STUDIES ON AUTOXIDATION AND DETERMINATION OF L-ASCORBIC ACID

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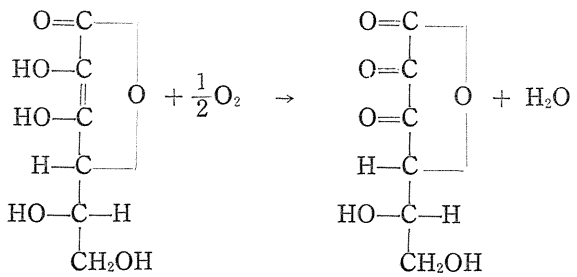
CONTENTS

1. Kinetics of Cupric Salt-catalysed Autoxidation of L-Ascorbic Acid in Aqueous Solutions[1]

1.1. Introduction

A number of workers have reported on the complexity of the metallic ion-catalysed autoxidation of L-ascorbic acid (Vitamin C, abbreviated as H_2A) to dehydroascorbic acid (DA).



(H_2A) (DA) (1)

The rate of reaction is known to depend on *p*H, catalyst, oxygen pressure, temperature, buffer, *etc.* Even in the well studied autoxidation catalysed by cupric ion at lower *p*H, there is a diversity of opinion among investigators. For example, the order with hydrogen-ion concentration has been reported to be -0.5[2], -0.7[3], -1[4], -2[5] and a non-integral number[6].

It has been said that the reaction is unimolecular in ascorbic acid, but Silverblatt *et al.*[3] have reported that the first-order constant varies with its initial concentration.

The present study was undertaken to obtain some information on the kinetics of this cupric ion-catalysed autoxidation in unbuffered aqueous solutions, especially as to the effect of the initial concentration of ascorbic acid, *p*H, temperature and also the concentration and nature of cupric ion.

1.2. Results and Discussion

Effect of cupric salts. The acceleration of the autoxidation of ascorbic acid by cupric salts such as $CuCl_2$[7], $Cu(NO_3)_2$[8] and $CuSO_4$[3] is well known.

The rates with various cupric ion concentration were measured and pseudo-first-order constants were calculated by means of an ordinary first-order equation.

$$k = \frac{2.303}{t} \log([H_2A]_0/[H_2A]) \qquad (2)$$

where $[H_2A]_0$ and $[H_2A]$ denote the concentration of ascorbic acid at reaction time zero and t, respectively. The results are presented graphically in Fig. 1. Apparently, the rate is proportional to the concentration of cupric ion as has been reported[7].

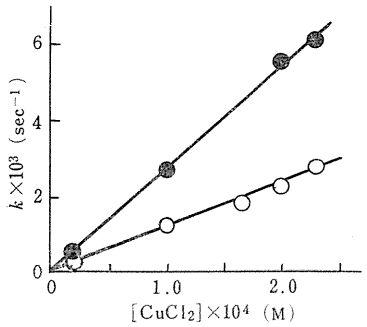


FIG. 1. Effect of cupric ion concentration of the first-order rate constant at 35° using $CuCl_2$ as catalyst. Initial concentration of ascorbic acid $[H_2A]_0$, ●, 1.15×10^{-2} M; ○, 2.3×10^{-2} M.

At very high concentration of $CuCl_2$, (*e.g.* above 2.3×10^{-3} M with $[CuCl_2]/[H_2A]_0$=*ca.* 0.1), an oxygen uptake increases by a factor of *ca.* 2 on account of the accumulation of hydrogen peroxide. The present kinetic experiments, however, were not extended to this concentration of $CuCl_2$.

The cupric ion-catalysed oxidation in hydrochloric acid is said to proceed *ca.* 50–100 times as fast as in nitric or perchloric acid of the same concentration[5], but it is not certain if there is a difference in the catalytic ability between cupric salts. The catalysis was compared using some cupric salts of different anions in the absence of excess acid or anion (Table 1).

TABLE 1. Effect of catalysts on rate constants in an aqueous solution at 35°

	$[H_2A]_0$ 2.3×10^{-2} M	5.0×10^{-2} M
$[Cu^{++}]$ 2.3×10^{-4} M	$10^3 k$ (sec^{-1})	$10^3 k$ (sec^{-1})
$CuCl_2$	3.22	1.36
$Cu(NO_3)_2$	2.56	1.29
$CuSO_4$	2.39	1.26

The Table shows that cupric nitrate and sulphate have virtually similar effects, but cupric chloride is a little more effective than the other two, especially at the lower concentration of ascorbic acid (2.3×10^{-2} M).

In order to obtain more accurate data at higher concentration of ascorbic acid, cupric chloride was used as a catalyst for all the experiments described.

Effect of the concentration of ascorbic acid. Most workers have reported the reaction to be first-order with ascorbic acid, *e.g.*, the pseudo-first-order constant was estimated by Dekker *et al.*[5] in 1.06×10^{-3} M perchloric acid at 24.9° to be 31–27×10^{-4} min^{-1} at different initial concentrations of ascorbic acid (1.46×10^{-4} M and 72.6×10^{-4} M). However, the reaction rate is controlled significantly by the stirring rate of the reaction mixture, and as pointed out by some workers[9][10] insufficient stirring is responsible for the many contradictory results found in the literature.

According to our observation, the reaction rate is independent of the ascorbic acid concentration, when the stirring rate is low (*e.g.*, below 300 rpm with a 35 mm Teflon magnetic stirrer in the reaction bulb of 45 mm diameter), but as the stirring becomes more vigorous (*e.g.* more than 500 rpm), the rate of reaction tends to depend on the ascorbic acid concentration. This means that vigorous stirring enables the dissolution of oxygen gas fast enough so that it cannot be rate-determining.

The observed first-order rate constant calculated by Eq. 2, however, tends to decrease with increasing initial concentration of ascorbic acid (Table 2).

TABLE 2. Effect of initial concentration of ascorbic acid (and *p*H) in unbuffered solutions with $[CuCl_2]$ 2.0×10^{-4} M at 35°

$[H_2A]_0\times10^2$ M	*p*H	10^3k (sec^{-1})
1.15	3.20	5.65
1.5	3.14	4.68
2.0	3.09	3.00
2.3	3.02	2.65
3.0	2.99	1.88
5.0	2.86	1.22
8.0	2.82	0.80
10.0	2.70	0.61

This decrease appears to be caused in part by an increase of the ionic strength and/or acidity of ascorbic acid itself, but even if the ionic strength and *p*H are changed by adding KNO_3 and HNO_3, respectively, with a constant concentration of ascorbic acid, such a sharp decrease as in Table 2 is not observed. This phenomena will be discussed later.

Effect of ionic strength. As described above, the increase of rate constant with the initial concentration of ascorbic acid may be due to the increase of ionic strength, *i.e.* the ions dissociated from substrate or catalyst may suppress the reaction. For the examination of the effect of ionic strength, the rates were measured in solutions with various ionic strength using KNO_3. The results are shown in Table 3.

As is obvious from the Table, the k value is not affected by the ionic strength, hence the effect of initial concentration of ascorbic acid cannot be ascribed to the ionic strength.

TABLE 3. Effect of ionic strength with $[CuCl_2]$ 2.0×10^{-4} M at 35°

	$[H_2A]_0$ 2.3×10^{-2} M	8.0×10^{-2} M
KNO_3 (M)	$10^3 k$ (sec^{-1})	$10^3 k$ (sec^{-1})
0	2.65	0.80
10^{-3}	2.62	0.73
10^{-2}	2.72	0.78

Effect of acidity. Table 2 lists the *p*H values in unbuffered aqueous solutions of ascorbic acid together with the rate constant. The relation between hydrogenion concentration and first-order constant can easily be obtained by plotting *p*H *vs.* $\log k$ as a broken line in Fig. 2, indicating a linear relation with a slope of 2 in a range of *p*H 2.7–3.2.

$$\log k = 2\,p\mathrm{H} - 4.2$$

This effect of acidity can be checked by varying *p*H alone with constant concentration of ascorbic acid. This change of *p*H was done by adding nitric acid because of no appreciable influence of the nitrate ion as shown in Table 3. Plots of $\log k$ *vs.* *p*H (real lines in Fig. 2) at higher acidity on addition of various amounts of nitric acid for the acid source gave lines having a slope of about unity at lower *p*H and 0.63–0.83 at higher *p*H.

