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CATALYTIC KINETIC SPECTROPHOTOMETRIC DETERMINATION OF TRACE COPPER WITH COPPER(II)-*p*-ACETYLCHLOROPHOSPHONAZO-HYDROGEN PEROXIDE SYSTEM

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ABSTRACT. Copper(II) catalyzes the oxidation of *p*-acetylchlorophosphonazo(CPA*p*A) by hydrogen peroxide in 0.10 M phosphoric acid. A novel catalytic kinetic-spectrophotometric method is proposed for the determination of copper based on this principle. Copper(II) can be determined spectrophotometrically by measuring the decrease in the absorbance of CPA*p*A at the wavelength of 554 nm using the fixed-time method. The optimum reaction conditions are as follows: H_3PO_4 (1.00 M) 1.0 mL, CPA*p*A (2.19×10^{-4} M) 1.5 mL, H_2O_2 (4.30×10^{-2} M) 1.2 mL, reaction temperature 100 °C and reaction time 13 min. The linear range for the determination of copper(II) is 0.020-0.30 μg/mL. The limit of detection is 10.94 ng/mL. The method was satisfactorily used to determine copper in tomato and cucumber samples. The relative standard deviation of thirteen replicate determinations was 1.20-1.34% and the recovery of the method was 99.5-103.9%.

KEY WORDS: Copper determination, Catalytic kinetic spectrophotometry, *p*-Acetylchlorophosphonazo, Hydrogen peroxide

INTRODUCTION

Copper is an indispensable material in the life of animal, plant and humankind. It is an important part of composition of the proteins and enzymes in body. Many important enzymes need copper to enlist for activation. Some enzymes provide the energy that biochemical reactions need in body. Some enzymes participate in the formation and transform of skin color pigment. Another enzyme can help to form the crosslink between collagen protein and elastic protein, and hence the join between cell tissues are kept or repaired. This is especially important to heart and artery blood vessel. The deficiency of copper is an important factor resulting coronary artery heart diseases and can promote the connection between sugar molecules and protein molecules. Moreover, the saccharification of protein results in tissue damnification in diabetes people [1, 2]. As the age of a person increases, the saccharification also speeds up. A human body needs copper element to inhale oxygen gas, and energy produces in cells to help nervous system transmit signals to various tissues. This is advantageous to metabolize iron element, develop blood vessel, form skin, muscle sinew and hair [1]. However, if content of the copper element in a body is too high, it results in poisoning [2]. Copper is an essential element for life and that too little or too much copper is detrimental to health. Mankind absorbs copper via the food chain.

Some methods have been proposed for the determination of copper, such as atomic absorption spectrometry [3], inductively coupled plasma (ICP)-atomic emission spectrometry (AES) [4], chromatography [5], neutron activation analysis [6], and inductively coupled plasma (ICP)-mass spectrometry (MS) [7]. However, these methods have the disadvantages that the operation of the instrumentation used is complex and the price of the instrumentation is expensive compared with kinetic spectrophotometry. Catalytic kinetic spectrophotometry has a series of outstanding advantages such as low detection limit, high sensitivity, and simplicity of instrumentation operation [8, 9]. It is especially suitable for the determination of trace

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component. Although catalytic kinetic spectrophotometry has been used in the determination of copper, the selectivity of this kind of systems is poor [5, 6] and it is still necessary to develop a new catalytic kinetic spectrophotometric method for copper. *p*-Acetylchlorophosphonazo (CPA*p*A, $C_{24}H_{18}ClN_4O_{12}PS_2$) [10] is a brown black powder. It is easily soluble in water and its aqueous solution is purple red. Its structural formula is shown in Figure 1.

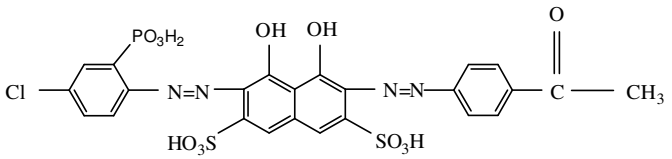
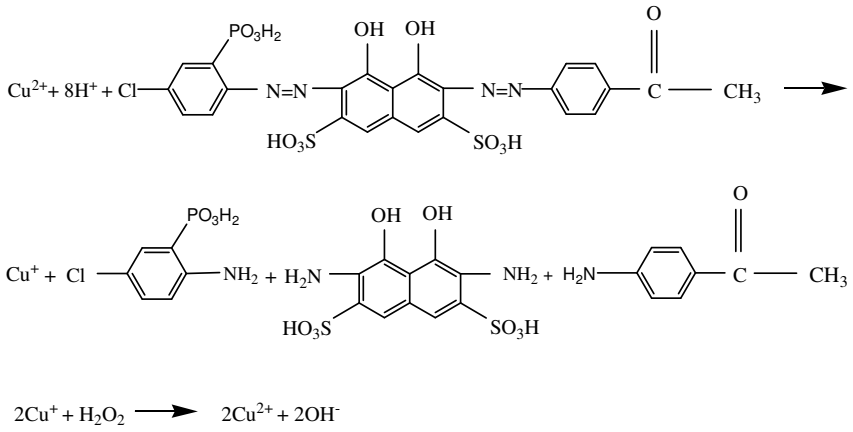


