

Kinetic spectrophotometric determination of trace copper(II) ions by their catalytic effect on the reduction of brilliant cresyl blue by ascorbic acid

Halil İbrahim ULUSOY*, Ramazan GÜRKAN, Mehmet AKÇAY

University of Cumhuriyet, Faculty of Science, Department of Chemistry

58140, Sivas-TURKEY

e-mail:hiulusoy@yahoo.com

Received: 21.01.2011

In the present study, a novel catalytic-kinetic spectrophotometric method was developed for the determination of trace amounts of copper. It is based on the catalytic effect of Cu(II) on the reduction of brilliant cresyl blue (BCB) by ascorbic acid in pH 5.0 acetate buffer medium. The reaction was monitored spectrophotometrically by measuring the decrease in absorbance of BCB at 624 nm. The operating conditions regarding concentration of reagents, pH, time, and temperature were established and optimized in detail. The working curve was linear in the concentration range of 0.1-5.0 $\mu\text{g L}^{-1}$. The maximum percentage error and standard deviation for 5 replicate determination of the 3 $\mu\text{g L}^{-1}$ Cu(II) were 3.20 and 0.45, respectively. The detection limit and quantification limit were 0.025 and 0.086 $\mu\text{g L}^{-1}$, respectively. The procedure was successfully applied to determination of dissolved copper levels of 3 different natural spring waters and artificially prepared wastewater samples. The accuracy of the proposed kinetic method was tested by means of 5 replicate analyses of the certified standard materials, SRM 1640, based on 2 independent methods.

Key Words: Cu(II), kinetic spectrophotometry, fixed-time method, ascorbic acid, brilliant cresyl blue, catalytic effect

Introduction

Nowadays, there is ever-increasing concern about toxicity of trace metals. As a consequence of rapid industrialization, the concentrations of many metals and other toxic chemical species have been increasing in the

*Corresponding author

environment and accelerating grievous pollution of the precious gifts of nature, namely air, water, and land. Copper is one of these pollutants. The increase in copper concentrations in the environment results from industrial and domestic waste discharge, disposal of mining washings, refineries, and the use of copper as a base material for antifouling paint. Copper is essential to human health, but only in limited amounts. Hereditary diseases such as Wilson's disease retain excessive amounts of copper in the body and cause fatal consequences like liver damage.¹ Violent tendencies in males, depression, and schizophrenia also have links to high copper levels.² Thus, copper has a drastic effect on human health and is a potent environmental pollutant. It is therefore extremely important to develop a cost-effective, sensitive, selective, and rapid analytical method for trace determination of copper.

Due to various biological roles of copper(II), its determination in biological samples in particular is very important. Moreover, copper is emitted into the environment due to its wide uses in various industries.³ Therefore, determination of copper in environmental sample is also important. In recent years, many methods for the determination of trace copper have been reported, such as electrothermal atomic absorption spectrometry,⁴ spectrophotometric flow injection method,⁵ phosphorescence quenching method,⁶ atomic absorption spectrometry after on-line solid phase extraction,⁷ graphite furnace-atomic absorption spectroscopy,⁸ novel quantum dot as fluorescent sensor,⁹ cloud point extraction, and after that spectrometric determination,¹⁰ flow-injection chemiluminescence,¹¹ optical fiber reflectance sensor,¹² and thermospray flame furnace-atomic absorption spectroscopy after on-line sorbent preconcentration.¹³

The development of new kinetic methods in analytical chemistry can be attributed to the need to analyze very small amounts of substance, to gain better knowledge of reaction mechanisms, and especially the great advancement in instrumental techniques, particularly in the field of computerization. These methods have the advantages of high sensitivity, extremely low detection limit, good selectivity, rapid analysis rate, and inexpensive instruments. Many kinetic methods have been proposed for Cu(II) determination at trace levels based on the catalytic effect of this ion on various inorganic and organic indicator reactions.¹⁴ However, few of them have been proposed for the determination of copper in real samples. The chief reason is that, in most of the kinetic catalytic methods that have been proposed for the determination of copper, high sensitivity is accompanied by poor selectivity,¹⁵ usually because several transition-metal ions show similar catalytic effects. Methods with good selectivity usually have poor limits of detection.¹⁶ Hence, the development of more sensitive and selective catalytic methods for the kinetic determination of copper is still sought. Recently, many analytical method development studies about the kinetic determination of trace level copper(II) concentrations have been given in the literature in detail.^{17–27}

The main aim of the present work was to develop a simple, selective, and sensitive system for determination of Cu(II) at trace levels by a kinetic spectrophotometric method based on its catalytic effect on the reduction of brilliant cresyl blue (BCB) with ascorbic acid in pH 5 acetate buffer media. The fixed-time approach of 7 min was adopted for the measurement of absorbance at (λ_{\max} : 624 nm) wavelength of maximum absorption of BCB at pH 5.0. The results obtained led to the development of a new kinetic method for the determination of Cu(II) with a wide linear range of 50-fold, good reproducibility, and low detection limit. The procedure was successfully applied for determination of Cu(II) in natural waters, artificially prepared water samples, the certified standard reference materials such as SRM 1640. The accuracy and precision of results were comparable to those obtained by flame atomic absorption spectroscopy (AAS).

Experimental

Reagents and solutions

Ascorbic acid solution (0.04 M) was prepared by dissolving a suitable amount of solid reagent and diluting with deionized water. A stock solution of BCB (1×10^{-3} M) was prepared by dissolving it in deionized water. The acetate buffer solution, pH 5.0, was prepared by dissolving solid CH_3COONa at the known amounts with HCl via a pH meter. The solution of CuCl_2 (1000 mg L^{-1}) was prepared by dissolving solid CuCl_2 . The working solutions of CuCl_2 were prepared by step dilution immediately before use. All chemicals were of analytical reagent grade and were provided by Merck unless indicated otherwise. All stock solutions were stored in polyethylene containers. All labware used for handling solutions was cleaned with detergent solution, soaked in 1:1 (v/v) HCl, followed by vigorous shaking, and rinsed thoroughly with deionized, distilled water.

