Determination of Trace Amounts of Copper(II) by Using Catalytic Redox Reaction between Methylene Blue and Ascorbic Acid

M. Nasiruddin KHAN[†] and Anila SARWAR

Department of Chemistry, University of Karachi, Karachi-75270, Pakistan

A sensitive and selective kinetic-spectrophotometric method is proposed for the determination of $\mu\text{g mL}^{-1}$ amounts of Cu^{2+} based on its catalytic effect on the oxidation of L-ascorbic acid by Methylene Blue in a strongly acidic medium. The reaction is monitored spectrophotometrically by measuring the decrease in color intensity of Methylene Blue at 665 nm. The analysis of Cu^{2+} ion is performed by a fixed-time method. At a given time of 2 min at pH 2.20 and 32°C, the detection limit is 10 ng mL^{-1} and the relative standard deviation for 0.4 $\mu\text{g mL}^{-1}$ Cu^{2+} is 3.60% ($n = 6$). The method is free from most of the interferences and the effect of diverse ions on the determination of Cu^{2+} is also reported. The proposed method is virtually specific to copper and has been satisfactorily applied to its determination in electric copper wire samples and pharmaceutical products. Results were also verified by the atomic absorption spectrometry technique (AAS).

(Received January 29, 2001; Accepted June 20, 2001)

Introduction

The physiological role of copper as a trace element is well established. It is important to carry out the proper functioning of different metalloenzymes, such as ascorbic oxidase (oxidation of ascorbic acid). Evidence has been presented that at low levels copper is an essential element in the diet, whereas at higher concentrations it is toxic. Because of its toxicity and cumulative effect in the body, copper presents a health problem when it occurs in food or water, even in minute amounts.

A number of sensitive analytical methods are available for the determination of copper. Some of the most commonly used methods are spectrophotometric,¹⁻³ fluorometric,⁴ thermometric,⁵ potentiometric,⁶ biamperometric⁷ and flow-injection determination⁸ methods.

Catalytic kinetic methods⁹ are an attractive alternative for the determination of trace amounts of copper. Such methods have the general advantage of combining high sensitivity with relatively simple procedures and apparatus. There are many kinetic methods for the determination of Cu^{2+} at trace levels based on the catalytic effect of its ion on various inorganic and organic indicator reactions.¹⁰⁻¹² However, few of them have been used for the determination of copper in real samples, or samples of analytical interest. The chief shortcoming of a kinetic catalytic determination of copper is that in most cases highly sensitive methods are poorly selective, or *vice versa*. Hence, the development of more sensitive and selective catalytic methods for the kinetic determination of copper is still sought.

Our work enhanced the scope of kinetic studies based on the copper(II) catalyzed oxidation of ascorbic acid by Methylene

Blue in strongly acidic media. The system forms the basis for an assay relying on spectrophotometric measurements at the wavelength of maximum absorption of the oxidation product of ascorbic acid. The proposed method permits the determination of Cu^{2+} at trace levels in different samples of analytical interest.

Experimental

Reagents and chemicals

L-Ascorbic acid (H_2A) was of BDH AnalR grade and its solution was always freshly prepared. A buffer solution of citric acid-disodium hydrogenphosphate was used as a solvent. Stock solutions of Methylene Blue (M.B.) (5×10^{-5} mol L^{-1}) and ascorbic acid (5×10^{-3} mol L^{-1}) were prepared in a buffer solution of pH 2.20.

An assay solution of 1 g mL^{-1} of copper wire was prepared by dissolving 1.000 g copper foil (99.99%) in nitric acid (1:1) and heated to expel nitrogen oxide, then diluted with distilled water.

Stress Tabs (manufactured by Cyanamid Pakistan Ltd.) and Theragran Stress (manufactured by Bristol-Myers SQUIB Pakistan Ltd.) tablets were purchased from local markets. First, ten Stress Tabs were weighed and pulverized. About 100 mg of tablet powder was accurately weighed and transferred to a 100 mL volumetric flask, which already contained a 50 mL buffer solution of pH 2.20. The mixture was sonicated for 5 min at room temperature and centrifuged for 10 min at 3500 rpm. Diluted solutions were used to determine the copper content in the drug.

