ml/min. The effects of loading flow rate are shown in Table 3.45 and Figure 3.38. It was found that the optimum loading flow rate ranging from 2.4-3.4 ml/min is chosen as appropriate flow rate.

Table 3.45 Effect of flow rate of sample on peak height.

Flow-rate of sample (ml/min)	Peak height*(mV) obtained from the standard Cu(II) (mg/l)	
	0.04	**0.08**
1.9	6.0	13.7
2.4	6.9	13.8
2.9	9.1	16.4
3.4	12.4	18.9
4.0	13.5	20.8

(*average of triplicate injections)

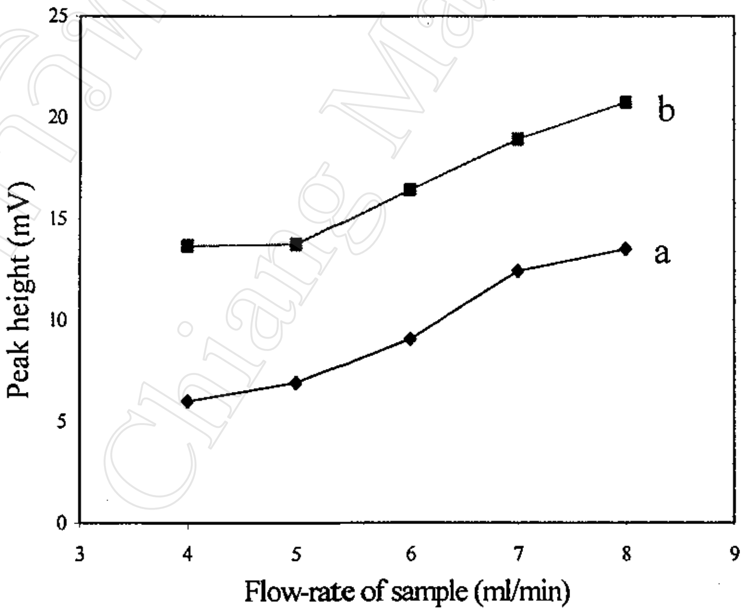


Figure 3.38 Effect of flow rate of sample on peak height [Cu(II) standard solution (a) 0.04 and (b) 0.08 mg/l].

3.3.3.10 Effect of Reagent Flow rate

Effect of reagent flow rate was evaluated over the range 1.6-4.0 ml/min at a sample flow rate of 3.1 ml/min. The results are shown in Table 3.46 and Figure 3.39. It was found that increasing the reagent flow rate from 1.6 to 2.8 ml/min resulted in an increase in peak height. A higher flow rate the peak height decreased.

Table 3.46 Effect of flow rate of reagent on peak height.

Flow rate of reagent (ml/min)	**Peak height*(mV) obtained from the standard Cu(II) (mg/l)**	
	0.04	**0.08**
1.6	13.2	26.2
2.2	14.7	27.2
2.8	15.2	27.7
3.4	10.8	22.8
4.0	8.7	21.2

(*average of triplicate injections)

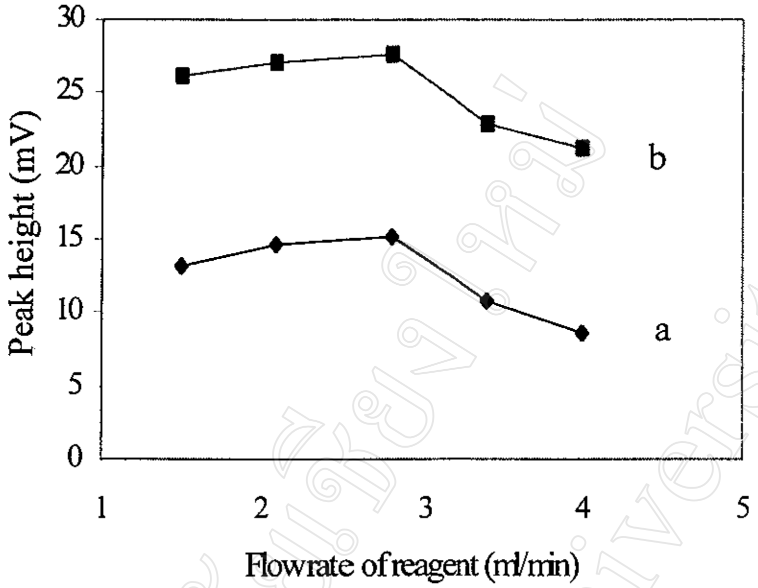


Figure 3.39 Effect of flow rate of reagent on peak height [Cu(II) standard solution (a) 0.04 and (b) 0.08 mg/l].

