
Takashi Tomiyasu, Satoshi Aikou, Katsuro Anazawa, and Hayao Sakamoto

Department of Earth and Environmental Sciences, Faculty of Science, Kagoshima University, Korimoto, Kagoshima 890-0065, Japan

A catalytic spectrophotometric method for the determination of traces of copper(II) is proposed. 3-Methyl-2-benzothiazolinone hydrazine (MBTH) is oxidized by hydrogen peroxide to form a yellowish-brown compound. The reaction is accelerated by trace amounts of copper(II), and can be followed by measuring the increase in the absorbance at 390 nm. Since the absorbance at 40 min from the reaction start increases with an increase in the copper(II) concentration, the absorbance value is used as a parameter for copper(II) determination. Under the optimum experimental conditions (8.4 \times 10^{-3} \text{ mol dm}^{-3} \text{ MBTH}, 0.7 \text{ mol dm}^{-3} \text{ hydrogen peroxide, pH 5.2, 35 }^\circ\text{C}), copper(II) can be determined in the range 0 – 50 \mu g dm^{-3}. The relative standard deviations are 6.9, 3.5, 2.7% for 2, 20 and 40 \mu g dm^{-3}, respectively. The detection limit of this method (3\sigma) is 0.27 \mu g dm^{-3}. It was successfully applied to a determination of copper(II) in river water, tap water and ground-water samples. According to the results of a kinetic study, a mechanism is proposed which leads to the following rate equation: \( R_{\text{cat}} = k K_{\text{Cu}} [\text{MBTH}] [\text{H}_2\text{O}_2] / \{1 + K_1 [\text{H}_2\text{O}_2] [\text{H}^+]\} \).

(Received January 11, 2005; Accepted May 26, 2005)

Introduction

Since copper occurs at low concentration levels in many natural water samples, a sensitive method is required for the determination of trace copper ions in such samples. Many methods\(^1\)-\(^13\) have been reported for copper determination, which include spectrophotometric detection,\(^1\) flow system with in-line separation/preconcentration coupled to graphite furnace atomic-absorption spectrometry,\(^2\) and atomic absorption spectrometry.\(^3\)-\(^6\) Among them, the kinetic-catalytic method gives high sensitivity and sufficient accuracy without the need for expensive and special equipment.\(^7\)-\(^13\) Various indicator reactions for the kinetic-catalytic determination of copper have been reported. Lopez et al. proposed a method for the determination of 25 – 380 \mu g dm^{-3} levels of copper(II) by using an aerial oxidation of dianolened bisguanylhydrazine in the presence of pyridine as an activator.\(^7\) Teshima et al. reported a method for the determination of copper(II) in the range 0.1 – 10 \mu g dm^{-3} based on its catalysis on the redox reaction of cystein with iron(III) in the presence of 1,10-phenanthroline.\(^8\) Nakano et al. have developed a catalytic method for the determination of 0.1 - 2.0 \mu g dm^{-3} of copper(II) by using the color formation of N-phenyl-p-phenylene diamide with m-phenylene diamine in the presence of pyridine and ammonia as activators.\(^9\)

On the other hand, most of the studies on kinetic-catalytic methods have mainly been directed at the development of better analytical procedures and their practical applications, and not much work has been done on a complete understanding of the reaction based on mechanistic studies. The interdependence of the reaction variables in the kinetic methods is commonly rather complicated, but a kinetic mechanistic study gives a better understanding of the reaction process, which may extend the analytical utility of the reactions.\(^14\)-\(^18\)

In this study, we found that MBTH is oxidized by hydrogen peroxide to form a product having an absorption maximum at 390 nm; this oxidation reaction is catalyzed by trace amounts of copper. Consequently, a kinetic spectrophotometric method for the determination of copper based on its catalytic effect on the MBTH-hydrogen peroxide reaction was developed. The resulting method is both highly sensitive and reproducible. This method has been applied to the determination of copper(II) in tap, river and ground-water samples. This paper also presents the results of a kinetic study of this catalyzed reaction. A mechanism is proposed that is consistent with the experimental results. The proposed mechanism was utilized to derive a rate equation that describes the quantitative behavior of the reaction throughout the range of the conditions studied.

