



DETERMINATION OF COPPER (II) BY GLYCINE IN FLOW INJECTION AND SEQUENTIAL INJECTION TECHNIQUES

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ABSTRACT

Two new, simple and rapid methods are reported for the accurate and precision spectrophotometric determination of copper (II) using flow injection analysis (FIA) and sequential analysis (SIA). Glycine has been used for determination of copper (II). Various parameters, physical and chemical, affecting the determination have been investigated such as flow rate, reaction coil, volume of reagent (glycine), volume of sample, pH and concentration of glycine. The calibration curve was prepared and the dispersion coefficient, reproducibility, interferences and application were studied. Two methods, flow injection and sequential injection analysis were compared for determination of copper (II) by glycine spectrophotometrically at 650 nm. The linear range was (1-170 mg/L), (0.01-110 mg/L) at sampling rate of 120, 100 sample per hour, the detection limits (0.1 mg/L), (0.01 mg/L) for FIA and SIA, respectively. Relative standard deviations for (10 mg/L), $n = 3$ for both methods were found (0.85% for FIA and 0.00% for SIA). Dispersion coefficient was also measured for the two methods.

Key words: Copper, Sequential, Determination, Flow injection, Glycine.

INTRODUCTION

Copper (II) forms stable complexes with amino acids through chelating. Sometimes, copper and amino acids form oxo complexes, which also feature copper (III)¹. Copper is an essential trace element, Complex plays a role in most living organisms. It also known as one of the toxic elements in the environment. In order to assess its accumulation or deficiency in biological and environmental samples, sensitive, reproducible and accurate analytical methods are required². Copper is one of the important and essential nutrients for human health as well as for the growth of animals and plants, Copper is required for normal metabolic processes. Copper combines with certain proteins to produce enzymes that act as catalysts to help a number of body functions. The lowest level, at which these adverse effects occur, has not been well defined. People with Wilson's disease, a rare genetic disorder, are more sensitive to the effects of copper and anemia, Hair kinky, while excess of copper can result into a disease like, jaundice³⁻⁷. Flow injection analysis (FIA) was first described by Ruzicka and Hansen in Denmark and Stewart and coworkers in United States in the middle of 1970⁸. Flow analysis represents a good alternative over the traditional methods used in the environmental field due to its characteristics such as low volumes of sample and reagents consumption, high sample throughput and mainly the possibility to develop automated monitoring systems adapting different

techniques and instruments⁹. The second generation of FI techniques, termed sequential injection analysis (SIA), was established in 1990 by Ruzicka and Marshall at the University of Washington. This methodology was proposed as a new feasible and mechanically simpler alternative to FIA for handling solutions. This technique for automatic sample analysis is based on the same principles as FIA (controlled partial dispersion and reproducible sample handling)¹⁰. Determination of copper ions by flow injection technique by discoverer (Adem and others) was done by using reagent 4-benzyl piperidine-dithio carbonate 4-(BPDC) with linear range (2.5-100 $\mu\text{g/L}$), detection limit less than 0.35 $\mu\text{g/L}$ and R.S.D. 0.89%¹¹, while flow injection technique by discoverer (Camargo and others) used reagent 2,6-dichlorophenolindophenol with can linear range (0.3-100 ppm), detection limit 0.09 ppm and R.S.D. less than 2%¹². Flow injection technique allows determination of copper(II) ion by utilize reagent p-anisidine with N,N-dimethyl aniline in presence of hydrogen peroxide in the range of 0.05-5 ppb. The relative standard deviation was 0.78% for 10 determination of 0.5 ppb of copper (II)¹³.

EXPERIMENTAL

Apparatus

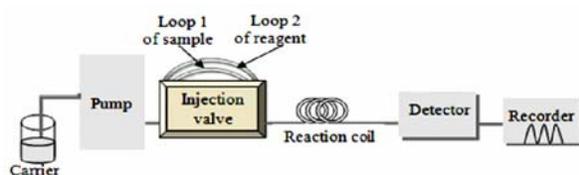
Analytical balance sensitive Denver Instrument, Spectrophotometer Labomed.inG single beam, USA, and a spectrophotometer Shimadzu UV-1700 spectrophotometer, Recorder Pen Siemens C 1032, Hitter thermal Ardeas 51, peristaltic pump Germany, Ismatic, files Interaction with the radius of 0.5 mm, homemade valves, pipes load of Teflon, flow cell volume of 450 μL , pH meter.