Figure 1. Molecular structure of CPA*p*A.

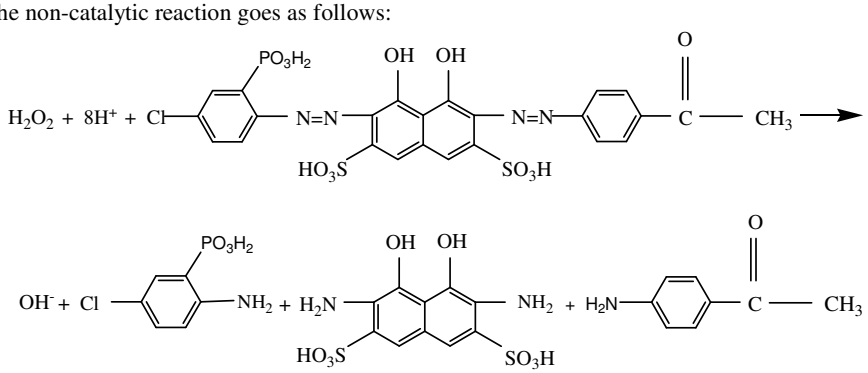
It can be seen from Figure 1 that the aromatic ring in CPA*p*A carries -OH, -SO_3H, -PO_3H_2, and -N=N- groups and has the potential of being a multidentate ligand. The reagent not only has a strong complex ability and forms various water-soluble complexes with metal ions, but also the -N=N- group itself can produce color. When the ligand is oxidised or reduced, the -N=N- group is destroyed, which results in the color of solution becoming shallow, or even colorless. CPA*p*A has been used in the photometric determination of rare earths [10], aluminium [11], platinum group elements [12], and protein [13]. It is found in this paper that in the medium of 1.0×10^{-3} M H_3PO_4, trace copper(II) catalyzes the fading reaction of CPA*p*A oxidized by H_2O_2 and based on this principle a new method for the determination of trace copper was developed. The present method is characterized by high sensitivity, operation simplicity, and low analytical cost. It has been successfully used for the determination of copper in tomato and cucumber samples.

In this paper CPA*p*A was used as the chromogenic agent, H_2O_2 as the oxidant, Cu(II) as the catalyst. Based on the principle of catalytic reaction [14], the author proposes the reaction mechanism as follow:

The catalytic reaction goes as follow:



The non-catalytic reaction goes as follows:



EXPERIMENTAL

Reagents

Cu(II) standard solution: 1.000 mg/mL. 0.3929 g of $CuSO_4 \cdot 5H_2O$ (Shenyang Third Plant for Reagent, China) was dissolved in the water acidified by 0.1 mL of concentrated H_2SO_4 and the solution was diluted with water to 100 mL. When needed, the copper(II) stock solution was diluted with water to 2.0 μg/mL working solution. *p*-Acetylchlorophosphonazo (CPA*p*A, Shanghai Changke Research Institute for Reagent, China) solution: 2.19×10^{-4} M. 0.0150 g of CPA*p*A was weighed and dissolved in definite amount of water and then the solution was transferred to a 100 mL volumetric flask and then diluted with water to the constant volume. H_3PO_4 (Beijing Chemical Plant, China) solution: 1.00 M. H_2O_2 (Beijing Chemical Plant, China) solution: 4.30×10^{-2} M. Unless specially stated, all reagents were of analytical grade. The water used for preparations was deionised distilled water.

Apparatus

A 722S spectrophotometer (Shanghai Lingguang Technique Co., Ltd, China), with 1 cm optical glass cells, was used for absorbance and spectra measurements. A HH-2 thermostat water bath kettle (Jiangsu Jintan Ronghua Apparetus Manufacture Co., Ltd, China) was used for temperature control.

