Development of a New spectrophotometric based flow injection analysis method for the determination of copper (II)

Sama Omarova a, Serkan Demir b and Müberra Andac a

aDepartment of Chemistry, Faculty of Arts and Sciences, Ondokuz Mayis University, Samsun, Turkey; bDepartment of Industrial Engineering, Faculty of Engineering, Giresun University, Giresun, Turkey

ABSTRACT
A new and highly sensitive spectrophotometric based flow injection analysis method has been developed for the determination of copper (II). N, N’-o-phenylene-bis (3-methoxysalicylideneimine) (H2IF) was used as a new chromogenic reagent. The absorbance was measured at \( \lambda_{\text{max}} = 420\) nm. Various factors affecting complex formation have been investigated. For example, the pH effect, reagent concentration was studied. Under optimum conditions, Beer’s law was met in the range of 3.18–318 \( \mu \)g L\(^{-1}\) copper (II) concentration. The system required no mixing chamber and allowed a sample throughput > 60 sample per hour for 20 \( \mu \)L injection volume. The interaction of the various ions was investigated and the experimental results were reported. The proposed method was successfully applied to the determination of the amount of copper (II) found in pharmaceutical samples and water samples. The validity and applicability of the method was confirmed by AAS and standard reference material SPS-WW2 (Wastewater) analysis.

ARTICLE HISTORY
Received 27 December 2017
Revised 13 August 2018
Accepted 3 September 2018

KEYWORDS
Spectrophotometry; FIA; copper (II) determination; pharmaceutical sample analysis

1. Introduction
Heavy metal pollution is a serious global environmental problem because it affects genetic change in all living things. Nowadays, efforts have been accelerated to remove these harmful content. Rapid industrialization and the development of technology have increased the need for high purity materials. Contamination of air, water and soil increases the importance of trace element determinations of the negative effects of this pollution on all living things and the increasing importance of environmental problems. At the same time, the effects of trace elements on human metabolism have made trace element determinations even more important [1].

The copper determination is important because they relate to a variety of areas such as industry, environment, clinics, and food. Since long time unconscious industrialization studies have resulted in incredible levels of pollution. All living things in the land are affected by harmful chemicals in various ways. These materials can be produced both by people and by natural means. Examples are medicines, commercial chemicals, industrial wastes, pesticides used in agriculture, industrial wastes and hydrocarbon combustion products, harmful chemical substances. As the number and variety of chemicals used together with the developing industry increases, people and the environment are more exposed to such chemical substances. Due to this situation which causes many diseases, the poisoning of chemical substances becomes a matter of research increasing day by day.

From the point of view of human health, metal poisoning is extremely important. Qualitative and quantitative determinations of metals in tissues are needed for the identification of these diseases and many spectroscopic methods are being developed for metal determinations.

Atomic absorption spectrometry [2,3], inductively coupled plasma emission spectrometry [4], inductively coupled plasma-mass spectroscopy [5], spectrophotometry, spectrofluorimetry [6] and electrochemical methods [7] are the most commonly used methods for Cu (II) determination. These methods are the right methods for the determination of copper (II) ions. However, these methods have many disadvantages: there has been a need to develop alternative methods for these methods, such as the use of expensive apparatuses, time consuming, labor-intensive and requiring high amounts of organic solvent use and well controlled experimental conditions. Flow injection techniques offer many advantages over manuel procedures since its simplicity and speed [8]. Numerous analytical reagent and flow procedures for the copper (II) determination have been presented in the literature. But some procedure involves critical reaction conditions or tedious sample preparation steps [9–12].

In the present work, a new reagent, N, N’-o-phenylene-bis (3-methoxysalicylideneimine) (H2IF) was used...
for the determination of copper (II) by spectrophotometric flow injection analysis. This reagent is readily available and has a potential application for rapid, selective and precise detection of Cu (II) in aqueous media. The developed method is simple and allows accurate measure the concentration (II) of the complex at a distinct wavelength of 420 nm. The developed methodology has been applied for the copper (II) determination in water samples and pharmaceutical samples.

2. Material and methods

2.1. Reagents and solutions

Analytical reagent grade chemistry was used in the study.

The metal solutions were prepared by dissolving each metal salt and diluting it to $2.5 \times 10^{-4}$ M concentration.

Absorption spectra were taken by adding ligand onto metal solutions.

Acetate buffer solutions were prepared at pH range 2.20–5.60 by mixing appropriate proportions of sodium acetate solution (0.1 M) and acetic acid (0.2 M).

