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Kinetic Determination of Ultramicro Quantities of Copper. Application of Copper-Ion-Catalyzed Oxidation of Ascorbic Acid

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A procedure for the determination of ultramicro quantities of copper ion was developed. The oxidation of ascorbic acid was catalyzed with copper ion, and the reaction rate followed first order kinetics up to about 60% oxidation of the acid at constant pH. As pseudo first order rate constant was proportional to the concentration of copper ion, 10^{-6}~10^{-5}M of copper was measured at pH 5.0, and 10^{-7}~10^{-6}M at pH 7.0.

INTRODUCTION

It has been well known that much of enzymatic reactions in vivo require metal ions for the activation of enzymes, and the metal ions are effective even in micro-quantities and are very specific for the activations.[1] Though this specificity of metal ions in enzymatic reactions show many possible application for the analysis of trace metals, a little work has been reported. This may be owing to the fact that activities of enzymes are substantially influenced by the purities and the purification processes, and species of enzyme easily obtained are extremely limited.

The troubles, however, may be minimized if enzymemodels are used. Though ascorbate oxidase requires copper ion for the activation,[2] ascorbic acid can be also oxidized by many metal ions themselves without the enzyme.

In this research, the determination of ultramicro quantities of copper ion was carried out by applying copper-ion-catalyzed oxidation of ascorbic acid.

EXPERIMENTAL

Apparatus and Materials. Spectrophotometric measurements were made with a Shimadzu spectrophotometer, QV-50. A Hitach-Horiba glass electrode pH meter was used for the pH measurements.

As buffer solution, acetic acid-sodium acetate (2×10^{-3}M), potassium dihydrogen phosphate-disodium hydrogen phosphate (2×10^{-3}M) or ammonium chloride-ammonia were used. Chemicals used were the reagent grade, and redistilled water was used.

Procedure. Two tenth milliliters of a copper solution are added to an absorption cell containing 4 ml of ascorbic acid solution (5×10^{-5}M) having a desired pH value. The mixed solution is instantly stirred for a few second and the absorbance at 265 nm, the maximum wavelength of characteristic bond of ascorbic acid, is measured at appropriate time intervals.

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RESULTS AND DISCUSSION

Autoxidation of ascorbic acid. As shown in Fig. 1, autoxidation of ascorbic acid was facilitated with the increase of pH value, and therefore, it is preferable the pH value is as low as possible minimize the autoxidation error. The autoxidation was extremely inhibited by adding EDTA: Ascorbic acid in the presence of EDTA was hardly oxidized at pH 7.0 even after standing for over night. This phenomenon indicates that the autoxidation of ascorbic acid is probably caused by the presence of trace amount of metal ions in the test solution.

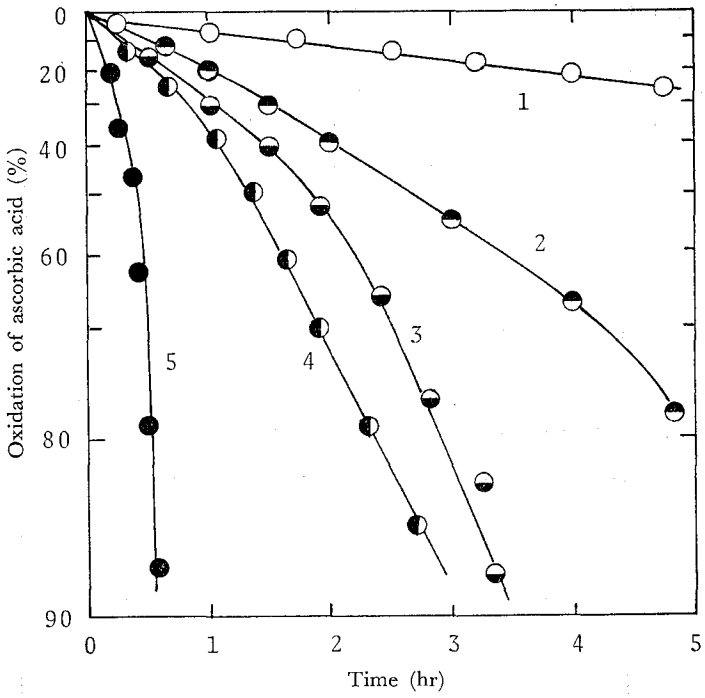

Fig. 1. Autoxidation of ascorbic acid
1: non-buffered, 2: pH=7.0,
3: pH=8.0, 4: pH=9.0,
5: pH=10.0
ascorbic acid: 5×10^{-5}M, temp.: 21~23°C

Effect of pH on copper-ion-catalyzed oxidation of ascorbic acid. As shown in Fig. 2, the oxidation of ascorbic acid catalyzed by copper ion followed first order kinetics up to about 60% oxidation of ascorbic acid; above 60% it somewhat deviated toward the rapid oxidation. The rate constants of pseudo first order reaction were determined from the initial linear portion.