Chemicals

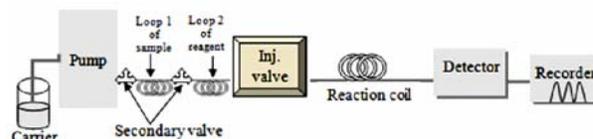
- (i) Distilled water was used for preparation of all solutions in the experiments.
- (ii) Copper ion 1000 ppm as stock solution was prepared by dissolving 0.2685 g of copper chloride salt in 100 mL of distilled water. Then other solution were prepared at using dilution law.
- (iii) 1 M of glycine were prepared by dissolving 7.507 g glycine in a beaker in 25 mL of distilled water, transferred to volumetric flask of 100 mL and then made up to mark with distilled water.
- (iv) Buffer solution of prepared pH = 8 was [0.1 M citric acid $\text{C}_6\text{H}_8\text{O}_7$ + 0.2 M disodium hydrogen phosphate, Na_2HPO_4] 0.4803 g citric acid was dissolved in distilled water in beaker 10 mL. It was transferred to volumetric flask of capacity 25 mL and made up to the mark with distilled water. Then 2.8392 g Na_2HPO_4 was dissolved in distilled water in a beaker (25 mL). It was transferred to volumetric flask of capacity 100 mL and made up to the mark with distilled water¹⁴.

Unit

The various parameters affecting the unit have been investigated and selected for a final method evaluation. The following results allow the operator to choose different operation conditions. Used new designs as shown in the design 1 and 2.



Designer 1: New design of FIA unit



Designer 2: New design of SIA unit

RESULTS AND DISCUSSION

Physical parameters

Effect of the flow rate

The effect of the flow rate on the peak height was studied in the range of 2.0-11.2 mL min⁻¹ (Table 1 and Fig. 1). Lower flow rate cause doublet peaks, possibly due to the fact that the carrier solution did not sufficiently disperse into the middle of the sample zone¹⁵. On other hand, the peak height decreased with the increasing flow rate¹⁶. Taking into consideration of the stability of the pump, peak shape and sampling time, the flow rate of the carrier solution was adjusted to 9 mL min⁻¹ for subsequent measurement due to highest sensitivity.

Table 1: Effect of the flow rate on the peak height; Cu (II) conc. = 10 ppm, R. C. (Reaction coil) = 30 cm, [Gly] = 1 M, and sample loop (L₁) = reagent loop (L₂) = 25 cm

S. No.	Flow rate of pump	Cal. Flow rate mL/min	Peak height (cm)			Mean	Notes	SD	RSD%
1	10	2.000	0.314	0.313	0.213	0.286	Double peak	0.058	20.279
2	20	4.000	0.354	0.353	0.354	0.353	-	0.001	0.283
3	30	5.600	0.324	0.377	0.325	0.342	-	0.030	8.771
4	40	7.500	0.512	0.411	0.362	0.428	-	0.076	17.757
5	50	9.000	0.388	0.484	0.439	0.437	-	0.048	10.983
6	60	11.20	0.380	0.316	0.256	0.317	-	0.062	19.558

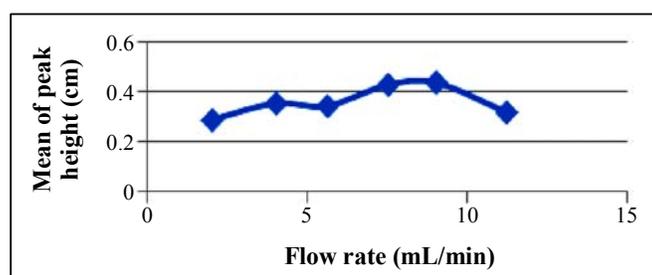


Fig. 1: Change of peak height with flow rate in FIA unit

Effect of the reaction coil length

Table 2 and Fig. 2 shows effect of the reaction coil length on the peak height in the range (without-80) cm it was seen that the suitable reaction coil length was 50 cm, since it provided the greatest sensitivity.

