



## Kinetic Spectrophotometric Determination of Copper in Food

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Copper is an important metallic element in nature. It is also one kind of important trace element for life body. The study and establishment of a method for the determination of copper has important significance. A new kinetic-spectrophotometric method is proposed for the determination of copper in present study. The method is based on the catalytic effect of copper(II) on the oxidation of dibromo-*p*-chloro-chlorophosphonazo (DBC-CPA) by hydrogen peroxide in 1 mol L<sup>-1</sup> phosphoric acid medium. Copper can be spectrophotometrically determined by measuring the decrease in the absorbance of dibromo-*p*-chloro-chlorophosphonazo at the wavelength of 550 nm using the fixed-time method. The linear range of the present method is 0.1-2.0 μg in 10 mL of solution and the limit of detection is 9.05 ng mL<sup>-1</sup>. The method has been successfully applied to the determination of copper in apple and banana. The relative standard deviations of 13 determinations of the method were 0.82-1.03 %. The recoveries of the present method were 101.3-103.6 %.

**Key Words:** Kinetic spectrophotometry, Copper, Dibromo-*p*-chloro-chlorophosphonazo, Food.

### INTRODUCTION

Copper is one of indispensable microamount elements of human body. It is extremely important for maintaining human body's health. Copper has an important effect on the growth and the function of blood, central nervous system, immune system, hair, skin, skeleton organization, brain, liver and heart internal organs. The human body mainly absorbs copper through food. In addition, copper also has the fine physical and chemical characteristics, widely been applied to industry, agriculture, animal husbandry, medicine and in the daily life. Methods for the determination of copper have atomic absorption spectrometry, inductively coupled plasma (ICP)-atomic emission spectrometry (AES) method, liquid phase chromatography and extraction spectrophotometry<sup>1</sup>. Although these methods have their characteristics, they have their insufficiency. Cupferron spectrophotometric method for the determination of copper is a commonly used method, but the sensitivity of method is low<sup>2</sup>. Although in recent years certain spectrophotometric methods were proposed for the determination of copper<sup>3-9</sup>, the sensitivity of method for the determination of copper is not ideal. Kinetic photometric analysis has been received widespread recognition due to the simplicity of equipment, the highness of sensitivity and the simplicity of operation<sup>10,11</sup>. In recent years, related catalytic kinetic methods for the determination of trace amount of copper have been reported<sup>12</sup>, but most of the selectivities of the reported methods

are not ideal. Therefore, development of a new determination copper catalytic spectrophotometric system still has an important significance.

Dibromo-*p*-chloro-chlorophosphonazo, abbreviated as DBC-CPA, is a chromogenic agent. Its molecular formula is C<sub>22</sub>H<sub>13</sub>N<sub>4</sub>O<sub>11</sub>PS<sub>2</sub>Br<sub>2</sub>Cl<sub>2</sub> and its structural formula (Fig. 1) is:

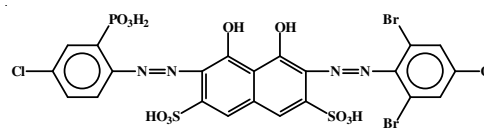


Fig.1. Molecular structure of DBC-CPA

Dibromo-*p*-chloro-chlorophosphonazo itself can produce colour. When it is oxidized or reduced, the -N=N- group is destroyed, which results in that the solution colour shoals even the solution is colourless. The study reports the fading reaction of dibromo-*p*-chloro-chlorophosphonazo oxidized by hydrogen peroxide and catalyzed by Cu<sup>2+</sup> in 0.1 mol L<sup>-1</sup> phosphoric acid medium and based on this a kinetic spectrophotometric method for the determination of copper has been established. Operation of this method is simple and the sensitivity is high. It has been applied to determination of copper in apple and banana samples with good results.

### EXPERIMENTAL

A 722S spectrophotometer (Shanghai Lingguang Technique Co. Ltd., China), with 1 cm cells, were employed

to measure absorbance. A HH-2 digital constant temperature water bath (Jiangsu Jintan Ronghua Apparatus Manufacture Co. Ltd., China) was used to control temperature.

