ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
27 (2002) 183–189

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Spectrophotometric determination of copper in pharmaceutical and biological samples with 3-{2-[2-(2-hydroxyimino-1-methyl-propylideneamino)-ethylamino]-ethyl-imino}-butan-2-one oxime

Ömer Dalman, Mehmet Tüfekçi, Sinan Nohut, Saadettin Güner, Serdar Karaböcek *

Department of Chemistry, Karadeniz Technical University, 61080 Trabzon, Turkey

Received 10 March 2001; received in revised form 29 May 2001; accepted 1 July 2001

Abstract

3-{2-[2-(2-hydroxyimino-1-methyl-propylideneamino)-ethylamino]-ethyl-imino}-butan-2-one oxime, (H_2mdo) reacts with copper(II) to form a highly stable 1:1 complex in alkaline medium at room temperature. The complex gives a maximum absorption at 570 nm with a molar absorptivity coefficient of 0.16×10^4 l mol^{-1} cm^{-1}. A spectrophotometric method using this ligand was developed and optimized in terms of pH, stability of the complex, amount of reagent required, sensitivity, linearity and tolerance limits of various foreign ions. The linear range for copper determination is 0.2–225 mg l^{-1}. The method is sensitive, accurate and tolerant to many foreign substances, and, all the reagents used are stable under the conditions. Moreover, the method is easy to perform for the determination of copper in pharmaceutical and biological samples.

Keywords: Dione dioxime; Foreign ions; Spectrophotometric copper determination

1. Introduction

The determination of Cu(II) in environmental and biological samples can be of interest in biochemical research since copper traces promote rancidity and off-flavors in foods and beverages. Moreover, copper accumulation in liver is a characteristics of Wilson's disease producing neurologic and psychiatric defects [1].

Although there are rapid and sensitive methods for copper(II) determination such as atomic absorption spectrophotometry [2], liquid chromatography [3], sorbent extraction [4,5], and solid phase

* Corresponding author. Tel.: +90-462-377-2598; fax: +90-462-325-3195.

0731-7085/02/$ - see front matter
PII: S0731-7085(01)00519-2

extraction [6], colorimetric methods are often preferred since they involve less expensive instrumentation and provide better sensitivity when appropriate chromogenic reagents are available [7]. However, the use of a preconcentration step prior to copper determination in these methods is usually necessary since the presence of copper in environmental and biological samples are at low levels. There are many effective spectrophotometric methods for the determination of trace amounts of Cu(II) most of which are based on reactions with suitable color producing reagents such as dithiocarbamates [8], 2,6-dichlorophenolindophenol [9], semicarbazones [10], 3-thiobenzoyl-1-*p*-tolylthiocarbamide [11], 1,5-*bis*(di-2-pyridylmethylene)-thiocarbonohydrazide [12], antipyrinylazo/pentane-2,4-dione [13], 1-(2-pyridylazo)-2-naphthol [14,15], pyridylazo dye[16], *N*,*N*′-diphenylbenzamidine [17], naphthazarin [18]. However, many of these reagents require steps of difficult and tedious organic syntheses. Furthermore, most of the existing methods suffer from limitations such as weak stability of colored complexes and interferences from Fe(III), Ni(II), Zn(II) or Al(III). Although these compounds are considered to be promising reagents for various metal ions, they react with these metal ions to give complexes that are insoluble in aqueous phase [19,20].

Schiff base and oxime compounds have been extensively studied because of their biological and structural importance [21–23] which lies mainly in their specific and selective reactions with metal ions. Therefore, these compounds have been proposed as spectrophotometric reagents for several metal ions [19,24]. The aim of this work was to develop a highly sensitive, efficient and direct spectrophotometric method for copper determination in aqueous media by using a new chromogenic reagent containing a Schiff base and an oxime moiety. The conditions for the direct spectrophotometric determination of copper(II) with 3-{2-[2-(2-hydroxyimino-1-methyl-propylideneamino)-ethylamino]-ethyl-imino}-butan-2-one oxime, (H_2mdo) were described. Various factors influencing the sensitivity of the proposed method such as wavelength, pH, stirring time, effect of foreign ions and ranges of applicability of Beer's law on the determination of copper are also included. The method was applied to some pharmaceutical and biological samples as well.