Table 2: Effect of the reaction coil length on the peak height; Cu (II) conc. = 10 ppm, flow rate (9 mL min⁻¹), [Gly] = 1 M, and sample loop (L₁) = Reagent loop (L₂) = 25 cm

S. No.	R. C. (cm)	Peak height (cm)			Mean	SD	RSD%
1	Without	0.345	0.345	0.336	0.342	0.005	1.461
2	30	0.388	0.484	0.439	0.437	0.048	10.983
3	50	0.702	0.702	0.702	0.702	0.000	0.000
4	80	0.429	0.429	0.429	0.429	0.000	0.000

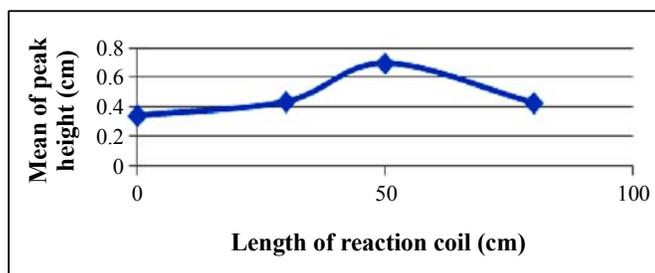


Fig. 2: Change of peak height with the reaction coil length in FIA unit

Effect of the sample volume

The influence of the sample volume on the peak height was investigated by injecting different volumes (78.5-392.5) μL . The peak height increased to the maximum at 314 μL and after that volume, the peak height decreased. So 314 μL was chosen for further work (Table 3 and Fig. 3).

Table 3: Effect of the volume sample on the peak height; Cu (II) conc. = 10 ppm, flow rate (9 mL min⁻¹), [Gly] = 1 M, reagent loop (L₂) = 25 cm and R.c = 50 cm

S. No.	L of Cu(II) (cm)	V (μL)	Peak height (cm)			Mean	SD	RSD%
1	10	78.50	0.245	0.295	0.347	0.295	0.051	17.288
2	25	196.25	0.702	0.702	0.702	0.702	0.000	0.000
3	40	314.0	1.257	1.258	1.200	1.238	0.033	2.665
4	50	392.5	0.517	0.517	0.374	0.469	0.082	17.484

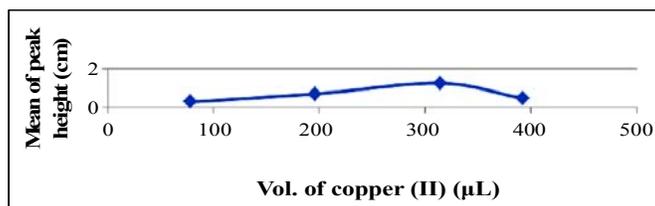


Fig. 3: Change of peak height with sample volume in FIA unit

Effect of glycine volume

The influence of the various volume (78.5-314 μL) of glycine was observed. Glycine volume that exhibited the greatest peak height was found to be 196.25 μL and it was chosen as the optimum (Fig. 4 and Table 4).

Table 4: Effect of the reagent volume on the peak height; Cu (II) conc. = 10 ppm, flow rate (9 mL min⁻¹), [Gly] = 1 M, sample loop (L₁) = 40 cm and R.c = 50 cm

S. No.	L of Gly (cm)	V (μL)	Peak height (cm)			Mean	SD	RSD%
1	10	78.50	0.415	0.415	0.297	0.375	0.068	18.133
2	25	196.25	1.257	1.258	1.200	1.238	0.033	2.665
3	40	314.0	0.367	0.294	0.294	0.318	0.042	13.207

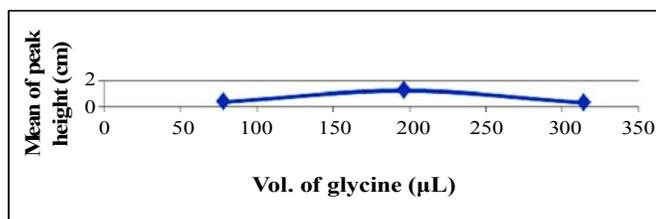


Fig. 4: Change of peak height with the reagent volume in FIA unit

Chemical parameters

Effect the pH

The formation of the complex was studied in the different pH (6-10). The optimum pH was 8. The suitable buffer was prepared from disodium hydrogen phosphate (Na_2HPO_4) and citric acid ($\text{C}_6\text{H}_8\text{O}_7$), to adjust the pH of medium. (Fig. 5 and Table 5).