Instrumentation

A Shimadzu Model UV-1800 spectrophotometer equipped with a constant temperature cell was used for absorbance measurements. This spectrophotometer has a wavelength accuracy of ± 0.2 nm and a bandwidth of 2 nm in the wavelength range of 190-1100 nm. An atomic absorption spectrometer (Shimadzu AAS-6300), equipped with a copper hollow cathode lamp and an air-acetylene flame atomizer, was also used for additional analysis of real samples in order to check the accuracy of the kinetic method. The wavelength, lamp current, slit width, burner height, and acetylene, air, and aspiration flow rates used were 324.8 nm, 4 mA, 0.5 nm, 13.5 mm, 2.00 L min^{-1} , 13.50 L min^{-1} and 3.2 L min^{-1} , respectively. A pH meter consisting of a glass-calomel electrode double was used to determine pH values of the solutions. All solutions were preheated to a working temperature of 40°C before the initiation of the indicator reaction.

General procedure

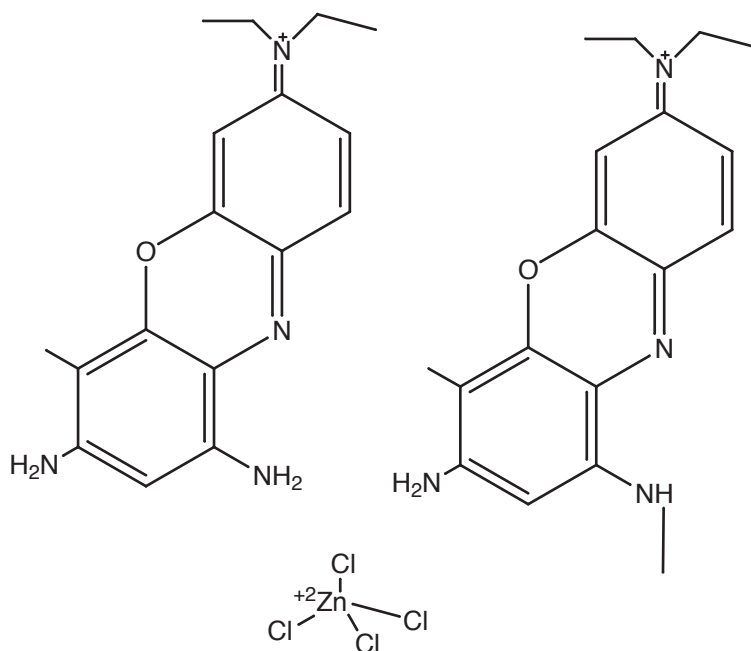
In a 10.0 mL volumetric flask were mixed 1.5 mL of acetate buffer (pH 5.0), 1.3 mL of 2.5×10^{-4} M BCB solution, and a sample solution containing $0.1\text{-}5.0 \mu\text{g L}^{-1}$ Cu(II); the solution was then diluted to about 8 mL with distilled water, and placed in a 40°C water bath. After that, 1 mL of 0.04 M ascorbic acid solution was added to the solution; it was made up to the mark with distilled water. The absorbance change at 624 nm was recorded 7 min after the initiation of the reaction. The zero-time was taken as the moment at which the last drop of indicator dye was added. A blank solution was prepared in a similar way without copper. The corrected absorbance change (the difference between the absorbance change of the blank solution, ΔA_0 and the solution containing copper as catalyst, ΔA_c , $\Delta(\Delta A)$) was plotted as a function of the copper(II) concentration.

Results and discussion

BCB is a quinine-imide dye with a planar structure and many analytical applications.²⁸ The open molecular formula of BCB is shown in the Scheme.

Ascorbic acid reduces BCB, being a basic oxazine group cationic dye, at pH 5.0, but the indicator reaction is very slow. However, in the presence of Cu(II) ions at trace levels as a catalyst the indicator reaction

is strongly catalyzed. The catalyzed-reaction was completed and reached equilibrium in 7 min. The indicator reaction system showed an absorption maximum at a characteristic wavelength of 624 nm in the visible region. The absorbance change in the Cu(II)-catalyzed system with intervals of 1 min in the first 7 min as a function of reaction time can clearly be seen in Figure 1a. In addition, a calibration spectrum was scanned at 624 nm by analysis of standard Cu(II) solutions changing in the range of 0-4 $\mu\text{g L}^{-1}$ under the optimized experimental conditions. The calibration spectrum obtained is given in Figure 1b. Under the conditions where the other experimental variables were kept optimum, the effect of heating time was investigated. A heating time of 1, 3, 5, 7, 9, 12, and 15 min was used consecutively and contrasted with blank reagent. The results showed that a linear relationship was present between ΔA and t over the range 1-7 min. At 7 min, ΔA was a maximum. Therefore, a heating time of 7 min was chosen. The regression equation calculated was $\Delta A = 0.0725t$ (min) $- 0.0612$ with a correlation coefficient of 0.9974. The rate constant of the catalyzed-reaction was $k = 1.25 \times 10^{-3} \text{ s}^{-1}$. The half-life period was 3.45 min. Therefore, 7 min was selected for further studies.



Scheme. The open molecular structure of BCB (BCB^+ , λ_{max} : 624 nm).

Effect of pH and buffer concentration

In order to determine the lowest possible detectable concentration for copper, the catalytic reaction variables need to be optimized. Therefore, the dependence of both the uncatalyzed- and catalyzed-reaction rates upon each reagent concentration was identified. With all other experimental parameters kept constant, the dependence of the indicator system on pH using the different buffers changing between 1.0 and 10.0 such as citrate buffer, acetate buffer, phosphate buffer, and $\text{NH}_3/\text{NH}_4\text{Cl}$ buffer solutions was examined. The results are shown in Figure 2a in which the indicator reaction has the highest sensitivity in the presence of the pH 5.0 acetate buffer.