3.3.3.11 Effect of Flow rate of Eluent

The eluent flow rate was varied over the range of 1.6-4.4 ml/min and its effect on the peak height of copper(II) complex was determined. It is seen that the peak height increases with increasing the eluent flow rate of 2.4 ml/min afterwards the peak height decreases with decreasing the eluent flow rate (see Table 3.47 and Figure 3.40). The eluent flow rate of 2.4 ml/min was selected for further studies. At this flow rate, 30 s was found to be sufficient for quantitative elution of adsorbed copper(II) into the nitroso-R stream.

Table 3.47 Effect of flow rate of eluent on peak height.

Flow rate of eluent (ml/min)	**Peak height*(mV) obtained from the standard Cu(II) (mg/l)**			**y = a(x) + b**	**r^2**
	0.02	**0.04**	**0.06**		
1.9	6.3	11.5	19.7	y=333.50(x)-0.84	0.9834
2.4	7.7	15.8	23.5	y=395.75(x)-0.16	0.9997
2.9	7.2	14.3	21.0	y=345.75(x)+0.34	0.9996
3.4	5.7	13.2	22.0	y=408.25(x)-2.72	0.9978
4.0	6.5	13.8	22.2	y=391.75(x)-1.50	0.9987

(*average of triplicate injections)

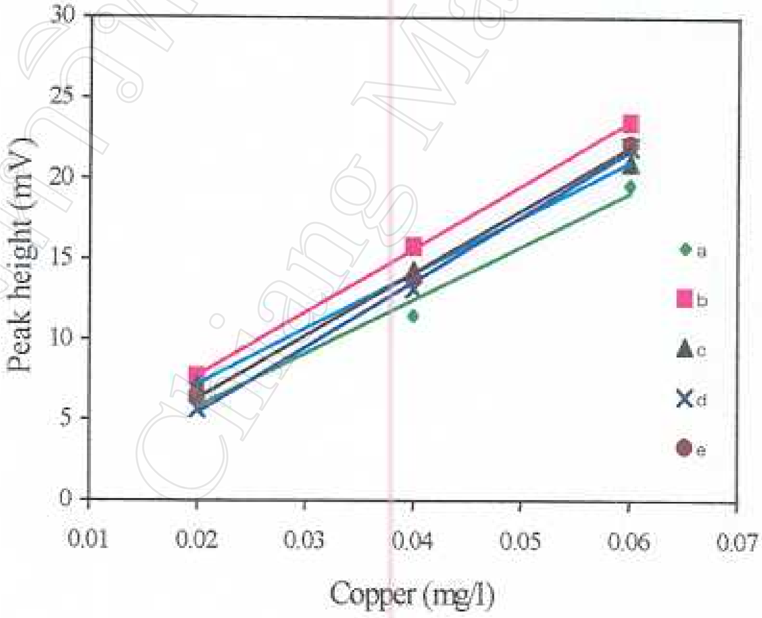


Figure 3.40 Effect of flow rate of eluent on peak height [eluent flow rate (a) 1.9, (b) 2.4, (c) 2.9, (d) 3.4 and (e) 4.0 ml/min].

3.3.4 Effect of preconcentration time on peak height

The effect of preconcentration time on the peak height of copper(II) complex was studied by passing appropriate volume of standard solutions containing 0.005, 0.01, 0.05, 0.1 and 0.2 mgCu(II)/l respectively with a constant flow rate of 3.1 ml/min through the microcolumn by varying the preconcentration times for each concentration. The results are shown in Table 3.48.

Table 3.48 Effect of preconcentration time on peak height.

mgCu(II)/l	Preconcentration time (s)	Cu(II) (μg)	Peak height* (mV)
0.005	120	0.031	0.2
	180	0.046	1.2
	240	0.062	2.5
0.01	60	0.031	0.2
	120	0.062	1.2
	180	0.093	2.7
	240	0.124	3.7
0.05	30	0.078	1.2
	60	0.155	5.7
	90	0.232	9.3
	120	0.310	12.8
	180	0.465	18.3
	240	0.620	23.0
0.1	30	0.155	4.7
	40	0.207	7.2
	50	0.258	10.0
	60	0.310	13.0
	90	0.465	20.7
	120	0.620	26.8
	180	0.930	38.3
0.2	30	0.310	11.3
	40	0.413	16.3
	50	0.517	20.7
	60	0.620	24.3
	90	0.930	34.5
	120	1.240	41.5
	180	1.860	52.8