Table 5: Effect of the pH on the peak height; Cu(II) conc. = 10 ppm, flow rate (9 mL min^{-1}), [Gly] = 1 M, sample loop (L_1) = 40 cm, reagent loop (L_2) = 25 cm and R.c = 50 cm

S. No.	pH	Peak height (cm)			Mean	SD	RSD%
1	6	0.571	0.355	0.477	0.467	0.108	23.641
2	7	1.257	1.258	1.200	1.238	0.033	2.665
3	8	2.095	2.095	1.998	2.062	0.056	2.715
5	9	0.551	0.824	0.648	0.674	0.138	20.474

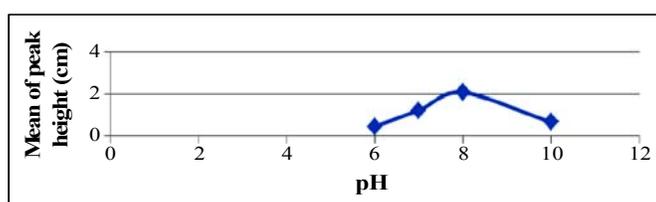


Fig. 5: Change of peak height with pH in FIA unit

Effect of the reagent concentration

The reagent concentration was varied in the range $0.1\text{-}1.5 \text{ mole L}^{-1}$ in order to maximize the peak height. Table 6 and Fig. 6 show the effect of reagent concentration on the peak height of the copper (II). The maximum peak height was obtained with 1.0 mole L^{-1} reagent and therefore, the 1.0 mole L^{-1} reagent was chosen for further work.

Table 6: Effect of the reagent concentration on the peak height; Cu (II) conc. = 10 ppm, R.C. (Reaction coil) = 50 cm, sample loop (L_1) = 40 cm, reagent loop (L_2) = 25 cm, pH = 8 and flow rate = 9 mL min^{-1}

S. No.	[Gly] (mole L^{-1})	Peak height (cm)			Mean	SD	RSD %
1	0.1	0.116	0.118	0.117	0.117	0.001	0.854
2	0.5	0.484	0.634	0.618	0.578	0.082	14.186
3	1.0	2.095	2.095	1.998	2.062	0.056	2.716
4	1.5	2.542	3.367	4.891	3.600	1.191	33.083

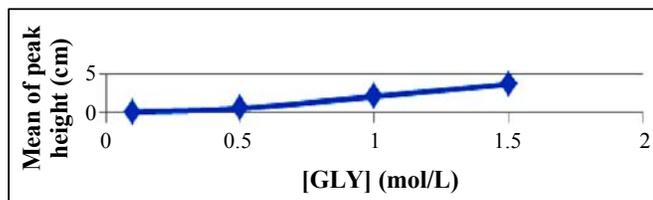


Fig. 6: Change of peak height with reagent concentration in FIA unit

Study of the dead volume

To ensure accurate results obtained from this unit, dead volume must be studied. Wherever, the dead volume is small, it means best result. Two experiments were done (i) in the first, the reagent was injected in one loop and in the another inject loop H_2O instead of sample $Cu(II)$ and there was no response and (ii) in the second experiment sample was injected and in another loop H_2O instead of reagent and there was no response. At concentration 0.2 M glycine, peak produced was drawn by the recorder at height (0.198 cm) and therefore, glycine concentration (0.1 M) has been used optimum and equal dead volume.

Calibration curve in FIA method

Calibration curve was prepared at the optimum conditions of complexation and change in the metal ion concentration. The result are shown in Table 7 and Fig. 7. The calibration curve is linear in the range of 1-170 $mg L^{-1}$ and the detection limit is 0.1 $mg L^{-1}$.

Table 7: Effect of the concentration of Cu (II) conc. with peak height; flow rate = 9 mL/min, pH $[Na_2HPO_4 (0.2 M) (18.5 mL) + Citric acid (0.1 M) (1.5 mL)] = 8$, R.c = 50 cm, sample Loop (L_1) $Cu^{2+} = 40$ cm, $[Gly] = 0.1 M$, and reagent Loop (L_2) of Gly 25 cm

S. No.	Cu(II) (ppm)	Peak height (cm)	Mean	SD	RSD%
1	1	0.075	0.081	0.010	11.904
2	5	0.112	0.112	0.000	0.000
3	10	0.116	0.117	0.001	0.854
4	20	0.204	0.204	0.000	0.000
5	30	0.245	0.244	0.001	0.408
6	40	0.329	0.327	0.001	0.304
7	50	0.424	0.423	0.001	0.236
8	60	0.438	0.437	0.001	0.228
9	70	0.453	0.524	0.082	16.400
10	80	0.573	0.573	0.000	0.000
11	90	0.628	0.628	0.000	0.000
12	100	0.743	0.743	0.000	0.000
13	110	0.819	0.819	0.000	0.000
14	120	0.827	0.827	0.000	0.000
15	130	0.942	0.944	0.001	0.106
16	140	1.003	1.003	0.000	0.000
17	150	1.033	1.033	0.000	0.000
18	160	1.136	1.088	0.022	1.964
19	170	1.167	1.167	0.000	0.000

