

Spectrophotometric Determination of Copper(II) Using Diamine-Dioxime Derivative

Khajadpai Thipyapong and Chomchai Suksai*

Department of Chemistry, Faculty of Science, Burapha University, Chonburi 20130, Thailand

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A simple, rapid and sensitive spectrophotometric method is herewith proposed for the determination of copper(II) by using diamine-dioxime ligand, *meso*-3,6,6,9-tetramethyl-4,8-diazaundecane-2,10-dione dioxime or *meso*-HexaMethyl Propylene Amine Oxime (*meso*-HMPAO). This method is based on the formation of a stable 1 : 1 red-pink complex between copper(II) and *meso*-HMPAO in aqueous solution. Absorption measurements were carried at 497 nm, with a molar absorptivity value of 338 L mol⁻¹ cm⁻¹. Beer's law was obeyed over the concentration range of 0.5-370 μg mL⁻¹ with a Sandell's sensitivity value of 0.18 μg cm⁻². The proposed method has been successfully applied for determination of copper(II) in foodstuffs and pharmaceutical samples. The results obtained from this method are comparable with those obtained AAS.

Key Words : Copper, *meso*-HMPAO, Foodstuffs, Pharmaceutical samples

Introduction

The separation and determination of metal ions have been one of the most important topics of analytical chemistry. Developing highly functional chelating agents has been a great concern of many analytical chemists. Investigations into developing new types of chelating agents have produced many functional results having high selectivity and/or sensitivity.

The amount of copper that contaminates various biological and environmental substances are of concern since copper traces promote rancidity and off-flavors in foods and beverages. The levels of copper in biological samples may indicate malefaction or contamination. In addition, the accumulation of copper in the human liver is a characteristic of Wilson's disease, which produces neurologic and psychiatric defects.¹ Hence, rapid and sensitive methods for the determination of copper in environmental and biochemical research are in great demand. There are conventional methods for copper(II) determination, such as spectrophotometry,² chromatography³ and extraction.⁴ However, the colorimetric methods are often preferred due to the fact that they involve less expensive instruments and show rapid results.

In this research, an interested spectrophotometric agent, dioxime compound HMPAO (Hexamethyl Propylene Amine Oxime or 3,6,6,9-tetramethyl-4,8-diazaundecane-2,10-dione dioxime), derived from PnAO (Propylene Amine Oxime), has been chosen for study. This compound exists in the form of *d,l*- (*ca.* 5%) and *meso*- isomers (*ca.* 95%) from synthesis. Only *d,l*-HMPAO is known as an important radiopharmaceutical agent for imaging the cerebral perfusion, when complexed with ^{99m}Tc.⁵ While other applications of *meso*-HMPAO have not been reported. Then, from the structure of this oxime compound, *meso*-HMPAO may be a chelating ligand for many metal ions.⁶⁻¹³

Concerning the present investigation, we report here on a

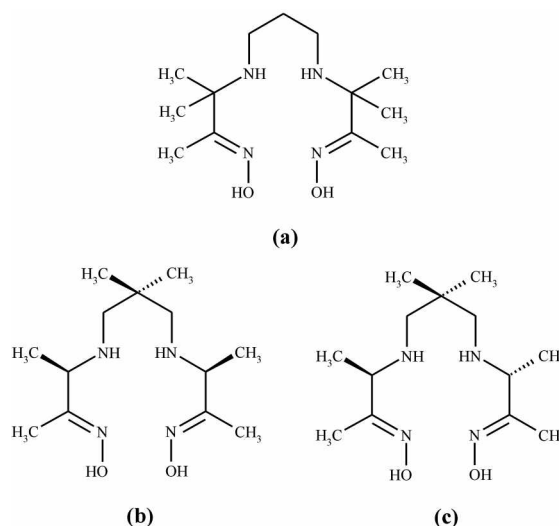


Figure 1. Structure of (a) PnAO, (b) *meso*-HMPAO and (c) *d,l*-HMPAO.

simple, rapid and adequately sensitive spectrophotometric method for determination of copper(II) in foodstuffs and pharmaceutical samples, using *meso*-HMPAO.

Experimental Section

Apparatus. A Beckman 34 pH meter was used for pH measurements. A Hewlett Packard 8453 and Jasco UV-Visible spectrophotometer with a quartz cell of 10 mm path length was used for the absorption spectra and the absorbance measurements.