The same procedure was adopted to prepare the assay of a Theragran Stress tablet.

Equipment

A temperature-controlled UV-visible spectrophotometer (160-A Shimadzu) was used for an absorbance measurement. The

[†] To whom correspondence should be addressed.
E-mail: nkhan210@yahoo.co.uk

Fig. 1 Catalytic effect of $[Cu^{2+}]$ on the oxidation of ascorbic acid by Methylene Blue. (a) Absorbance change vs. time plot: (1) blank (uncatalyzed reaction). $[Cu^{2+}]/\mu g mL^{-1}$: (2) 0.2 (3) 0.4 (4) 0.6 (5) 0.8 (6) 1.0 (7) 2.0 (8) 3.0 (9) 4.0 (10) 5.0. All other conditions are described under procedure. (b) The inset provides details for the rate vs. $[Cu^{2+}]$ plot.

pH was measured with a digital Orion 710 pH-meter (± 0.01 pH). The temperature ($\pm 0.1^\circ C$) was maintained in the reaction cell with a circulating water bath. In a 10 mL volumetric flask, 3 mL of $5 \times 10^{-3} mol L^{-1}$ ascorbic acid and an appropriate amount of Cu^{2+} standard solution were placed and kept in a water bath ($32^\circ C$) for 3 min. Finally, a 2.0 mL volume of $5 \times 10^{-3} mol L^{-1}$ Methylene Blue was added to the same flask; 2.0 mL of the reaction mixture was transferred to a spectrophotometric cell and the variation of absorbance vs. time was recorded immediately. The absorbance was measured at 665 nm against a buffer solution.

Results and Discussion

There are several methods, such as fixed-time, fixed-concentration, initial-rate and rate-constant methods, for measuring the catalytic species. In this work we checked three methods:

1. The initial rate was plotted against $[Cu^{2+}]$. The resulting calibration curve was linear over the range $0.2 - 1.0 \mu g mL^{-1}$ and the relative standard deviation (r.s.d.) was 3.8%.
2. Figure 1a shows that the difference in the absorbance of a blank (A_0) and a sample (A) increased as the reaction time increased. The most suitable reaction time was 2 min after the addition of Methylene Blue, based on its correlation coefficient. A calibration curve was obtained by plotting the instantaneous rate against the Cu^{2+} concentrations. The curve was linear over the range $0.2 - 2.0 \mu g mL^{-1}$ with an r.s.d. of 3.6% (Fig. 1b).
3. In the fixed-concentration method the time needed to decrease a preset absorbance (0.250) was taken as a basis. The determination range for copper between $0.2 - 0.8 \mu g mL^{-1}$ was accomplished with an r.s.d. of 6.3%.

The fixed-time method was selected on the basis of its wider determination range and smaller r.s.d. compared to the other two.

Table 1 Correlation coefficients for the calibration curves at different reaction times after the addition of Methylene Blue

Reaction time/min	Regression coefficient (<i>r</i>)
0.5	0.984
1.0	0.987
1.5	0.988
2.0	0.999
2.5	0.994
3.0	0.983
3.5	0.980
4.0	0.974
4.5	0.973
5.0	0.971

The influence of different parameters on the reaction rate was studied to establish the best reaction conditions and maximum sensitivity.

The initial concentrations of H_2A in the range of $(0.5 \text{ to } 3.0) \times 10^{-2} mol L^{-1}$ were used to study the influence of $[H_2A]$ with fixed $1.0 \times 10^{-5} mol L^{-1}$ Methylene Blue. The pseudo first-order rate constant increases with increasing $[H_2A]$. A plot of k_{obs} vs. $[H_2A]$ was linear with a small intercept. This result implies that the reaction is first order with respect to $[H_2A]$. The plot of k_{obs} vs. Methylene Blue concentration ranging from $(0.5 \text{ to } 2.0) \times 10^{-5} mol L^{-1}$ shows a linear relationship between k_{obs} and $[M.B.]$. This fact reveals a first-order dependence in the oxidant as well.