Certified materials C12X3500 and SPS-WW2 were supplied from MBH Analytical Ltd and LGC Group (UK).

2.2. Synthesis of $H_2IF$

0.305 g (2 mmol) o-vanillin and 0.108 g (1 mmol) o-phenylenediamine were refluxed in absolute methanol for 30 min and bright orange precipitate of the Schiff base was formed. Upon cooling the mixture, the precipitate was filtered, washed with cold ethanol and dried in air. Yield: 93%. Anal. Calc. C, 70.20; H, 5.36; N, 7.44. Found: C, 69.21; H, 5.19; N, 7.02. IR (KBr, cm$^{-1}$): 3463 $\nu$ (OH); 3058–3011 $\nu$ (ar(CH)); 1623 $\nu$ (C=N); 1490 $\nu$ (C=C). 1H-NMR (Scheme 1, 400MHz, ppm, CDCl3): 13.56 (m, 2H, (H6, O–H..N)) 8.66 (s, 2H, (H5, CH=N)); 7.06–7.28 (m, 6H, aldehyde); 6.80–6.94 (m, 4H, diamine); 3.97 (s, 6H, methyl).

The structure of the ligand is given in Figure 1.

The stock solution of ligand $1 \times 10^{-3}$ M was prepared by weighing 0.0376 g of $H_2IF$ in ethanol and dissolving in 100 ml. The working standard resolutions were done by diluting the stock solution at the appropriate ratios.

2.3. Apparatus

An HPLC (DIONEX ultimate 3000) pump was used to propel the samples and reagent solutions. Samples were injected into the carrier stream by a Rheodyne injection valve with a 20 μl loop. In the FIA system, the absorbance of the coloured complex formed was measured with a UV-visible spectrophotometer (Variable Wavelength Detector VWD-3100). The Chromeleon software was used to control the system and observe the all flowgrams.

UV-visible spectra of metal-IF complexes were taken with a UV-Visible Spectrophotometer (Thermo Array Evolution, USA)

A glass pH electrode (Schott) connected to a Jenway 3040 model Ion Analyzer was used for pH measurements of the buffer solutions.

AAS (Shimadzu AA-6800 G) was used as a reference method for determination of copper(II) in order to compare the results obtained from the developed method by using the Student’s t-test at 95% confidence level.

2.4. FIA procedure for determination of copper(II)

Standard solutions and samples were analyzed using the flow system that is shown diagrammatically in Figure 2. Two channel HPLC pump was used for propelling the carrier solution at a flow rate of 2 ml min$^{-1}$. The copper(II) standard or sample solution (20 μl) was injected into a carrier stream consisting of acetate buffer solution at pH 4.5 and $5 \times 10^{-6}$ M $H_2IF$ solution via an injection valve. After that, the solutions were mixed together at the reaction coil (1 m long) forming the copper(II) complex. The flow gram plotted between the absorbance of the complex at 420 nm against time was monitored by a spectrophotometer. A calibration graph was obtained by plotting the peak heights against various concentrations of the standard copper(II).
2.5. Sample collection and treatments

2.5.1. Water samples
The water samples, taken from the different waste stream, were filtered over a 0.45 μm filter from Millipore (Milford MA).

2.5.2. Pharmaceutical samples
Two commercially available pharmaceutical samples such as Supradyn and Decavit were selected for the determination of copper(II). A known weight of sample was crushed and then heated in a furnace at 400°C for 2 hours. The residue was dissolved in 5 mL HCl and filtered through Whatmann filter paper No. 1. The obtained filtrate was diluted with water to 100 mL in a calibrated flask [13].

2.6. Interferences and precision study
The general procedure was applied for interference studies. Different amounts of ions possibly present in analysed samples were added to the standard solution containing 31.8 μg L⁻¹ Cu(II).

The repeatability of the method, replicate samples (n = 3) containing Cu(II) 15.9 and 31.8 μg L⁻¹, respectively, were prepared and analyte contents were determined by proposed methodology.

3. Results and discussion

3.1. Spectral characteristics of the H₂IF and [Cu-IF] complex
The H₂IF ligand forms a stable complex with the Cu (II) ion. The complex is highly soluble in the ethanol–water mixture. The proposed structure of the complex is depicted in Figure 2.

All metal ions react with H₂IF in aqueous medium to form complexes with different stoichiometry. The absorption profiles of free ligand and its complexes with Cu²⁺, Co²⁺, Ni²⁺, Pb²⁺, Zn²⁺, Mn²⁺, and Cd²⁺ ions are superimposed and given in Figure 3.