Experimental

Apparatus and reagents

A Japan Spectroscopic Ubest-35 spectrophotometer was used with a thermostated cell holder coupled with a Japan Spectroscopic PTL396S plotter. The temperature was controlled with a Shibata Science Instrument control unit (CU-85) circulating thermostat bath. For the measurement of absorbance, 1-cm quartz cells were used. In the kinetic study a remote-controlled magnetic Acrobat stirrer (MS Instrument, Osaka, Japan) was installed at the side of the cell holder in the spectrophotometer for mixing a solution in a 1-cm quartz cell.

The water used to prepare the solutions was purified with a
Procedure (8.4 × 10^{-3} mol dm^{-3}) MBTH, 0.7 mol dm^{-3} hydrogen peroxide, pH 5.2, 35˚C). 0.3 cm³ of copper(II), MBTH and hydrogen peroxide solution was added respectively at the arrow in (a).

Milli-Q SP system (Millipore) just before use. Reagent-grade chemicals were used throughout.

A MBTH solution (5.9 × 10^{-2} mol dm^{-3}) was prepared by dissolving 1.4 g of 3-methyl-2-benzothiazolinone hydrazone hydrochloric acid monohydrate in water and diluted to 100 cm³ with water. A commercial 31% solution of hydrogen peroxide (10.2 mol dm^{-3}) was used. The concentration of this solution was checked by permanganate titration. A copper(II) standard solution (1000 mg dm^{-3}, Cu(NO₃)₂ in 0.1 mol dm^{-3} HNO₃) was obtained from Wako Pure Chemical. Working solutions were prepared by diluting this solution with 0.1 mol dm^{-3} HNO₃. A copper(I) standard solution (1000 mg dm^{-3}, CuCl in 0.1 mol dm^{-3} HCl) was prepared by dissolving 0.156 g of copper(I) chloride in 10 cm³ of 1 mol dm^{-3} hydrochloric acid, and diluting to 100 cm³ with water. Working solutions were prepared by diluting this solution with water. An acetate buffer solution (pH 6.7) was prepared by mixing 0.1 cm³ of 2.0 mol dm^{-3} acetic acid and 10 cm³ of a 2.0 mol dm^{-3} sodium acetate solution.

Procedure for the kinetic determination of copper(II)

To 5.0 cm³ of a sample solution in a glass-stoppered tube, 0.5 cm³ of acetate buffer was taken. The mixed solution was then kept at 35˚C in a water bath in order to achieve the required temperature. Then, 1.0 cm³ of a 5.9 × 10^{-2} mol dm^{-3} MBTH solution (35˚C) and 0.5 cm³ of 10 mol dm^{-3} hydrogen peroxide (35˚C) were added into the solution in this order, and the solution was thoroughly mixed. The absorbance of the solution after 40 min from the hydrogen peroxide addition was measured at 390 nm against a water reference. When the reaction was started every 1 min by adding the hydrogen peroxide, forty samples could be treated within 39 min, followed by absorbance measurement every 1 min.

Results and Discussion

Oxidation of MBTH by hydrogen peroxide and the catalytic effect of copper(II)

MBTH was oxidized by hydrogen peroxide to form a yellowish-brown compound. The formation of an oxidized product was accelerated by trace amounts of copper. The reaction could be followed by measuring the absorbance of the product at 390 nm. The absorbance for the copper solution increased with an increase in the reaction time, and reached a maximum value at a given time after adding the hydrogen peroxide solution (Fig. 1 (a), curves I, II). On the other hand, the absorbance for the blank solution increased gradually with the reaction time (Fig. 1(a), curve III). Since the maximum absorbance increased with the increase in the copper(II) concentration, this value was used as a parameter for the copper determination.