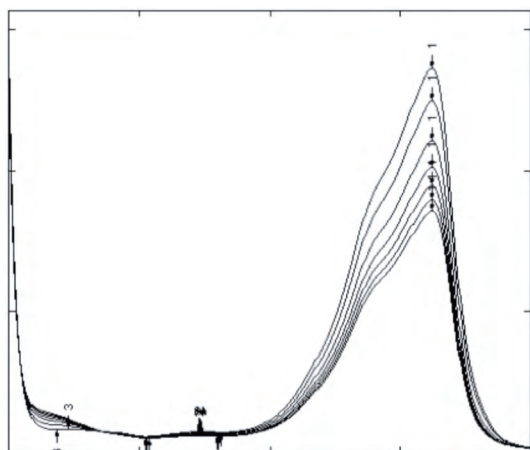


Figure 1a. The change in absorbance with time.^a

^aOptimal conditions: 1.5 mL pH 5.0 acetate buffer, 1.3 mL BCB, 3 $\mu\text{g L}^{-1}$ Cu(II), 1.0 mL 0.04 M ascorbic acid for fixed time of 7 min at 40 °C.

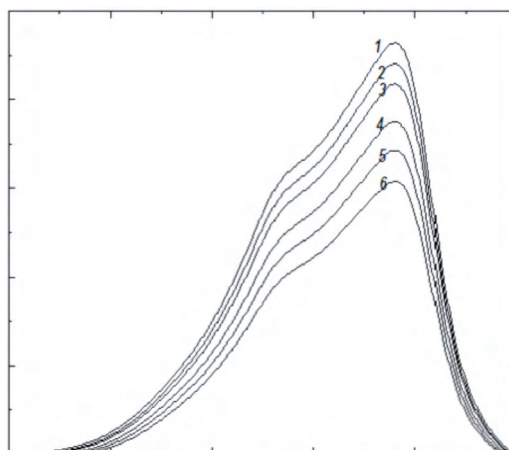


Figure 1b. The calibration spectrum obtained by means of standard Cu(II) solutions changing in the range of 0-4 $\mu\text{g L}^{-1}$ Cu(II) as a catalyst.^b

^bOptimal conditions: 1.5 mL pH 5.0 acetate buffer, 1.3 mL BCB, 3 $\mu\text{g L}^{-1}$ Cu(II), 1.0 mL 0.04 M ascorbic acid for fixed-time of 7 min at 40 °C. (1. Reagent blank 2. 0.5 $\mu\text{g L}^{-1}$ Cu(II) 3. 1.0 $\mu\text{g L}^{-1}$ Cu(II) 4. 2.0 $\mu\text{g L}^{-1}$ Cu(II) 5. 3.0 $\mu\text{g L}^{-1}$ Cu(II) 6. 4.0 $\mu\text{g L}^{-1}$ Cu(II))

The effect of the concentration of the buffer solution was also examined. From the experimental results, it was found that $\Delta(\Delta A)$ increased with increasing buffer concentration up to 1.5 mL and stayed constant and gradually decreased. When the volume of buffer was 1.5 mL, $\Delta(\Delta A)$ showed a maximum and constant value. Therefore, 1.5 mL of acetate buffer (pH 5.0) was used as the optimal value in the next experimental step (Figure 2b).

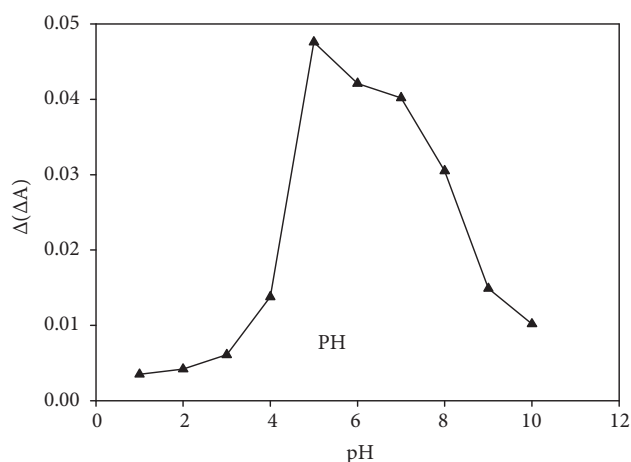


Figure 2a. Effect of pH on analytical sensitivity.

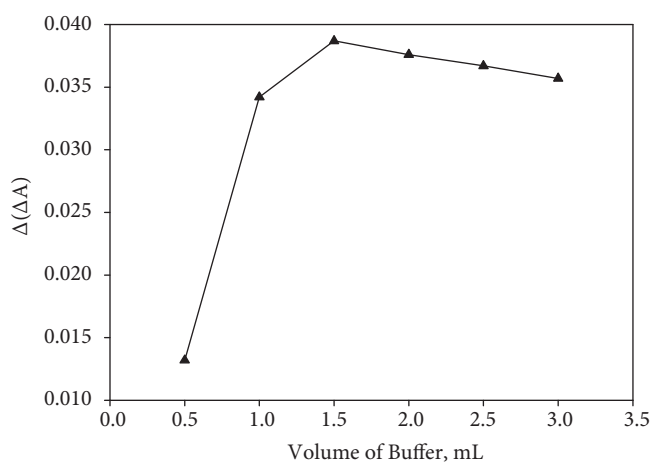


Figure 2b. Effect of buffer volume on analytical sensitivity.

Effect of ascorbic acid concentration

The effect of ascorbic acid concentration on analytical sensitivity was examined in the range of $(0.4-6.0) \times 10^{-3}$ M. The results showed that $\Delta(\Delta A)$ increased with the increase in the concentration of ascorbic acid at the beginning and then gradually reduced. When the concentration of ascorbic acid was 2.0×10^{-3} M, $\Delta(\Delta A)$ gave a maximum value. Therefore, an ascorbic acid concentration of 2.0×10^{-3} M was used as the optimal value for further studies (Figure 3a).