Preparation of ligand. The preparation of 3,6,6,9-tetramethyl-4,8-diazaundecane-2,10-dione dioxime, HMPAO, consisted of processing in two steps from commercially available materials.¹⁴ Bisimine, 3,6,6,9-tetramethyl-4,8-diazaundecane-3,8-diene-2,10-dione dioxime was prepared by refluxing a mixture of 2,3-butanedione monooxime (0.50 mol, 50.55 g) and *p*-toluene sulfonic acid monohydrate (0.15 g) in benzene under nitrogen atmosphere. To this mixture

*Corresponding author. Tel: +66-038-745900 Ext. 3069; Fax: +66-038-390354, e-mail: jomjai@buu.ac.th

was slowly added a solution of 2,2-dimethyl-1,3-propanediamine (0.31 mol, 31.7 g) in benzene (75 mL) over a period of one hour. Heating of the resulting mixture was continued at reflux for 16 hours and stirred at room temperature for 12 hours. The white powder was obtained by chilling the mixture at 4 °C for 16 hours. Then the solid was filtered and washed with cold benzene. The crude product was recrystallized in acetonitrile and dried under reduced pressure.

Found: 24.09 g, 41.59% yield, mp. 118-121 °C. δ_{H} (60 MHz, DMSO d_6 , reference TMS) 1.0 (6H, s, 2CH₃-C), 1.9 (12H, s, 4CH₃-C=N), 3.2 (4H, s, 2-CH₂N-) and 10.3 (2H, s, -OH) and CHN analysis found: C, 56.23, H, 9.87, N, 20.90 CHN requires C, 58.18, H, 9.01, N, 20.88.

HMPAO was prepared by reducing bisimine (75 g, 0.28 mol) in ethanol by adding NaBH₄ (10.9 g, 0.28 mol) over one hour at 0-5 °C. The reaction mixture was stirred at that temperature for 2 hours. Water (230 mL) was added and stirring continued for 3 hours to heat to room temperature. Ethanol was removed under reduced pressure, and the resulting solution was adjusted to pH 11 with sodium hydroxide. The slurry mixture was chilled in an ice bath, filtered, washed with cold water and dried under vacuum. The product was recrystallized in ethylacetate, filtered and dried under reduce pressure.

Found: 69.68 g, 91.5% yield, mp. 130-135 °C. δ_{H} (300 MHz, DMSO d_6 , reference TMS) 0.78 (6H, s, 2CH₃-C), 1.05 (6H, s, 2CH₃-C=N), 1.64 (6H, s, 2-CH₂N-), 2.04 (4H, s, 2-CH₂N-), 3.12-3.30 (m) (4H, CH₂N-O-) and 10.29 (2H, s, -OH) and CHN analysis found: C, 57.30; H, 10.40; N, 20.85 CHN requires C, 57.32; H, 10.36; N, 20.57.

Chemicals and reagents. All chemicals used in this study were analytical reagent grade (Fluka, Merck and BDH). All solutions were prepared with Milli-Q water.

The stock standard copper(II) solution was prepared by dissolving 0.6708 g of copper (II) chloride dihydrate in water and diluting to 250 mL. The solution was standardized titrimetrically by the known method.¹⁵

A 0.0360 M stock ligand solution was prepared by dissolving 1.0476 g *meso*-HMPAO in 10 mL dimethylsulfoxide, and this solution was diluted with methanol to 100 mL.

The buffer solution was prepared by dissolving 64.16 g of ammonium chloride in water, and the pH was adjusted to 9.0 by addition of 65.2 mL concentrated ammonium hydroxide, and the solution was diluted to 1 L with water.

Determination of copper(II). For the determination of copper(II), the solution containing an amount of copper(II) within the range recommended for the method was transferred into a 25 mL volumetric flask, 5 mL of 0.036 M ligand solution was added after addition of 2 mL buffer solution. The absorbance was measured after 5 min at the wavelength of maximum absorbance. The amount of copper(II) in the sample solution was deduced from the calibration curve.

Analysis of copper(II) in foodstuff and pharmaceutical samples. For foodstuffs, rice, wheat flour and starch were dried at 90 °C for 24 hours. A 20 g sample of foodstuff was

digested with conc. HNO₃ and H₂O₂ (1 : 1), and heated gently on a hot plate to dryness. The residue was dissolved in 20 mL conc. HNO₃, filtered and made up to 50 mL with water.

For pharmaceutical samples, ten tablets of Centrum, Supradyn and Revicon were powdered and ignited at 800 °C for 3 hours. The ash was digested with conc. HNO₃ and H₂O₂ (1 : 1) and heated gently on a hot plate to dryness. The residue was taken up in 20 mL conc. HNO₃, filtered and made up to 50 mL with water.