Procedure for copper determination

In the following order, 1.5 mL of 2.19×10^{-4} M CPA*p*A solution, 1.0 mL of 1.00 M H_3PO_4 solution, and 1.2 mL of 4.30×10^{-2} M H_2O_2 solution were subsequently placed into two 10 mL comparison tubes, respectively. In the one calibrated flask, an appropriate amount of Cu(II) solution was added to the mixed solution (for variable optimization conditional experiments: 2.0 μg), while in the another one, the copper(II) was not added to the mixture. The mixtures were diluted up to the mark with water, shaken and then heated with a boiling water bath of 100 ^{o}C for 13 min. Then the mixtures were taken out and cooled down for 10 min by running water to terminate the reaction. The absorbance A_0 of the uncatalyzed reaction solution, and the absorbance A of the catalytic reaction solution, were recorded at 554 nm in 1 cm cells against water, then $\Delta A = A_0 - A$ or $\lg A_o/A$ was calculated.

Procedure for the determination of copper in tomato or cucumber

100 g of tomato or cucumber sample was accurately weighed and washed. After it was cut into smaller pieces, the sample was dried in an oven for 6 h at a constant temperature of 110 ^{o}C. The sample was then placed in a muffle oven at 680 ^{o}C for incineration for 8 h. After the incineration was complete, the sample was cooled down to a room temperature. A few drops of water were added to wet it. Along the vessel 4-5 mL of hydrogen chloride (1+1, v/v) and 4-5 mL of nitric acid (1+1, v/v) was added drop wise. The content was transferred to a-50 mL volumetric flask, diluted to the constant volume by water. 2.00 mL of the above test solution was taken out and placed in a 10 mL comparison tube. Then, the determination of copper was made according to the procedure described above.

Procedure for the recovery of copper in tomato or cucumber

The recovery test was made according to the following procedure. Into two 10-mL comparison tubes were successively placed 1.5 mL of 2.19×10^{-4} M CPA*p*A solution, 1.0 mL of 1.00 M H_3PO_4 solution, and 1.2 mL of 4.3×10^{-2} M H_2O_2 solution, respectively. In the one calibrated flask, 2.00 mL of the test solution and 0.200 μg of standard Cu(II) solution were put in the mixed solution, while in the another one, the test solution and the standard Cu(II) solution were not put in the mixture. Then determination of copper was carried out according to the procedure described above. The total amount of copper was calculated according to the linear regression equation. The total amount, which was obtained from the above calculation, subtracts the amount in the testing solution to obtain the amount of the recovered copper. This value was divided by the added amount of copper to obtain the recovery.

RESULTS AND DISCUSSION

Absorption spectra

The absorption spectra of different reaction systems are shown in Figure 1. The curves A and B are the absorption of CPA*p*A versus water and CPA*p*A + H_2O_2 versus water, respectively. From the curves it can be seen that the addition of H_2O_2 can make the absorbance of CPA*p*A decrease to some extent, indicating that the H_2O_2 can oxidize CPA*p*A to fade under the acidity condition, but the change of peak value is not large. The curve c is the spectrum of system CPA*p*A + H_2O_2 + Cu(II) (1.0 μg) versus water. A comparison between curve B and C showed that Cu(II) has a catalytic effect of the fading reaction of CPA*p*A oxidized by H_2O_2. The curve D is the spectrum for the reaction of CPA*p*A + H_2O_2 + Cu(II)(2.0 μg) versus water. From the Figure it can be seen that both the peak values of curve C and D decreased. Especially, the decrease is more obvious for curve D. It indicates that more the addition amount of catalyst Cu(II) was, more the fading change of the reaction was. Over a definite range of concentration, a linear relationship is obtained between the addition amount of Cu(II) and fading degree ΔA. This is the quantitative base for the determination of copper(II). The experiments of absorption spectra of the catalytic reaction and non-catalytic reaction solutions were made according to the procedure described above. The results showed that under the test conditions both maximum absorption wavelengths are 554 nm. At this wavelength the difference of absorbance was maximum. Thus, 554 nm was selected as the measurement wavelength.