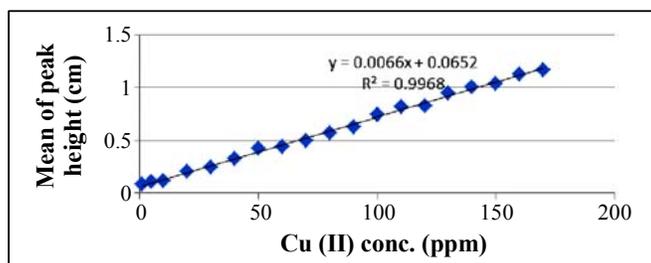


Fig. 7: Calibration curve of Cu (II) with glycine in FIA method

Reproducibility

Precision range and effective method in determination of copper (II) was studied through reproducibility, injection and measure for multitudes, using 60 ppm concentration of glycine, so that amount of standard deviation for (60 mg/L) was $n = 10$ (0.0004) and amount of relative standard deviation was 0.090% for accuracy and effective system for determination of copper ion. The results are shown in Table 8 and Fig. 8.

Table 8: Reproducibility for 60 ppm of copper (II) in FIA unit

S. No.	1	2	3	4	5	6	7	8	9	10	Mean	S.D.	RSD%
Peak height (cm)	0.443	0.442	0.442	0.442	0.442	0.442	0.442	0.443	0.443	0.442	0.4423	0.0004	0.090

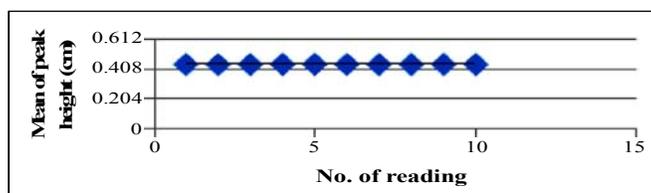


Fig. 8: Reproducibility of copper (II) for 60 ppm with glycine

Sequential injection technique (SIA)

Chemical parameters

Effect the pH

The formation of the complex was investigated in the different pH (5-8). The optimum pH was 7. The suitable buffer was prepared from disodium hydrogen phosphate (Na_2HPO_4) and citric acid ($\text{C}_6\text{H}_8\text{O}_7$) to adjust of the pH medium (Fig. 9 and Table 9).

Table 9: Effect of the pH on the peak height; Cu (II) conc. = 30 ppm, R.C. (reaction coil) = 30 cm, [Gly] = 0.1 M, sample loop (L_1) = 40 cm, reagent loop (L_2) = 25 cm, and flow rate = 9 mL/min

S. No.	pH	Peak height (cm)			Mean	SD	RSD%
1	5	0.277	0.277	0.277	0.277	0.000	0.000
2	6	0.277	0.277	0.277	0.277	0.000	0.000
3	7	0.496	0.496	0.496	0.496	0.000	0.000
4	8	0.277	0.277	0.277	0.277	0.000	0.000

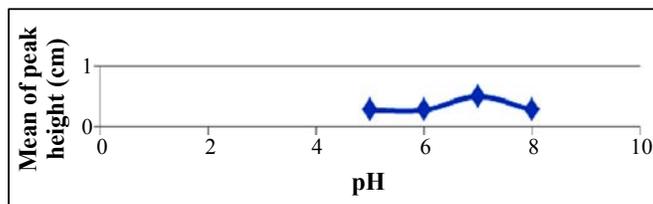


Fig. 9: Change of peak height with pH in SIA unit

Effect of the reagent concentration

At concentration 0.2 M of the reagent (Gly), peak produced was recorded in the recorder at height (0.198 cm) and therefore, glycine concentration (0.1 M) was used optimum and equal dead volume.

Physical parameters

Effect of the flow rate

The effect of the flow rate on the peak height was studied in the range of 1.0-13 mL min⁻¹ (Table 10 and Fig. 10). Taking into consideration the stability of the pump, peak shape and sampling time, the flow rate of the carrier solution was adjusted to 9 mL min⁻¹ for subsequent measurement due to highest sensitivity.