Effect of BCB concentration

The effect of BCB concentration was investigated at $(0.75-7.5) \times 10^{-5}$ M. The results showed that high sensitivity could be obtained when the concentration of BCB was in the range of $3.25-5.0 \times 10^{-5}$ M, whereas the analytical sensitivity was lower at the higher concentrations. When the concentration of BCB was high, a large analytical signal error was easily caused and the repeatability was getting worse due to aggregation of dye. In order to guarantee high sensitivity and a suitable linear detection range, an indicator dye concentration of 5.0×10^{-5} M was taken into consideration as the optimal value in this study (Figure 3b).

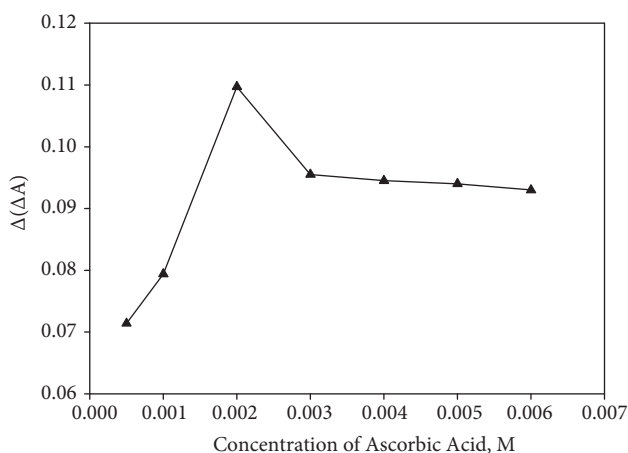


Figure 3a. Effect of ascorbic acid concentration on analytical sensitivity.

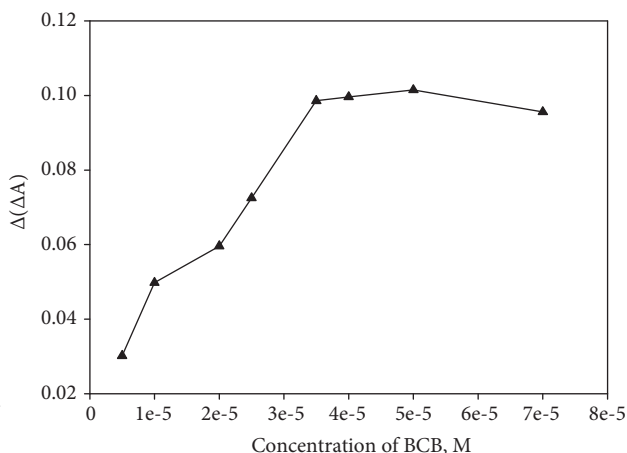


Figure 3b. Effect of BCB concentration on analytical sensitivity.

Effect of reaction temperature

Under the selected optimum conditions, the effect of reaction temperature on analytical sensitivity, $\Delta(\Delta A)$, was examined in the range of 25-60 °C. The results showed that the reaction rate increases with a low slope with temperature rising up 55 °C and that catalyzed reaction rate or analytical sensitivity increased with a high slope with increasing temperature after this temperature. The cause of this is the fact that the speed of the catalyzed reaction is progressing faster than the speed of the uncatalyzed reaction. A reaction temperature of 40 °C was taken into consideration as an optimal value as it gave stable and sufficient analytical sensitivity (Figure 3c).

Effect of reaction media

Under the optimal conditions selected, the effect of ionic strength intensity on the analytical sensitivity, $\Delta(\Delta A)$, was examined in the concentration range of 0.001-0.500 M NaCl. The results showed that the reaction rate changed very little with increasing concentration until 0.100 M; after this concentration it exhibited a negative change with increasing inclination. This case implied that the catalyzed-indicator reaction would give the right response for catalyst in real life samples with a low matrix. It can be expressed that inert salt effect should be checked in matrix systems with a high salt content, such as sea water (Figure 3d).

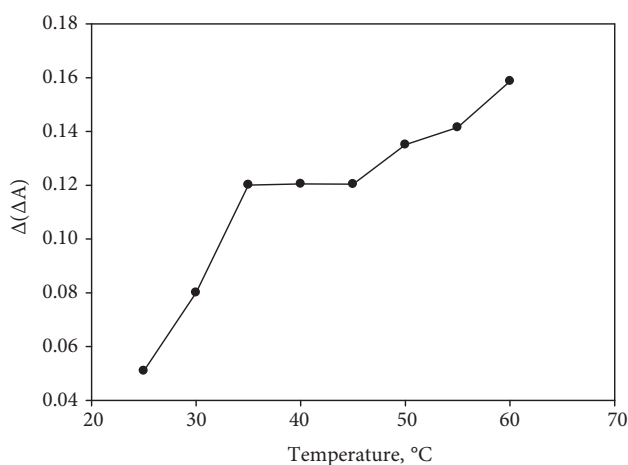


Figure 3c. Effect of reaction temperature on analytical sensitivity.

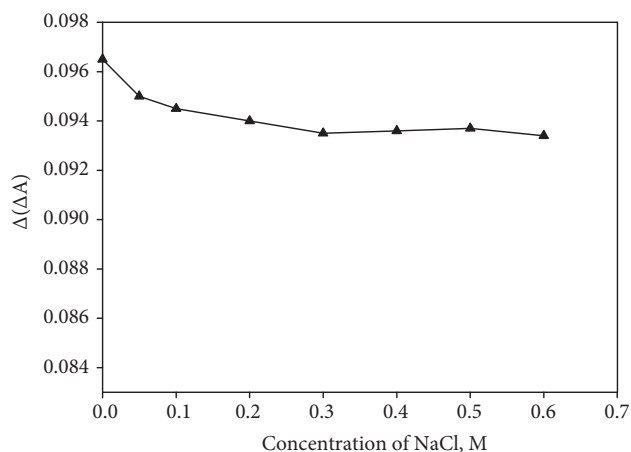


Figure 3d. Effect of ionic intensity of environment on analytical sensitivity.