An overall second-order rate equation will be expressed in the form

$$-d[M.B.]/dt = k_{obs}[M.B.] = k_2[H_2A]_t[M.B.].$$

Here, k_2 is the second-order rate constant; $[H_2A]_t$ indicates the total concentration of all forms of L-ascorbic acid, and can be set as $[H_2A] + [HA^-]$ at the working pH.

The experimental results showed that the blank and catalytic reactions could proceed only in strongly acidic media (pH < 3.00). Therefore a pH of 2.20 was selected for this work.

The influence of the temperature was studied in a range $20 - 40^\circ C$. A temperature of $32 \pm 0.2^\circ C$ was selected for better sensitivity and a lower detection limit.

The rate equation of the catalyzed reaction is

$$\text{Rate} = -d[M.B.]/dt = k[Cu^{2+}][M.B.]^m[H_2A]^n, \quad (1)$$

where k is the rate constant. By integrating Eq. (1) and incorporating Beer's law, we obtain the final expression under a fixed initial concentration of $[M.B.]$ and $[H_2A]$,

$$\ln(A_0/A) = k'[Cu^{2+}]_t,$$

where t is the reaction time.

Rate vs. $[Cu^{2+}]$ linear graphs were obtained for solutions containing $0.1 - 1.0 \mu g mL^{-1} Cu^{2+}$ with the regression equation $v = 3 \times 10^{-4}[Cu^{2+}] + 0.0005$ with $r = 0.9992$. The accuracy and precision for this analysis of six replicate measurements with various concentrations of Cu^{2+} are given in Table 2.

The detection limit, calculated as the value corresponding to three-times the standard deviation of the blank (uncatalyzed reaction), is $10 ng mL^{-1}$. The relative standard deviation of six replicate determinations is 3.6% for $0.4 \mu g mL^{-1}$.

The possible potential interference of 25 foreign cations and anions in the solution was examined. Solutions of copper and

Table 2 Accuracy and precision obtained by the recommended procedure

Amount of Cu ²⁺ present/ μg mL ⁻¹	Relative error of Cu ²⁺ found, %	Relative standard deviation (n = 5), %
0.2	-2.3	0.67
0.4	+2.2	0.52
0.6	+1.9	0.47
0.8	+0.9	0.21

Table 3 Selectivity achieved in the kinetic determination of 0.4 μg mL⁻¹ of copper

Ions tested	Tolerated ratio
SO ₄ ²⁻ , NO ₃ ⁻	3000
Pb ²⁺ , Ni ²⁺ , Zn ²⁺ , Mg ²⁺ , TI ⁺ , Ca ²⁺ , K ⁺ , Na ⁺ , Co ²⁺ , Br ⁻ , HCO ₃ ⁻ , F ⁻ , S ₂ O ₃ ²⁻ , HCOO ⁻ , PO ₄ ³⁻	1000
NH ₄ ⁺	200

each ion tested were mixed to obtain samples containing 1 μg mL⁻¹ of Cu²⁺ and up to 3000 μg mL⁻¹ of the foreign ion. The maximum amount of a substance causing an error of less than 5% in the determination of 1 μg mL⁻¹ Cu²⁺ was taken as the tolerance limit (Table 3). Fe³⁺, Fe²⁺, V⁵⁺, Cl⁻, and Ag⁺ interfered in the reaction.

Ce³⁺ alone has no catalytic effect, but in the presence of Cu²⁺, after reaching the threshold level, it promotes the rate of copper catalyzed reaction significantly, whereas 0.4 μg mL⁻¹ Mn²⁺ and Cr⁶⁺ show 12% and 15% inhibition for 0.4 μg mL⁻¹ Cu²⁺ ion, respectively.