The amount of copper(II) present in each sample was determined by taking a suitable amount of the samples and treating them according to the present method and AAS.

The effect of diverse ions. The interference studies were investigated by using a standard 8 $\mu\text{g mL}^{-1}$ copper(II) solution with varying concentrations of other species added, as listed in Table 2. The tolerance limit of ions was taken as the maximum amount ($\mu\text{g mL}^{-1}$) causing an error not greater than $\pm 2\%$.

Results and Discussion

The ligand *meso*-HMPAO reacts with copper(II) to form a stable red-pink complex species, which is highly soluble in water. The absorption spectrum of the copper(II)-*meso*-HMPAO complex in aqueous solution was studied over the wavelength range of 300-800 nm. The complex exhibited the absorption maximum at 497 nm due to the *d-d* transitions (Fig. 2), indicating the formation of a complex between the ligand and copper(II). The molar absorptivity of complex calculated from the absorbance data was found to be 338 L mol⁻¹ cm⁻¹ at 497 nm.

The IR spectrum of the ligand shows a sharp band at 3084 cm⁻¹, which can be attributed to the -OH groups, and this band disappears and a broad band appears around 1770 cm⁻¹ after complexation with copper(II). This indicated that the intramolecular hydrogen bond (O -- H -- O) between two terminal oxime groups had occurred (Fig. 3).¹⁶

The effect of pH on the determination of copper(II) in aqueous solution medium was investigated spectrophotometrically. For this purpose, the solution containing 200 μg

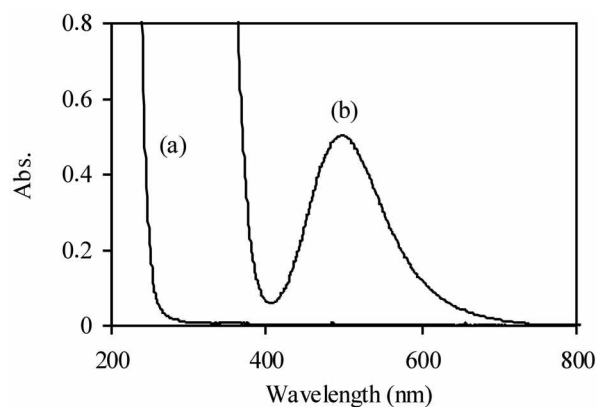


Figure 2. Visible absorption spectrum of (a) *meso*-HMPAO and (b) copper(II)-*meso*-HMPAO complex.

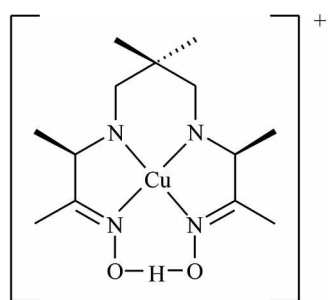


Figure 3. Proposed structure of copper(II)-*meso*-HMPAO complex.

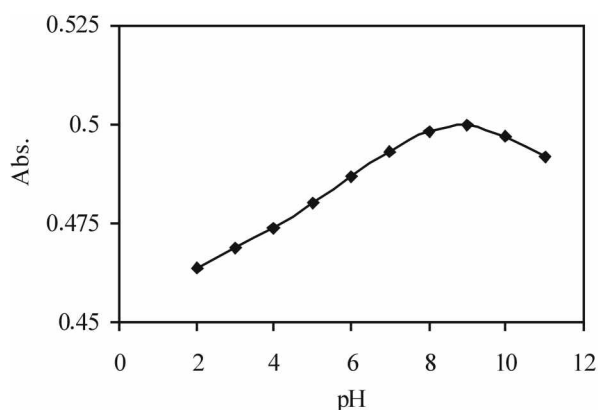


Figure 4. Effect of pH on the formation of copper(II)-*meso*-HMPAO complex.

mL^{-1} of copper(II) was measured in the pH range 2-11 at 497 nm. As is clearly seen from Figure 4, the amount of copper can quantitatively be determined at pH 9.

Job's method of various variation was applied to ascertain the stoichiometric composition of the complex, and the 1 : 1 (copper(II) : *meso*-HMPAO) complex was indicated by this method (Fig. 5). The color of the copper(II) complex was attained within 5 min after the addition of copper (II), and the intensity remained constant for at least 24 h.