The apparent disagreement of *p*H-rate profiles is observed as shown in real and broken lines in Fig. 2. This fact may be explained by assuming that the rate constant k is inversely proportional to the ascorbic acid concentration, because of (i) association of ascorbic acid (a strong Raman line[11] near 1700 cm^{-1} and a shift of UV absorption spectra) or (ii) transformation into keto form and (iii) the effect of chloride ion as shown in Table 1, and/or (iv) the complex dependency of the rate equation on $[H_2A]$ as shown in Eq. 13, if the stoichiometric concentration of total copper is taken into account.

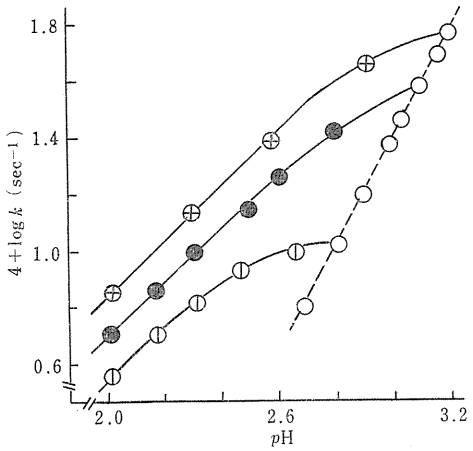


FIG. 2. Effect of *p*H of solution caused by the initial concentration of ascorbic acid (○) and added HNO_3 with initial concentration of ascorbic acid (⊕ 1.15×10^{-2} M, ● 2.3×10^{-2} M and ⊖ 5.0×10^{-2} M) on the first-order rate constant at 35°.

Effect of oxygen pressure and temperature. All experiments described were conducted by supplying pure oxygen. In order to examine the effect of partial pressure of oxygen, a mixture of oxygen and nitrogen was used for the oxidation keeping the initial total pressure in the reaction vessel to be 1.0 atm, which is the sum of partial pressures of O_2. N_2 and vapourized solvent. Effect of oxygen pressure was investigated at 30°, 35°, 40° and 45°, the results are shown in Table 4 and Fig. 3.

TABLE 4. Effect of oxygen pressure, p (atm) and reaction temperature of the first-order rate constant, k (sec^{-1}) with $[CuCl_2]$ 2.0×10^{-4} M and $[H_2A]_0$ 2.3×10^{-2} M

30°	p	0.958	0.757	0.592	0.362
	$10^3 k$	1.69	1.47	1.10	0.80
35°	p	0.945	0.822	0.592	0.362
	$10^3 k$	2.65	2.39	1.95	1.30
40°	p	0.927	0.855	0.658	0.395
	$10^3 k$	3.98	3.53	2.96	2.48
45°	p	0.905	0.822	0.592	0.290
	$10^3 k$	5.50	4.54	4.10	2.96

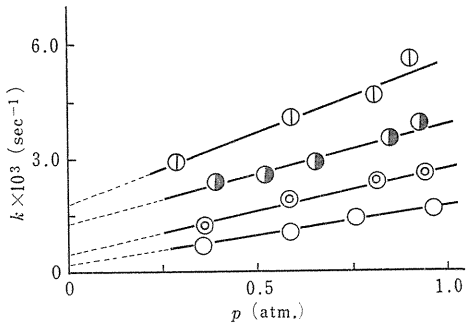


FIG. 3. Effect of partial pressure of oxygen. Total pressure, 1.0 atm; $[H_2A]_0$ 2.3×10^{-2} M, $[CuCl_2]$ 2.0×10^{-4} M, ○ 30°, ◎ 35°, ◑ 40°, ⓛ 45°.

Weissberger *et al.*[3] have found that the oxidation rate of divalent ascorbate ion is proportional to the partial pressure of oxygen (p) but that of monovalent ascorbate ion is independent of p. A number of investgators[3,6,7,13] reported that the oxygen dependency of reaction rate in an acidic solution deviates from proportionality.

As shown in Fig. 3, straight lines do not pass through the origin. This means that the reaction involves two simultaneous steps, *i.e.*, a step dependent on p and a step independent of p. The comparison of these steps will be described later.

Mechanism. The rate data together with the other known facts suggests the following mechanism for the autoxidation at pH *ca.* 3, $[H_2A]_0$ of *ca.* 10^{-2}—10^{-} M and *ca.* 35°.

$$H_2A \overset{K_3}{\rightleftarrows} H^+ + HA^- \tag{3}$$

$$HA^- + Cu(II) \overset{K_4}{\rightleftarrows} [HACu(II)]^- \tag{4a}$$

$$[\mathrm{HACu(II)}]^- \underset{\text{slow}}{\overset{k_4}{\longrightarrow}} \mathrm{HA^{\cdot} + Cu(I)} \tag{4b}$$

$$\mathrm{HA^- + Cu(II) + O_2} \overset{K_5}{\rightleftarrows} [\mathrm{HACu(II)O_2}]^- \tag{5a}$$

$$[\mathrm{HACu(II)O_2}]^- \underset{\text{slow}}{\overset{k_5}{\longrightarrow}} \mathrm{HA^{\cdot} + Cu(II) + O^{\cdot -}} \tag{5b}$$

The monodehydroascorbate radical ($HA^{\cdot}$) formed is then transformed into dehydroascorbic acid (DA).

$$\mathrm{HA^{\cdot}} \overset{\text{Base}}{\rightleftarrows} \mathrm{A^{\cdot -}} \overset{\mathrm{Cu(II)}}{\longrightarrow} \mathrm{DA + Cu(I)} \tag{6}$$

and/or

$$\mathrm{HA^{\cdot}} \longrightarrow \mathrm{DA}(+\mathrm{H_2A}) \tag{7}$$

Ascorbic acid is stable in pure water[14]. In our experiments without copper catalyst at 35°, pseudo-first-order constant k in pure water was *ca.* $10^{-6}\,\mathrm{sec^{-1}}$ or less than 1/1000 of k with a catalyst of 2.0×10^{-4} M $CuCl_2$ under the same conditions. It is plausible that the Cu(II) catalysed autoxidation proceeds by an electron transfer from ascorbate ion to Cu(II).

Although the electron transfer may be easier between Cu(II) and divalent ascorbate ion(A^{2-}) which is richer in electrons, the dissociation constant (first and second dissociation constants are *ca.* 4×10^{-5} and 2×10^{-12}, respectively) indicates that the concentration of A^{2-} is extremely low at *p*H *ca.* 3.

The reactive species HA^- donates an electron to Cu(II) forming probably a complex as shown in Fig. 4 or 5, where R is $CH(OH)CH_2OH$ and L_1, L_2 and L_3 are ligands which may be Cl, H_2O or O_2.

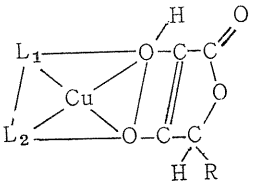


FIG. 4
[HACu(II)]$^-$ complex

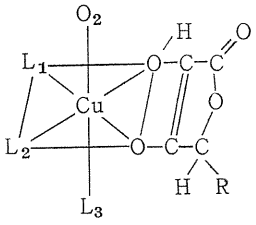


FIG. 5
[HACu(II)O_2]$^-$ complex

It is known that in the absence of oxygen, ascorbic acid can reduce cupric salts to give cuprous salts[15]. Hence, the complex in Fig. 4 is conceivable, at least, in an insufficient supply of oxygen in a solution. The complex in Fig. 5 is similar to that for the cupric ion-catalysed autoxidation of *o*-phenylenediamine[16].

Both compexes are subject to rate-determining decomposition expressed as Eqs. 4b and 5b. Khan and Martell[4] proposed that the reaction proceeds by way of only Eq. 5a based on their results at 25° and 0.4°. However, as was discussed in the previous section, the rate equation includes a term independent of oxygen

pressure at a lower concentration of ascorbic acid.

The slightly S-shaped conversion curve of this reaction suggests the presence of a short induction period which is probably caused by the formation of hydrogen peroxide during this period according to 4 b and 5 b together with the steps described below.

$$Cu(I) + O_2 + H^+ \rightarrow Cu(II) + HO_2^{\cdot} \quad (8)$$

$$O_2^{\cdot-} + H^+ \rightarrow HO_2^{\cdot} \quad (9)$$

$$2\,HO_2^{\cdot} \rightarrow H_2O_2 + O_2 \quad (10)$$

All these steps are very fast and hydrogen peroxide reacts with ascorbic acid in the stoichiometry:

$$H_2O_2 + H_2A \rightarrow DA + 2\,H_2O \quad (11)$$

Eq. 11 was confirmed by the reaction of 1.08×10^{-2} M hydrogen peroxide with ascorbic acid of various concentrations (2.0×10^{-2}, 4.0×10^{-2} and 10^{-1}M) at 35°.

