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Extraction and spectrophotometric determination of copper(II) with *S*,*S*′-bis(2-aminophenyl)oxalate

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Abstract

A new selective reagent, *S*,*S*′-bis(2-aminophenyl)oxalate (H_2L), for the extractive spectrophotometric determination of copper has been prepared. The ligand, H_2L, forms a 1:1 complex with copper(II) in methanol. The molar absorptivity of Cu(II)-*S*,*S*′-bis(2-aminophenyl)oxalate complex in methanol is 5365 M^{-1} cm^{-1} at 504 nm. The method has been applied for the determination of copper in pharmaceutical formulations, environmental and foodstuff samples.

Keywords: *S*,*S*′-bis(2-aminophenyl)oxalate; Spectrophotometric determination of copper(II); Preconcentration; Determination of trace elements

1. Introduction

Preconcentration has been used in the determination of trace amounts of elements in organic and inorganic precipitates for some time [1,2]. Trace amounts of copper in various biological and environmental substances are vital since copper traces promote rancidity and off-flavours in foods and beverages. Moreover, the levels of copper in biological samples may indicate malfunction or contamination. Hence, rapid and sensitive methods for its determination in environmental and biochemical research are in great demand. At the present time, the most widely used methods are atomic emission or absorption, and colorimetric methods. Although colorimetric methods require appropriate chromogenic reagents, they are generally preferred since they provide better sensitivity and involve less expensive instrumentation. Most of the chromogens developed for extraction and determination copper are reagents prepared by difficult and tedious organic syntheses. Some of best known colorimetric reagents are sodium diethyldithiocarbamate [3], 5-bromosalicylaldoxime [4], *N*-*m*-tolyl-*p*-methoxybenzohydroxamic acid [5], picolinamidoxime [6], dithizone [7], 4,7-diphenyl-1,10-phenantroline [8], etc.

This paper describes a colorimetric procedure for the pre-concentration and determination of trace amounts of copper in different matrices. The

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purpose of this work was to investigate various factors influencing the sensitivity and specificity of the developed method such as wavelength, pH, stirring time, the effect of foreign ions and the ranges of applicability of Beer's law on the determination of copper.

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 was also used for the absorption spectra and the absorbance measurements, with a quartz cell of 10 mm path length.

2.2. Preparation of ligand

The ligand, *S*,*S*′-bis(2-aminophenyl)oxalate, H_2L, was prepared by two steps. First, potassium ethoxide, produced by mixing 1.56 g (40 mmol) metallic potassium with 50 ml absolute ethanol under nitrogen atmosphere, was reacted with 5.01 g (40 mmol) 2-amino thiophenol. Second, 2.6 g (20 mmol) oxalyl chloride was slowly added to this solution, and mixed for 2 h at room temperature, then was refluxed for 2 h. The product (H_2L) was filtered while hot, and evaporated, then crystallized in ethanol. The pale-yellow, air-stable, crystalline solid (yield, 80%) was insoluble in water, and soluble in methanol, ethanol, and chloroform. The purity of this reagent was checked by physical data; melting point range, 90–92°C; anal. calcd. for $C_{14}H_{12}O_2N_2S_2$: C, 55.25; H, 3.95; N, 9.20; S, 21.05, found: C, 55.15; H, 4.05; N, 9.10; S, 20.90. IR (ν cm^{-1}) 3377–3295 (NH_2), 1608 (C═O). ^{1}H-NMR, δ(ppm/$CDCl_3$): 4.10 (NH_2, 4H); 6.30–7.20 (Ar—H, 8H).

2.3. Chemicals and reagents

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

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 standardised titrimetrically by a known method [9]. The working standard solutions were prepared by suitable dilution of the stock solution.

A stock complexing agent solution (0.0157 M) was prepared by dissolving 1.19 g H_2L in 250 ml MeOH.

Buffer solution pH (5.5) was prepared by dissolving 400 g of ammonium acetate in water and adjusting by addition of 350 ml concentrated ammonium hydroxide, and diluted to 1000 ml with demineralized water.

Validation of the method described here was performed by using five Certified Reference Materials (CRMs) from the Community Bureau of Reference (BCR), International Atomic Energy Agency (IAEA), Office of Standard Reference Materials (NBS) and National Institute for Environmental Japan Environment Agency (NIES).

2.4. Procedure

An aliquot of the metal solution was transferred into a separatory funnel to which the desired volume of buffer solution was added. The total volume was brought to 10 ml with distilled water, then an excess of *S*,*S*′-bis(2-aminophenyl)oxalate solution was added. This solution was then equilibrated for 45 min. The mixture containing copper was extracted three times with 10 ml of chloroform. Organic phases were combined in a beaker, dried over anhydrous sodium sulfate and transferred into the measuring cells. The absorbance of the extract was measured against the reagent blank at 504 nm.

