The Spectrophotometric Determination of a Small Amount of l-Ascorbic Acid with the Iron(III)-1,10-Phenanthroline Reagent

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(Received March 24, 1964)

Though several methods[1-4] have been reported for the spectrophotometric determination of ascorbic acid on the basis of the oxidation-reduction reaction between *l*-ascorbic acid and the coloring agents, they seem to be either complicated or unreliable because the absorptivity of the colored solution varies with time. The purpose of the present study is to establish a simple and accurate spectrophotometric method, which is indispensable for the investigation of the catalytic effects of metal complexes on the autoxidation of *l*-ascorbic acid.

It is well known that the iron(II) - 1,10-phenanthroline complex shows an intense red color and is more stable than the corresponding ferric complex over a wide pH range. On the other hand, the ferric complex has been found by the authors to be quantitatively reduced to the ferrous complex by *l*-ascorbic acid. Use is made of these facts for the present purpose. Because of the instability of the colored solution, no report has been presented for the determination of *l*-ascorbic acid using the ferric complex as a coloring agent. The instability of the colored solution seems to be caused by the photo-reduction of the ferric complex, because the iron(III) - 1,10-phenanthroline solution gradually shows a red color in visible light.[5]

The photo-reduction of the ferric complex, however, has been avoided by adding a chelating agent such as ethylenediaminetetraacetic acid (EDTA) to mask excessive ferric iron after the color development. The method has been further applied to the measurement of the catalytic activities of metal complexes for the autoxidation of *l*-ascorbic acid.

Experimental

Apparatus.—Absorbance mearurements were made with a Shimadzu spectrophotometer, Model QR-50, using 1 cm. glass cells.

Reagents. — *l-Ascorbic Acid Solution.* — For the determination, 0.2 g. of l-ascorbic acid manufactured by Merck & Co. is dissolved in water, and the solution is diluted to 100 ml. A 10 ml. portion is pipetted out and diluted to 500 ml. For the catalytic reaction, 0.2 g. of *l*-ascorbic acid is directly dissolved in 500 ml. of water. These solutions are freshly prepared for every use.

1,10-Phenanthroline Solution.—Prepared by dissolving 1.4401 g. of 1,10-phenanthroline hydrochloride in water, and by diluting this to 1000 ml. with water.

Ferric Iron Solution. — Prepared by dissolving 0.9858 g. of ferric ammonium sulfate in 1000 ml. of water.

EDTA Solution.—Prepared by dissolving 9.2555 g. di-sodium salt of EDTA in water, and by diluting it to 500 ml.

Metal Salt Solutions.—Prepared as 10^{-4}M solutions.

Buffer Solution.—Prepared by mixing each of 1 M acetic acid and sodium acetate solution at a moderate ratio.

Water.—Prepared by redistilling ordinary distilled water with an all-glass-jointed Pyrex glass apparatus, and by saturating it with purified air.

Procedure. — Take 5.0 ml. each of iron(III) and buffer solutions in a 50 ml. measuring flask, add a sample solution containing *l*-ascorbic acid, and, then 5.0 ml. of the 1,10-phenanthroline solution. Add 1.0 ml. of the EDTA solution after 1 min., dilute to the mark with water, and measure the absorbance of the colored solution at 514 mμ against a reagent blank solution within an hour.

Results and Discussion

Absorption Spectra.—The absorption spectra of the blank, iron(II)- and iron(III)-complexes are shown in Fig. 1. The maximum absorbances for both A and B in Fig. 1 are observed at 514 mμ, where the blank shows a slight absorption. The shapes of the absorption spectra are quite similar for A and B, and the λ_{max} gives a good agreement with the values reported in the literature.

The Effect of pH. — The absorbance of the mixture of 5.0 ml. each of iron(III) and 1,10-phenanthroline solutions and of 10 ml. of a *l*-ascorbic acid solution was measured at various pH values. The results obtained are shown in Fig. 2. The absorbances of the colored solutions are almost the same at pH 3.6~5.8.

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1) J. A. Owen, B. Iggo and D. B. Horn, *Nature*, **174**, 701 (1954).

2) M. Schmall, C. W. Pifer, E. G. Wollish et al., *Anal. Chem.*, **26**, 1521 (1954).

3) H. Fukuba, J. Yamazaki and N. Inagaki, *Vitamin (Japan)*, **9**, 312 (1955).

4) C. G. King, *Ind. Eng. Chem.*, **13**, 225 (1942).

5) Cf. C. C. Tsen, *Anal. Chem.*, **33**, 849 (1961).

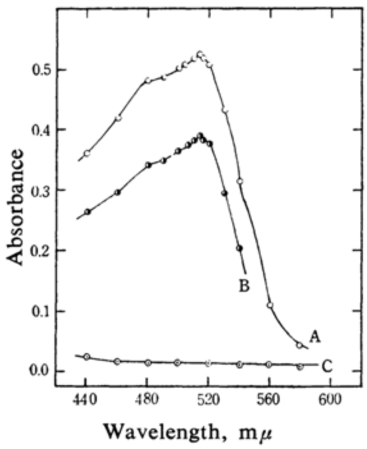

Fig. 1. Absorption spectra.