(*average of triplicate injections)

Figure 3.41 shows a calibration curves constructed by plotting various amounts of copper(II) against peak heights using varying concentrations of single standard solutions. The experimental results show that all curves yield the same linearity and the linear range can be obtained at least up to nearly 1 μgCu(II). Hence, a particular quantity of copper(II) loads onto column from standard solution of different concentrations always gives peaks of nearly the same height. It can be noticed that a series of different concentrations of standard copper(II) solutions passing through the column for the same loading time exhibits linear relationship as conventional calibration.

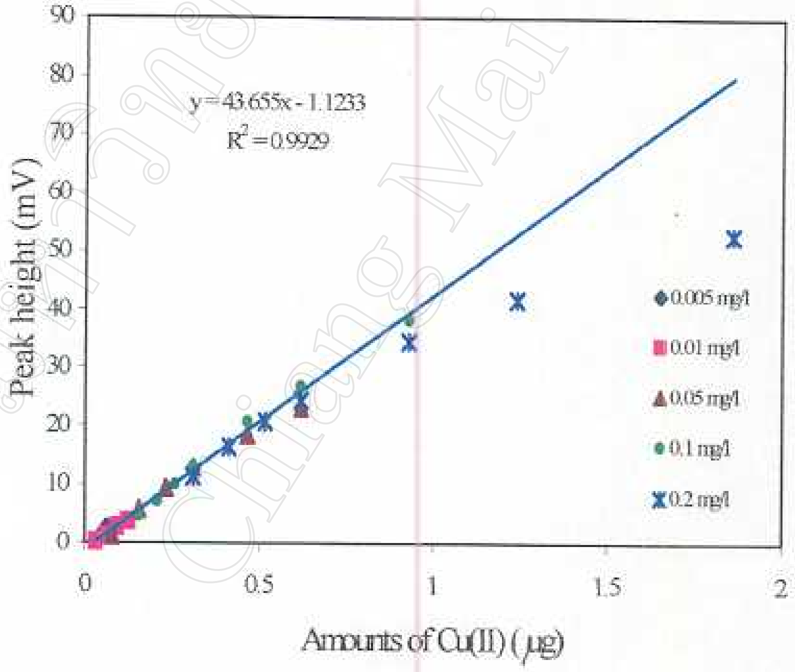


Figure 3.41 The calibration curve using different amounts of copper(II) standard solutions.

3.3.5 Calibration Curve

A calibration curve was obtained by injecting copper(II) standard solutions into the recommended FIA manifold (see Figure 2.2) under the optimum conditions. All measurements were made in triplicate injections. These results are shown in Table 3.49, Figure 3.42 and Figure 3.43. The calibration curve was linear over the range 0.02-0.16 mg/l and the linear regression equation of best fit was y = 245.3x + 0.0357 (where x is the concentration of copper(II) in mg/l). The correlation coefficient for this line was 0.9981.

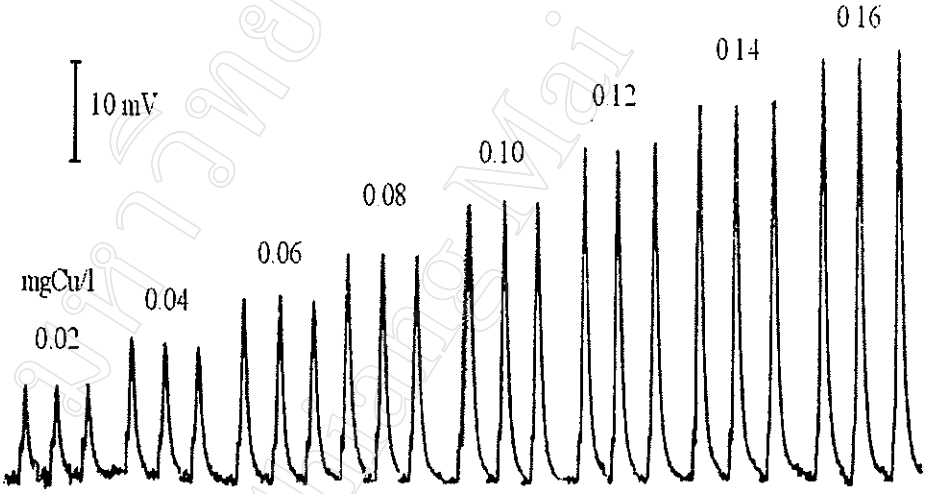


Figure 3.42 FIA-gram of standard copper(II) solution.