2.4.1. Interference studies

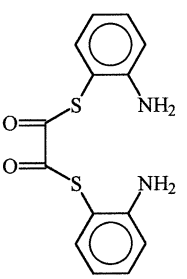
A standard 20.00 μg ml^{-1} copper solution was used for all interference work, with varying concentrations of other species added as listed in Table 2.

3. Results and discussion

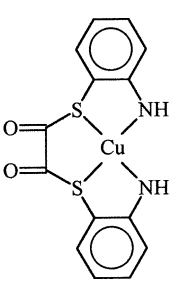
3.1. Absorption spectra

The ligand (Fig. 1) reacts with divalent copper

to form a stable brownish complex species which is very soluble in chloroform. The absorption spectra of H_2L and the copper(II)–H_2L complex are shown in Fig. 2. The absorption spectrum of the copper(II)–H_2L complex in chloroform was studied over the wavelength range 300–600 nm. The absorption maximum of the ligand is at 340 nm and that of the complex is at 504 nm. Absorption due to the ligand at 504 nm was negligible. Therefore, all absorbance measurements were performed at 504 nm. The molar absorptivity of the complex calculated from the absorbance value was found to be 5365 M^{-1} cm^{-1} at 504 nm. Copper contents were estimated by stirring the aqueous phase containing 20 μg ml^{-1} of copper and buffer solution with H_2L for varying time periods from 1 to 60 min. The absorbance of each case was measured. The absorbance value remained constant when the stirring period was 45 min or more.



H_2L



L-Cu

Fig. 1. Proposed structure of (A) ligand and (B) its copper(II) complex.

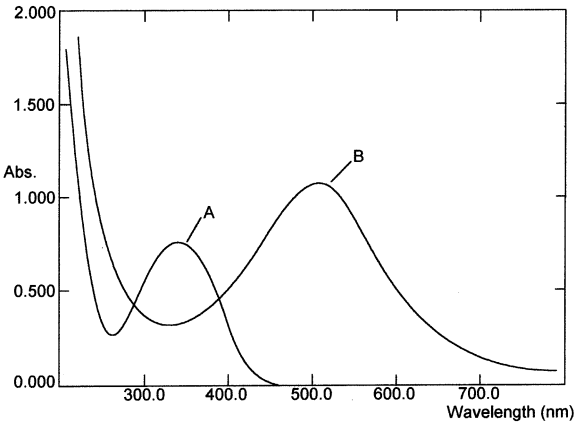


Fig. 2. Visible absorption spectrum of ligand (H_2L) (A), and its copper complex (B).

3.2. Nature and stability of the complex

Job's method of continuous variation and the molar-ratio method were applied to ascertain the stoichiometric composition of the complex. A 1:1 (Cu: H_2L) complex was indicated by both methods. The complex formation was examined by varying time periods from 1 to 60 min. The absorbance of each extract at 504 nm was measured. The absorbance value remained constant after 45 min and was stable for at least 20 h.

The UV–Vis spectra of the ligand (H_2L) showing a maximum (λ_{max}) at around 340 nm with that of the Cu(II) complex at 504 nm indicate the complex formation between the ligand and the metal ion. The energy observed at around 504 nm belonging to d–d transitions indicates that the geometry is distorted tetragonal. IR spectrum of the ligand shows a doublet at ca. 3377 and 3295 cm^{-1} which is attributed to the —NH_2 groups of the free ligand. After complexation, the doublet belonging to the free ligand disappeared and a singlet was observed at ca. 3400 cm^{-1}, instead. This observation indicates that one of the hydrogen atoms of each —NH_2 group is released as free protons. Moreover, lower water-solubility of the complex was evaluated as due to its greater covalent character.

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3.3. Effect of pH

The effect of pH on the determination of copper in aqueous medium was spectrophotometrically investigated. For this purpose, the absorbances of the complex solution containing 20 μg ml^{-1} of Cu(II) were measured at a pH range of 0–14 and at 504 nm. The most stable complex formation was observed at a wide pH range of 3.0–8.0 as it is clearly seen in Fig. 3. Most of the present methods, however, are only applicable at a limited pH range [3–8].

3.4. Effect of solvents

A H_2L (0.0157 mol l^{-1}) solution was prepared in different solvents (chloroform, methylenechloride, carbontetrachloride, hexane) to find out the effect of solvents on the absorbance value at 504 nm for copper(II) determination, and was used as the reagent phase for extracting an aqueous phase containing 20 μg of copper in buffer solution. Of the solvents examined, the absorbance value as well as percentage extraction of copper decreased in the order for chloroform (99.7%), methylenechloride (99.5%), carbontetrachloride (40.2%), and hexane (30%).