A: Fe(III) + (ascorbic acid) + 1, 10-phenanthroline)
B: Fe(II) + (1, 10-phenanthroline)
C: Fe(III) + (1, 10-phenanthroline)

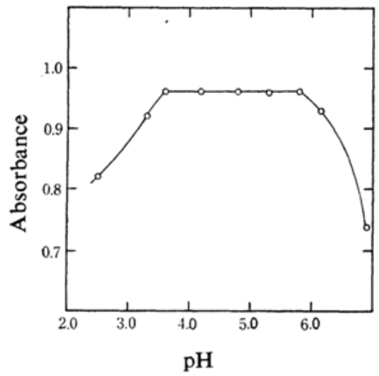

Fig. 2. Relationship between pH and absorbance of the colored solution.

The Stability of the Colored Solution.—The relations between the absorbance and the time after the color development are shown in Fig. 3. It is apparent from Fig. 3 that the absorbance of the colored solution increases with time in the absence of a chelating agent, while it is almost constant in the presence of a moderate amount of EDTA or phosphoric acid. The increase in the absorption mentioned above seems to be due to the photo-reduction of the ferric complex, because the mixed solution of iron(III) and 1, 10-phenanthroline easily turns red under sunlight, and the stable coloration seems to be based on the masking of the excessive iron(III) with EDTA or phosphoric acid. The coloration is stable for at least an hour in the presence of a chelating agent.

Calibration Curve. — Solutions containing various amounts of *l*-ascorbic acid were treated by the preceding procedure; the results are plotted in Fig. 4. As can be seen from Fig. 4, up to 8.0 p.p.m. *l*-ascorbic acid obeys Beer's law.

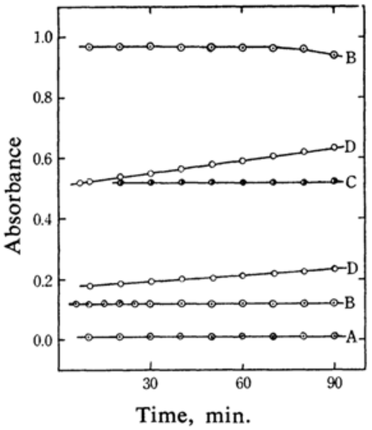

Fig. 3. Stability of the colored solution.

A: Fe(III) + (1, 10-phenanthroline) + (1 ml. of 0.05 M EDTA)
B: Fe(III) + (1, 10-phenanthroline) + (different amount of *l*-ascorbic acid) + (1 ml. of 0.05 M EDTA)
C: Fe(III) + (1, 10-phenanthroline) + (*l*-ascorbic acid) + (1 ml. of 0.15 M H_3PO_4)
D: Fe(III) + (1, 10-phenanthroline) + (*l*-ascorbic acid)

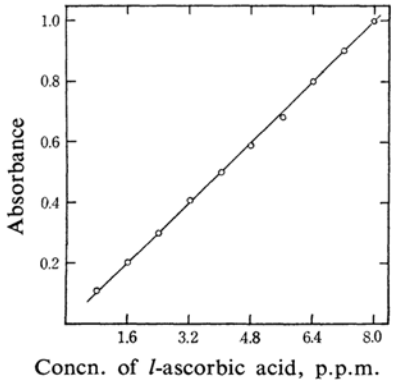

Fig. 4. Calibration curve for *l*-ascorbic acid at 514 mμ.

Diverse Ions.—The method is interfered with not only strong oxidizing or reducing agents, but also by small amounts of cations. The effects of metal ions on the catalytic oxidation of *l*-ascorbic acid were measured by the following procedure: Take a definite amount of metal ions in a test-tube fitted with a glass-stopper, adjust the pH to 4.3 with the acetate buffers, and dilute to 90 ml. with water. Take an aliquot volume of an *l*-ascorbic acid solution in another test-tube. Stopper both tubes and place them in a thermostat held at 25±0.1°C for about two hours. Pipette out 10 ml. of the *l*-ascorbic acid solution, add it to the other test-tube, mix, and let it stand in the thermostat. Pipette a 10 ml. portion out of the solution as soon as possible, and estimate the amount of *l*-ascorbic acid in it by the preceding method, where the time is regarded as zero. The residual amounts of *l*-ascorbic acid in the mixed solution are determined by the same treatment after a definite interval.