Calibration curve, precision, and detection limit

Different Cu(II) standard calibration solutions were sampled to measure net absorbance change, $\Delta(\Delta A)$ (that is, $\Delta A_0 - \Delta A_C$), of noncatalytic and catalytic systems according to the optimal experimental conditions. The results showed that $\Delta(\Delta A)$ versus concentration of Cu^{2+} ion in the range of 0.1-5.0 $\mu\text{g L}^{-1}$ obeyed a very wide linear relation. The following regression equation was obtained in this linear range:

$$\Delta(\Delta A) = 0.2920[\text{Cu(II)}, \mu\text{g L}^{-1}] + 0.0155 \quad (\text{N: } 7, \text{r: } 0.9983, \text{S}_r: 0.0021, \text{S}_a: 0.0035, \text{S}_b: 0.0108)$$

in which S_a , S_b , and S_r are uncertainties in slope, intercept, and regression coefficient for the calibration curve, respectively. The detection and quantification limits of the developed kinetic method, as LOD and LOQ respectively, were 0.025 and 0.086 $\mu\text{g L}^{-1}$, calculated by dividing 3σ and 10σ by the slope of calibration curve ($3\sigma/m$ and $10\sigma/m$) for 10 replicate measurements of analyte blank solution without catalyst. Five replicate determinations at different concentration levels in the range of 0.1-5.0 $\mu\text{g L}^{-1}$ were carried out to test the precision and accuracy of the proposed method. The relative standard deviation (RSD) and analytical error (RE) are shown in Table 1.

Table 1. Accuracy and precision obtained by the proposed kinetic method.

Added Cu(II), $\mu\text{g L}^{-1}$	Found Cu(II), $\mu\text{g L}^{-1}$	*Relative error (RE%, N: 5)	*Relative standard deviation (RSD%, N: 5)
0.3	0.293 ± 0.002	-2.33	0.68
0.5	0.511 ± 0.003	+2.20	0.59
0.8	0.815 ± 0.004	+1.88	0.49
1.0	1.017 ± 0.003	+1.70	0.29
3.0	3.026 ± 0.007	+0.87	0.23

*The RE and RSD are a measure of accuracy and precision of the method for 5 replicate measurements of fixed Cu(II) solutions at concentrations varying in the range of 0.3-3.0 $\mu\text{g L}^{-1}$

Method robustness was tested by making small incremental changes in BCB and ascorbic acid concentrations and reaction time were altered. To check the ruggedness, analysis was performed by 2 different analysts and on 2 different spectrophotometers by the same analyst. The robustness and the ruggedness were checked at 3 different copper levels: 1.0, 3.0 and 5.0 $\mu\text{g L}^{-1}$. The intermediate precision, expressed as percent RSD, which is a measure of robustness and ruggedness was within the acceptable limits as shown in Table 2.

Table 2. Robustness and ruggedness expressed as intermediate precision (%RSD).

The proposed kinetic method	Cu(II) taken, $\text{L}^{-1} \mu\text{g}$	Method robustness			Method ruggedness	
		Parameter altered				
		BCB (2.5×10^{-4} M) mL^a RSD, % (n = 3)	Ascorbic acid (0.04 M) mL^b RSD, % (n = 3)	Reaction time ^c RSD, % (n = 3)	Inter- analysts RSD, % (n = 5)	Inter- instruments RSD, % (n = 5)
	1.0	2.31	2.24	2.39	2.09	2.24
	3.0	2.16	2.18	2.35	2.13	2.30
	5.0	2.21	1.87	2.18	1.98	2.17

^a BCB volumes used were 0.8, 1.0 and 1.2 mL. ^b Ascorbic acid volumes used were 0.8, 1.0 and 1.2 mL. ^c Reaction times of 3, 5 and 7 min in the developed kinetic method were employed

Interference study

In order to determine the selectivity of the kinetic method, the effect of anionic and cationic interfering species on the catalytic reaction rate was studied at constant copper concentration of 1.0 $\mu\text{g L}^{-1}$. The results are given in Table 3. The results showed that when determining 1.0 $\mu\text{g L}^{-1}$ copper in a final volume of 10 mL under the optimal reaction conditions and when the relative error is below $\pm 5\%$, the following concurrent ions had no interference in kinetic measurements.

Table 3. Effect of some interfering species on the determination of $1.0 \mu\text{g L}^{-1}$ Cu(II) by the present kinetic method.

Interfering Species	*Tolerance Level, [$C_{interfering}/C_{analyte}$]
Na^+ , K^+ , Li^+ , NH_4^+ , Cl^- , F^- , $\text{C}_2\text{O}_4^{2-}$	2000
SeO_4^{2-} , SO_3^{2-} , HPO_4^{2-} , SO_4^{2-} , Ca^{2+}	1000
SeO_3^{2-} , Cd^{2+} , Ni^{2+} , Cr^{3+} , Zn^{2+} , Mn^{2+}	250
Ce^{3+} , Fe^{3+} , Ag^+ , VO^{2+} , Hg_2^{2+}	100
Hg^{2+} , Cu^{2+} , NO_2^- , I^- , CN^-	50
VO_2^+ , Br^- , Cr(VI)	10

*Tolerance level is described as concentration ratio of interfering ion to analyte when the relative error in determination of $1.0 \mu\text{g L}^{-1}$ Cu(II) is below $\pm 5\%$

Analytical applications

To evaluate the analytical applicability of the method, the proposed procedure was applied to the determination of copper in artificially prepared wastewater and natural spring water samples using the calibration curve method, and the standard addition curve method to suppress the matrix effect when necessary. The water samples were preconcentrated in order to obtain reliable results by AAS,²⁹ whereas this treatment is not required in the kinetic method. For comparing the results of the kinetic method with those of an established standard method (AAS method), we applied Student's t-test to compare the 2 sample means. It can be concluded from Table 4 that there is no significant difference between the results obtained by the 2 methods. By using F-test it was shown (Table 4) that there is no significant difference in the precision of the 2 methods. The results show that the current kinetic method is suitable for the analysis of real samples.