The reaction rate may be expressed as:

$$-d[H_2A]/dt = \frac{3}{2}\{k_4[HACu(II)]^- + k_5[HACu(II)O_2]^-\} \quad (12)$$

Eq. 12 can be expressed using the total stoichiometric copper concentration $[Cu]_T$ as follows.

$$-d[H_2A]/dt = \frac{3}{2}\{K_3[Cu]_T(k_4K_4 + k_5K_5p)/[H^+]\} \times \left\{[H_2A]/\left(1 + \frac{K_3(K_4 + K_5p)}{[H^+]}[H_2A]\right)\right\} \quad (13)$$

Eq. 13 seems to explain the observed facts, *i.e.*, the deviation of kinetics for Eq. 2 at lower acidity, especially at higher cnceontration of ascorbic acid, and the observed constancy of k with various concentrations of ascorbic acid at higher acidity as obvious in Fig. 2.

Reactions 6 or 7 are considered for the formation of dehydroascorbic acid (DA) from radical HA·. Reactions between radical anion $A^{\cdot-}$ and C(II) giving DA should be rapid, although the concentration of $A^{\cdot-}$ is low at lower *p*H. On the other hand, the concentration of HA· should be higher, hence its reaction with oxygen molecule[17] and the disproportionation reaction[18] between two HA· to give DA and H_2A may occur.

Apparent activation energy was 14.4 kcal mole^{-1} estimated from Fig. 6.

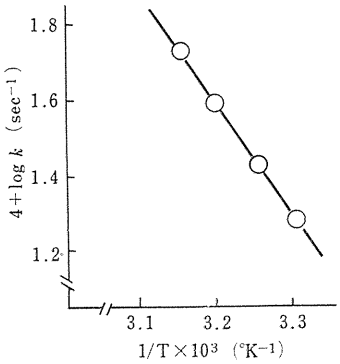


FIG. 6. Arrhenius plot for apparent activation energy.

1.3. Experimental Section

Materials. Ascorbic acid was of 99.5% pure with decomposition point of 190.9° (lit.[19] 190–192°) and optical rotation of $[\alpha]_D^{25} + 20.94°$

(lit.[19] $[\alpha]_D^{25}$ 20.5-21.5°). Used water was passed through a cation exchange resin, Amberlit MB-1, followed by the distillation in a glass-joint flask. O_2 and N_2 were of over 99.7% and 99.95% pure, respectively. Cupric chloride, nitrate, sulphate, potassium nitrate and nitric acid were of guaranteed reagent grade.

Reaction products criterion. The cupric salt-catalysed autoxidation products obtained from ascorbic acid at *p*H *ca.* 3 were analysed for content by the well established methods, *i.e.*, titration with 2, 6-dichloro-phenolindophenol[15)a]. At an initial stage of reaction no hydrogen peroxide could be detected by iodometry with taking into account a content of ascorbic acid. Cuprous ion was virtually absent in the reaction mixture. The hydrogen sulfide reduction of reaction mixture followed by filtration and iodometry proved that dehydroascorbic acid was a main oxidation product, *i.e.*, a total percentage of ascorbic acid and dehydro-ascorbic acid in the reaction mixture was 100±5%. The other by-products could not be detected. These facts were also confirmed by the amount of O_2 consumed, and was in accordance with literature on the autoxidation at a lower *p*H, *i.e.*, the stoichiometry is expressed as Eq. 1.

Kinetic pocedure. A reaction vessel shown in Fig. 7 was ued in a thermostat in which the difference of pressures in both bulbs can be read as the difference of mercury levels of both capillary tubes. An aqueous solution of cupric salt, whose copper concentration was estimated by iodometry, was introduced into the right-side bulb (B) and a solution of ascorbic acid of a measured amount in another bulb (A) which can be stirred by a Teflon stirrer. After evacuation of the vessel by an aspirator, a solution of cupric salt (10 m*l*) was also poured from a burette into the left-side bulb. The vessel was again evacuated, and then O_2 gas was introduced into both bulbs. The cocks A_1 and B_1 were quickly closed and the reaction was started with vigorous magnetic stirring. The difference of levels of manometer at definite intervals of time gave the estimation of O_2 pressure. The *p*H was measured by a Hitachi-Horiba Type-4 *p*H meter calibrated at three points.

The consumed molarity of O_2 (Δm) can be calculated by Eq. 14.

$$\Delta m = \Delta h V / 760\, RT \tag{14}$$

where Δh is the manometric difference, V, the volume of reaction bulb (74.4 cm^3), R, the gas constant and T, the reaction temperature in Kelvin.

The stoichiometric Eq. 1 affords the amount of remaining ascorbic acid [H^2A] at time t as follows:

$$[H_2A] = [H_2A]_0 - 2\,\Delta m \tag{15}$$

Here, $[H_2A]_0$ is the initial concentration of ascorbic acid. Some examples of conversion curve and plot of $\log[H^2A]$ *vs.* t were shown in Figs. 8 and 9. The first-order kinetic law with ascorbic acid was almost atisfied at lower concentration of ascorbic acid with stirring of 800 rpm.

1.4. Summary

The kinetics of the cupric salt-catalysed autoxidation of L-ascorbic acid has been studied in unbuffered aqueous solutions at *ca.* 35°. The catalytic ability is

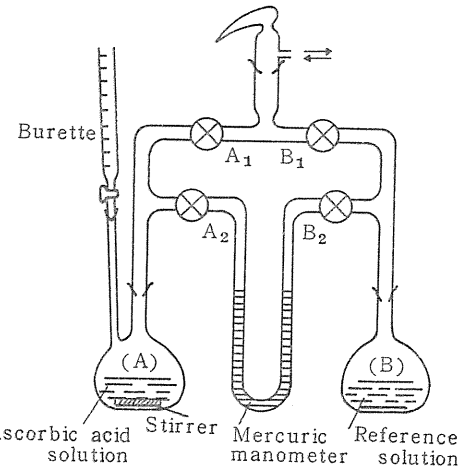


FIG. 7. Apparatus for the kinetic experiment for the autoxidation of ascorbic acid.

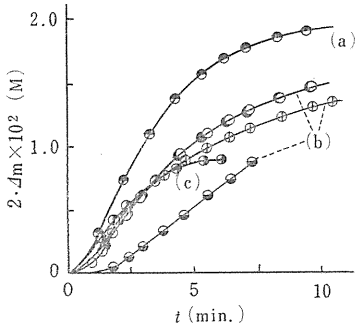


FIG. 8. Conversion curves.
Initial concentration of ascorbic acid: (a) 10^{-1} M (stirring of 800 rpm), (b) 2.3×10^{-2} M (○ 800 rpm, ● 500 rpm, ⓘ 300 rpm, ◒ 100 rpm) and (c) 1.15×10^{-2} M (800 rpm).

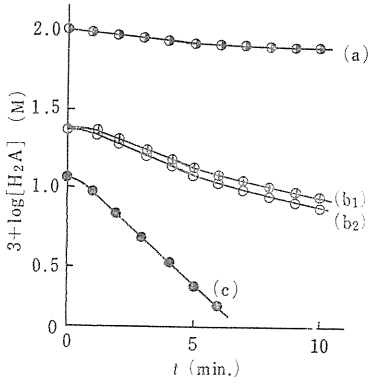


FIG. 9. Plots of log $[H_2A]$ *vs.* time.
Initial concentration of ascorbic acid: (a) 10^{-1} M (stirring of 800 rpm), (b_1) 2.3×10^{-2} M (300 rpm), (b_2) 2.3×10^{-2} M (800 rpm) and (c) 1.15×10^{-2} M (800 rpm).

in the order $CuCl_2 \geq Cu(NO_3)_2 \sim CuSO_4$. The effect of ionic strength is negligible. The observed effects of *p*H, cupric ion concentration and partial pressure of oxygen at various temperatures on the reaction rate suggests a mechanism which may involve two courses for the formation of mono-dehydroascorbate radical ($HA^{\cdot}$):

$$H_2A \rightleftarrows H^+ + HA^-$$

$$HA^- + Cu(II) \rightleftarrows [HACu(II)]^- \xrightarrow{\text{slow}} HA^{\cdot}$$

$$HA^- + Cu(II) + O_2 \rightleftarrows [HACu(II)O_2]^- \xrightarrow{\text{slow}} HA^{\cdot}$$

The final product, dehydroascorbic acid (DA), may be formed from both $HA^{\cdot}$ and $A^{\cdot-}$ (radical anion). The resulting hydrogen peroxide reacts readily with ascorbic acid, giving also DA.