2. Experimental

2.1. Apparatus

An Orion 601/A pH meter was used for pH measurements. A Unicam UV2-100 double beam UV–Visible spectrophotometer equipped with a quartz cell of 10 mm path length was used for the absorption spectra and the absorbance measurements.

2.2. Preparation of ligand

The ligand, 3-{2-[2-(2-hydroxyimino-1-methyl-propylideneamino)-ethylamino]-ethyl-imino}-butan-2-one oxime, (H_2mdo) was prepared according to the procedure reported previously [25]. Diethylenetriamine (4.12 g, 40 mmol) and 2,3-butanedione monoxime (8.10 g, 80 mmol) were mixed together in absolute EtOH (25 cm^3). After stirring for 1 h at room temperature, the resulting white precipitate was isolated immediately by vacuum filtration, washed well with Et_2O, and dried in air. The ligand was used without further purification. The product is a white, air-unstable, crystalline solid soluble in dimethylsulfoxide, but its 1:1 copper complex is highly hydrosoluble. The purity of this reagent was checked by physical and spectral data; ^{1}H-NMR (ppm): 2.0 (s. CH_3-1, 6H), 1.88 (s. CH_3-4, 6H), 3.44 (t. CH_2-5, 4H), 2.88 (t. CH_2-6, 4H), 10.45 (br. s. –NH, 1H), 11.5 (br.s. –OH, 2H); ^{13}C-NMR (ppm): C(1)13.2, C(2)164.2, C(3)156.2, C(4)8.8, C(5)51.16, C(6)49.8; m.p. 138–141 °C.

2.3. Chemicals and reagents

All chemicals used were of analytical reagent grade (BDH and Merck). All solutions were prepared with distilled demineralized water. The

stock standard copper(II) solution was prepared by dissolving 0.6393 g of copper(II) chloride dihydrate in distilled water and diluting to 250 ml. The solution was standardized titrimetrically by a known method [26]. The method basically depends on the titration of evolving iodine in the presence of starch by the reduction of Cu^{+2} to Cu^{+} in the mixtures containing iodide and copper(II) salts. The working standard solutions were prepared by suitable dilution of the stock solution. A 0.0157 M stock complexing agent solution was prepared by dissolving 1.06 g ligand in 20 ml dimethylsulfoxide and this solution was diluted with ethanol to 250 ml. The buffer solution was prepared by dissolving 94.16 g of ammonium chloride in water and its pH was adjusted to 9.0 by addition of 65.2 ml concentrated ammonium hydroxide and was diluted to 1 l with demineralized water.

Validation of the method described here was performed by using two Certified Reference Materials Office of Standard Reference Materials (NBS) and National Institute for Japan Environmental Agency (NIES).

2.4. Procedure

For the determination of Cu(II), an aliquot containing an amount of copper(II) within the range recommended for the method was transferred into a 25 ml volumetric flask, 5 ml of 0.0157 M complexing agent solution was added after addition of 1 ml buffer solution. The absorbance change was measured after 30 min at the wavelength of maximum absorbance against a reagent blank as a reference. Copper contents were estimated by stirring the aqueous phase containing 20 mg l^{-1} of copper and buffer solution with H_2mdo for varying time periods from 1 to 60 min and the absorbance in each case was measured. Interference studies were investigated by using a standard 20.00 mg l^{-1} copper solution with varying concentrations of other species added as listed in Table 2.

2.5. Effect of diverse ions

Under the optimum conditions, the effects of various foreign ions on the determination of 20 mg l^{-1} of Cu(II) was studied separately according to the procedure described above. An aliquot containing an amount of copper(II) within the range recommended for the method was transferred into a 25 ml volumetric flask, 5 ml of 0.0157 M complexing agent solution was added after addition of 1 ml buffer solution. Then different amounts of diverse ions were added to the above mixture. The tolerance limit of an ion was taken as the maximum amount (mg) causing an error not greater than 2%.