In order to test the validation of the newly developed kinetic method, the method was also applied to the determination of copper in a certified standard water sample. The standard reference material, SRM 1640, was employed without any pretreatment procedure. The recovery of spiked Cu(II) was found to be quantitative and the reproducibility was satisfactory. It was observed that the result was in good agreement with the certified value. For comparison of the results found using the present method, the certified sample was also analyzed by means of the standard AAS method. It was found that the results obtained by the present method were in agreement with those of the AAS method. The results are summarized in Table 4. It was observed that the results obtained by the 2 independent methods were in good agreement with the certified value after multiplying the obtained value by a dilution factor of 1000. The reason for the difference in mean value obtained by the calibration curve method for the certified sample may be the interfering effect arising from matrix components at ultra-trace levels. This effect was greatly controlled by using the standard addition curve method. Another reason for the difference in the mean and its standard deviation may be either its dilution effect at 1:1000 ratio prior to kinetic analysis or not sufficiently good reproducibility as a measure of reliability at very low concentrations. As a result, it can be concluded that the proposed kinetic method for determining trace levels of copper available in real samples is a useful, economic, fast, and applicable method.

Table 4. Determination of copper ($\theta \text{ g L}^{-1}$) in water samples by using the present kinetic method and standard AAS method.

Samples	Added Cu(II), $\mu\text{g L}^{-1}$	The proposed kinetic method		AAS method ^{a,29}		Student's t-test**	F-test***
		Found \pm SD, $\mu\text{g L}^{-1}$	Recovery%	Found \pm SD*, $\mu\text{g L}^{-1}$	Recovery%		
Commercially marketed spring water samples							
S ₁	-	ND	-	ND	-	-	-
	0.5	0.478 \pm 0.017	95.60	0.490 \pm 0.013	98.00	0.89	1.71
	1.5	1.529 \pm 0.020	101.93	1.540 \pm 0.012	102.67	0.77	2.78
S ₂	3.0	2.963 \pm 0.024	98.77	2.980 \pm 0.012	99.33	1.01	4.00
	-	ND	-	ND	-	-	-
	0.5	0.466 \pm 0.016	93.20	0.480 \pm 0.013	96.00	1.05	1.58
S ₃	1.5	1.499 \pm 0.017	99.93	1.520 \pm 0.012	101.33	1.56	1.96
	3.0	2.961 \pm 0.018	98.70	2.970 \pm 0.012	99.00	0.62	2.25
	-	ND	-	ND	-	-	-
Artificially prepared waste water sample ^b	0.5	0.491 \pm 0.019	98.20	0.500 \pm 0.013	100.00	0.62	2.25
	1.5	1.503 \pm 0.018	100.20	1.520 \pm 0.012	101.33	1.24	2.25
	3.0	2.963 \pm 0.018	98.77	2.980 \pm 0.012	99.33	1.24	2.25
SRM 1640 ^c (After diluting at a ratio of 1:1000)	3.0	3.09 \pm 0.020	103.00	3.10 \pm 0.013	103.33	0.76	2.34
	0	0.073 \pm 0.020 (0.081 \pm 0.019) ^e	85.68 ^d 95.07	0.085 \pm 0.013	99.76 ^d	0.83	2.30
	0.5	0.571 \pm 0.019	99.60	0.580 \pm 0.013	99.00	0.62	2.27
	1.0	1.063 \pm 0.018	99.00	1.080 \pm 0.012	99.50	1.24	2.24
	3.0	3.063 \pm 0.018	99.67	3.080 \pm 0.012	99.83	1.24	2.23

^aAAS values for the copper content of different water samples using the extractive preconcentration method based on the stable Cu(II)-pyrrolidinedithiocarbamate complex formation
^bContained 3 $\mu\text{g L}^{-1}$ Cu²⁺, plus 25 $\mu\text{g mL}^{-1}$ of Fe³⁺, Ag⁺, VO²⁺, and Hg²⁺, 100 $\mu\text{g mL}^{-1}$ of Cd²⁺, Ni²⁺, Cr³⁺, Zn²⁺, and 500 $\mu\text{g mL}^{-1}$ of HPO₄²⁻, SO₄²⁻, Ca²⁺, AAS values for the copper content of different water samples using the extractive preconcentration method based on the stable Cu(II)-pyrrolidinedithiocarbamate complex formation
^cStandard reference material, SRM 1640 (Trace element in Natural Water) issued by National Institute of Standard and Technology (NIST). The certified value of copper is 85.2 \pm 1.2 $\mu\text{g L}^{-1}$
^dThe percent recoveries, based on the consideration of certified value for analysis of samples by 2 independent methods before standard addition method
^eThe result given in parentheses is the mean value and its standard deviation obtained by means of standard addition curve method

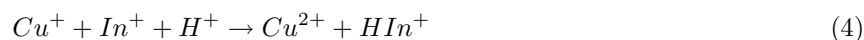
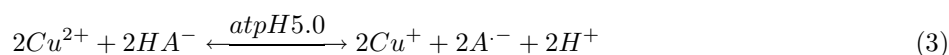
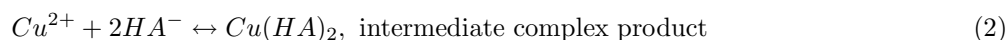
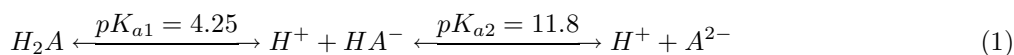
*Average value plus standard deviation of 5 replicate measurements

**Statistical t-value for 8 degrees of freedom (N₁ + N₂ - 2) at 95% confidence level (2.31)

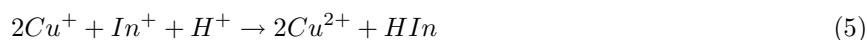
***Statistical F(4,4)-value for 4 degrees of freedom at 95% confidence level (6.39)