Table 3.49 Peak height for calibration curve.

Cu(II) (mg/l)	**Peak height (mV)***
0.02	5.7
0.04	9.7
0.06	14.7
0.08	18.8
0.10	24.0
0.12	29.8
0.14	34.7
0.16	39.5

(* average of triplicate injections)

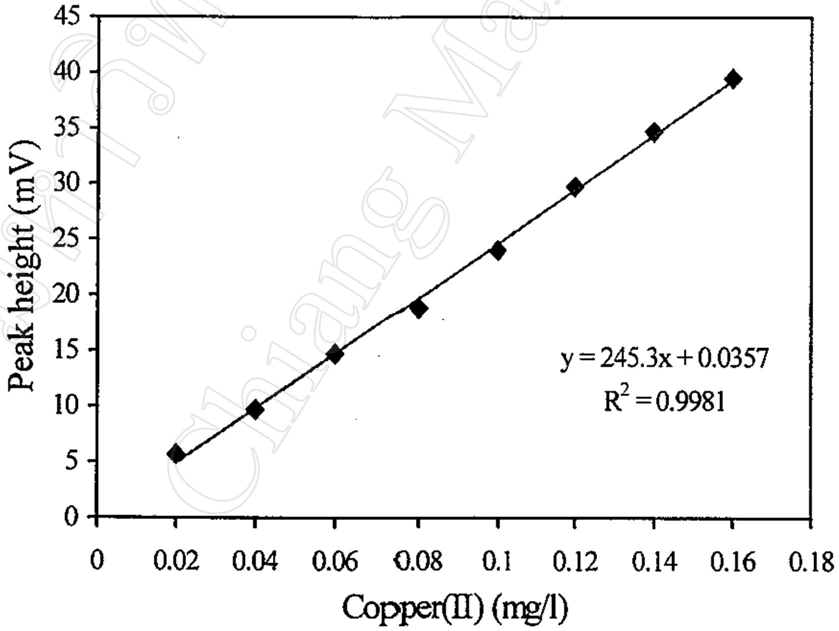


Figure 3.43 Calibration curve of on-line microcolumn preconcentration for trace copper(II) determination.

3.3.6 Precision and Detection Limit

The precision of the proposed method was checked by taking 11 replicate measurements on standard solutions each containing 0.08 mg/l of copper(II). The results are shown in Table 3.50. The relative standard deviation is 0.98% and the mean recovery (n=11) is 102%.

Table 3.50 The precision study using 0.08 mg/l standard copper(II).

Expt. No.	Peak height (mV)*	Cu(II) taken (mg/l)	Cu(II) found (mg/l)
1	16.23	0.08	0.083
2	16.06	0.08	0.082
3	16.06	0.08	0.082
4	16.06	0.08	0.082
5	15.80	0.08	0.081
6	18.89	0.08	0.081
7	15.89	0.08	0.081
8	15.80	0.08	0.081
9	16.23	0.08	0.083
10	15.89	0.08	0.081
11	16.06	0.08	0.082
			$\bar{X}$ = 0.082 SD = 0.0008 %RSD = 0.98
			%Recovery = 102

(* average of triplicate injections)

The detection limit of the method, which were obtained using the preconcentration procedure and the blank signal (results are shown in Table 3.51), were 0.007 mg/l (based on three times the standard deviation of 11 replicate measurements). The detection limit of the method could be furthers decreased by increasing the sample volume. The results show that this system can execute a series of preconcentration and determination procedures automatically with high precision.

Table 3.51 The study of detection limit.

Expt. No.	Peak height of blank peak (mV)*
1	3.0
2	3.0
3	2.5
4	3.0
5	3.0
6	3.0
7	2.5
8	3.0
9	3.0
10	3.0
11	3.0
	$\bar{X}$ = 2.9 SD = 0.2
	Detection limit = 0.007 mg/l

(* average of triplicate injections)

3.3.7 The recommended Manifold and Conditions

By using the FIA manifold shown in Figure 2.2 the recommended conditions are proposed as shown in Table 3.52.

Table 3.52 Optimum conditions for on-line preconcentration of copper(II).