When a small amount of Cu (II) ion is added to the ligand solution, the colour of the solution turns from light yellow to light brown. At 420 nm a distinct absorption band is formed. In this range none of the complexes formed of free ligand and other metals were observed, making Cu (II) determination possible.

3.2. Optimization of the experimental parameter
H₂IF concentration, the effect of common interfering ions on the copper (II) analysis, the working pH range, the complexity of complex formation between copper (II) and H₂IF in time has been studied in detail.

To investigate the effect of H₂IF concentration, a study was carried out in the range of 1 × 10⁻⁵ to 1 × 10⁻⁴ M. When the concentration of the reagent was increased, an increase in the absorbance of the complex was observed. At 5 × 10⁻⁵ M concentration of H₂IF, the absorbance reached maximum.

The selectivity of the proposed method for Cu(II) determination was evaluated with various ions. The tolerance limit was taken as the amount causing the ± 5% absorbance error. The determination of Cu(II) in the determination of the excess of NH₄⁺, Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻, NO₃⁻, SO₄²⁻, 400 times more Co²⁺ and 40 times more Mn²⁺, Ni²⁺, Cd²⁺, Zn²⁺, Pb²⁺.

Several buffer types were used, ranging in pH values from 3.00 to 8.00. It was determined that the highest absorbance of the acetate buffer at 420 nm was observed. The pH values were changed between 3.50 and 5.50 as shown in Figure 4. The optimum value of 4.50 was selected.

The effect of complex formation reaction between H₂IF and Cu (II) was investigated at the same time. The solution colour changed from pale yellow to light brown upon addition of the reacted Cu (II) ion. The absorbance of the complex at 420 nm reached its maximum and did not increase with time.

The calibration curve for the Cu (II) spectrum was obtained under optimum conditions. Experimental
Table 1. Experimental conditions and analytical parameters for Cu(II) determination in FIA analyzes.

| Parameters               | Optimal conditions          |
|--------------------------|-----------------------------|
| pH                       | 4.50                        |
| Acetate buffer concentration | $1.65 \times 10^{-2} \text{ mol L}^{-1}$ |
| $\text{H}_2\text{IF}$ concentration   | $5 \times 10^{-6} \text{ mol L}^{-1}$ |
| LOD                      | $2.38 \times 10^{-5} \text{ mol L}^{-1}$ |
| LOQ                      | $1 \times 10^{-6} - 1 \times 10^{-4} \text{ mol L}^{-1}$ |
| $R^2$                    | 0.9897                      |

3.3. Determination of the stoichiometry of the complex

Job method was applied for the determination of the stoichiometry of $[\text{Cu–IF}]$ complex. Equimolar ($2.5 \times 10^{-4} \text{ mol L}^{-1}$) solutions of copper(II) and $\text{H}_2\text{IF}$ was prepared and then copper(II) and ligand solutions were mixed in constantly ranging proportions and ensuring the total volume of metal and ligand constant at 4.0 mL. The absorbance values of the prepared solutions were measured at 420 nm at a constant pH value of 4.5. The changes of absorbance values are shown in Figure 5. The maximum absorbance value was read at around 2 ml ligand volume as deduced from Figure 5 and $[\text{Cu–IF}]$ complex formation stoichiometry is proposed to be 1:1 proportion.

3.4. Application of the proposed work and its validation

Using the developed spectrophotometric method, Cu(II) analysis was carried out on two commercially available vitamin samples and water samples. The results obtained for the determination of copper ions in the sample by the proposed method are shown in Table 2. Calibration curves were used to determine copper (II) content in real samples. Concentration values were calculated from the calibration graph against the obtained absorbance values and the relationship between the actual values was examined. The results obtained for the copper (II) content in the vitamins were also verified by the AAS method. In addition, t-test was applied. A comparison of the results indicates a good correlation between the proposed procedure and the AAS method. There was also good correlation between method and labelled values. The calculated t values for vitamin samples were found to be 0.9532 and 0.8533, respectively (lower than the critical value ($t_{4.95\%}$, 95% confidence level)).

Certified reference materials, including alloys and water samples, have been tested for Cu determination using the recommended method for testing the validity of the method. The calculated t values for aluminum and alloy samples were lower than the critical value ($t = 4.30$, 95% confidence level) to 0.00557 and 1.5867.

These satisfactory results showed that FIA method could be used for rapid and sensitive detection of vitamin (II) in vitamin samples and water samples.

The flow gram curves obtained with FIA for vitamin and water samples are shown in Figures 6–8.