Dibromo-*p*-chloro-chlorophosphonazo solution:  $1.78 \times 10^{-4}$  mol L<sup>-1</sup> solution; Cu(II) standard solution:  $0.5 \mu\text{g mL}^{-1}$  working solution; H<sub>2</sub>O<sub>2</sub> solution:  $2.15 \times 10^{-2}$  mol L<sup>-1</sup> solution; H<sub>3</sub>PO<sub>4</sub> solution: 1 mol L<sup>-1</sup>. All reagents used in the experiment were of analytical grade and water was distilled water.

In two 10 mL calibrated flasks, 1 mL DBC-CPA solution, 1 mL H<sub>3</sub>PO<sub>4</sub> solution, 2 mL H<sub>2</sub>O<sub>2</sub> solution were respectively added. Then, in the one calibrated flask, an appropriate amount of Cu(II) solution was put in the mixed solution (catalytic reaction), while in the another one, the copper(II) was not put in the mixed solution (non-catalytic reaction). The solutions were diluted with water to the mark and shaken well. They were heated at a boiling water-bath of 100 °C for 13 min. Then, they were taken out and cooled down by running water for 13 min. The absorbance of catalytic reaction (A) and the absorbance of non-catalytic reaction (A<sub>0</sub>) were determined at the wavelength of 550 nm *versus* water using 1 cm cells and then  $\Delta A = A_0 - A$  was calculated.

## RESULTS AND DISCUSSION

**Absorption spectra:** The absorption curves of different reaction systems are shown in Fig. 2. The curves (a), (b) in the figure showed that H<sub>2</sub>O<sub>2</sub> has an oxidation effect on DBC-CPA. The further decrease in absorbance of curve (c) suggested that Cu<sup>2+</sup> has a catalytic effect on the oxidation of DBC-CPA by H<sub>2</sub>O<sub>2</sub>. Compared with curve (c), the absorbance of curve (d) is that the absorbance still lowered, indicating that with the increase in Cu<sup>2+</sup> concentration the catalytic effect is further enhanced. Fig. 3 showed that under the test conditions the maximum absorption wavelengths of both catalytic and non-catalytic reactions are 550 nm. At this wavelength,  $\Delta A$  was maximum, showing that the sensitivity of the catalytic kinetic spectrophotometric determination of copper is maximum. Therefore, 550 nm was chosen as the measurement wavelength in this study.

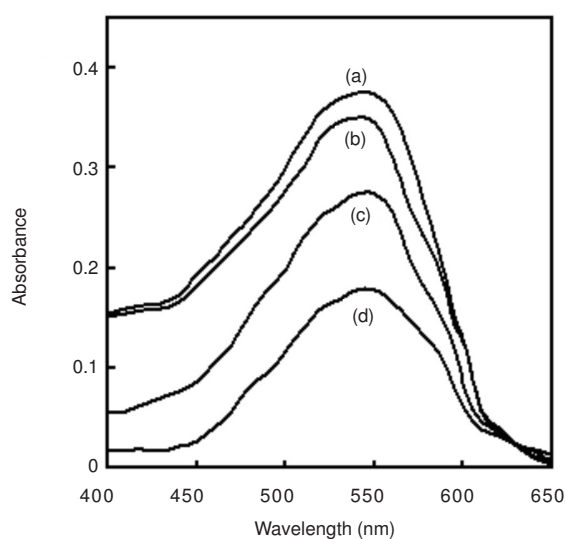


Fig. 2. Absorption spectra: (a) DBC-CPA, (b) (DBC-CPA)-H<sub>2</sub>O<sub>2</sub>, (c) (DBC-CPA)-H<sub>2</sub>O<sub>2</sub>-1.0  $\mu\text{g Cu}^{2+}$ , (d) (DBC-CPA)-H<sub>2</sub>O<sub>2</sub>-2.0  $\mu\text{g Cu}^{2+}$ , [DBC-CPA] =  $1.78 \times 10^{-5}$  mol L<sup>-1</sup>, [H<sub>2</sub>O<sub>2</sub>] =  $4.30 \times 10^{-3}$  mol L<sup>-1</sup>, [H<sub>3</sub>PO<sub>4</sub>] = 0.1 mol L<sup>-1</sup>; heating temperature: T = 100 °C; heating time: t = 13 min