Variable	Optimum conditions
Buffer solution	0.5 M acetate buffer pH 6-7
pH of sample	2.5-3.0
Coloring agent	0.02% (w/v) nitroso-R solution
Mixing coil length	20 cm
Eluent	2% (w/v) sodium nitrate
Flow-rate of reagent	2.6 ml/min
Preconcentration flow rate	3.1 ml/min
Flow rate of eluent	2.4 ml/min
Microcolumn	4 cm packed with synthetic zeolite from natural perlite (45-80 mesh) 0.15 g
Preconcentration time	2 min
Elution time	0.5 min

3.3.8 Interference Studies

The effect of diverse ions in the determination of 0.08 mgCu(II)/l was studied according to the procedure described earlier. Most ions studied do not interfere with the determination of copper(II); as shown in Table 3.53 and 3.54. The tolerance limit of an ion was taken as the maximum amount (mg/l) causing an error not greater than 10%.

Table 3.53 Effect of interference study for 0.08 mgCu(II)/l.

Ions	Added chemical form	Ion:Cu(II) weight ratio	Peak height (mV)*	% Relative error
none	-	-	20.5	-
Pb^{2+}	$Pb(NO_3)_2$	10	20.5	0.0
		20	19.4	-5.4
		50	18.9	-7.8
		70	18.6	-9.3
Cr^{3+}	$Cr(NO_3)_3$	4	20.5	0.0
		5	20.0	-2.4
		10	18.2	-11.2
		20	6.3	-69.8
Mn^{2+}	$Mn(NO_3)_2$	10	20.0	-2.4
		20	19.2	-6.3
		30	18.6	-9.3
		50	17.4	-15.1
Mg^{2+}	$Mg(NO_3)_2$	10	19.3	-5.8
		20	17.5	-14.6
		30	13.2	-35.6
		50	10.1	-50.7
Cd^{2+}	$Cd(NO_3)_2$	10	20.0	-2.4
		30	19.4	-5.4
		40	19.1	-6.8
		50	17.6	-14.2
Zn^{2+}	$Zn(NO_3)_2$	50	20.3	-1.0
		100	20.0	-2.4
		150	18.5	-9.8
		200	17.1	-16.6

Table 3.53 (Continued).

Ions	**Added chemical form**	**Ion:Cu(II) weight ratio**	**Peak height (mV)***	**% Relative error**
Ca^{2+}	$Ca(NO_3)_2$	10	20.0	-4.6
		20	18.5	-13.9
		40	16.3	-22.2
Fe^{3+}	$Fe(NO_3)_3$	0.25	21.1	+2.9
		0.5	22.9	+11.7
		1	25.9	+26.3
Ni^{2+}	$Ni(NO_3)_2$	0.25	22.4	+9.3
		0.5	25.7	+25.4
		1	31.3	+32.9
Co^{2+}	$Co(NO_3)_2$	0.25	28.5	+32.6
		0.5	33.5	+55.8
		1	43.5	+102.3
K^+	KCl	10	20.5	0.0
		30	19.7	-3.9
		50	19.1	-6.8
		60	18.8	-8.3
NO_2^-	$NaNO_2$	10	20.5	0.0
		30	20.2	-1.5
		50	19.7	-3.9
		70	19.2	-6.3
SO_4^{2-}	Na_2SO_4	10	20.5	0.0
		30	20.0	-2.4
		50	19.6	-4.4
		70	18.5	-9.8

Table 3.53 (Continued).

Ions	Added chemical form	Ion:Cu(II) weight ratio	Peak height (mV)*	% Relative error
PO_4^{3-}	NaH_2PO_4	10	20.5	0.0
		30	20.2	-1.5
		50	19.5	-4.9
		70	18.6	-9.3

(* average of triplicate injections)

Table 3.54 Summary of the interference effects of some ions on the response obtained from 0.08 mgCu(II)/l.

Interference ions	Tolerable concentration ratio*(mg/l) of ion/Cu(II)
Zn^{2+}	150
NO_2^-, PO_4^{3-}, SO_4^{2-}, Pb^{2+}	70
K^+	60
Cd^{2+}	40
Mn^{2+}	30
Mg^{2+}, Ca^{2+}	10
Cr^{3+}	5
Ni^{2+}, Fe^{3+}	< 1

* The concentration of an ion is considered to be interfering when causing a relative error of more than ±10 % with respect to the signal of copper(II) alone.