2.6. Analysis of pharmaceutical samples

The proposed method was applied to the determination of copper in pharmaceutical samples such as Supradyn, Megadyn and Vitadyn tablets. In each case, ten tablets were powdered and the required amount of the powder was ignited in a muffle furnace at 400 °C for 2 h. The ash was dissolved in 5 ml of conc. HCl, then the final solution was filtered and diluted to 100 ml with distilled water. The copper concentration was determined by the present method taking suitable aliquots of the above sample solution and the results are compared with the certified values (Table 2).

2.7. Analysis of biological samples

The proposed method was applied for the determination of copper(II) in biological samples as tomato leaves (NBS 1573), tea leaves (NIES No.7), Turkish tea leaves and hazelnut (*C. avellena var. Pontica*). The foodstuffs were dried at 90 °C for 24 h. A 20–50 g sample was digested with nitric acid and perchloric acid (1:1) and heated gently on a hot plate to dryness. The ash was taken up with 50 ml of HCl (10%) and evaporated to dryness. The residue was taken up in 20 ml conc. HCl, filtered and made up to 100 ml with distilled water. An aliquot of each solution was then treated according to the present method and the Bathocuproin method, then compared with the certified values. The results obtained are given in Table 2.

3. Results and discussion

3.1. Absorption spectra

The ligand and its metal complexes were dissolved in DMSO and show characteristic UV–Vis spectra of the oxime groups. The absorption maximum of the free ligand, H_2mdo was seen at ≈ 350 nm and is attributable to $\pi-\pi^*$ transition

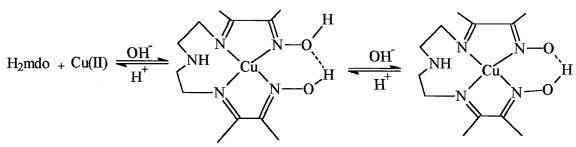

Fig. 1. Behaviour of H_2mdo:copper(II) complex in acidic/alkaline media.

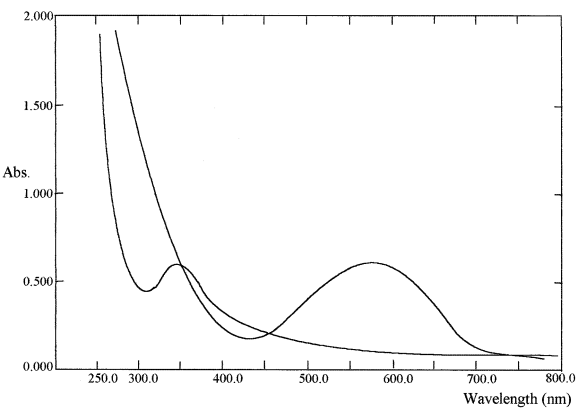

Fig. 2. Visible absorption spectra of the ligand, H_2mdo (A) and its copper(II) complex (B).

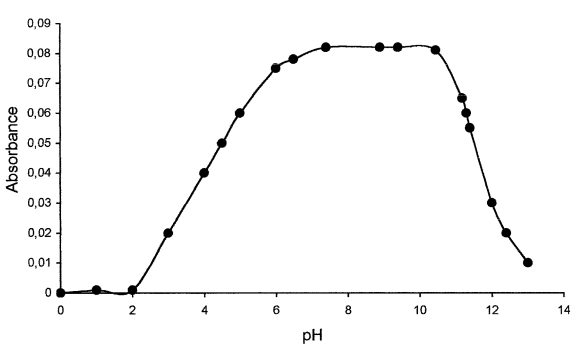

Fig. 3. Effect of pH on the formation of copper(II)–H_2mdo complex.

Table 1
Conditions for the spectrophotometric determination of copper(II) by H_2mdo ligand

Wavelength of maximum absorbance (nm)	570
Limit of detection (LOD) (mg l^{-1})	0.19
Limit of quantification (LOQ) (mg l^{-1})	0.42
LOD/LOQ	0.46
Range of linearity (mg l^{-1})	0.2–225
Molar absorptivity (l mol^{-1} cm^{-1})	0.16×10^4
Optimum pH	7–11
Calibration graph	
Slope	0.004885
Intercept	0.000542
Correlation coefficient (r)	0.9999
Relative standard deviation (%)	0.25

of the C = N group. The ligand (Fig. 1) reacts with divalent copper to form a stable violet complex species, which is highly soluble in water and may be used for the direct determination of copper in aqueous media. The absorption spectrum of the copper(II)–H_2mdo complex in aqueous solution was studied over the wavelength range of 300–700 nm. The complex exhibited absorption maxima at 570 nm due to the d–d transitions (Fig. 2) indicating the formation of a complex between the ligand and copper(II).