Table 10: Effect of the flow rate on the peak height; Cu (II) con. = 30 ppm, R.C. (Reaction coil) = 30 cm, [Gly] = 0.1 M, sample loop (L₁) = 40 cm, reagent loop (L₂) = 25 cm, and pH = 7 [Na₂HPO₄ (0.2 M) (15 mL) + C₆H₈O₇ (0.1 M) (5 mL)]

S. No.	Flow rate on pump	Cal. flow rate	Peak height (cm)			Mean	S.D.	RSD%
1	5	1.000	0.118	0.117	0.119	0.118	0.001	0.847
2	10	2.000	0.275	0.275	0.275	0.275	0.000	0.000
3	30	5.600	0.275	0.275	0.275	0.275	0.000	0.000
4	50	9.000	0.496	0.496	0.496	0.496	0.000	0.000
5	60	11.20	0.357	0.357	0.357	0.357	0.000	0.000
6	70	13.00	0.341	0.341	0.341	0.341	0.000	0.000

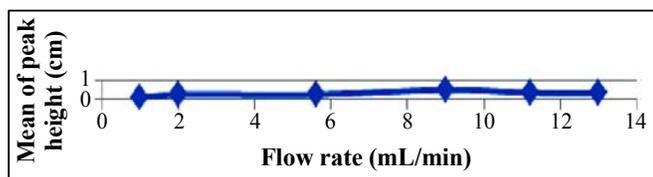


Fig. 10: Change of peak height with flow rate in SIA unit

Effect of the reaction coil length

Table 11 and Fig. 11 show the effect of the reaction coil length on the peak height in the range (0-50 cm). It was seen that the suitable reaction coil length was 30 cm, since it provided the greatest sensitivity.

Table 11: Effect of the reaction coil length on the peak height; Cu(II) conc. = 30 ppm, flow rate (9 mL min⁻¹), [Gly] = 0.1 M, sample loop (L₁) = 40 cm, reagent loop (L₂) = 25 cm, and pH = 7 [Na₂HPO₄ (0.2 M) (15 mL) + C₆H₈O₇ (0.1 M) (5 mL)]

S. No.	R.c. (cm)	peak height (cm)			Mean	SD	RSD%
1	Without	0.344	0.340	0.339	0.341	0.002	0.586
2	30	0.496	0.496	0.496	0.496	0.000	0.000
3	50	0.339	0.339	0.339	0.339	0.000	0.000

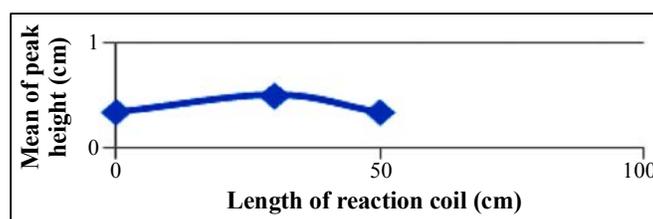


Fig. 11: Change of peak height with the reaction coil length in SIA unit

Effect of glycine volume

The influence of the various volume was observed in the ranged (78.5-314 μL). The glycine volume that exhibited the greatest peak height was found to be 78.5 μL and it was chosen as the optimum (Fig. 12 and Table 12).

Table 12: Effect of the reagent volume on the peak height; Cu(II) conc.= 30 ppm, flow rate (9 mL min⁻¹), [Gly] = 0.1 M, sample loop (L₁) = 40 cm, R.c. = 30 cm, and pH = 7 [Na₂HPO₄ (0.2 M) 15 mL + C₆H₈O₇ (0.1 M) 5 mL]

S. No.	L of Gly.	V (μL)	Peak height (cm)			Mean	SD	RSD%
1	10	78.50	0.540	0.540	0.540	0.540	0.000	0.000
2	25	196.25	0.496	0.496	0.496	0.496	0.000	0.000
3	40	314.0	0.471	0.471	0.471	0.471	0.000	0.000

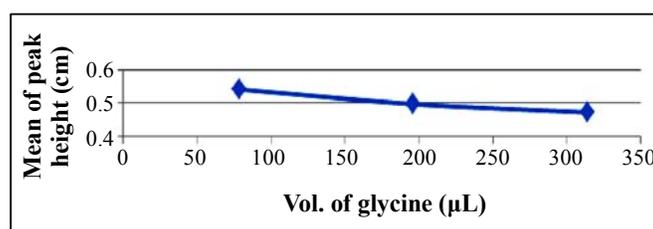


Fig. 12: Change of peak height with the reagent volume in SIA unit

Effect of the sample volume

The influence of the sample volume on the peak height was investigated by injecting different volumes (78.5-471 μL). The peak height increased to the maximum at 392.5 μL after that volume, the peak height decreased, so 392.5 μL was chosen for further work (Table 13 and Fig. 13).