Analytical features. The red-pink color species obeyed Beer's law in the concentration range 0.5-370 ppm with a molar absorptivity value of $338 \text{ L mol}^{-1} \text{ cm}^{-1}$ at 497 nm. Sandell's sensitivity value, as calculated from Beer's law data, was found to be $0.18 \mu\text{g cm}^{-2}$. A regression analysis of

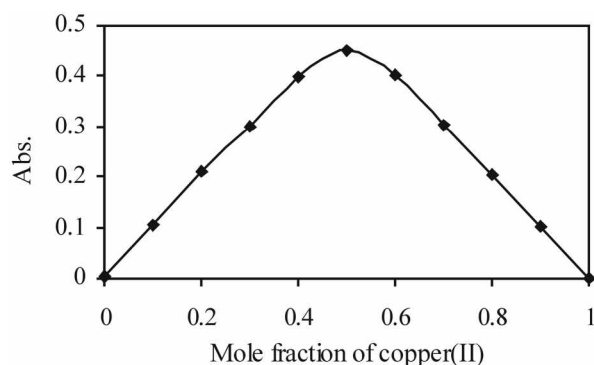


Figure 5. Job's method of copper(II)-*meso*-HMPAO complex at pH 9.

Table 1. Effect of foreign ions on the determination of copper(II) by *meso*-HMPAO

Ion	Added as	Tolerance limit of ions (interfering ion $\mu\text{g mL}^{-1}$)
Ni^{2+}	$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	8
Fe^{3+}	$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	3
Co^{2+}	$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	2
Mg^{2+}	$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	40
Ca^{2+}	CaCl_2	80
Cd^{2+}	$\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$	20
Zn^{2+}	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	30
Nitrate	NaNO_3	100
Sulphate	Na_2SO_4	80
Chloride	NaCl	100
Oxalate	Oxalic acid	60
Fluoride	NaF	100
Phosphate	Na_3PO_4	70

Table 2. Analytical results of copper(II) ion in foodstuffs and pharmaceutical samples

Samples	Total weight of the tablet (g)	Label Claim (mg)	Amount of copper(II) found in sample ^a	
			AAS ($\bar{X} \pm \text{RSD}\%$)	This Method ($\bar{X} \pm \text{RSD}\%$)
Centrum ^{b*}	1.50	3.0	2.98 ± 0.20	3.01 ± 0.18
Supradyn ^{c*}	1.30	0.4	0.39 ± 0.58	0.40 ± 0.54
Revicon ^{d*}	0.90	2.5	2.46 ± 0.26	2.51 ± 0.28
Rice ^e	-	-	31.42 ± 0.42	31.85 ± 0.58
Wheat flour ^e	-	-	20.32 ± 0.37	19.26 ± 0.29
Starch ^e	-	-	37.63 ± 0.54	38.29 ± 0.48

^aAverage for five determinations. ^bProduced by White Hall, Thailand. ^cProduced by Roche, Germany. ^dProduced by Olic, Thailand. ^e $\mu\text{g g}^{-1}$. *mg/tablet.

Beer's law plot at 497 nm revealed a good correlation ($r^2 = 0.9999$). A graph of the absorbance *versus* the concentration shows low intercept value (0.0005) and slope (0.0025); these are described by a regression equation $Y = a + bX$ (where Y is the absorbance, a is the intercept, b is the slope and X is the concentration of copper(II) in $\mu\text{g mL}^{-1}$), obtained by the least squares method. The precision of the method was checked by taking six replicate measurements of $0.5 \mu\text{g mL}^{-1}$ of copper(II). For the relative standard deviation value (0.90%) and the range of 95% confidence level (0.59%), the limit of detection (LOD) is $0.5 \mu\text{g mL}^{-1}$.

Effect of foreign ions. To assess the analytical potential of the proposed method, the effect of some foreign ions, which often accompany copper(II), were examined by carrying out the determination of $8 \mu\text{g mL}^{-1}$ of copper(II) in the presence of a number of other ions. The results presented in Table 1, indicate most of the ions do not interfere with the determination of copper(II). Although cobalt(II) and iron(III) compete with copper(II), this interference can be eliminated by using an excess of reagent in the procedure.

The proposed method for the determination of copper(II), using *meso*-HMPAO as a spectrophotometric agent and flame AAS detection were examined for foodstuffs and

pharmaceutical samples (Table 2). Thus, the proposed method was shown to be an alternative method for an investigation of the amount of copper(II) in foodstuffs and pharmaceutical samples.

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