The possible catalytic reaction mechanism

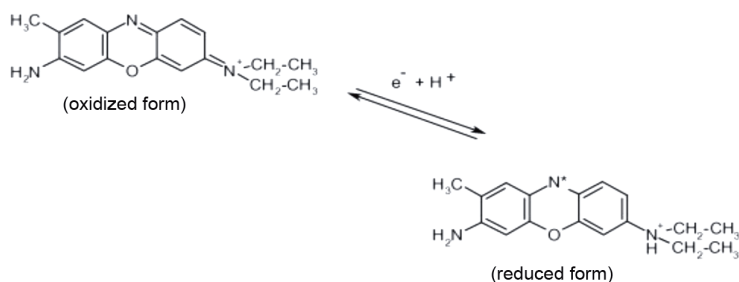
The possible catalytic reaction mechanism for the indicator system at pH 5.0 may be indicated by a series of reactions as follows:



or



The general reduction reaction of BCB^+ with ascorbic acid in the presence of trace amounts of copper(II) as a catalyst at pH 5.0 can be represented as follows:



where In^+ and HIn^+ are the oxidized and reduced forms of the monomeric indicator dye, and HA^- and A^{2-} are the oxidized and reduced forms of ascorbic acid in the redox reaction. L-ascorbic acid exists mainly in 3 different ionized forms: the fully protonated L-ascorbic acid (H_2A), L-ascorbic acid monoanion (HA^-), and the dianion (A^{2-}). All 3 forms are capable of reducing both indicator dye and Cu^{2+} ion to afford reducing products and forming an intermediate complex with Cu^{2+} ions; however, from the results of pH variation it is quite clear that HA^- species are probably the most reactive species in the optimum reaction conditions. It was found that the addition of Cu^{2+} at trace amounts was very effective at decomposing dye molecule to produce reduced products according to those of only ascorbic acid. This is clearly indicated by a dramatic decrease in absorbance of indicator dye at the working wavelength of 624 nm.

Table 5. Comparison of the proposed kinetic method with the existing Cu(II)-catalyzed kinetic methods mentioned in the literature.

Indicator reaction	Dynamic range ($\mu\text{g L}^{-1}$)	Detection limit ($\mu\text{g L}^{-1}$)	Conditions (Temperature, λ_{max} , method)	Type of samples	Kinetic parameters and remarks	References
Rhodamine B + H_2O_2	0.8-5.6	-	555 nm, 100 °C fixed-time method, 17 min	Serum		16
Oxidative coupling reaction of 3-methyl-2-benzothiazolone hydrazine with N-ethyl-N-(2-hydroxy-3-sulfo-propyl)-3,5-dimethoxyaniline	0.005-0.75	-	525 nm, pH 5.9, micellar medium, 60 °C	Pepperbush certified reference material	RSD, 0.21% and 2.0% for 0.5 and 0.05 $\mu\text{g L}^{-1}$	17
Hexacyanoferrate (III) + cysteine (RSH)	0-6.35	0.15	420 nm, 25 °C, short analysis time of 1 min, fixed time method	Synthetic and real samples		18
Dibromo-p-chloro-arsenazo (DBC-ASA) + H_2O_2	0.1-1.2 $\mu\text{g mL}^{-1}$	3.2	514 nm, 1.2×10^{-3} M H_3PO_4 medium, 100 °C, 8 min	Water samples		19
p-acetylchlorophosphonazo (CPApA) + H_2O_2	0.02-0.3 $\mu\text{g mL}^{-1}$	10.94 $\mu\text{g L}^{-1}$	554 nm, 0.1 M H_3PO_4 , 100 °C, 13 min	Tomato and cucumber samples	RSD, 1.20%-1.34% (n: 13)	20
Sulfamic acid + H_2O_2	0.5-6 $\mu\text{g mL}^{-1}$	0.028 $\mu\text{g mL}^{-1}$	330 nm, 25 °C, pH 6.0 fixed-time method, 5 min	Human hair	RSD, 1.6% for 0.02 $\mu\text{g mL}^{-1}$	21
Azure B + Sulfide + CTAB	50-1600	9.2	647 nm, 60 °C fixed-time method, 10 min	Water samples	RSD, 0.9% for 400 $\mu\text{g L}^{-1}$	22
Nuclear fast red + KIO_4 + Tween 20	0.05-20	0.0144	530 nm, pH 7.20 $\text{KH}_2\text{PO}_4/\text{NaOH}$ buffer	Pharmaceutical products and electric copper wire	RSD, below 4.6%	23
3-Methyl-2-benzothiazolinone hydrazone + H_2O_2	0-50	0.27	390 nm, 35 °C fixed-time method, 40 min	River water and ground water		24
Weak acid brilliant blue dye (RAWL) + H_2O_2	0-12	0.011	626 nm, 80 °C, fixed-time method of 20 min	Freshwater and seawater samples	RSDs, 3.2% and 2.3% for 2 and 8 $\mu\text{g L}^{-1}$	25
Thionine + H_2O_2 + 1,10-Phenanthroline	1-160	0.7	600 nm, alkaline medium	Real samples	RSD, 0.75%-1.71% for 2, 20, 50, and 100 $\mu\text{g L}^{-1}$	26
Thioglycolic (TGA)/thioloacetic acid (TLA) + $\text{K}_2\text{Cr}_2\text{O}_7$	8-1900 (TGA), 150-1920 (TLA)	65 for TGA, 80 for TLA	345 nm, 20 °C	River and wastewater		27
Xylydyl blue + H_2O_2	2.5-75	0.85	570 nm, 30 °C fixed-time method, 3 min	Tap water, wastewater and drinking water	E_a : 50.70 kJ mol^{-1} and λ_{cat} : - 100.46 $\text{J K}^{-1} \text{mol}^{-1}$ for the Cu(II) catalyzed-reaction	30
Brilliant cresyl blue + Ascorbic acid	0.1-5	0.025, 0.086 $\mu\text{g L}^{-1}$	570 nm, 40 °C fixed-time method, 7 min	Artificially prepared wastewater and natural drinking water	$t_{1/2}$: 3.45 min, k: $1.25 \times 10^{-3} \text{ s}^{-1}$ for the Cu(II) catalyzed-reaction	The present method

Conclusions

In order to determine copper at trace levels the sensitivity and selectivity of the newly developed kinetic method are very good except for Cr(VI) ion. The method is based on the catalytic effect of copper on the reduction of BCB by ascorbic acid at pH 5.0 acetate buffer. Its applicability was quantitatively verified with the results of the analysis of artificially prepared waste water and 3 different drinking water samples. The data presented in Table 4 to determine copper in real samples show that the performance of the method is very good. The method is comparable with many of the spectrophotometric and kinetic spectrophotometric methods reported in the literature in terms of detection limit, linear range, repeatability, reproducibility, sensitivity, and selectivity, and so it can be successfully implemented for trace analysis of copper in real samples.