The absorbance-time curves for the copper solution reached the maximum according to the copper concentration, and the values were kept for several tens of minutes. This fact suggests that the catalytic effect of copper ion had been lost at the maximum absorbance. The inactivation of the catalytic effect of copper ion was confirmed by following experiments. To the reaction mixtures for both uncatalyzed and catalyzed reactions, copper, MBTH and hydrogen peroxide solution were added, respectively, at 120 min after the reaction started (arrows in Fig. 1(a)). Although an additional increase in the absorbance took place upon the addition of a copper solution, no additional increase in the absorbance was observed upon adding the hydrogen peroxide and MBTH solution (Fig. 1 (a), dotted line).

After several tens of minutes from the reaction start, a brownish substance could be found in the reaction mixture, which may have been an oxidation product of MBTH. It can be considered that the copper ion was adsorbed on the substance and lost its catalytic activity.

Effect of reaction variables

The influence of the temperature on the maximum absorbance was studied in the range 25 - 45˚C under conditions otherwise as in the recommended procedure. Although higher sensitivity was obtained at higher temperature, at higher than 40˚C the absorbance for the uncatalyzed reaction became considerably higher. A temperature of 35˚C was chosen because of the lower blank value and of the convenience for adjusting the temperature throughout the year. The time required for the absorbance to reach the maximum was 40 min at 35˚C; the time was gradually reduced with an increase in temperature. A reaction time of 40 min was selected.

The effect of the pH on the maximum absorbance was examined over the range 4.2 - 5.5. The results are shown in Fig. 2(a). The maximum absorbance for the copper increased with increasing pH, and became constant at pH values higher than 5.0, while no significant changes in the maximum absorbance for a blank solution were observed over the pH range examined. A pH of 5.2 was chosen. The maximum absorbance rose with increasing the MBTH concentration in both the presence of copper and in its absence. Since the effect was more pronounced for a catalyzed reaction, a higher sensitivity could be realized at higher MBTH concentrations (Fig. 2(b)). A value of 8.4 × 10^{-3} mol dm^{-3} was chosen by considering the sensitivity and the solubility of this reagent. The maximum absorbance increased with an increase in the hydrogen peroxide concentration (Fig. 2(c)). The sensitivity of this method increased with an increase in the hydrogen peroxide concentration in the range of 0.1 - 0.4 mol dm^{-3}; above 0.4 mol dm^{-3} the sensitivity remained nearly constant. A hydrogen peroxide concentration of 0.7 mol dm^{-3} was chosen.

Kinetics of the copper-catalyzed reaction between MBTH and hydrogen peroxide

Since the inactivation of copper ion during the reaction was suggested, a kinetic investigation of the copper-catalyzed reaction was carried out by the initial-rate method, in which the initial slopes of the reaction curves (d(Abs.)/dτ = R₀) were manually determined, and then used as a measure of the initial reaction rate (Fig. 1(b)). All concentrations given in figures in the kinetic study were the initial analytical concentrations in the reaction mixture at the initiation of the reaction. To determine
the dependence of $R_0$ upon the copper concentration, a series of experiments was performed in which the copper concentration was varied while the MBTH, hydrogen ion and hydrogen peroxide concentrations were held constant. The plots of $R_0$ versus the concentration of copper [Cu(II)] were linear in the range of $0 - 4.5 \times 10^{-7}$ mol dm$^{-3}$ Cu(II) (Fig. 3). The copper-catalyzed reaction was first order in copper; $R_0 = k_{app}[Cu(II)]_0 + a$, (1) where $k_{app}$ is the apparent rate constant and $a$ is the intercept on the ordinate, which corresponds to the rate of the uncatalyzed reaction. The $k_{app}$ was determined at different MBTH concentrations in the range $1.7 \times 10^{-3} - 8.4 \times 10^{-3}$ mol dm$^{-3}$. As can be seen in Fig. 4, a straight line obtained by plotting $k_{app}$ versus MBTH concentration shows that the relation is given by $k_{app} = b[MBTH]$, where $b$ is the slope of the line. Thus, the initial rate of the catalyzed reaction, $R_{0cat} = R_0 - a$, is rewritten as $R_{0cat} = b[MBTH][Cu(II)]_0$. (2)

