*Journal of Pharmaceutical & Biomedical Analysis*
Vol. 6, Nos 6–8, pp. 1019–1021, 1988
Printed in Great Britain

0731–7085/88 $3.00 + 0.00


# Spectrophotometric determination of copper in biological and pharmaceutical samples*

F. MOLINA,† D. G. FERNANDEZ,‡§ J. M. BOSQUE-SENDRA† and P. ESPINOSA†

† *Department of Analytical Chemistry,* ‡ *Department of Chemical Engineering, University of Granada, 18071 Granada, Spain*

**Keywords**: *Copper determination; Schiff bases; liquid–liquid extraction; spectrophotometry; biological and pharmaceutical samples.*

## Introduction

Copper is an essential element present in all organisms. However, elevated serum copper levels in humans are manifest in certain disease processes such as infections, leukemias, Hodgkin's disease, hemochromatosis, etc. Decreased serum copper levels and increased urinary copper excretions are associated with Wilson's disease and nephrosis. Copper deficiencies also have been observed in infants when the usual diets (e.g. milk preparations) are deficient in this element. The determination of serum and urinary copper content is regarded to be a reliable diagnostic tool [1].

Schiff bases have been shown to be potential analytical reagents in both inorganic and organic analysis [2, 3]. 2-(*o*-Hydroxyphenyliminomethyl)pyrrole (HL) forms coloured complexes with several metal ions that can be used in the separation and spectrophotometric determination of the metals [4].

The present paper describes the development of a new method for the extractive spectrophotometric determination of Cu(II) based on its reaction with HL.

## Experimental

*Reagents and equipment*

The chemicals used were of analytical reagent grade. Standard copper(II) solution was prepared from $Cu(NO_3)_2.3H_2O$ and standardized titrimetrically with EDTA. Working solutions of 2-(*o*-hydroxyphenyliminomethyl)pyrrole (HL) were prepared in chloroform as required.

The apparatus used included a Spectronic 2000 (Bausch–Lomb) spectrophotometer, a Crison Digit-501 pH-meter and a Perkin–Elmer 2380 AA spectrophotometer.

*Presented at the "International Symposium on Pharmaceutical and Biomedical Analysis, September 1987, Barcelona, Spain.

§To whom correspondence should be addressed.

*Analytical procedure*

An aliquot of the sample solution containing not more than 30 μg of copper was transferred into a 100 ml separating funnel and the pH was adjusted to 8.40 with a borate buffer solution. The volume of the aqueous phase was then made up to 40 ml. Next, 10 ml of HL in chloroform $3.5 \times 10^{-4}$ M were added and the mixture shaken for 5 min. The organic phase was then separated, centrifuged and measured at 410 nm against a spectrophotometric blank of HL prepared in the same way but in the absence of copper.

*Preparation of sample solutions*

For blood serum samples proteins were precipitated by heating to 70–80°C and the lipids removed by extraction with chloroform (1:1 v/v chloroform:blood serum). With powdered milk samples a suitable weight was calcinated at 540°C and the ash thus obtained dissolved in $HNO_3$. In the case of pharmaceutical samples a suitable weight was transferred into a 100 ml Kjeldahl flask, treated with 10–15 ml concentrated sulphuric acid and 1–2 g anhydrous $K_2SO_4$ and warmed until the mixture became a clear solution. Finally, the solutions obtained after the above treatments were neutralized (as necessary) and diluted to a known volume in a graduated flask.

## Results and Discussion

*Absorption spectrum*

The absorption spectrum of the yellow complex formed between Cu(II) and HL exhibits an absorption maximum at 410 nm when it is extracted in chloroform.

*Effect of pH*

The optimum pH range for the determination of copper was found to be 5.0–9.5. The pH of the aqueous phase was ultimately adjusted to 8.4 with borate buffer.

*Effect of the reagent concentration*

The extraction was found to be quantitative from a reagent concentration of $2.4 \times 10^{-4}$M. A HL concentration of $3.5 \times 10^{-4}$M was selected as the optimum.

*Phase–volume ratio*

This can be varied from 1:1 to 4:1 a.ph./o.ph. without any variation in the absorbance values or extraction efficiency.

*Shaking time*

Trial experiments revealed that shaking for 5 min was sufficient for the quantitative extraction of copper.

Furthermore it is found that the absorbance of the organic phase remains constant for at least 24 h.

*Extraction efficiency*

In order to determine the extraction efficiency, Cu(II) was extracted by the proposed procedure, and subsequent back-extracted with HCl 6 M. The Cu(II) content of the aqueous phase was then determined by atomic absorption to reveal that the percent extraction was greater than 97.

*Nature of the extracted species*

The combining ratio of Cu(II) to HL in the extracted species was determined by the molar ratio and the slope ratio methods, modified for a two-phase system. These methods gave results consistent with a 1:3 |Cu(II):ligand| complex. However, plotting log D versus pH for constant reagent concentration, over the pH range 3.4–4.6, gave a straight line with a slope of 2.0, showing that two hydrogen ions are released for each metal ion complexed. From these results it may be concluded that the probable composition of the extracted species is $|CuL_2(HL)|$ where the neutral molecule of the ligand (HL) seems to act as a synergistic agent enhancing the extraction efficiency.

*Calibration range, sensitivity and precision*

Beer's law was obeyed over the range 0.05–0.7 μg $ml^{-1}$ of Cu(II) in the aqueous phase. The optimum copper concentration range was 0.07–0.45 μg $ml^{-1}$ according to Ringbom's plot. The molar absorptivity at 410 nm was $1.4 \times 10^5$ l $mol^{-1}$ $cm^{-1}$ (Sandell's sensitivity 0.00045 μg $Cu/cm^2$ [5]. The IUPAC detection limit was 0.004 μg $ml^{-1}$ of copper for a value of $K = 3$ (confidence level 99.86%). The precision of the method was evaluated from the analysis of ten different samples each containing 0.3 μg $ml^{-1}$ of copper. The relative standard deviation was found to be ±1.3%.

*Effect of foreign ions*

The effects of various elements on the extraction and determination of copper under the conditions mentioned were investigated. There was no interference from $Ca^{2+}$ and $Mg^{2+}$ in at least 8000-fold mass ratio to Cu(II); $Cd^{2+}$ and $Cl^-$ at a 2000-fold ratio; $Ni^{2+}$ at a 1000-fold ratio; $F^-$ at a 400-fold ratio; $Pb^{2+}$ at a 30-fold ratio; $Co^{2+}$ and $Fe^{3+}$ at a 15-fold ratio; and $Zn^{2+}$ at a 5-fold ratio.

*Applications*

The recommended procedure was tested with several biological and pharmaceutical samples. The results given in Table 1 are the mean values of four determinations with different sample solutions prepared from the same sample. As shown, the results are in good agreement with those obtained by AAS and the reported values, showing the reliability of the proposed method.

**Table 1**
Determination of copper in different samples

| Sample | AAS | Proposed method |
|---|---|---|
| Serum | 0.65 ± 0.02 | 0.66 ± 0.03 g $ml^{-1}$ |
| Powder milk | 6.65 ± 0.04 | 6.50 ± 0.08% |
| Vitaminic complex* | 2.08 ± 0.03 | 2.03 ± 0.05% |

*Composition (% Cu): 2.12.

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[*Received for review 23 September 1987*]