Table 13: Effect of the volume sample on the peak height; Cu(II) conc. = 30 ppm, flow rate (9 mL min⁻¹), [Gly] = 0.1 M, Reagent loop (L₂) = 10 cm R.c = 30 cm, and pH = 7 [(Na₂HPO₄ (0.2 M) 15 mL + C₆H₈O₇ (0.1 M) 5 mL]

S. No.	L of Cu (II) cm	V (μL)	Peak height (cm)			Mean	SD	RSD%
1	10	78.50	0.426	0.426	0.426	0.426	0.000	0.000
2	25	196.25	0.494	0.494	0.494	0.494	0.000	0.000
3	40	314.0	0.540	0.540	0.540	0.540	0.000	0.000
4	50	392.5	0.591	0.591	0.591	0.591	0.000	0.000
5	60	471.0	0.576	0.576	0.576	0.576	0.000	0.000

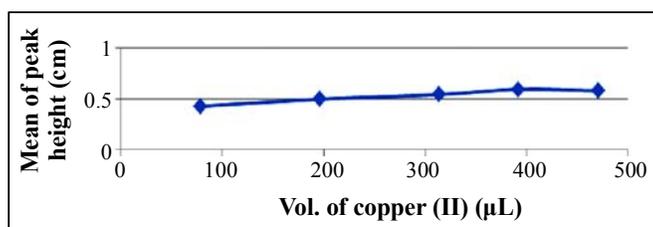


Fig. 13: Change of peak height with sample volume in SIA unit

Calibration curve in SIA method

Calibration curve was prepared at the optimum conditions of complexation and change in the metal concentration (Table 14 and Fig. 14). The calibration curve is linear in the range of 0.01-110 mg L⁻¹ and the detection limit is 0.01 mg L⁻¹.

Table 14: Effect of the concentration Cu (II) conc. with peak height; flow rate = 9 mL/min, pH [Na₂HPO₄ (0.2 M) (15 mL) + Citric acid (0.1 M) (5 mL)] =7, sample Loop (L1) of Cu (II) = 50 cm, reagent Loop (L2) of Gly = 10 cm, R. C. = 30 cm, [Gly] = 0.1 M

S. No.	Cu (II) (ppm)	Peak height (cm)			Mean	SD	RSD%
1	0.01	0.103	0.103	0.103	0.103	0.000	0.000
2	0.05	0.105	0.105	0.105	0.105	0.000	0.000
3	0.1	0.105	0.105	0.105	0.105	0.000	0.000
4	0.5	0.127	0.127	0.127	0.127	0.000	0.000
5	1	0.135	0.135	0.135	0.135	0.000	0.000
6	5	0.185	0.185	0.185	0.185	0.000	0.000
7	10	0.291	0.291	0.291	0.291	0.000	0.000
8	20	0.451	0.451	0.451	0.451	0.000	0.000
9	30	0.591	0.591	0.591	0.591	0.000	0.000
10	40	0.691	0.691	0.691	0.691	0.000	0.000
11	50	0.837	0.837	0.837	0.837	0.000	0.000
12	60	1.051	1.051	1.051	1.051	0.000	0.000

Cont...

S. No.	Cu (II) (ppm)	Peak height (cm)			Mean	SD	RSD%
13	70	1.129	1.129	1.129	1.129	0.000	0.000
14	80	1.332	1.332	1.332	1.332	0.000	0.000
15	90	1.484	1.484	1.484	1.484	0.000	0.000
16	100	1.640	1.640	1.640	1.640	0.000	0.000
17	110	1.769	1.769	1.769	1.769	0.000	0.000

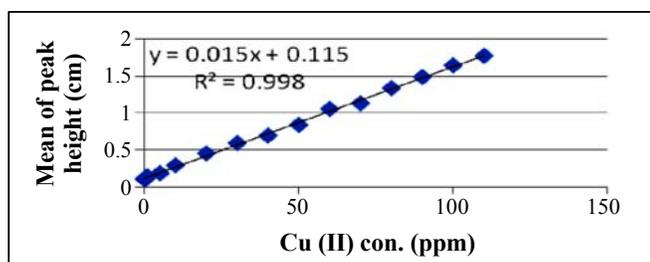


Fig. 14: Calibration curve of Cu (II) with glycine in SIA method

Reproducibility

For study Precision range and effective method in determination of copper (II) was studied through reproducibility injection and measure for multitudes, using 30 ppm concentration of glycine, so that amount of standard deviation for (30 mg/L) was $n = 10$ (0.002) and amount of relative standard deviation was 0.366% for accuracy and effective system for determination of copper ion. The results are shown in Table 15 and Fig. 15.