Table 2. The results obtained for the determination of copper ions in the sample by the proposed method.

| Samples         | Proposed method | AAS measurement | Label Values | t value |
|-----------------|-----------------|-----------------|--------------|---------|
| Supradyn        | 1.13 ± 0.27     | 1.13 ± 0.25     | 1.16         | 0.9532  |
| Decavit         | 1.73 ± 0.18     | 1.29 ± 0.19     | 1.64         | 0.8533  |
| C12X3500        | 20.00 ± 5.24    | 25.00           | 1.5867       |
| SPS-WW2         | 1999.85 ± 41.36 | 2000.00         | 0.00557      |

$^a$ Average of three determinations ± S.D.: t value calculated versus label values.
3.5. Comparison with the current literature

In summary, a relevant benchmark of spectrophotometric methods from the attached literature [13–20] and from present study is compiled and given in Table 3. All parameters in Table 3 are nearly comparable within a reasonable data range and seem not superior to each other in general trend. The developed methodology has remarkable advantage over classical FIA spectrophotometric determination of Cu (II) using H2IF as colorimetric reagent which is the possibility to carry out the analytical process without use of a solvent extraction step before absorbance measurement. This fact yields an analytical methodology with fewer risks to the analyst and to the environment, since the handling of toxic organic solvents is not needed.

4. Conclusions

In this study, a novel and simple, selective and easily applicable flow injection spectrophotometric method for the determination of Cu (II) was developed. The method is based on the spectrophotometric monitoring of Cu (II) ions using N, N’-o-phenylene-bis (3-methoxysalicylideneimine) (H2IF) as a novel chromogenic reagent. This ligand has been found to be a sensitive and selective spectrophotometric reagent for Cu (II) ions. H2IF exhibits better selectivity and sensitivity to Cu (II) than other common ions.

The spectrophotometric method developed within the scope of the study seems to be more advantageous in terms of the short duration of the reaction period than other existing methods [13–20]. Experimental findings have shown that in aqueous media the reagent is a potential application for the sensitive, rapid and selective perception of Cu (II).

Table 3. Comparison of the present FIA method to others in determination of copper(II).

| Reagent used                                                                 | \( \varepsilon \) (L mol\(^{-1}\) cm\(^{-1}\)) | Beer’s law range               | \( \lambda_{\text{max}} \) nm | Ref |
|------------------------------------------------------------------------------|-----------------------------------------------|--------------------------------|-------------------------------|-----|
| 4-(2-Pyridylazo)-resorcinol Disodium                                         | \( 4.0 \times 10^4 \)                         | 0.063–1.58 mg/mL              | 510                           | 13  |
| Bromo-2-benzothiazolylazo pyrogallol                                          | \( 0.4 \times 10^5 \)                         | 0.01–7.0 μg/mL                | 570                           | 14  |
| N,N’-bis[(E)-(4-fluorophenyl)methylidene] thiocarbonohydrazide                 | \( 1.08 \times 10^4 \)                        | 0.31–6.35 mg L\(^{-1}\)       | 390                           | 15  |
| S-Bromosalicylaldehyde                                                       |                                               |                                |                               |     |
| Thiosemicarbazone                                                             |                                               |                                |                               |     |
| Oxalic acid bis(cyclohexylidene hydrazide)(cuprizone)                        |                                               |                                |                               | 16  |
| 1-(2,4-dinitroaminophenyl) 4,4,6-trimethyl-1,4 dihydropyrimidine-2-thiol     | \( 0.87 \times 10^3 \)                        | 10–80 μg mL\(^{-1}\)          | 445                           | 17  |
| 4-AAP and thymol                                                             |                                               |                                |                               |     |
| N,N’-bisalicylidene-2,3-diaminopyridine                                      | \( 1.46 \times 10^3 \)                        | 5–70 μg/mL                    | 490                           | 19  |
| N, N’-o-phenylene-bis (3-methoxysalicylideneimine)                           | 6538                                          | 3.18–318 μg L\(^{-1}\)        | 420                           | This work
Improved flow injection analysis has been successfully applied in vitamin samples and water samples.

Acknowledgements
The authors are thankful to the Ondokuz Mayıs University for the Equipment support.

Disclosure statement
No potential conflict of interest was reported by the authors.