2. Solvent Effect on the Autoxidation of L-Ascorbic Acid[10]

2.1. Introduction

Only few reports are available concerning the solvent effects on the oxidation of L-ascorbic acid (H_2A), *e.g.*, retardation of enzymatic oxidation in acetic acid[21] and the retardation of copper-catalysed autoxidation in pyridine which forms a complex with cupric ion[22]. The latter solvent effect is similar to the retardation of metaphosphoric acid or N- or S-containing compounds which complex with copper. The rates of oxidation by molecular oxygen[23], or iodine[24] or potassium permanganate[24] are lowered in the reaction in a solution of alcohol or acetone, and no suitable explanation has been offered for this.

The authors[25] have stated the kinetic and mechanistic study of the cupric salt-catalysed autoxidation of L-ascorbic acid to dehydroascorbic acid (DA) in unbuffered aqueous solutions.

Here, we describe the solvent effects on the rate of cupric chloride-catalysed autoxidation, and its relation to the mechanism.

2.2. Results and Discussion

Autoxidation of ascorbic acid in ethylene glycol. The effect of initial concentrations of ascorbic acid ($[H_2A]_0$), $CuCl_2$ and oxygen on the rate at various reaction temperatures was measured in ethylene glycol as a standard organic solvent. The oxygen consumption curves are similar to those of the reactions in water[25]. Therefore, the reactions in ethylene glycol may also follow Eq. 1.

$$H_2A + \frac{1}{2}O_2 \rightarrow DA + H_2O \tag{1}$$

In fact, plots of $\log([H_2A]_0/[H_2A])$ *vs.* t give a straight line, where $[H_2A]_0$ and $[H_2A]$ denote the concentrations of ascorbic acid at time zero and t, respectively. Pseudo-first-order rate constant (k) of Eq. 2 was calculated from the straight lines. Eq. 2 was also confirmed by UV spectrophotometry described in the Experimental Section.

$$-d[H_2A]/dt = k[H_2A] \tag{2}$$

The effect of the initial concentration of ascorbic acid is shown in Fig. 10. The decrease of k value with an increase of $[H_2A]_0$ is observed, although the trend is less remarkable than in an aqueous solution. The decrease may be due to the association of the acid or to the transformation of the enolic acid into its keto form and the presence of zeroth-order term in the kinetic equation (the second term in the right side of Eq. 8)[25].

The effect of $[CuCl_2]$ in a range of $2.3\times10^{-5}-1.1\times10^{-3}$ M with 2.3×10^{-2} M initial concentration of ascorbic acid ($[H_2A]_0$) is given in Fig. 11. It is obvious that the rate constant k increases proportionally to $[CuCl_2]$ up to 1.1×10^{-3} M or *ca.* 1/5 times of $[H_2A]_0$. Higher concentration of $CuCl_2$ accelerates the reaction and the saturation of the solution with $CuCl_2$ may cause experimental errors.

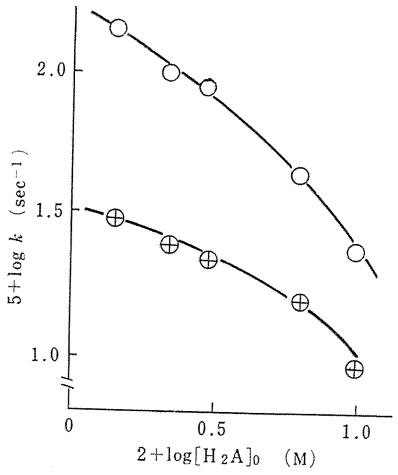


FIG. 10. Effect of initial concentration of ascorbic acid ($[H_2A]_0$) on the rate constants (k) for its autoxidation in the presence of 2.3×10^{-4} M $CuCl_2$ at 25°; ○ in water, ⊕ in ethylene glycol.

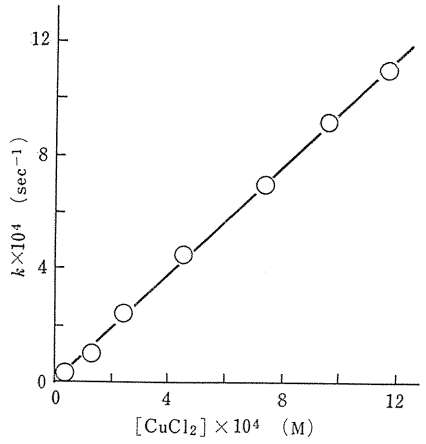


FIG. 11. Effect of $CuCl_2$ concentration on the first-order rate constant for the autoxidation of ascorbic acid ($[H_2A]_0=2.3\times10^{-2}$ M) in ethylene glycol at 25°.

These results together with the effect of oxygen pressure and temperature, which will be described later, suggest that the reaction in ethylene glycol may also proceed by Eqs 3–7 as postulated with an aqueous solution[25].

$$\text{associated } H_2A \rightarrow H_2A \underset{}{\overset{K_3}{\rightleftarrows}} H^+ + HA^- \tag{3}$$

$$HA^- + Cu(II) \overset{K_4}{\rightleftarrows} [HACu(II)]^- \tag{4a}$$

$$[HACu(II)]^- \overset{k_4}{\longrightarrow} HA^{\cdot} + Cu(I) \tag{4b}$$

$$HA^- + Cu(II) + O_2 \overset{K_5}{\rightleftarrows} [HACu(II)O_2]^- \tag{5a}$$

$$[HACu(II)O_2]^- \overset{k_5}{\longrightarrow} HA^{\cdot} + Cu(II) + O_2^{\cdot-} \tag{5b}$$

$$HA^{\cdot} \underset{+H^+}{\overset{\text{Base } (-H^+)}{\rightleftarrows}} A^{\cdot-} \xrightarrow{Cu(II)} DA + Cu(I) \tag{6}$$

and/or

$$HA^{\cdot} \rightarrow DA(+H_2A) \tag{7}$$

General solvent effect. The reaction rates were measured in some other organic solvents, *e.g.* methanol, *n*-butanol, aqueous ethanol and dimethylformamide (DMF). Conversion curves at initial stages for various solvents are similar to those in water and in ethylene glycol. Therefore, the rate equation should have the same form as that in an aqueous solution[25].

$$-\mathrm{d}[H_2A]/\mathrm{dt} = \frac{3}{2}\frac{K_3[Cu]_T(k_4K_4+k_5K_5p)}{[H^+]} \times \frac{[H_2A]}{1+\dfrac{K_3(K_4+K_5p)[H_2A]}{[H^+]}} \qquad (8)$$

Here, $[Cu]_T$ is the total stoichiometric concentration of copper, p is the partial pressure of oxygen, and factor 3/2 corresponds to the effect of hydrogen peroxide produced in the oxidation of cuprous ion to cupric ion or in the reaction of radical anion ($O_2^{\cdot-}$) with a proton $\left(O_2^{\cdot-}+H^+ \rightarrow {}^{\cdot}O_2H \rightarrow \frac{1}{2}O_2+\frac{1}{2}H_2O_2\right)$. The concentration of ascorbic acid remaining was estimated by Eq. 1 by means of the oxygen consumption and the concentration was also confirmed by the UV method[26]. This suggests that hydrogen peroxide is consumed according to Eq. 9.

$$\frac{1}{2}H_2A+\frac{1}{2}H_2O_2 \rightarrow \frac{1}{2}DA+H_2O \qquad (9)$$

The second term on the right-hand side of Eq. 8, $K_3(K_4+K_5p)[H_2A]/[H^+]$, may be neglected except at higher concentration of ascorbic acid; plots of $\log[H_2A]$ *vs.* t gave straight lines, the proportionality of k to $[Cu]_T$ being observed in ethylene glycol (Figs. 10 and 11). Eq. 8 can be approximated to Eq. 10.