The proposed method was applied to an analysis of Cu²⁺ in two pharmaceutical products and three electric copper wire samples. The obtained results are summarized in Table 4. The proposed kinetic method is in good agreement and comparable in accuracy and precision with a reference method, *i.e.* atomic absorption spectrometry (AAS).

The method was also checked against synthetic samples with a known amount of Cu²⁺. The recovery of added Cu²⁺ was 92 – 95% (Table 4).

The data given in Tables 4 and 5 show good performance and selectivity of the proposed kinetic method for the determination of Cu²⁺ in a variety of samples with a significant advantage to determine copper rapidly in samples over a wider concentration range.

Conclusion

The kinetic method proposed for the determination of Cu²⁺ is a sensitive and selective method. It is comparable in accuracy and precision with the reference method and other procedures described in the cited reference.

The procedure is also suitable for the analysis of Cu²⁺ in pharmaceuticals, because there is no interference from the excipients normally found in the commercial preparations. In addition, its high sensitivity permits its use in the determination

Table 4 Determination of Cu²⁺ in pharmaceuticals and electric wire samples

Sample	Cu ²⁺ content per tablet/mg Reference method	Proposed method ^a
Stress tabs	2.84	2.53 ± 0.11
Theragran stress	3.05	2.89 ± 0.15
Wire sample 1	0.477	0.51 ± 0.07
Wire sample 2	0.50	0.52 ± 0.04
Wire sample 3	0.52	0.54 ± 0.05

a. Mean of five-determination ± relative standard deviations.

Table 5 Determination of Cu²⁺ in synthetic samples

Sample	Added/ μg mL ⁻¹	Found/ μg mL ⁻¹	Recovery, %	Relative error, %
1	0.2	0.184	92.0	-8.00
2	0.2	0.189	94.5	-5.50
3	0.3	0.280	93.3	-6.62

Sample 1: 0.2 μg mL⁻¹ of Cu²⁺ + 10 μg mL⁻¹ of Zn²⁺ and TI⁺.

Sample 2: 0.2 μg mL⁻¹ of Cu²⁺ + 10 μg mL⁻¹ of Zn²⁺, TI⁺ and Ni²⁺.

Sample 3: 0.3 μg mL⁻¹ of Cu²⁺ + 10 μg mL⁻¹ of Zn²⁺, TI⁺, Ni²⁺ and Pb²⁺.

of industrial copper products.

Acknowledgements

The authors are grateful to the Dean, Faculty of Science, University of Karachi, for financial support.

References

1. J. F. Van Staden and A. Botha, *Talanta*, **1999**, *49*, 1099.
2. S. Nohut, S. Karaboecek, S. Guener, and Y. Goek, *J. Pharm. Biomed. Anal.*, **1999**, *20*, 309.
3. Y. Zhou and H. Jiang, *Fenxi Huaxue*, **1992**, *20*, 1228.
4. J. Medina-Escriche and P. Hernandez, *Analyst* [London], **1985**, *110*, 1457.
5. I. Bargallo, F. Borrull, V. Cerda, and J. Guasch, *Thermochim. Acta*, **1987**, *117*, 283.
6. H. P. Chen, *Lihua Jianyan, Huaxue Fence*, **1999**, *35*, 371.
7. L. Huang and H. Gao, *Gaodeng Xuexiao Huaxue Xuebao*, **1992**, *13*, 179.
8. N. Teshima, H. Katsumata, M. Kurihara, T. Sakai, and T. Kawashima, *Talanta*, **1999**, *50*, 41.
9. H. A. Mottola, "Kinetic Aspects of Analytical Chemistry", **1988**, John Wiley and Sons, New York, Chichester, Brisbane, Toronto, Singapore, 37.
10. D. Perez-Bendito and M. Silva, "Kinetic Methods in Analytical Chemistry", **1988**, Harwood, Chichester.
11. H. A. Mottola, D. Perez-Bendito, and H. B. Mark, Jr., *Anal. Chem.*, **1988**, *60*, 181 R.
12. N. Pourreza and M. Behpour, *Anal. Sci.*, **1998**, 997.