The dependence of $b$ on the hydrogen peroxide concentration was determined at a constant hydrogen ion concentration. The $b$ value increased with an increase in the hydrogen peroxide concentration and a straight line was obtained by plotting $b$ versus $[H_2O_2]$ (Fig. 5(a)). The linearity of the plots leads to the following relation:

$$b = [H_2O_2]/(c + d[H_2O_2]),$$

(3)

where $c$ is the slope of the line and $d$ is the intercept on the ordinate of $b$ versus $[H_2O_2]$ plots. Thus, the following relation is obtained:

$$R_{0cat} = [H_2O_2][MBTH][Cu(II)]/[H_2O_2].$$

(4)

Equation (4) means that in the lower concentration range of $H_2O_2$ ($c \approx d$ [H$_2$O$_2$]), the reaction rate increases with an increase in the $H_2O_2$ concentration, and in the higher $H_2O_2$ concentration range ($c \gg d$ [H$_2$O$_2$]), the reaction rate is independent of $H_2O_2$ concentration change. Thus, the complex formation of $H_2O_2$ with copper,

$$Cu(II) + H_2O_2 \rightleftharpoons [Cu(II)·H_2O_2],$$

was suggested. In the lower concentration range of $H_2O_2$, the equilibrium is shifted to the right with an increase in the $H_2O_2$ concentration. Finally, almost all copper ions had formed complexes with $H_2O_2$ and a further increase in the $H_2O_2$ concentration had no effect on the reaction rate. The obtained values of $c$ and $d$ were $1.6 \times 10^{-6}$ mol$^2$ dm$^{-6}$ s and $8.7 \times 10^{-6}$ mol dm$^{-3}$ s, respectively.

The dependence of hydrogen ion on $b$ was determined at constant hydrogen peroxide concentration. As can be seen in Fig. 5(b), a straight line obtained by plotting $b$ versus $[H^+]^{-1}$ shows that the relation is given by $b = e/[H^+]$, where $e$ is the slope of the line, which is $8.8 \times 10^{-2}$ s$^{-1}$. The linearity of the plots leads to the following relation:

$$R_{0cat} = e[MBTH][Cu(II)]/[H^+].$$

(5)
This kinetic behavior suggests a release of hydrogen ion.

**Mechanism of the copper-catalyzed reaction between MBTH and hydrogen peroxide**

The mechanism which is consistent with the rate measurements is thought to be the following:

\[
\text{MBTH} \rightleftharpoons \text{MBTH}^\prime + \text{H}^+ \quad \text{(rapid, } K_1),
\]

\[
\text{Cu(II)} + \text{H}_2\text{O}_2 \rightleftharpoons [\text{Cu(II)-H}_2\text{O}_2] \quad \text{(rapid, } K_2),
\]

\[
\text{MBTH}^\prime + [\text{Cu(II)-H}_2\text{O}_2] \rightarrow \text{P} \quad \text{(rate determining, } k_1),
\]

\[
\text{P} \rightarrow \text{yellowish-brown compound} + \text{Cu(II)} \quad \text{(rapid),}
\]

where P may be an intermediate converting rapidly to the yellowish-brown product and copper ion through several steps.

The formation of the yellowish-brown compound proceeds in accordance to the pathway shown by Eqs. (6) – (9). The rate equation implied by this sequence, \(d[P]/dt = k[M\text{BTH}^\prime] \times [\text{Cu(II)}] \times [\text{H}_2\text{O}_2] \), can be rewritten as follows:

\[
d[P]/dt = k K_1 K_2 [\text{MBTH}][\text{H}_2\text{O}_2][\text{Cu(II)}]/[\text{H}^+] ,
\]

where \( K_1 = [\text{MBTH}^\prime][\text{H}^+]/[\text{MBTH}] \) and \( K_2 = [\text{Cu(II)}-\text{H}_2\text{O}_2]/[\text{H}_2\text{O}_2][\text{Cu(II)}] \).