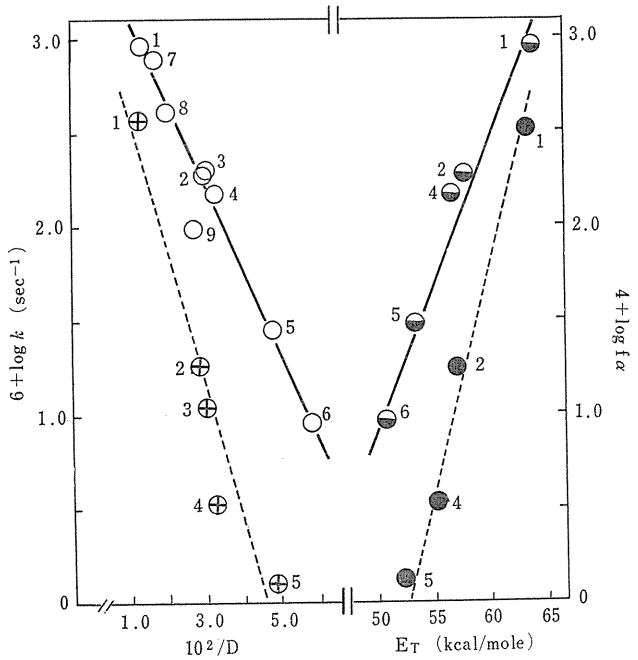


FIG. 12. Relation between rate constant (k) or degree of ionization ($\simeq f\alpha$) and solvent polarity with $[H_2A]_0$ of 2.3×10^{-2} M in various solvents at 25°: —○— $\log k$ *vs.* reciprocal of dielectric constant (D), —◒— $\log k$ *vs.* E_T value, ---⊕--- $\log f\alpha$ *vs.* reciprocal of dielectric constant, ---●--- $\log f\alpha$ *vs.* E_T value.

1, H_2O; 2, Ethylene glycol; 3, DMF; 4, MeOH; 5, EtOH; 6, *n*-BuOH; 7, 20% EtOH sq; 8, 50% EtOH aq; 9, 80% EtOH aq.

$$-d[H_2A]/dt = \frac{3}{2}\frac{K_3[Cu]_T(k_4K_4 + k_5K_5p)}{[H^+]}[H_2A] \quad (10)$$

The solvent effect (or polar effect of solvent) on the overall reaction is presented in Fig. 12 in real lines.

Dielectric constant (D) and E_T values[27] of solvents were found effective for the presentation of relationship between rate and solvent polarity, while other empirical parameters[27] such as X, Y, Z and S values were unsatisfactory. D values of aqueous alchol at 25° are 35.5, 52.5, and 68.5 for 80%, 50% and 20% ethanol solutions, respectively. These values were estimated by using data of Amis[28], Koelichen[29] and Landlt-Börnstein's Table[30].

Ionization degree of ascorbic acid. The degree of ionization of ascorbic acid (H_2A) or the concentration of ascorbate ion (HA^-), which is the actual reactive species (Eq. 4 or 5), was measured by means of electrical conductance. The degree of ionization is not expressed by K_3 but by K_{obs} in which association of ascorbic acid is also taken into account.

$$K_{obs} = \frac{(fc\alpha)^2}{(1-f)\cdot c + fc\cdot(1-\alpha)} = \frac{f^2\cdot c\cdot\alpha^2}{1-f\alpha} \quad (11)$$

Here, f is a fraction of monomolecular H_2A, $1-f$ is a fraction of associated H_2A, α is the ionization degree of H_2A and c is the stoichimetric concentration of H_2A. The value of $f\alpha$ in Eq. 11 is given by the ratio of electrical equivalent conductance at concentration c (Λ_c) to that at infinite dilution (Λ_0). It is difficult to obtain Λ_0 by the ordinary methods because of poor solubilities of salts of H_2A in organic solvents. Therefore, Onsager's theory[31] is appropriate for the estimation of Λ_0.

$$\Lambda_0' = \frac{\Lambda_c + \sigma C^{1/2}}{1-\theta C^{1/2}} \quad (12)$$

Here, σ and θ are constants characteristic of solvents (the values of constants are shown in Table 5).

Values of Λ_0' in Eq. 12 were measured at various concentrations of ascorbic acid (C) and extrapolated to infinite dilution, *i.e.*, C=0. The value of Λ_0 or Λ_0' at infinite dilution is presented in Table 5.

TABLE 5. Solvent constant (σ and θ) and ionization degree ($\simeq f\alpha$) of 2.3×10^{-2} M ascorbic acid at 25°

Solvent	σ (*)	θ (**)	Electrical equivalent conductance (Ω^{-1}) Λ_c	Λ_0	$f\alpha$
H_2O	60.2	0.229	17.5	488	3.52×10^{-2}
Ethylene glycol	4.43	0.669	0.15	68.3	2.20×10^{-3}
DMF	97.14	0.691	0.153	109.0	1.40×10^{-3}
MeOH	156.1	0.923	0.14	386.0	3.63×10^{-4}
EtOH	89.7	1.33	0.052	397.3	1.31×10^{-4}

* $l^{1/2}\Omega^{-1}\,mol^{-3/2}\,cm^2$

** $l^{1/2}\,mol^{-1/2}$

The combination of Eqs. 10 and 11 leads to Eq. 13.

$$-\mathrm{d}[\mathrm{H_2A}]/\mathrm{dt} = \frac{3}{2}\frac{f\alpha}{1-f\alpha}[\mathrm{H_2A}][\mathrm{Cu}]_T k_{14}K_{14} \tag{13}$$

where

$$k_{14}K_{14} = k_4K_4 + k_5K_5p \tag{14}$$

The combination of Eqs. 2 and 13 gives the rate constant k.

$$k = \frac{3}{2}\frac{f\alpha}{1-f\alpha}[\mathrm{Cu}]\, k_{14}K_{14} \tag{15}$$

The solvent effect on the ionization degree of ascorbic acid was examined by plotting $\log f\alpha$ *vs* $1/D$ or E_T which corresponds to the solvent polarity and is shown in Fig. 12 as dotted lines. It is apparent from Fig. 12 that the solvent effect on the ionization step (Eq. 3) is more remarkable than the other steps. No effect of ionic strength is found in an aqueous solution on addition of KNO_3[25], and this fact may suggest that the solvent effect on Eqs. 4 b and 5 b is small.

Effect of partial pressure of oxygen. Each constants k_4, K_4, k_5 and K_5 could not be estimated independently, but $k_{14}K_{14}$ values for reactions in various solvents

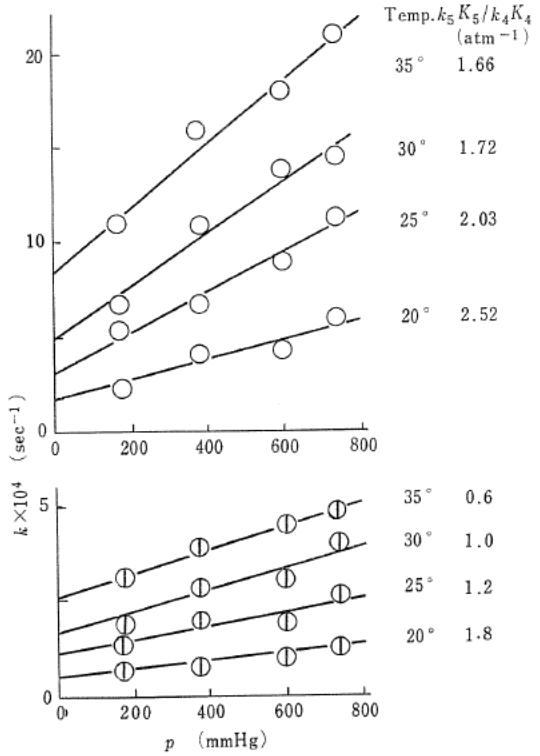


FIG. 13. Effect of partial pressure of oxygen (p), and values of k_5K_5/k_4K_4; ○ in water, ⦶ in ethylene glycol.

were estimated by means of Eq. 15 to be 1.05×10^2 for H_2O, 2.96×10^2 for ethlene glycol, 5.11×10^2 for DMF, 1.18×10^3 for MeOH and 8.41×10^2 for EtOH.

Ratio of k_5K_5 to k_4K_4 is also obtained with variation of oxygen pressures (p). Experiments were done in water and ethylene glycol with a mixture of oxygen and nitrogen keeping the initial total pressure in the reaction vessel to be 1.0 atm, which was the sum of partial total pressures of oxygen, nitrogen and vapourized solvent. The results are shown in Fig. 13.

The observed linerity between the partial pressure of oxygen and k suggests that Eqs. 4 b and 5 b occur simultaneously, and an equilibrium between these two complexes, *i.e.* one with oxygen molecules and the other without it, would exist as follows.

$$[HACu(II)]^- \underset{-O_2}{\overset{O_2}{\rightleftarrows}} [HACu(II)O_2]^- \qquad (16)$$

The ratio, k_5K_5/k_4K_4 in Fig. 13 was calculated by the ratio of the slopes to the intercepts of lines. The decrease of the ratio with temperature implies the shift of the equilibria of Eqs. 5 a and 16 to the left sides. Both k_5K_5 and k_4K_4 are affected by partial pressure of oxygen and temperature more in water than in ethylene glycol. This is consistent with the decrease of $k_{14}K_{14}$ value (*i.e.* the stability of complex) with an increase of solvent polarity under the same conditions.