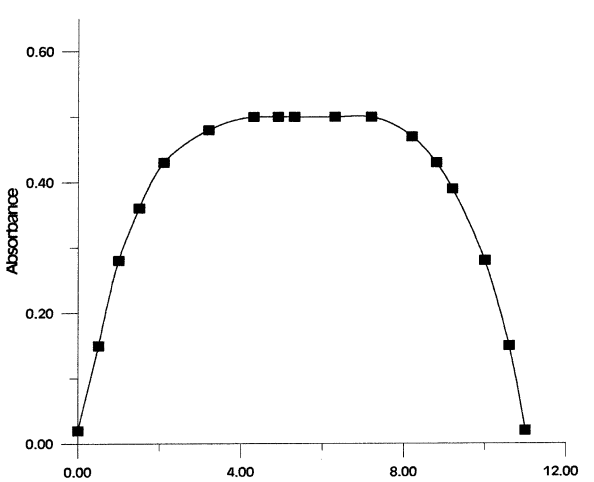

Fig. 3. Effect of pH on formation of copper complex of ligand.

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

Wavelength of maximum absorbance (nm)	504
Limit of detection (LOD) (μg ml^{-1})	11.5
Limit of quantification (LOQ) (μg ml^{-1})	30.0
LOD/LOQ	0.4
Range of linearity (μg ml^{-1})	0.4–150
Molar absorbtivity (M^{-1} cm^{-1})	5365
Optimum pH	3.0–8.0
Calibration graph	
Slope	0.005365
Intercept	−0.00122
Correlation coefficient (r)	0.9998
Relative standard deviation (RSD) (%)	0.66

3.5. Beer's law and sensitivity

A calibration curve for the determination of copper was prepared under optimum experimental conditions (1 ml buffer solution, pH 5.5, 0.0157 M H_2L in aqueous medium). Beer's law is obeyed within the range 0.4–150 μg ml^{-1} of copper at 504 nm (Table 1), which is much greater than the detection range for most of the methods reported earlier [3–8]. The calibration curve can be represented by a linear regression equation:

$$y = 0.005365x - 0.00122 \qquad (r^2 = 0.9998)$$

where y is the absorbance and x the concentration of Cu(II) in μg ml^{-1}. The molar absorptivity is 5365 M^{-1} cm^{-1} and the Sandell's sensitivity calculated on the basis of total copper present is 0.015 μg cm^{-2}. Conditions for the spectrophotometric determination of the copper(II) are shown in Table 1.

3.6. Effect of diverse ions

The effect of diverse ions (Table 2) on the determination of 20 μg of Cu(II) was studied according to the procedure described under Section 2. The tolerance limit of an ion was taken as the maximum amount (mg) causing an error not greater than 2%. Most of the ions studied do not form complexes and therefore do not interfere with the determination of copper, as shown in Table 2. Although cobalt salts form complexes

with the new analytical reagent in competition with copper, they show absorbance at 540 nm ($\varepsilon = 257\ M^{-1}\ cm^{-1}$). However, this can be eliminated by using excess reagent.

3.7. Precision and accuracy

The precision of the method was checked by taking 10 replicated measurements on solutions each containing 2 μg of Cu(II). The relative standard deviation (10 determinations with 2 μg of Cu(II), 95% confidence level) is $\pm 0.66\%$. Limit of detection (LOD) is 11.5 μg Cu/L, limit of quantification (LOQ) is 30 μg Cu/L, and the LOD/LOQ ratio is 0.4. Altogether, these data indicate the robustness of the procedure. The accuracy of the method was checked by an atomic absorption method as described previously [10]. The experimental data (Table 3) show a good agreement between the results obtained by the two methods.