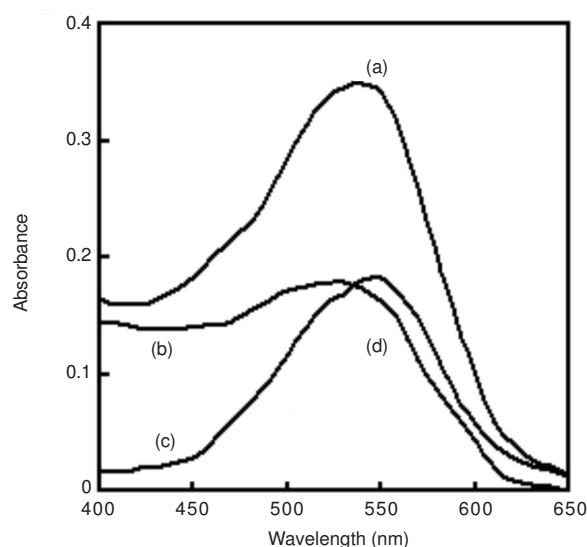


Fig. 3. Absorption spectra: (a) (DBC-CPA)-H<sub>2</sub>O<sub>2</sub>, non-catalytic reaction-A<sub>0</sub>; (b) Cu<sup>2+</sup>-(DBC-CPA)-H<sub>2</sub>O<sub>2</sub>, catalytic reaction-A; (c) net catalytic reaction -  $\Delta A$ ; [Cu<sup>2+</sup>] =  $1.10 \times 10^{-6}$  mol L<sup>-1</sup>; [DBC-CPA] =  $1.78 \times 10^{-5}$  mol L<sup>-1</sup>; [H<sub>2</sub>O<sub>2</sub>] =  $4.30 \times 10^{-3}$  mol L<sup>-1</sup>; [H<sub>3</sub>PO<sub>4</sub>] = 0.1 mol L<sup>-1</sup>; heating temperature: T = 100 °C; Heating time: t = 13 min

**Amount of phosphoric acid:** The experimental results showed that over the range of 0-1.0 mL,  $\Delta A$  increased with the increase of the amount of phosphoric acid. When the amount of H<sub>3</sub>PO<sub>4</sub> was 1 mL,  $\Delta A$  was maximum and the sensitivity of the reaction was maximum.  $\Delta A$  began to decline when the amount of H<sub>3</sub>PO<sub>4</sub> was more than 1 mL. In this work, 1 mL of 1 mol L<sup>-1</sup> H<sub>3</sub>PO<sub>4</sub> solution was chosen. At this time, the concentration of phosphoric acid in the system was 0.1 mol L<sup>-1</sup>.

**DBC-CPA dosage:** The experimental results showed that over the range of 0-0.9 mL,  $\Delta A$  increased with the increase of the amount of DBC-CPA. When the amount was in the range of 0.9-2.0 mL,  $\Delta A$  was maximum. After it,  $\Delta A$  lowered as increase in the amount of chromogenic agent. In the experimnt 1 mL of  $1.78 \times 10^{-4}$  mol L<sup>-1</sup> solution was selected.

**H<sub>2</sub>O<sub>2</sub> dosage:** The experimental results showed that over the range of 0-1.8 mL,  $\Delta A$  increased with the increase of the amount of H<sub>2</sub>O<sub>2</sub>. When the amount was in the range of 1.8 - 3.0 mL, the sensitivity of catalytic reaction was maximum. After it, the sensitivity decreased as increase in the amount of H<sub>2</sub>O<sub>2</sub>. 2.0 mL was recommended for further study.