Activation parameter. Rate constants at various temperatures gave Arrhenius plots which give apparent energies of activation; 15.1 and 14.7 kcal mol^{-1} for the reactions in water and in ethylene glycol, respectively (Fig. 14). These values are very close to the value (14.4 kcal mol^{-1}) in aqueous solution at 30–45°[25].

Using values $k_{14}K_{14}$ and similar treatments, $\log k_5K_5$ or $\log k_4K_4$ *vs* 1/T was

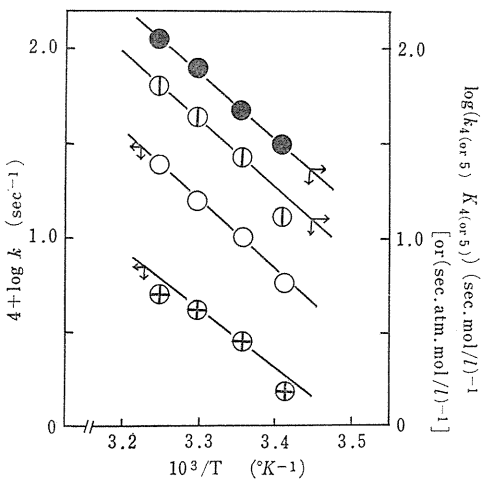


FIG. 14. Arrhenius plots for the reaction in water (—○—) and in ethylene glycol (—⊕—), and the effect of temperature on k_4K_4 (—◑—) or k_5K_5 (—●—).

plotted for the reaction in the aqueous solution, the plots being shown in Fig. 14 (● and ⓘ). These two lines have the same slopes as obvious in Fig. 14. This may mean that both pathways (4a–4b) and (5a–5b) are possible, the energy barriers being similar. This is another proof for the mechanism of Eq. 3–7.

2.3. Experimental Section

Materials. Ethylene glycol (b.p. 197°), DMF (b.p. 152.5°), MeOH (b.p. 64.6°), EtOH (b.p. 78.3°) and *n*-BuOH (b.p. 117°) were dried and fractionated by ordinary methods and qualified by the measurements of electrical conductances. Other reagents were of the same grade as in the previous report[25].

Kinetic procedure. The consumption rate of O_2 was measured by the manometric method[26] and stoichiometric Eq. 1 afforded the concentration of remaining ascorbic acid at time t, $[H_2A]$. The values of $[H_2A]$ at various times during the oxidation were also confirmed by UV spectrophotometry. The first-order kinetic law with ascorbic acid (Eq. 2) was satisfied.

UV spectrophotometry[26]. The reaction mixture at time t was diluted to *ca.* 5×10^{-5} M ascorbic acid with a buffer cosisted of a mixture of 0.4 M KCl and 0.4 N HCl and its absorbance at 244 mμ ($\log\varepsilon=4$) was determined at 20°. It was confirmed that the presence of catalyst copper salts and dehydroascorbic acid did not disturb this spectrophotometric estimation.

Electrical conductance measurements. Resistance of an ascorbic acid solution was measured in an usual conductance cell with Pt electrodes (the cell constant of 0.38 cm^{-1}) in *p*-xylene thermostated to 0.05°. The combination of an *a–c* bridge (1000 c/s) and a galvanometer for zero point indicator was employed.

2.4. Summary

The kinetics of cupric chloride-catalysed autoxidation of L-ascorbic acid has been studied in a number of organic solvents. The solvents effect can be expressed as a linear function of $\log k$ *vs.* reciprocal of dielectric constant ($1/D$) or Reicherdt's empirical parameter of solvent polarity (E_T)[27]. The electrical conductance measurement shows that ionization of ascorbic acid to ascorbate ion is affected by the solvent polarity. The stability of ascorbate-copper complex seems to be lower in more polar solvents. The concentration of the complex combined with an oxygen molecule is higher at lower temperature. The mechanism of the autoxidation is discussed in comparison with that in aqueous solutions[25].

3. Salt Effect on Metallic ion-Catalysed Autoxidation of L-Ascorbic Acid[32]

3.1. Iutroduction

Autoxidation catalysts such as Cu, Ni, Co and Fe salts are widely used, but no convincing reason for the specific abilities and selectivities is known; their redox potentials, given in ordinary textbooks, are not always the satisfactory measure. Another interesting and important feature in the autoxidation is the acceleration and retardation affected by some other ions such as halide ion. An example is the Cu(II)-catalysed autoxidation of ascorbic acid in the presence of other additives, where the retardation mechanism may be classified into two

types; (i) Chelating agents such as EDTA and metaphosphate form with Cu(II) stable complexes which are no longer effective catalysts. (ii) Some compounds, *e.g.* carbon monoxide, form stable complexes with Cu(I) which resist oxidation to Cu(II), *i.e.* an effective catalyst.

There may be, however, another effect which affects the redox potential of the metallic ion. We wish to call it "ligand effect", which brings about acceleration and also retardation depending on the nature and amount of added salts as has been reported with halide[33], azide and cyanide salts[34].

Following is an attempt for the elucidation of the effect of added salts in the $Cu(NO_3)_2$-catalysed autoxidation of ascorbic acid in aqueous solutions.

3.2. Results and Discussion

Effect of additives on the rate. The effect of Cl^-, Br^-, I^-, SCN^- and CN^- on the rate of $Cu(NO_3)_2$-catalysed autoxidation of ascorbic acid is shown in Fig. 15. The kinetics were investigated in aqueous solutions, in order to avoid interference of buffering anions. Fig. 15 shows plots of psuedo-first-order rate constant (k) *vs.* log [additives].

The figure shows Cl^- and Br^- ions have an acceleration effect, especially Cl^- accelerates the autoxidation about three fold at optimum conditions. There was no effect of ionic strength for the addition of KNO_3, *i.e.*, rate constants ($10^4 k$ sec^{-1}) at 0, 0.1 and 1.0 M were 9.10, 9.07 and 9.17, respectively. Dekker *et al.*[35] reported, without describing their experimental conditions, that Cu(II)-catalysed autoxidation of ascorbic acid in HCl aq. proceeds 50–100 times as fast as in nitric or perchloric acid of the same concentration. The authors reexamined this effect by using HCl and HNO_3, the results are shown in Fig. 16.

The feature observed on addition of HNO_3 agrees with our previous mechanism[36]. On the contrary, the rate constants show a complex variation as the concentration of HCl varies. This result may be caused by Cl^-.

The difference between the effects of Cl^- and Br^- (Fig. 15) may be ascribed to the "ligand effect" which will be mentioned in the next section. Mapson[33] has observed the acceleration with KI in an acidic buffer. Since I^- has a poor coordination ability and a high trans effect described below, the acceleration is attributable to the oxidation of ascorbic acid by molecular iodine formed.

$$2I^- + 2H^+ + \frac{1}{2}O_2 \rightarrow I_2 + H_2O \quad (1)$$

$$I_2 + H_2A \rightarrow DA + 2HI \quad (2)$$

The hydrogen iodide produced is autoxidized to iodine again in an acidic solution.

Alternatively, I_2 may be formed by Eq. 3 which corresponds to the iodometry of cupric ion.

$$2Cu(II) + 4I^- \rightarrow 2CuI + I_2 \quad (3)$$

But the formation of CuI should retard the reaction. In fact, the reaction mixture at high concentration of I^- became turbid by the formation of CuI. Thus the effect of I^- shown in Fig. 15 is explicable with ligand effect and/or an unusual reaction (Eq. 3).

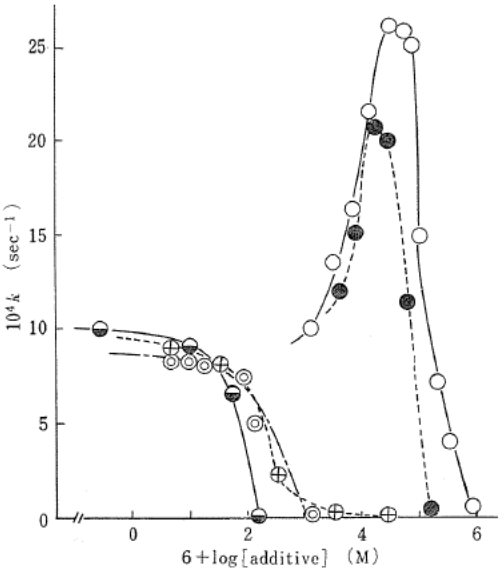


FIG. 15. Effect of concentration of additives on the first-order rate constant (k) with initial [ascorbic acid], 3×10^{-2} M; [$Cu(NO_3)_2$], 3×10^{-4} M and partial pressure of oxygen, 1 atm at 25°. ○ KCl, ● KBr, ⊕ KI, ◎ KSCN, ◒ CN^- from $K_3Fe(CN)_6$. k in the absence of additives was 9.1×10^{-4} sec^{-1}.