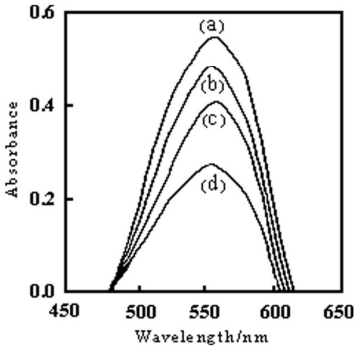


Figure 2. Absorption spectra. a-CPA*p*A (against water), b-CPA*p*A + H_2O_2 (against water), c-CPA*p*A + H_2O_2 + 1.0 μg Cu(II) (against water), d-CPA*p*A + H_2O_2 + 2.0 μg Cu(II) (against water). [CPA*p*A] = 3.29×10^{-5} M; [H_2O_2] = 5.16×10^{-3} M; [H_3PO_4] = 0.10 M; heating temperature T = 100 °C; heating time t = 13 min.

Optimization of reaction conditions

Effect of reaction acidity

Under the conditions where the other experimental variables were kept optimum, the effect of the amount of phosphoric acid was investigated. 0.2, 0.3, 0.5, 0.8, 1.0, 1.2, 1.5 and 2.0 mL of 1.00 M H_3PO_4 solution was, respectively, added and contrasted with blank reagent. The results showed (Figure 3) as the amount of H_3PO_4 increased over the range 0.2-1.0 mL, ΔA increased. When the amount of H_3PO_4 was at 1.0 mL, ΔA was a maximum and the reaction sensitivity was the highest. After the amount of H_3PO_4 was more than 1.0 mL, ΔA decreased. Thus, 1.0 mL of 1.00 M H_3PO_4 solution was selected. At this time, the concentration of H_3PO_4 was 0.10 M.

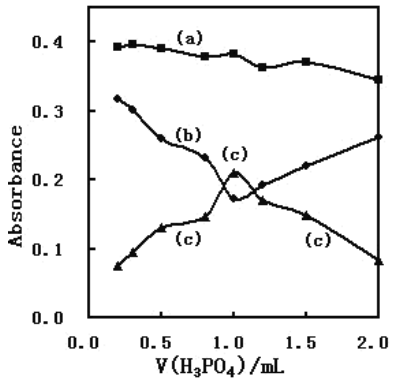


Figure 3. Effect of acidity. a-CPA*p*A + H_2O_2 (against water), b-Cu(II) + CPA*p*A + H_2O_2 (against water), c-Cu(II) + CPA*p*A + H_2O_2 (against reagent blank). [Cu^{2+}] = 1.10×10^{-6} M; [CPA*p*A] = 3.29×10^{-5} M; [H_2O_2] = 5.16×10^{-3} M; λ = 554 nm; heating temperature T = 100 °C; heating time t = 13 min.

Effect of the amount of CPApA

Under the conditions where the other experimental variables were kept optimum, the effect of the amount of CPA*p*A was investigated. 0, 0.2, 0.3, 0.5, 0.8, 1.0, 1.5, 2.0, 2.2 and 2.5 mL of 2.19×10^{-4} M CPA*p*A solution was, respectively, added and contrasted with blank reagent. The results showed (Figure 4) as the amount of CPA*p*A increased over the range 0-1.0 mL, ΔA increased. When the amount of CPA*p*A was 1.0-2.2 mL, ΔA was a maximum. After 2.2 mL, ΔA decreased. Thus, 1.5 mL of 2.19×10^{-4} M CPA*p*A solution was selected.

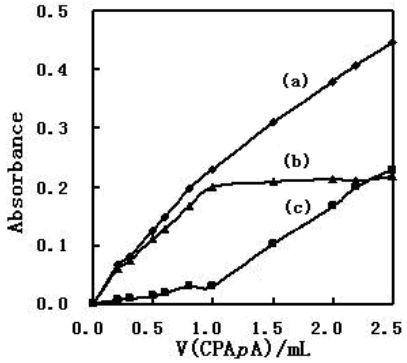


Figure 4. Effect of amount of CPA*p*A. a-CPA*p*A + H_2O_2 (against water), b-Cu(II) + CPA*p*A + H_2O_2 (against reagent blank), c-Cu(II) + CPA*p*A + H_2O_2 (against water). $[Cu^{2+}] = 1.10 \times 10^{-6}$ M; $[H_2O_2] = 5.16 \times 10^{-3}$ M; $[H_3PO_4] = 0.1$ M; $\lambda = 554$ nm; heating temperature T = 100 °C; heating time t = 13 min.