Equation (10) is combined with the expression for the total analytical concentration of copper,

\[
[\text{Cu(II)}] = [\text{Cu(II)}]_0 + [\text{Cu(II)}-\text{H}_2\text{O}_2],
\]

from which

\[
[\text{Cu(II)}] = [\text{Cu(II)}]_0/(1 + K_2[\text{H}_2\text{O}_2])
\]

leads to

\[
R_{0cat} = k K_1 K_2 [\text{MBTH}][\text{H}_2\text{O}_2][\text{Cu(II)}]/((1 + K_2[\text{H}_2\text{O}_2])[\text{H}^+]).
\]

At constant \([\text{H}^+]\), Eq. (11) is of the same form as the experimentally observed Eq. (4), with \(c\) and \(d\) given by

\[
c = [\text{H}^+]/k K_1 K_2,
\]

\[
d = [\text{H}^+]/k K_1.
\]

At constant \([\text{H}_2\text{O}_2]\), Eq. (11) is of the same form as the experimentally observed Eq. (5), with \(e\) given by

\[
e = k K_1 K_2 [\text{H}_2\text{O}_2]/((1 + K_2[\text{H}_2\text{O}_2])).
\]
Calibration graph and reproducibility

A series of standard solutions of copper was treated as in the recommended procedure. The resulting graph showed a slightly negative deviation (Fig. 7). The relative standard deviations for 12 replicate determinations of 40, 20 and 2 \( \mu \text{g dm}^{-3} \) copper were 2.7, 3.5 and 6.9%, respectively. Copper(I) is oxidized by hydrogen peroxide to copper(II); therefore, copper(I) can also be determined. The calibration graphs for copper(I) were almost identical to those for copper(II) in the same concentration range within the experimental error. The detection limit of this method (3\( \sigma \)) is 0.27 \( \mu \text{g dm}^{-3} \) of copper.

Effect of foreign ions

The effect of various foreign ions on the determination of 20 \( \mu \text{g dm}^{-3} \) copper(II) was examined by the proposed method. The following ions showed no interference up to at least the concentrations (mg dm\(^{-3}\)) indicated in parentheses: Ca(II) (1000); Mn(II) (500); Br\(^-\) (200); Cl\(^-\) (150); Li(I), K(I), Cd(II), Pb(II), Na(I), BrO\(_3\)-, ClO\(_4\)-, IO\(_3\)-, HCO\(_3\)-, S\(_2\)O\(_3\)\(_2\)- (100); Al(III), As(III), Mg(II), Mo(VI), NH\(_4\)+, Ni(II), Sr(II), W(VI), Zn(II) (10); Co(II), Ag(I), Fe(III), Hg(II), PO\(_4\)\(^3-\), SCN\(^-\) (1); F\(^-\), V(IV) (0.1). The lowest tolerance limit of 0.1 mg dm\(^{-3}\) was observed for iodide and vanadium. However, the interference from these ions can be considered as being not serious, because their content is usually very low in natural water; the iodide\(^{-19}\) and vanadium\(^{20}\) concentrations of 0.0004 – 0.0039 mg dm\(^{-3}\) and 0.0023 – 0.013 mg dm\(^{-3}\) were reported for natural water samples, respectively. In some catalytic method for copper determination, iron shows serious interference and a masking agent is used to eliminate it.\(^7\) In the proposed method, iron(III) at a concentration of up to 1 mg dm\(^{-3}\) did not interfere with the determination of copper.

Application to water analysis

The present method was applied to the determination of copper in tap, ground, river and well-water samples without any pretreatment. The determinations were made by diluting the water samples at different times. To examine the recovery of copper, known amounts of copper(II) were added to the samples. The results are given in Table 1. The values corrected for dilution showed good agreement, and the recoveries of added copper(II) were 93.7 and 105% for tap and ground-water samples, respectively. These samples were also analyzed by atomic absorption spectrometry (AAS) and inductivity coupled plasma spectrometry (ICP). As can be seen in Table 1, the results obtained by the proposed method showed good agreement with that obtained by AAS and ICP.

References

1. J. J. Pinto, C. Moreno, and M. G. Vargas, *Talanta*, 2004,