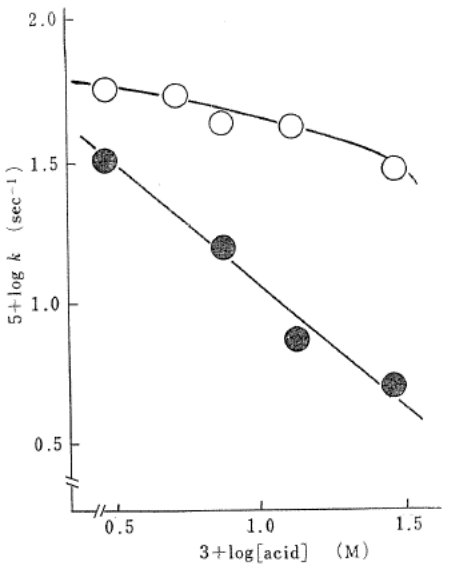


FIG. 16. Effect of acid concentration on the first-order rate constant (k) with initial [ascorbic acid], 3×10^{-2} M; [$Cu(NO_3)_2$], 3×10^{-4} M and partial pressure of oxygen, 1 atm at 25°. ● HNO_3, ○ HCl.

It is evident[37] that thiocyanate and cyanide salts suppress the autoxidation effectively, Fig. 15 showing that both ions have almost the same inhibition effect, if their complete dissociation at low concentration occurs. It is rather strange that Butt *et al.*[34] have observed *ca.* 1.3 fold acceleration by using $Ca(OH)_2-Cu(CN)_2$ at higher *p*H of 5.5.

In the present experiment, potassium ferricyanide was used as a source of CN^- ion. The catalytic ability of Fe(III) is much less than that of Cu(II) and the ligand (CN^-) exchange between Fe(III) and Cu(II) should be very fast, since the colour change of the solution from light yellow to pink is very fast. The same CN^- effect was observed with potassium ferrocyanide [Fe(II) is a quite weak catalyst]. The effect of SCN^- and CN^- on the autoxidation is also explained by the ligand effect described below.

TABLE 6. Effect of cations of additives on the first-order rate constant (k) at 25°. Initial concentration of ascorbic acid, 3.0×10^{-2} M; [$Cu(NO_3)_2$], 3.0×10^{-4} M; Partial pressure of oxygen, 1 atm

KBr (M)	10^4k (sec^{-1})	NaBr (M)	10^4k (sec^{-1})
0.003	11.7	0.003	11.8
0.03	19.3	0.03	20.3
0.3	0.68	0.3	0.68

Effect of cation of additives were examined with NaBr in place of KBr. Virtually no effect of cation is observed as shown in Table 6.

Ligand effect. Solvent and oxygen molecules and others can coordinate competitively with metallic ion, resulting in the change of the redox potential of the metallic ion. The ligand field theory (LFT)[33)39)] and crystal field theory (CFT)[33)39)] may be applicable to this case and the resulting phenomena would be simply called as "ligand effect" in this paper. The effect may be composed of several factors: (i) Spectrochemical series or Fajans-Tuchida series[33)] which indicates the order of separation energy (Δ) between two energy levels, e_g and t_{2g}. The series is shown in the following and is almost equal to the coordination ability with metallic ions.

$$CN^- > NO_2^- > H_2O > NO_3^- > F^- > N_3^- \sim SCN^- \sim Cl^- > Br^- > I^-$$

(ii) The trans effect has the following order[8)].

$$CN^- > NO_2^- \sim I^- \sim SCN^- > Br^- > Cl^- > H_2O$$

If a substrate in a metal complex is sited on the trans position to the ligand anion having stronger trans effect, the substrate is easily liberated from the central metal in the oxidation.

(iii) Instability effect, which is newly proposed, implies the mobility of ligand from a complex and, therefore, is related to the instability constant of the complex. The order of mobility, *i.e.*, leaving ability, is in the order[39)40)].

$$NO_3^- > H_2O > Cl^- > F^- > SCN^- > NO_2^- > CN^-$$

The order of this effect with an exception of NO_3^- is almost the reverse of the trans effect.

(iv) The cis effect[39)] is much less important than trans effect, and steric effect[39)] is negligible in the case of the anions studied here.

The effect on the rate, shown in Fig. 15, may be explained in view of the ligand effect. The order of spectrochemical series is $H_2O > NO_3^- > Cl^- > Br^- > I^-$, but a part of the halide ions can coordinate with Cu(II), if they are in high concentration. The nitrate ion, which was used for the proof of the absence of ionic strength effect in the autoxidation, is higher in the spectrochemical series than Cl^- or Br^-, but the instability order is $NO_3^- > H_2O > Cl^- > Br^-$. Therefore, aquation predominates over the coordination of NO_3^- ion. The cyanide ion having strong spectrochemical and trans effects can coordinate strongly to Cu(II) and expels effectively other ligands including substrate and halide ions. The autoxidation was inhibited by addition of over 3×10^{-3} M CN^-, even when optimum condition for acceleration by 3×10^{-2} M KCl (*cf.* Fig. 15) was used.

Ligand effect was further examined by changing the ratios $[H_2A]_0/[Cu(II)]$ or $[H_2A]_0/[Cl^-]$ using KCl, where $[H_2A]_0$ is initial concentration of ascorbic acid (Fig. 17).

Although the acceleration is not remarkable at lower concentration of the complex, a maximum of raction rate constant exists at almost the same ratio (*ca.* 1) of $[H_2A]_0/[Cl^-]$. This fact suggests that these two ligands, *i.e.*, ascorbate

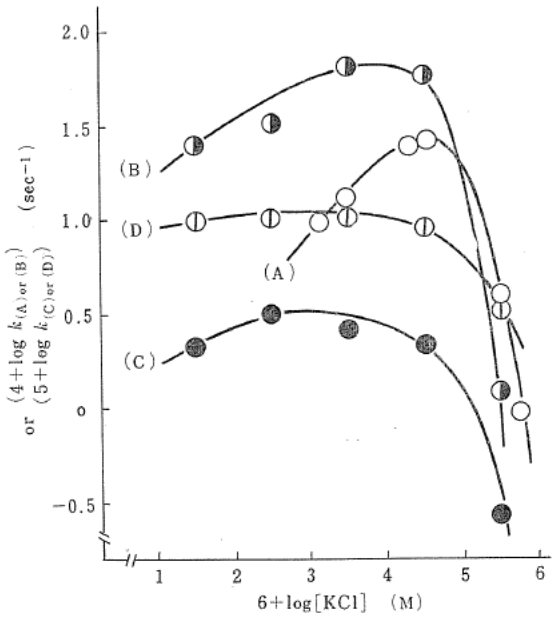


FIG. 17. Effect of ratios $[H_2A]_0$ *vs.* [Cu(II)] and $[H_2A]_0$ *vs.* $[Cl^-]$ on the first-order rate constant ($k_{(A)\sim(D)}$) with (A) $[H_2A]_0$, 3×10^{-2} M; $[Cu(NO_3)_2]$, 3×10^{-4} M; (B) $[H_2A]_0$, 3×10^{-3} M; $[Cu(NO_3)_2]$, 3×10^{-4} M; (C) $[H_2A]_0$, 3×10^{-2} M; $[Cu(NO_3)_2]$, 3×10^{-6} M; (D) $[H_2A]_0$, 3×10^{-3} M; $[Cu(NO_3)_2]$, 3×10^{-6} M at 25° ($[H_2A]_0$: Initial concentration of ascorbic acid).

monoanion and Cl^-, coordinate to Cu(II) in a certain ratio to cause favourably an electron transfer for the oxidation of ascorbate monoanion.

Unusual action of additives. An abnormal effect of additives was also observed in the following two cases. Firstly, the NO_2^- ion, which is high in the order of both spectrochemical and trans effects and is analogous to the CN^- ion, does not show the ligand effect, but rapid oxidation of ascorbic acid was observed even in the absence of copper catalyst. This may be ascribed to the following mechanism.

$$KNO \rightleftarrows K^+ + NO_2^- \tag{4}$$

$$NO_2^- + H^+ \rightleftarrows HNO_2 \tag{5}$$

$$2\,HNO_2 \rightleftarrows NO + H_2O + NO_2 \tag{6}$$

$$NO + \frac{1}{2}O_2 \rightleftarrows NO_2 \tag{7}$$

$$NO_2 + H_2A \rightarrow NO + DA + H_2O \tag{8}^{41)}$$

Secondly, the F^- ion accelerates the autoxidation, but the rate is not first-order in ascorbic acid and the oxidation also occurs without copper catalyst. There is still no rational explanation[42] for this abnormality of the F^- ion.