Effect of the amount of H_2O_2

Under the conditions where the other experimental variables were kept optimum, the effect of the amount of H_2O_2 was investigated. 0, 0.5, 1.0, 1.2, 1.5 and 2.0 mL of 4.30×10^{-3} M H_2O_2 solution was, respectively, added and contrasted with blank reagent. The results showed (Figure 5) as the amount of H_2O_2 increased over the range 0-1.0 mL, the sensitivity of the catalytic reaction increased. When the amount of H_2O_2 was 1.0-1.5 mL, the reaction sensitivity was a maximum. When the volume of H_2O_2 was more than 1.5 mL, the reaction sensitivity decreased. Therefore, 1.2 mL of 4.30×10^{-3} M H_2O_2 solution was selected.

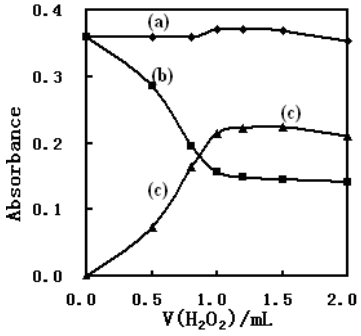


Figure 5. Effect of amount of H_2O_2. a-CPA*p*A + H_2O_2 (against water), b-Cu(II) + CPA*p*A + H_2O_2 (against water), c-Cu(II) + CPA*p*A + H_2O_2 (against reagent blank). $[Cu^{2+}] = 1.10 \times 10^{-6}$ M; [CPA*p*A] = 3.29×10^{-5} M; $[H_3PO_4] = 0.1$ M; $\lambda = 554$ nm; heating temperature T = 100 °C; heating time t = 13 min.

Effect of reaction temperature

Under the conditions where the other experimental variables were kept optimum, the effect of temperature was investigated. A water bath of 50, 60, 70, 75, 80, 85, 90, and 100 ^oC was used for heating, respectively, and contrasted with blank reagent. The results showed (Figure 6) under the test condition below 50^oC the catalytic reaction is negligible. As reaction temperature increased, ΔA gradually increased. When temperature of the water bath reached 100 ^oC, ΔA was a maximum and the reaction sensitivity was a maximum. Therefore, the temperature bath of 100 ^oC was chosen to heat the samples. To stop the reaction the samples were placed under running water to cool down. The data obtained over 70-100 ^oC was regressed and disposed to obtain a linear regression equation: $\log A_o/A = 3.5749 - 1180.992/T(K)$, with a correlation coefficient $r = 0.9965$. The apparent activation energy of the catalytic reaction was obtained to be E_a = 67.580 kJ/mol.

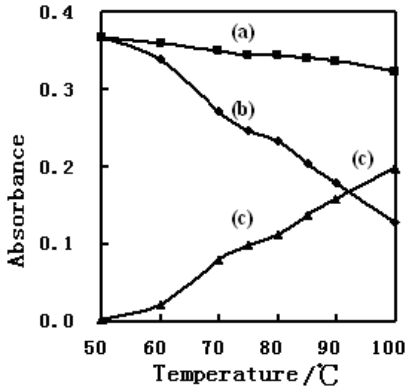


Figure 6. Effect of temperature. a-CPA*p*A + H_2O_2 (against water), b-Cu(II) + CPA*p*A + H_2O_2 (against water), c-Cu(II) + CPA*p*A + H_2O_2 (against reagent blank). $[Cu^{2+}] = 1.10 \times 10^{-6}$ M; [CPA*p*A] = 3.29×10^{-5} M; $[H_2O_2] = 5.16 \times 10^{-3}$ M; $[H_3PO_4]$ = 0.1 mol/L; λ = 554 nm; heating time t = 13 min.

Effect of heating time

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, 10, 13, 15, 17, 19 and 20 min was, respectively, made and contrasted with blank reagent. The results showed (Figure 7) that a linear relationship was presented between ΔA and t over the range 1 -13 min. At 13 min, ΔA was a maximum. Therefore, heating time was chosen to be 13 min. The regression equation calculated was ΔA = 0.0166t (min) + 0.0023, with a correlation coefficient γ = 0.9989. The rate constant of the reaction was $k = 2.77 \times 10^{-4}$ s^{-1}. The half-life period was 7.566 min.

Stability of system

Under the optimum experimental conditions, stability of the system was investigated. When the determination of 0.20 μg/mL copper(II) was made, the change of ΔA did not exceed 5% within 4 h and the system remained stable.