Table 2
Effect of diverse ions on the determination of copper(II)

Ion	Added as	Tolerance limit of ion (interfering ion mg^{-1})
Ni^{2+}	$NiSO_4 \cdot 7H_2O$	7.5
Fe^{3+}	$FeCl_3$	10.5
Fe^{2+}	$FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$	12.4
Zn^{2+}	$ZnSO_4 \cdot 7H_2O$	6.5
Al^{3+}	$Al(NO_3)_3$	15
Co^{2+}	$Co(NO_3)_2 \cdot 6H_2O$	0.5
Pb^{2+}	$Pb(NO_3)_2$	1.0
Mn^{2+}	$MnSO_4 \cdot 4H_2O$	5.5
Cr^{6+}	$K_2Cr_2O_7$	2.5
Mg^{2+}	$MgSO_4$	15
Ca^{2+}	$CaCl_2$	15
Sr^{2+}	$SrCl_2 \cdot 6H_2O$	10.5
Ba^{2+}	$BaCl_2 \cdot 2H_2O$	15
Cd^{2+}	$CdSO_4$	2.5
Sn^{2+}	$SnCl_2 \cdot 2H_2O$	5.5
Thiosulphate	$Na_2S_2O_3 \cdot 2H_2O$	2.6
Phosphate	Na_3PO_4	6.4
Tartarate	Tartaric acid	5.5
Oxalate	Oxalic acid	6.0
Nitrate	$NaNO_3$	4.5
Chloride	NaCl	5.5
Sulphate	Na_2SO_4	9.5
Fluoride	NaF	14
EDTA	EDTA·2Na	10

3.8. Applications

3.8.1. Analysis of pharmaceutical samples

The determination of copper in pharmaceutical samples such as Supradyn, Vi-mineral and Unicap therapeutic tablets was also studied by the present method. In each case, 10 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 solution, the mixture was filtered and then diluted to 100 ml with distilled water. The copper contents were determined by taking suitable aliquots of the sample. The results obtained are comparable with the certified values (Table 3).

3.8.2. Analysis of foodstuff

The present method was also applied to the determination of copper(II) content in foodstuffs including rice, wheat flour, cabbage and banana. After the foodstuffs were dried at 90°C for 24 h, a 5 g sample of foodstuff was digested with nitric acid and perchloric acid, and heated gently on a hot plate to dryness. The ash was then treated as mentioned in the analysis of pharmaceutical samples. The results obtained are given in Table 3.

3.8.3. Analysis of biological and environmental samples

The proposed method was also applied to the determination of copper(II) in biological (tea, hazelnut) and environmental samples (sediment, industrial effluent, etc.). Twenty to fifty grams of material were digested with an excess of perchloric and nitric acids. The mixture was centrifuged and filtered, and the filtrate was evaporated to dryness. The residue was dissolved in 1 M HCl, diluted to 100 ml with distilled water, and used for the determination of copper(II). The results are summarized in the Table 3.

4. Conclusion

The present method has several advantages, some of which are summarised here. The ligand, H_2L, is a product of a one-step addition reaction in high yields (80–85%). It can be easily purified

Table 3
Determination of copper in environmental and foodstuffs

Sample	Certified Cu content (μg g^{-1}) ($X \pm SD$)	Cu found (μg g^{-1})[a]	
		Proposed method (X (RSD%))	AAS[c] method (X (RSD%))
River sediment (NBS-SRM-1645)	109 ± 19	116(5.4)	111(4.3)
Sewage sludge (BCR-CRM-145)[b]	394 ± 12	400(5.5)	399(4.8)
Lake sediment (IAEA-SL-1)	30 ± 5.6	28(3.2)	27(2.7)
Tomato leaves (NBS 1573)	11 ± 1	12(2.5)	12(1.8)
Turkish Tea leaves	–	67.5(4.2)	64.2(2.6)
Hazelnut (*C. Avellena* var. Pontica)	–	14.7(2.2)	14.5(1.2)
Turkish tobacco	–	25.4(1.6)	21.2(1.8)
Supradyn (Roche, Turkey)[b]	1.0	0.94(1.7)	0.95(6.5)
Vi-Mineral (Deva, Turkey)[b]	0.07	0.12(2.5)	0.15(3.2)
Unicap-Therapeutic (Eczacibasi Turkey)[b]	0.40	0.45(3.3)	0.46(2.9)
Rice	–	30.25(2.20)	29.76(2.50)
Wheat flour	–	19.50(2.05)	19.19(1.75)
Banana	–	15.15(3.21)	13.98(2.70)

[a] Average of five determinations.
[b] Value per milligram.
[c] AAS, atomic absorption spectroscopy.

by ethanol crystallisation. Therefore, there is no need for any additional purification step. The H_2L forms a very stable complex with copper(II), which possess an absorbance signal at 504 nm, and the complex is highly soluble in common organic solvents. Although cobalt salts can form complexes with the ligand, they absorb at a different wavelength, 540 nm. The other ions examined do not interfere with copper(II). The ligand has been successfully applied to extraction and determination of copper(II) from aqueous media prepared by using different samples from various sources. The analytical results obtained are very reproducible. Therefore, this chromogen can be applied to rapid, simple, sensitive and reproducible determination of copper(II) in pharmaceutical, biological, and environmental samples.

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