**Reaction temperature:** The experimental results indicated that under the test conditions below 60 °C the catalytic reaction is almost negligible. As reaction temperature increased,  $\Delta A$  gradually increased. When temperature achieved 100 °C,  $\Delta A$  was maximum and the reaction sensitivity was maximum. In this study a bath of 100 °C was selected for heating and running water for cool was used to terminate the reaction. The data obtained over the range of 70-100 °C was regressed and disposed to obtain a regression equation:  $\log(A_0/A) = 3.5749 - 1180.992/T(K)$ , with a correlation coefficient of  $r = 0.9965$ . The apparent activation energy of catalytic reaction was  $E = 43.112 \text{ kJ mol}^{-1}$ .

**Heating time:** The experimental results indicated that a linear relationship was presented between  $\Delta A$  and t over the range of 1-13 min. At 13 min,  $\Delta A$  was maximum.  $\Delta A$  gradually

TABLE-1  
 ANALYTICAL RESULTS OF SAMPLES

Sample	Result determined ( $\mu\text{g g}^{-1}$ )	Average value ( $\mu\text{g g}^{-1}$ )	Relative standard deviation (RSD %)	Added ( $\mu\text{g g}^{-1}$ )	Recovered ( $\mu\text{g g}^{-1}$ )	Recovery (%)	Atomic absorption spectrometric contrast method ( $\mu\text{g g}^{-1}$ )
Apple	0.605, 0.598, 0.610, 0.596, 0.601, 0.603, 0.598, 0.603, 0.589, 0.593, 0.603, 0.601, 0.593	0.593	1.03	0.200	0.203	101.3	0.592
Banana	1.399, 1.404, 1.390, 1.385, 1.375, 1.408, 1.404, 1.408, 1.399, 1.385, 1.394, 1.390, 1.404	1.395	0.82	0.200	0.207	103.6	1.400

decreased after 13 min. In this study, heating time was employed to be 13 min. The regression equation of  $\Delta A$  versus  $t$  was  $\Delta A = 0.0148 t(\text{min}) + 0.0235$ , with a correlation coefficient of  $\gamma = 0.9931$ . The rate constant of the reaction was  $k = 2.37 \times 10^{-4} \text{ s}^{-1}$ . The half-life period was 7.8 min.

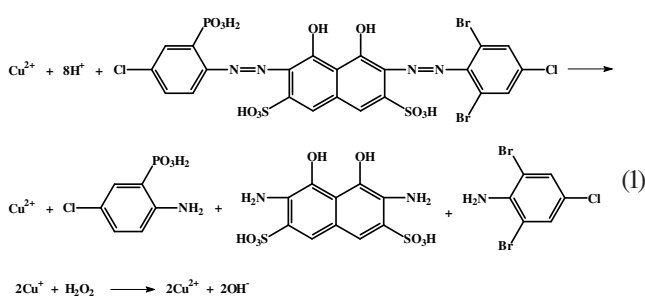
**System stability:** Under the optimal conditions, for the determination of  $0.1 \mu\text{g/mL Cu}^{2+}$ ,  $\Delta A$  achieved stable after the system was cooled for 13 min by running water. When a limit of the relative error of  $\Delta A$  change did not exceed 5 %, stability time of the system was 5.5 h.

**Working curve:** The experimental results showed that under the optimal experimental conditions in a 10 mL solution the amount of  $\text{Cu}^{2+}$  in the range of 0.1-2.0  $\mu\text{g}$  and  $\Delta A$  presented a good linear relationship. Its regression equation is:  $\Delta A = 0.1061C$  ( $C: \mu\text{g}/10 \text{ mL}$ ) - 0.0058, with a correlation coefficient of  $r = 0.9931$ . For eleven replicate determinations of  $1 \mu\text{g mL}^{-1} \text{Cu}^{2+}$  standard solution, the relative standard deviation was 1.5 %. By 11 times blank experiments, the detection limit of the method obtained is  $3S/K = 9.05 \text{ ng mL}^{-1}$  ( $S$  is the standard deviation of 11 blank solution determinations,  $K$  is the slope of the regression equation).