Table 15: Reproducibility for 30 ppm of copper (II) in SIA unit

S. No.	1	2	3	4	5	6	7	8	9	10	Mean	S.D	RSD%
Peak height (cm)	0.550	0.540	0.540	0.540	0.550	0.550	0.550	0.550	0.540	0.540	0.545	0.002	0.366

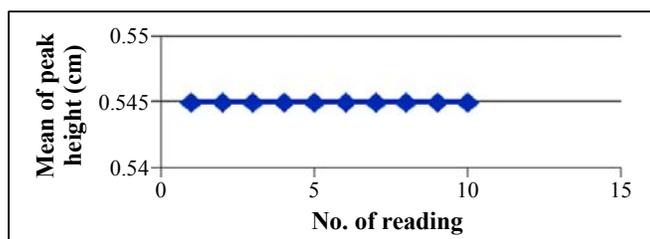


Fig. 15: Reproducibility of copper (II) for 60 ppm with glycine

Determination of dispersion

To measure the dispersion value in different sample zones of 30 and 80 ppm copper ion for FIA; (10 and 80 ppm) copper ions for SIA, two experiments were carried out.

In the first experiment, after mixing of reactants (glycine and copper ion) that passes through manifold unit gives continuous response; this indicates non-existence of dispersion effect by convection or diffusion. This measurement represents (H^0). The second experiment includes injecting different concentration of Cu ions (30 and 80 ppm) for FIA and Cu (II) (10 and 80 ppm) for SIA. The obtained value

from this experiment represents intensity response for sample injected (H_{\max})¹⁷⁻²⁰. The equation used to calculate dispersion (D) is: $D = \frac{H^{\circ}}{H_{\max}}$. These values fall in limit state of dispersion (Table 16 and 17).

Table 16: Determination of dispersion of Cu (II) in FIA method

Dispersion (D) $D = \frac{H^{\circ}}{H_{\max}}$	Response (cm)		Cu(II) concentration (ppm)
	H_{\max}	H°	
1.538	0.245	0.377	30
1.413	0.573	0.810	80

Table 17: Determination of dispersion of Cu (II) in SIA method

Dispersion (D) $D = \frac{H^{\circ}}{H_{\max}}$	Response (cm)		Cu(II) concentration (ppm)
	H_{\max}	H°	
1.099	0.291	0.320	10
1.051	1.332	1.400	80

Interference

Studies related to overlapping elements with copper in the composition of the copper complex at wavelength 650 nm were carried out. It was found to that both negative ions (Cl^{-} , CH_3COO^{-} , I^{-} , NO_3^{-} , CO_3^{2-} , F^{-} , SO_4^{2-}) and positive ions (Fe^{3+} , Zn^{2+} , CO_2^{2+} , Ni^{2+} , Mn^{2+}) did not interfere with the formation of copper complex.

Applications

In SIA system, when preparing 50 ppm of Kufa water, it was found that the average height of the top of the curve is equal to 0.837 cm, which is equivalent to 50 ppm as well as the same thing for Najaf water. It was same the FIA system (Table 18).

Table 18: Applications

The value in calibration curve	The found value for sample in FIA	Sample
50 ppm	51.92 ppm	Kufa water with 50 ppm Cu(II)
50 ppm	50.92 ppm	Najaf water with 50 ppm Cu(II)
The value in calibration curve	The found value for sample in SIA	Sample
50 ppm	50 ppm	Kufa water with 50 ppm Cu(II)
50 ppm	50 ppm	Najaf water with 50 ppm Cu(II)

Comparison between the two methods

The comparison between two methods for the determination of Cu (II) is given in Table 19.

Table 19: Optical characteristics, precision and accuracy of the spectrophotometric determination of Cu (II) with FIA and SIA method

SIA method	FIA method	Optical characteristics
0.01-110	1-170	Concentration linearity range (mg/L)
650.0	650.0	λ_{\max} (nm)
0.010	0.100	Detection limit (μ mg/L) practically
0.9986	0.9968	Correlation coefficient
0.0151	0.0066	Slope
0.000%	0.850%	Relative standard deviation (%), Determination for (10 mg/L), $n = 3$
1.051	1.413	Dispersion coefficient for concentration (80) mg/L
100.0	120.0	sampling rate (Sample/h)
392.5	314.0	Sample volume Cu(II) μ L

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