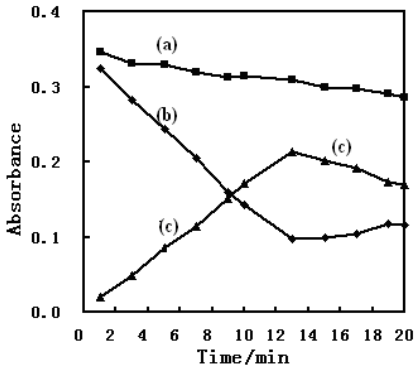


Figure 7. Effect of heating time. a-CPA*p*A + H_2O_2 (against water), b-Cu(II) + CPA*p*A + H_2O_2 (against water), c-Cu(II) + CPA*p*A + H_2O_2 (against reagent blank). [Cu^{2+}] = 1.10 × 10^{-6} M; [CPA*p*A] = 3.29 × 10^{-5} M; [H_2O_2] = 5.16 × 10^{-3} M; [H_3PO_4] = 0.10 M; λ = 554 nm; heating temperature T = 100 ^oC.

Effect of coexisting ions

Under the optimum conditions the effects of coexisting ions were investigated. When 2.0 μg of copper(II) was determined in a 10 mL of solution and a relative error was less than ± 5 %, the allowable amounts of coexisting ions were as follow (in mass multiple, m/m): SO_4^{2-}, PO_4^{3-} (1000); F^-, Cl^- (200); Ac^-, Zn^{2+}, B(III) (30); Mg^{2+}, W(VI) (25); Ag^+, Mn^{2+}, Cd^{2+} (20); VO_3^- (15); Mo(VI) (10); Bi^{3+} (5); MnO_4^-, Br^-, Ca^{2+} (2); Pb^{2+}, Ni^{2+}, Al^{3+} (1); Ce(IV), Cr(VI) (0.8); Eu^{3+}, Ti(IV), Th(IV) (0.5); Zr^{4+} (0.3); I^-, Cr^{3+}, Y^{3+} (0.1); Fe^{3+}, La^{3+} (0.05); Fe^{2+} (0.01).

Calibration curve

According to the proposed procedure described above, a series of different amounts of Cu(II) standard solution were respectively added to 10 mL comparison tubes and the absorbance was measured. The results showed that the linear range determined by present procedure was 0.20-3.0 μg/10 mL (0.020-0.30 μg/mL). The regression equation of the working curve is ΔA = 0.9870 C (C: μg/mL)–0.0049, with a correlation coefficient γ = 0.9950. For eleven replicate determinations of 0.20 μg/mL Cu(II), the relative standard deviation determined was 1.70%. This indicated that the present method has good precision. The precision of the method for real samples was checked by repetitive analyses (n = 13) of two samples. The results in Table 1 indicate satisfactory precision for the proposed method. By eleven replicate blank experiment determinations the detection limit of the method was calculated to be 10.94 ng/mL according to 3S/K method (S is the standard the deviation of eleven blank replicate determinations, K is the slope of calibration curve).

Analysis of sample

The present method was applied to the determination of tomato and cucumber real samples to test the suitability of the method. The results are listed in Table 1. From the table it can be seen that the results obtained by proposed method were in excellent agreement with those of dibromo-*p*-chloro-chlorophosphonazo spectrophotometric method [15]. The recovery of the method was in the range of 99.5-102.0% and the relative standard deviation of thirteen replicate

determinations were1.20-1.34%. The analytical results of the method proposed in this paper were quite satisfactory.

Table 1. Determination of copper in tomato and cucumber samples.

Sample	Found (μg/g)	Average (μg/g)	RSD (%)	Added (ng/g)	Recovered (ng/g)	Recovery (%)	Contrast method [15] (μg/g)
Tomato	0.712, 0.696, 0.724, 0.714, 0.701, 0.709, 0.714, 0.717, 0.722, 0.704, 0.717, 0.700, 0.700	0.710	1.34	20.0	20.4	102.0	0.712
Cucumber	0.501, 0.509, 0.491, 0.496, 0.509, 0.501, 0.494, 0.491, 0.496, 0.504, 0.501, 0.504, 0.504	0.501	1.20	20.0	19.9	99.5	0.500

CONCLUSIONS

The optimum experimental conditions of the catalytic kinetic spectrophotometric system copper(II)-*p*-acetylchlorophosphonazo-hydrogen peroxide were established. Under the optimum conditions, the linear range of the determination of copper(II) was 0.020-0.30 μg/mL and the regression equation was $\Delta A = 0.9870C$ (C: μg/mL) – 0.0049, respectively. The detection limit of the method was 10.94 ng/mL. The present method has been satisfactorily applied to the determination of trace copper in tomato and cucumber samples.

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