3.2. The effect of pH

The effect of pH on the determination of copper in aqueous medium was investigated spectrophotometrically. For this purpose, the solution containing 20 mg l^{-1} of Cu(II) was measured in the pH range of 0–14 at 570 nm. As it is clearly seen from Fig. 3, the amount of copper can quantitatively be determined in the pH range of 7–11.

3.3. Nature and stability of the complex

The stoichiometric composition (1:1 metal:ligand ratio) of the complex was ascertained by application of the Job's method of continuous variation and the molar-ratio method. The color of the copper(II) complex was attained within 10 min after the addition of copper(II) ion, and the intensity remained constant for at least 24 h.

3.4. Beer's law and sensitivity

Beer's law is obeyed within a wide range of 0.2–225 mg l^{-1} of copper at 570 nm (Table 1) under optimum experimental conditions. The calibration graph can be represented by a linear regression equation:

$$y = 0.004885x + 0.000542 \quad (r^2 = 0.9999)$$

Here, y is the absorbance and x the concentration of Cu(II) in mg l^{-1}. The molar absorptivity of the complex calculated from the absorbance value was found to be 0.16×10^4 l mol^{-1} cm^{-1} at 570 nm. Sandell's sensitivity calculated on the basis of

Table 2
Determination of copper in pharmaceutical and biological samples by H_2mdo ligand

Samples	Certified Cu content μg g^{-1} (X ± SD)	Cu found (μg g^{-1})[a]	
		Proposed method (X ± RSD%)	Bathocuproin method[b] (X ± RSD%)
Tomato leaves (NBS 1573)	11 ± 1	12.5 ± 2.8	14 ± 3.8
Tea leaves (NIES No. 7)	7.0 ± 0.3	5.6 ± 1.4	6.8 ± 4.6
Turkish tea leaves	–	72.6 ± 4.2	76.1 ± 6.2
Hazelnut (*C.avellena var. Pontica*)	–	11.8 ± 2.3	12.2 ± 4.0
Supradyn (Roche, Turkey)[c]	1.0	0.84 ± 1.5	0.87 ± 4.6
Megadyn (Mecom, Turkey)[c]	1.0	0.99 ± 1.8	0.95 ± 2.2
Vitadyn (Bilim, Turkey)[c]	1.0	1.04 ± 2.3	1.1 ± 2.5

[a] Average of five determinations.
[b] Spectrophotometric determination of copper with bathocuproine disulfonic acid was performed as described in reference [27].
[c] mg g^{-1}.

Table 3
Effect of diverse ions on the determination of copper(II) by H_2mdo ligand

Ion	Added as	Tolerance limit of ion (interfering ion mg^{-1})	Cu(II)/(added 20 mg l^{-1})	Recovery (%)
Ni^{+2}	$NiSO_4 \cdot 7H_2O$	0.5	21	105
Fe^{+3}	$FeCl_3$	2.5	20.5	102
Al^{+3}	$Al(NO_3)_3$	7.5	20	100
Co^{+2}	$Co(NO_3)_2 \cdot 6H_2O$	2.5	20.3	102
Pb^{+2}	$Pb(NO_3)_2$	7.5	19.9	99
Hg^{+2}	$Hg(NO_3)_2$	10	19.8	99
Mn^{+2}	$MnSO_4 \cdot 4H_2O$	10	19.7	98
Cr^{+3}	$CrCl_3 \cdot 6H_2O$	5	19.6	98
Mg^{+2}	$MgSO_4$	100	20	100
Ca^{+2}	$CaCl_2$	100	20	100
Sr^{+2}	$SrCl_2 \cdot 6H_2O$	100	20.2	101
Cd^{+2}	$CdSO_4$	10	20.2	101
I^-	KI	40	20	100
Nitrate	$NaNO_3$	200	20	100
Chloride	NaCl	200	19.9	99
Sulphate	Na_2SO_4	200	19.9	99
Fluoride	NaF	40	19.5	97
Acetate	NaAc	200	20	100
EDTA	EDTA·2Na	20	19.8	99