ORCID
Sama Omarova http://orcid.org/0000-0002-4738-0085
Serkan Demir http://orcid.org/0000-0003-3704-4800

References
[1] Förstner G, Wittmann T. Metal pollution in the aquatic environment. Newyork: Springer Verlag; 1981; 3, 21, 271–318.
[2] Welz B. Atomic absorption spectroscopy. Amsterdam: VCH; 1985.
[3] Carmo S.N. D, Damásio FQ, Alves VN, et al. Direct determination of copper in gasoline by flame atomic absorption spectrometry after sorption and preconcentration on Moringa oleifera husks. Microchem J. 2013;110:320–325.
[4] Ferreira SLC, Queiroz AS, Fernandes MS, et al. Application of factorial designs and Doehlert matrix in optimization of experimental variables associated with the preconcentration and determination of vanadium and copper in seawater by inductively coupled plasma optical emission spectrometry. Spectrochim Acta B. 2002;57:1939–1950.
[5] McLaren JW, Jarnis KE, Gray AL, et al. (Eds.), Handbook of inductively coupled plasma mass spectrometry. Glasgow: Blackie & Son Ltd.; 1992, p. 266.
[6] Tolg G, Klockenkamper R. The role of total-reflection X-ray fluorescence in atomic spectroscopy spectrochim. Acta B. 1993;48:111–127.
[7] Andac M, Coldur F, Bilir S, et al. Solid-contact polyvinyl chloride membrane electrode based on the bis[(2-hydroxyethyliminio)phenolato]copper(II) complex for trace level determination of copper ions in wastewater. Can J Chem. 2014;92:324–328.
[8]-valcárceL, castro L. Flow injection analysis of pharmaceuticals. J PharmaceutBiomed Anal. 1990;7(12):1291–1300.
[9] pinto jj, moreno c, García-Vargas M. A very sensitive flow system for the direct determination of copper in natural waters based on spectrophotometric detection. Talanta. 2004;64:562–565.
[10] Nohut S, Karaböcek S, Güner S, et al. Extraction and spectrophotometric determination of copper(II) with S,S'-bis(2-aminophenyl). J Pharm Biomed Anal. 1999;20(1-2):309–314.
[11] Pei L, Juan Y. Cloud point extraction preconcentration and spectrophotometric determination of copper in food and water samples using amino acid as the complexing agent. J Food Compos Anal. 2010;23(1):95–99.
[12] Fulmes CS, Bulatov AV, Yasakova OG, et al. Multicommutated stepwise injection analysis as new approach for simultaneous determination of nickel (II), copper (II) and zinc (II) in wet aerosols. Microchem J. 2013;110:649–655.
[13] Ramadan AA, Mandil H, Kamel AA. Spectrophotometric determination of copper(II) with 4-(2-pyridylazo)-resorcinol disodium in pharmaceuticals. Asian – J Chem. 2009;21(9):7367–7374.
[14] Nasser NA, Taha DN, Kahdim KH. A simple flow injection-spectrophotometric determination of copper (II) using 4-(6-bromo-2-benzothiazolylazo)pyrogallol as chromogenic agent. J Babylon Univ/Pure and Appl Sci. 2013;21(7):2544–2554.
[15] Nalawade RA, Nalawade AM, Kamble GS, et al. Rapid, synergistic extractive spectrophotometric determination of copper(II) by using sensitive chromogenic reagent N,N'-bis[(E)-(4-fluorophenyl)methylidene]thiocarbonylhydrazide. Spectrochim Acta A. 2015;146:297–306.
[16] Ramanjaneyulu G, Reddy PR, Reddy VK, et al. Direct and derivative spectrophotometric determination of copper(II) with 5-bromosalicylaldehyde thiosemicarbazone. Open Anal Chem J. 2009;2:78–82.
[17] Rumori P, Cerda V. Reversed flow injection and sandwich sequential injection methods for the spectrophotometric determination of copper(II) with cuprizone. Anal Chim Acta. 2003;486:227–235.
[18] Kamble GS, Kolekar SS, Anuse MA. Synergistic extraction and spectrophotometric determination of copper (II) using 1-(2,4-dinitro aminophenyl) 4,4,6-trimethyl-1,4 dihydropyrimidine-2-thiol: analysis of alloys, pharmaceuticals and biological samples. Spectro Chim Acta A. 2011;78:1455–1466.
[19] Al-Abachi MQ, Abed SS, Al-Najjar NA. A New chromogenic reagent for determination of copper (II) in water samples using flow injection-technique. Iraqi J Sci. 2017;58(1B):201–210.
[20] Babayeva K, Demir S, Andac M. A novel spectrophotometric method for the determination of copper ion by using a salophen ligand, N,N'-disalicylidene-2,3-diaminopyridine. J Taibah Univ Sci. 2017;11(S):808–814.