3.3.9 Application of the Proposed FIA with On-line Preconcentration Method

In order to test the reliability of the proposed methodology suitable for the assay of the two targeted copper(II) ions, it was applied to determine their concentration in drinking water and mining discharge water samples.

3.3.9.1 Drinking Water Samples

The drinking water samples were collected from the municipal commercial drinking water around Chiang Mai University. Two replicates of each sample were performed. Drinking water samples were prepared by using the procedure in section 3.3.7-(1). All of the samples were adjusted to pH 2.5-3.0 and analyzed by means of the proposed FIA on-line preconcentration procedure. Table 3.55 shows the results in comparison between the proposed method and the standard addition method.

Table 3.55 Determination of copper in drinking water samples by the proposed FIA and standard addition FIA.

Sample No.	Cu (mg/l)		%Recovery
	the proposed method	standard addition FIA	
D 1	0.018	0.014	105
D 2	0.008	0.004	109
D 3	0.001	0.003	106
D 4	0.010	0.014	103
D 5	ND	ND	-
D 6	ND	ND	-
D 7	ND	ND	-
D 8	ND	ND	-

ND is not detected because the concentration is less than detection limit.
(t-test value is 0.32 , at confidence interval of 95% t-test value in range)

3.3.9.2 Mining Discharge Water Samples

The proposed method has been also applied to the determination of copper(II) in twelve mining discharge water samples. Sample pretreatments were performed by using microwave digestion procedure, mentioned previously (section 2.5.3). A 50 ml of pretreated samples was adjusted to pH 2.5-3.0 followed by heating to near dryness and adding about 10 drops of 40% H_2O_2 before being diluted to the mark 50 ml with deionized water. The experimental process for copper(II) determination in mining discharge water samples were carried out by using the recommended manifold under the conditions in section 3.3.7. A comparative determination of copper(II) in mining discharge water samples by ICP-OES was also carried out. The differences between the means and the precision obtained from the proposed method and the reference method (ICP-OES) were evaluated by the student t-test. The calculated t-test value of the proposed-FIA/ICP-OES is 0.01. The critical value of t-test is 2.08 (22 degree of freedom) at the confidence interval of 95%. Therefore the results obtained by the proposed FIA method are not significantly different from those obtained by the reference method at the confidence interval of 95%. The results are shown in Table 3.56 and Figure 3.44.

Table 3.56 Comparison of copper concentration in mining discharge water samples with the proposed FIA and ICP-OES.

Sample No.	Cu (mg/l)	
	The proposed FIA	ICP-OES
W 1	0.019	0.020
W 2	0.272	0.280
W 3	0.137	0.140
W 4	0.107	0.110
W 5	0.056	0.060
W 6	0.118	0.115
W 7	0.048	0.045
W 8	0.036	0.032
W 9	0.057	0.055
W 10	0.014	0.012
W 11	0.056	0.055
W 12	0.018	0.020

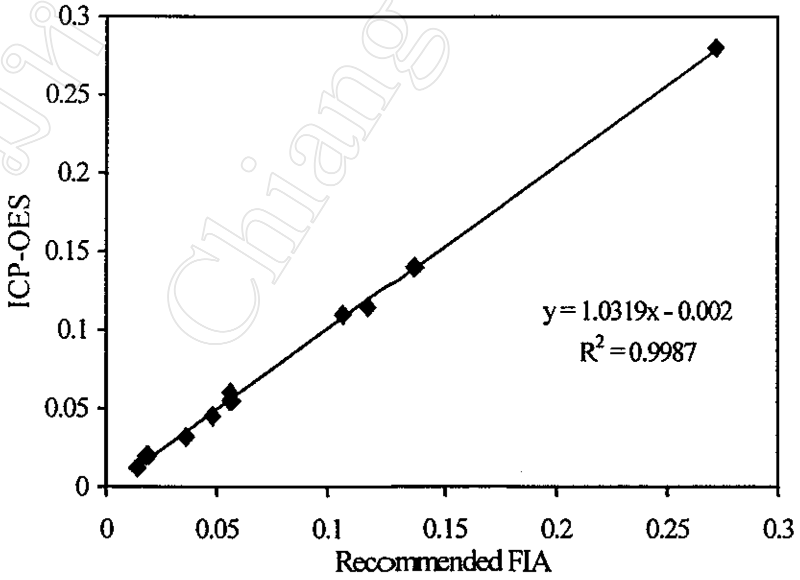


Figure 3.44 Comparison of copper concentration in mining discharge water samples with the proposed FIA and ICP-OES.