Table 4
Comparison of characteristic features of various spectrophotometric methods for the determination of copper(II)

Reagents	λ_{max} (nm)	Molar absorptivity, ϵ (l mol^{-1} cm^{-1}) $\times 10^5$	Linear dynamic range, μg ml^{-1}	Remarks	References
Dithiocarbamate	434	0.13	0.0544 ± 0.0017 (DL)	Poor sensitivity, Fe, Ni, Co, interfere, critical pH	[8]
2,6-Dichlorophenolindo - phenol	562	–	5–300	V(IV), Cd(II) interfere	[9]
Biacetyl-2-pyridylhydrazo n-ethylo-semicarbazone	520	0.56	–	Fe, Co, Ni interfere	[10]
3-Thiobenzoyl-1-*p*-tolyl-thio-carbamide	420	0.10	3–12	Poor sensitivity	[11]
1,5-*bis*(di-2-pyridylmethyl-ene)-thiocarbonohydrazide	420	0.42	0.1–1.3	Co(II), Ni(II), Fe(III), Hg(I), Hg(II) interfere	[12]
Antipyrinylazo/pentane-2, 4-dione	535	0.13	0.2–15	Poor sensitivity, Zn interfere	[13]
1-(2-Pyridylazo)-2-naphthol	595	–	0.5–3.6	Many metals interfere	[14]
Pyridylazo dye	532	0.47	–	Less sensitivity	[16]
N,*N*′-diphenylbenzamidine	520	1.14	0.05–5.0	Sensitive and selective, Zn, Cd interfere	[17]
Naphthazarin	330	0.18	0.9–4.5	Fe(III), Al(III), Cr interfere	[18]
H_2mdo	570	0.016	0.2–225	Sensitive and selective, Ni(II) interfere except access reagent	Present method

total copper present is 0.039 μg cm^{-2}, and other parameters are listed in Table 1.

3.5. Precision and accuracy

The precision of the method was checked by taking ten replicate measurements on solutions each containing 0.2 mg of Cu(II). The relative standard deviation (ten determinations with 0.2 mg l^{-1} of Cu(II), 95% confidence level) is $\pm$ 0.25%. The limit of detection (LOD) is 0.19 mg Cu l^{-1}, the limit of quantification (LOQ) is 0.42 mg Cu l^{-1}, and the LOD/LOQ ratio is 0.46 altogether indicating the robustness of the procedure. The accuracy of the method was checked by bathocuproine disulphonic acid method [27] and using two International Certified Reference samples; tomato leaves (NBS 1573), and tea leaves (NIES No. 7). The experimental data (Table 2) obtained by the two methods are consistent with each other.

3.6. Effect of diverse ions

Most of the ions studied do not interfere with the determination of copper, as shown in Table 3. Although nickel(II) competes with copper(II) for the dioxime ligand, this interference can be eliminated by using an excess of reagent in the procedure.

4. Conclusions

The ligand used in this study could be obtained at high yields by a very simple procedure of mixing initial reagents in ethanol for not more than 1 h. However, it might decompose in solu-

tion if kept more than a couple of days at room temperatures. Therefore, it should be prepared when it is needed and used freshly or kept below 4 °C in a colored bottle.

The reagent described here forms highly stable 1:1 complexes with Cu(II) in aqueous media. Although nickel(II) forms complexes with the same reagent, its absorbance maximum (λ_{max}: 540 nm) is significantly different from the one formed with Cu(II) (λ_{max}: 570 nm, ε_{max}: 0.16×10^4), therefore, does not interfere with copper. The complex becomes much more stable at alkaline media due to formation of hydrogen bridge by proton abstraction from the complex, and it has great stability even in the presence of EDTA.