The values of $\log C_0/C$ are plotted against the time, "t," and the rate constant, "k," is calculated from the equation, $k=(2.303/t) \times \log C_0/C$. The results obtained are shown in Table I and in Fig. 5. The catalytic effects of various metal ions are compared by the k values, the order of which is as follows:

$$\mathrm{Cu(II)} \gg \mathrm{Fe(II)} > \mathrm{Cd} > \mathrm{Zn} > \mathrm{Al} > \mathrm{Fe(III)}$$

Among these, copper(II) is the most active, followed by iron(II), iron(III), cadmium, zinc and aluminum. The other metal ions, such as silver(I), cobalt(II), mercury(II), magnesium, manganese(II), nickel(II), lead(II), palladium and chromium(III), do not show any remarkable activity. Since the linear relationships as shown in Fig. 5 are obtained between $\log C_0/C$ and t with regard to the active ions, the oxidation is consider to proceed as of the first order with respect to the ascorbic acid. The reaction rate of *l*-ascorbic acid against various pH values in the presence of a definite amount of copper(II) is shown in Fig. 6. Judging from the reaction mechanism reported in the literature,[6,7] the following conditions seem to be at least necessary for the catalytic oxidation of *l*-ascorbic acid with metal ions: (1) the coordination of *l*-ascorbic acid to the metal ions, and (2) the valency change of the metal ion by the coordination. Since the complex formation proceeds witd the pH increase, the results shown in Fig. 6 are understandable.

According to Mellor and Maley,[8] the stability of bivalent metal complexes is ordered as follows:

TABLE I. OXIDATION RATE OF *l*-ASCORBIC ACID IN THE PRESENCE OF VARIOUS KINDS OF METAL IONS (25±0.1°C, pH=4.3, metal ion: 5×10^{-6} M/l.)

Metal ion	Salt	Rate constant k, $\times10^3$ min^{-1}
—	—	3.14
Ag	$AgNO_3$	3.14
Cd	$Cd(NO_3)_2$	6.90
Co	$Co(CH_3COO)_2$	3.03
Cu	$CuSO_4$	33.7
Fe	$FeSO_4(NH_4)_2SO_4$	7.32
Hg	$Hg(NO_3)_2$	2.68
Mg	$MgCl_2$	2.74
Mn	$MnCl_2$	2.49
Ni	$NiCl_2$	4.22
Pb	$Pb(CH_3COO)_2$	4.23
Pd	$PdCl_2$	3.86
Zn	$ZnCl_2$	6.30
Al	$Al_2(SO_4)_3$	6.12
Cr	$Cr(NO_3)_3$	4.72
Fe	$Fe_2(SO_4)_3(NH_4)_2SO_4$	5.48

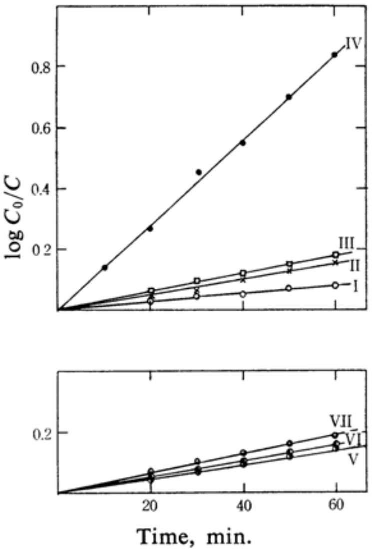

Fig. 5. Relationship between "t" and $\log C_0/C$.
I: blank, II: Zn, III: Cd, IV: Cu, V: Fe(III), VI: Al, VII: Fe(II)

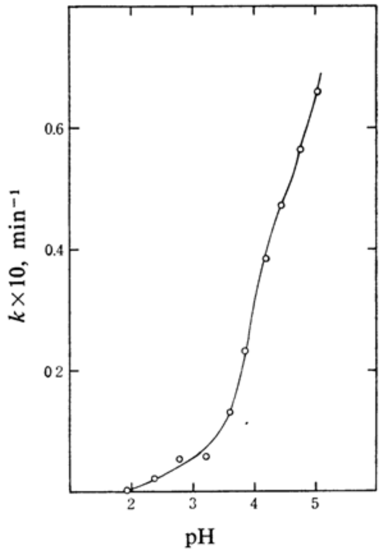

Fig. 6. Relationship between pH and "k" for the oxidation of *l*-ascorbic acid in the presence of a definite amount of Cu^{2+}, at 25°C.

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[6] A. Weissberger, J. E. LuValle and D. S. Thomas, Jr., *J. Am. Chem. Soc.*, **65**, 1934 (1943).

[7] A. Weissberger and J. E. LuValle, ibid., **66**, 700 (1944).

[8] D. P. Mellor and L. Maley, *Nature*, **159**, 370 (1947); **161**, 436 (1948).

$$\mathrm{Pd} > \mathrm{Cu} > \mathrm{Ni} > \mathrm{Pb} > \mathrm{Co} > \mathrm{Zn} > \mathrm{Cd} > \mathrm{Fe} > \mathrm{Mn} > \mathrm{Mg}$$

In view of this stability series and the reversibility of the valency change, the order of the catalytic activity is also understandable, except for iron(III). The inactivity of iron(III) seems to be closely related to the hydrolysis at pH 4.3. The inactivity of palladium is considered to be based on the irreversibility of its valency, since palladium(II) is easily reduced to palladium(0).

Summary

A simple and accurate spectrophotometric method with the iron(III) - 1, 10-phenanthroline reagent has been established for the determination of a small amount of *l*-ascorbic acid; it has been applied to the measurement of the catalytic effects of metal ions on the autoxidation of *l*-ascorbic acid.

The authors wish to express their heartly thanks to Miss Mikiko Kobata and Miss Masako Onishi for their skillful technical assistance.

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