Oxidation rate of ascorbic acid with copper ion increased with the rise of pH value, as indicated in Fig. 3, and subsequently a suitable rate could be selected by controlling pH value.

Effect of temperature. This reaction was considerably influenced by temperature and the rate increased about 50% with a 10°C rise (Table 1). Since

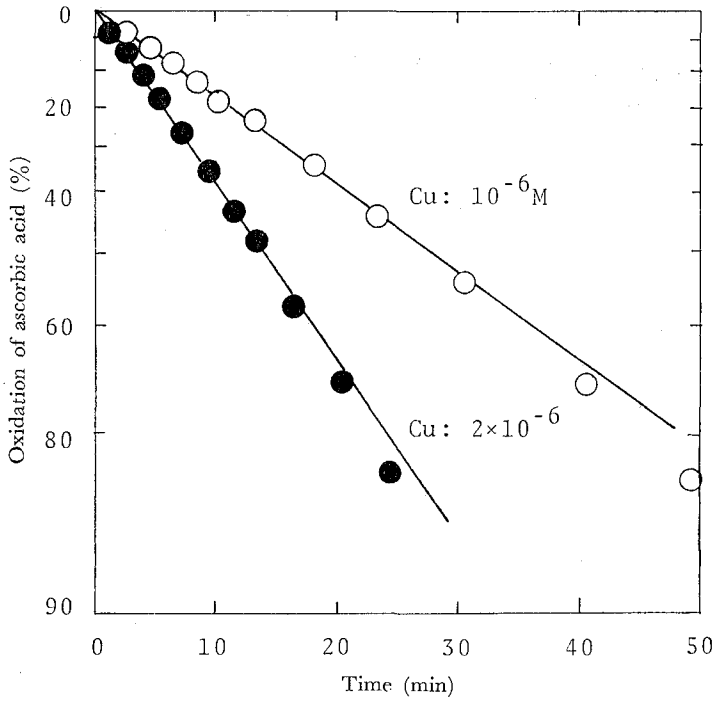

Fig. 2. First order reaction of oxidation of ascorbic acid with copper ion.
pH: 5.00, ascorbic acid: 5×10^{-5}M, temp.: 21～23°C

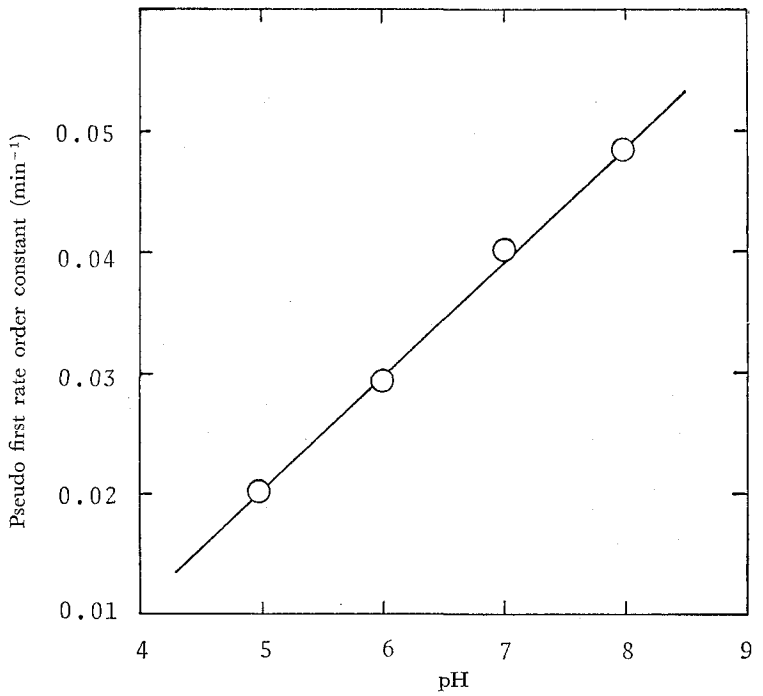

Fig. 3. Effect of pH on oxidation of ascorbic acid with copper ion.
ascorbic acid: 5×10^{-5}M, Cu^{2+}: 10^{-6}M, temp.: 21～23°C

Table 1. Effect of Temperature on Copper-Ion-Catalyzed Oxidation of Ascorbic Acid.

Temp. (°C)	k (min^{-1})
12	0.031
22	0.0405

ascorbic acid: 5×10^{-5}M
Cu^{++}: 10^{-6}M
pH: 7.0

experimental error inherent in catalyzed reactions are generally the order of several per cent, the temperature change within 2～3°C in the experiment may be allowed.