The proposed colorimetric method could easily be applied to various biological and pharmaceutical samples for determination of 0.2–225 mg l^{-1} copper. So, it could be regarded as rapid, simple, sensitive and selective for copper(II) and therefore very appropriate for direct copper(II) determination. Furthermore, it has several advantageous in terms of linearity, working pH range, interfering ions when compared with other methods described and applied to copper(II) determination previously (Table 4), but has a weak point as molar absorptivity.

Acknowledgements

This work was supported by The Research Fund of Karadeniz Technical University (Trabzon, Turkey).

References

[1] G.J. Brewer, V. Yuzbasiyan-Gurkan, Wilson disease, Medicine 71 (1992) 139.
[2] A.D. Eaton, L.S. Clesceri, A.E. Greenberg, Standard Methods for the Examination of Water and Waste Water, 19th ed., American Public Health Assoc, Washington, DC, 1995.
[3] Y. Shijo, H. Sato, N. Uehara, S. Aratake, Analyst 121 (1996) 325.
[4] Y.A. Zolotov, I.M. Maksimova, E.I. Morosanova, A.A. Velikorodny, Anal. Chim. Acta 308 (1995) 398.
[5] Y. Petit de Pena, M. Gallego, M. Valcarcel, Anal. Chem. 67 (1995) 2524.
[6] L.B. Björklund, G.M. Morrison, Anal. Chim. Acta 343 (1997) 259.
[7] C.B. Ojeda, F.S. Rojas, J.M.C. Pavon, Talanta 42 (1995) 1195.
[8] Li. Shine, Li. Shenquan, A. Chen, Talanta 40 (1993) 1085.
[9] M.I. Prodormidis, C.D. Stakilas, P.Th. Veltsistas, M.I. Karayannis, Talanta 41 (1994) 1645.
[10] V.D. Pillay, V.M. Shinde, Indian J. Chem. Sect. A 34 (1995) 401.
[11] D.M. Ambhore, A.P. Joshi, J. Indian Chem. Soc. 68 (1991) 175.
[12] A.M. Garcia Rodriga, F.Z.A. Garcia de Torres, J.M. Cano Pavon, C. Bosh Ojeda, Talanta 40 (1993) 1861.
[13] T. Inshizaki, H. Wada, G. Nakagawa, Anal. Chim. Acta 212 (1988) 253.
[14] A. Gallardo Malgarejo, A. Gallardo Cespedes, J.M. Cano Pavon, Analyst 114 (1989) 109.
[15] N.K. Agnihotri, K. Singh, H.B. Singh, Talanta 45 (1997) 331.
[16] O.M. Baudino, B.A. Gill, P.M. Molinsde, An. Quim. Ser. B 86 (1992) 613.
[17] M. Thakur, M. Kanti Deb, Talanta 49 (1999) 561.
[18] R. Chaisuksant, W. Palkawong-na-ayuthaya, K. Grudpan, Talanta 53 (2000) 579.
[19] M.S. El-Shahawi, Anal. Sci. 7 (1991) 443.
[20] S. Nohut, S. Karaböcek, S. Güner, Y. Gök, J. Pharm. Biomed. Anal. 20 (1999) 309.
[21] S. Karaböcek, S. Nohut, Ö. Dalman, S. Güner, Anal. Chim. Acta 408 (2000) 163.
[22] H. Koshima, H. Onishi, Talanta 27 (1980) 795.
[23] S. Ambe, J. Radional. Nucl. Chem. 81 (1984) 77.
[24] M. Shamsipur, A.R. Ghiasvand, H. Sharghi, H. Naeimi, Anal. Chim. Acta 408 (2000) 271.
[25] K. Serbest, S. Karaböcek, I. Degirmencioglu, S. Güner, F. Kormali, Transition Met. Chem. 26 (2001) 375.
[26] A.I. Vogel, A Text-Book of Quantitative Inorganic Analysis, third ed., Longmans, London, 1961, p. 441.
[27] T.S. West, H.W. Nürnberg, The determination of Trace Metals in Natural Waters IUPAC, Blackwell Scientific Publications, Oxford, 1988, p. 53.