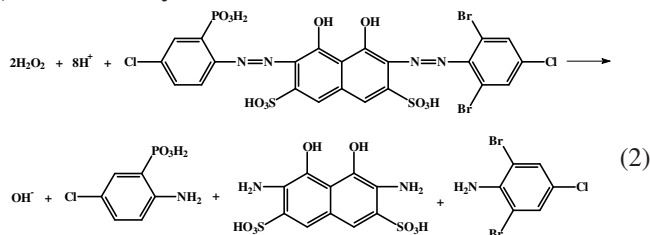
**Selectivity of method:** The effect of coexisting substances on the determination of  $0.1 \mu\text{g/mL Cu}^{2+}$  was studied under the optimal conditions. A  $\pm 5 \%$  deviation from  $\Delta A$  value expected for  $\text{Cu}^{2+}$  along was considered as the criterion of the interference. The allowable amounts of coexisting substances (multiple in mass, m/m) are as follows:  $\text{MnO}_4^-$  (5),  $\text{VO}_3^-$  (20),  $\text{Br}^-$  (3),  $\text{F}^-$  (250),  $\text{I}^-$  (0.1),  $\text{Cl}^-$  (300),  $\text{SO}_4^{2-}$  (900),  $\text{B}^{3+}$  (5),  $\text{PO}_4^{3-}$  (900),  $\text{HAc}$  (50),  $\text{Ag}^+$  (15),  $\text{Mg}^{2+}$  (20),  $\text{Ni}^{2+}$  (1),  $\text{Ca}^{2+}$  (2),  $\text{Zn}^{2+}$  (25),  $\text{Pb}^{2+}$  (1),  $\text{Fe}^{2+}$  (0.02),  $\text{Mn}^{2+}$  (25),  $\text{Cd}^{2+}$  (25),  $\text{Bi}^{3+}$  (3),  $\text{Fe}^{3+}$  (0.02),  $\text{Al}^{3+}$  (1),  $\text{La}^{3+}$  (0.2),  $\text{Cr}^{3+}$  (0.1),  $\text{Eu}^{3+}$  (0.2),  $\text{Y}^{3+}$  (0.1),  $\text{Zr}^{4+}$  (0.5),  $\text{Ti}^{4+}$  (0.3),  $\text{Th}^{4+}$  (0.2),  $\text{Ce}^{4+}$  (0.5),  $\text{W(VI)}$  (20),  $\text{Mo(VI)}$  (15),  $\text{Cr(VI)}$  (0.5).

**Mechanism of catalytic reaction:** In this study,  $\text{Cu(II)}$  was used as the catalyst,  $\text{H}_2\text{O}_2$  as the oxidant, DBC-CPA as the chromogenic agent, reaction mechanism proposed is as follows:

#### Catalytic reaction:



#### Non-catalytic reaction:



**Analysis of sample:** 100 g of apple or banana fruit sample was accurately weighed and placed in an oven at  $110 \text{ }^\circ\text{C}$  and dried to remove water for 5 h. Then, the sample was placed in a muffle furnace and ashed at  $650 \text{ }^\circ\text{C}$  for 6 h. After it, the door of the furnace was opened to cool down to a room temperature. A few drops of water were added to wet the sample. Along the vessel 1.0 mL of 1 + 1 (V/V) hydrochloric acid was added dropwise to dissolve leftover. The content was transferred to a 25 mL volumetric flask, diluted to the constant volume by water, shaken well as the test solution. 1.00 mL of the solution was accurately taken out and determined for copper according to the standard procedure. The analytical results are seen in Table-1. From the table it can be seen that the analytical results of the method agree with those of atomic absorption spectrometry. The analytical results are very excellent.

#### Conclusion

This article studied the optimum experimental conditions and its kinetic property of the oxidation reaction of dibromo-*p*-chloro-chlorophosphonazo by  $\text{H}_2\text{O}_2$  and catalyzed by copper(II). At 550 nm,  $\text{Cu}^{2+}$  amount over the range 0.1-2.0  $\mu\text{g}/10 \text{ mL}$  with the difference of absorbance present the linear relationship. Detection limit of the method is  $9.05 \text{ ng mL}^{-1}$ . The method has been successfully used in the determination of copper in apple and banana samples with good results.

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