Validation of the method was achieved by determination of Cu(II) present in the certified water sample by using the standard AAS method, and the results obtained using both methods statistically showed a good agreement in view of accuracy and precision. The current kinetic method is simple and fast, and can be successfully used to determine copper at trace/ultra-trace levels in poor surface waters of humic and fulvic acid. The method has the advantages of low cost, a linear range of 50-fold, reproducibility, accuracy, and, most importantly, low DLs, comparable with the DLs obtained by the existing catalytic kinetic techniques for the determination of trace quantities of Cu(II) present in water samples^{16-27,30} in Table 5. Furthermore, it has a reasonable reaction temperature and short reaction time compared to the other kinetic methods.

Acknowledgements

The authors are grateful to the Cumhuriyet University Research Council for partial support of the present study. We thank the referees for their helpful comments and scientific contributions.

References

1. Brewer, G. J. *Biol Med.* **2000**, *223*, 39-46.
2. Walsh, W. J.; Isaacson, H. R.; Rehman, F. *Physiol. Behav.* **1997**, *62*, 327-329.
3. Gad, S. C. *Encyclopedia of Toxicology*, Academic Press, New York, 2005.
4. Pinto, F. G.; Andrada, D.; Rey, U. V.; Ansaloni, L. M. S.; Silva, J. B. B. *Anal. Lett.* **2006**, *39*, 2441-2445.
5. Lunvongsa, S.; Takayanagi, T.; Oshima, M.; Motomizu, S. *Anal. Chim. Acta*, **2006**, *576*, 261-269.
6. Liu, J. M.; Lin, X.; Xu, S. S.; Lu, Q. M.; Zeng, L. Q.; Lin, S. Q. *Spectrosc. Lett.* **2006**, *39*, 311-320.
7. Anthemidis, N.; Ioannou, K. G. *Anal. Chim. Acta* **2006**, *575*, 126-131.
8. Minami, T.; Sohrin, Y.; Ueda, J. *Anal. Sci.* **2005**, *21*, 1519-1524.
9. Lai, Y.; Yu, Y.; Zhong, P.; Wu, J.; Long, Z.; Liang, C. *Anal. Lett.* **2006**, *39*, 1201-1206.
10. Lemos, V. A.; Santos, J. S.; Baliza, P. X. *J. Braz. Chem. Soc.* **2006**, *17*, 30-35.
11. Li, B.; Wang, D.; Liv, J.; Zhang, Z. *Talanta* **2006**, *69*, 160-165.
12. Sundari, R.; Ahmad, M.; Heng, L. Y. *Sensor. Actuat. B-Chem.* **2006**, *113*, 201-208.

13. Tarley, C. R. T.; Figueiredo, E. D. C.; Matos, G. D. *Anal. Sci.* **2005**, *21*, 1337-1345.
14. Mottola, H. A.; Perez-Bendito, D. *Anal. Chem.* **1992**, *54*, 407-411.
15. Safavi A.; Tavallali, H. *Anal. Sci.* **1995**, *11*, 453-55.
16. Khan, M. N.; Sarvar, R. A. *Anal. Sci.* **2001**, *17*, 1195-1197.
17. Kawashima, T.; Itabashi, H.; Teshima, N.; Kurihara, M. *Anal. Sci.* **1999**, *15*, 835-839.
18. Prasad, S.; Halafih, T. *Microchim. Acta* **2003**, *142*, 237-244.
19. Song, B. Q.; Zhai, Z. *Asian J. Chem.* **2009**, *21*, 5670-5676.
20. Zhai, Q. Z. *Bul. Chem. Soc. Ethiopia* **2009**, *23*, 236-240.
21. Ghasemi, J.; Kiaee, S. H.; Abdolmaleki, A.; Semnani, A. *Acta Chim. Slov.* **2008**, *55*, 184-185.
22. Safavi, A.; Mirzaee, M.; Hormozi Nezhad, M. R.; Saghiri, N. *Spectrosc. Lett.* **2005**, *38*, 13-22.
23. Du, B.; Liu, Y.; Ding, Y.; Duan, C.; Wei, Q. *Anal. Lett.* **2005**, *38*, 711-725.
24. Tomiyasu, T.; Aikou, S.; Anazawa K.; Samakoto, H. *Anal. Sci.* **2005**, *21*, 917-924.
25. Yanxia, Q.; Hongwei, J.; Huizhen X.; Li L. *J. Ocean Uni. China* **2007**, *6*, 143-150.
26. Bagheri, H.; Tabatabaee, M.; Kerdegari, A. A.; Shahvazian, M.; Shishehbore, M. R. *Asian J. Chem.* 2010, *22*, 661-668.
27. Safavi, A.; Maleki N.; Farjami, F. *Talanta.* **2001**, *54*, 397-402.
28. Kazemzadeh, A.; Moztarzadeh, F. *Sensor. Actuat. B-Chem.* **2005**, *106*, 832-836.
29. Greenberg, A. E.; Clesceri, L. S.; Eaton, A. D. *Standard methods for the examinations water and waste water* (18th ed.), American Public Health Association, Washington, 1992.
30. Gürkan, R.; Ulusoy, H. I.; Dönmez, M. *Intern. J. Environ. Anal. Chem.* 2011, *91*(9), 1-18 (In press).