Calibration curve of copper ion. Because copper ion down to 10^{-5}M can be easily determined by ordinary analytical methods,[3] attempts were made to determine 10^{-7}M～10^{-5}M of copper. Pseudo first order rate constants, k, at pH 5.00 were plotted against copper ion concentration in range of 10^{-6}M to 10^{-5}M in Fig. 4. The plots gave a straight line, and 10^{-6}M～10^{-5}M of copper can be successfully determined in this condition. Measurement time was about eight minutes for 10^{-5}M copper and about forty five minutes for 10^{-6}M copper. Since the time necessary for

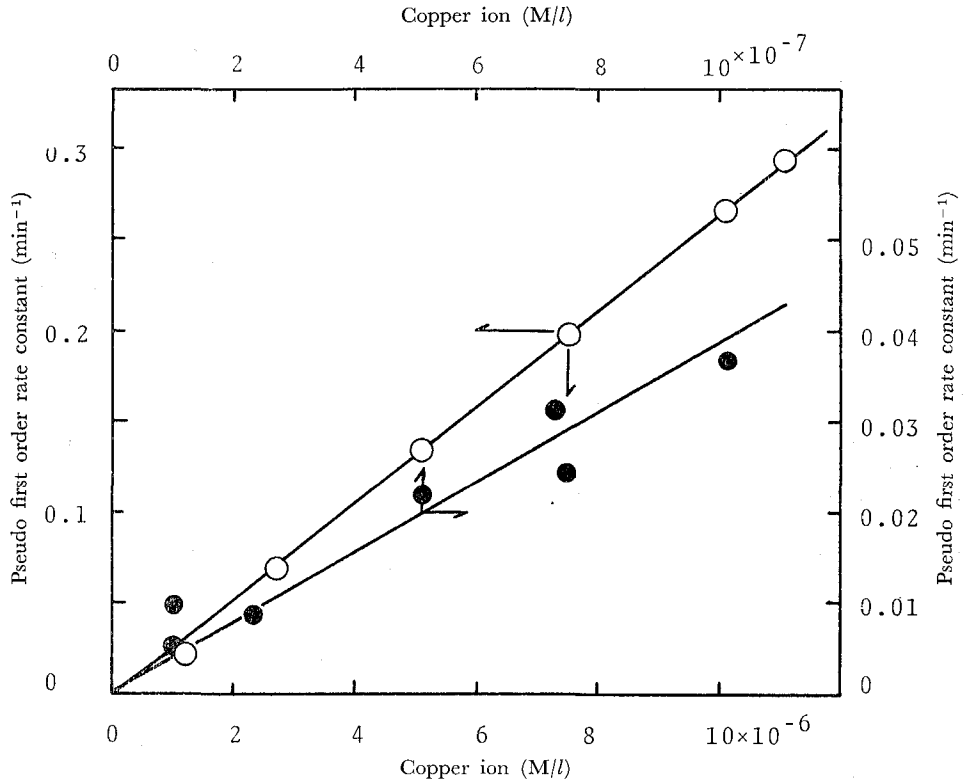

Fig. 4. Analytical curves for copper ion.
pH: 5.0 (-○-) and 7.0 (-●-),
ascorbic acid: 5×10^{-5}M, temp.: 21～23°C.

the accurate determination of less than 10^{-6}M of copper is too long to measure in the same condition, the measurements were carried out at a higher pH, pH 7.00. As shown in Fig. 4, the calibration also gave straight line from 10^{-7}M to 10^{-6}M of copper, though k values fluctuated moderately. The measurement time for 10^{-7}M copper ion was about fifty minutes. The determination of copper less than 10^{-7}M may be possible, if the reagents and glass ware used were not contaminated with any metal ions. To investigate the influence of several metal ions the measurements were made with solutions containing foreign cations and 10^{-6}M of copper ion. The results were summarized in Table 2. Cations tested did not so interfere the determination of copper: In the presence of about 50 times of copper ion relative errors

Table 2. Influence of Diverse Ions.

Ion	present (M/L)	Cu found (M/L)
Cr^{+++}	5×10^{-5}	0.94×10^{-6}
Mn^{++}	〃	1.15×10^{-6}
Fe^{++}	〃	1.09×10^{-6}
Fe^{+++}	〃	0.88×10^{-6}
Co^{++}	〃	1.13×10^{-6}
Ni^{++}	〃	1.22×10^{-6}
Zn^{++}	〃	1.11×10^{-6}
Mo^{6+}	〃	1.05×10^{-6}
Al^{+++}	〃	1.18×10^{-6}
Cd^{++}	〃	1.07×10^{-6}
Pb^{++}	〃	1.16×10^{-6}
Ag^{+}	〃	1.07×10^{-6}

were 10～20%, and it seems to be allowable in the trace analysis. It may be interesting that ferric and chromic ions, in contrast with most of cations, gave negative error, although the reason for this was not known.

REFERENCES

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(3) For example, Sandell, "Colorimetric Determination of Traces of Metals" 3rd. Edition, p. 449 (1959).