Effect of partial pressure of oxygen (p_0)[43]. In the Cu(II)-catalysed autoxidation

of ascorbic acid, two complexes I and II should exist, since a plotted line of k *vs* p_0 does not pass through the origin and the ratio of the slope to intercept varies with temperature and solvent[44].

$$H_2A \overset{K_9}{\rightleftarrows} HA^- + H^+ \tag{9}$$

$$HA^- + Cu(II) + O_2 \overset{K_{10}}{\rightleftarrows} \underset{(I)}{[HACu(II)O_2]^-} \tag{10}$$

$$HA^- + Cu(II) \overset{K_{11}}{\rightleftarrows} \underset{(II)}{[HACu(II)^-]} \tag{11}$$

Further support for complex II is the isolation of cuprous salt in the absence of oxygen[45].

A similar complex III is conceivable in the presence of Cl^-.

$$HA^- + Cu(II) + mCl^- \overset{K_{12}}{\rightleftarrows} \underset{(III)}{[HACu(II)Cl_m]^{n-}} \tag{12}$$

Assuming that the decomposition of these three complexes is rate-determining, the rate is expressed as Eq. 13, where k and K are rate and equilibrium constants of subscripted steps, respectively.

$$-d[H_2A]/dt = K_9\frac{[Cu(II)][H_2A]}{[H^+]} \times \{k_{10}K_{10}p_0 + k_{11}K_{11} + k_{12}K_{12}[Cl^-]^m\} \tag{13}$$

The effect of p_0 was examined at 25° with constant total pressure (1 atm) of oxygen and nitrogen (Fig. 18). The ratio of the slope *vs* the intercept in the plot of k *vs* p_0, $k_{10}K_{10}/(k_{11}K_{11}+k_{12}K_{12}[Cl^-]^n)$ at 0, 3×10^{-2} and 3×10^{-1} M KCl were 2.0[13], 4.7 and 0.13 (atm^{-1}), respectively. This rate behaviour is explicable by assuming that the coordination of a little Cl^- involves the coordination of O_2 to Cu(II) and accelerates the reaction, but the coordination of a large amount of Cl^- expels O_2 and retards the reaction. Hence Eq. 14 is an expression better than Eq. 12.

$$HA^- + Cu(II) + mCl^- + O_2 \overset{K_{14}}{\rightleftarrows} [HACu(II)Cl_mO_2]^{n-} \tag{14}$$

Since the ratio is small at high concentration of KCl, Eq. 15 cannot be rate-determining as reported by Mapson[46].

$$2\,Cu(I) + 2\,H^+ + O_2 \rightarrow 2\,Cu(II) + H_2O_2 \tag{15}$$

Comparison of catalysing metallic ions. If the ligand effect is operating for the complex formation and also for the smooth oxidation of substrate with an electron transfer from the substrate towards the metallic ion, the autoxidation of ascorbic acid in the presence of other transition metals may be accelerated or retarded by addition of suitable salts. Several examples are given in Table 7. Though Ni(II), Co(II) and Fe(III) should form a complex similar to that of Cu(II),

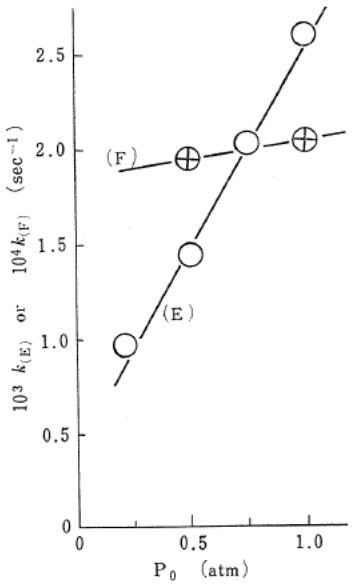


FIG. 18. Effect of partial pressure of oxygen (p_0) on the first-order rate constant ($k_{(E) \text{ or } (F)}$) with initial [ascorbic acid], 3×10^{-2} M; [$Cu(NO_3)_2$], 3×10^{-4} M at 25°. (E), [KCl] 3×10^{-2} M; (F), 3×10^{-1} M.

TABLE 7. Comparison of first-order rate constants ($10^5 k$ sec^{-1}) with some catalysts in the presence of KCl of various concentration. Initial concentration of ascorbic acid, 3×10^{-2} M; partial pressure of oxygen, 1 atm at 25°

Catalyst (10^{-4} M)	KCl (M)	0	3×10^{-2}	6×10^{-1}	5×10^{-6}*
$Cu(NO_3)_2$	3.0	91.0	266.0	9.7	89.0
$Ni(NO_3)_2$	3.0	1.9	1.9	—	—
$Ni(NO_3)_2$	30	6.0	6.4	—	—
$Co(NO_3)_2$	30	2.0	2.3	—	—
$Fe(NO_3)_3$	30	3.6	3.9	0.5	0.9

* $K_3Fe(CN)_6$ was added instead of KCl.

Ni(II) and Co(II) have poor oxidation potentials for the electron transfer from the substrate to the metal, while Fe(III) may form only a complex with a small stability constant. It is known that Cu(II) has a smaller ion radius and forms more stable complexes with many chelating agents than other transition metal ions such as Fe(III)[38a]. However, even the Cu(II)-catalysed autoxidation did not show a distinct ligand effect at low concentration of Cu(II). This may be due to the very low concentration of complex formed.

Autoxidation of catechol and phenol. Our suggestion of the complex formation and ligand effect in the autoxidation of ascorbic acid was examined in the autoxidation of catechol and phenol. The results were given in Table 8.

Although the stoichimetries of these reactions were not checked, the oxygen consumption at a given time increased on addition of KCl. It is known that catechol, an enediol-type compound, forms a green complex with Cu(II) and a red complex with Co(II)[47]. Phenol probably forms a similar complex, though its stability constant is small. This difference or the difference between bidentate

TABLE 8. Autoxidation of catechol and phenol in aqueous solutions at 50°

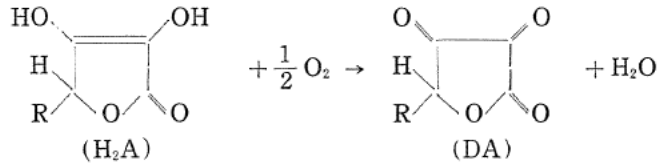
Substrate (mole/10 ml)	Catalyst (10^{-3} M)	Additive (M)	Consumed O_2 (10^{-5} mol)		
			5 hr	10 hr	15 hr
Catechol 3.0×10^{-3}	$Cu(NO_3)_2$ 3.0	none	3.2	—	—
	$Cu(NO_3)_2$ 3.0	KCl 0.2	18.9	—	—
	$Co(NO_3)_2$ 3.0	none	0	0.27	—
	$Co(NO_3)_2$ 3.0	KCl 0.2	1.2	3.7	—
	none	none	0	0.20	—
Phenol 3.0×10^{-3}	$Cu(NO_3)_2$ 3.0	none	0	—	1.2
	$Cu(NO_3)_2$ 3.0	KCl 0.2	1.04	—	2.3

anion of catechol and monodentate anion of phenol is caused by the difference of entropy increment at the metallic complex formation. The very small dissociation degree of catechol ($K_1=3.3\times10^{-10}$) and phenol ($K=1.3\times10^{-10}$) in an aqueous solution are not so favourable for the complex formation as ascorbic acid ($K_1=4\times10^{-5}$). In conclusion, the ligand effect of Cl^-, similar to that of ascorbic acid, is applicable to catechol and phenol which form metallic complexes.

3.3. Experimental Section

Materials. Ascorbic acid was of 99.5% pure with decomposition point of 190.9° (lit[48] 190–192° (dec.)) and optical rotation of $[\alpha]_D^{25}+20.94°$ (lit.[48] 20.5–21.5°). Catechol and phenol were commercial extra pure grade and used without further purification. Inorganic reagents were of commercial guaranteed reagent grade. O_2 and N_2 gases were of over 99.7% and 99.95% pure, respectively. Ion-exchanged water was used.

Kinetic procedure. This was the same as reported[36] and pseudo-first-order rate constant (k) was calculated by means of the usual first-order rate equation. The stoichiometry in general was as follows:



(16)

where $R=-CH(OH)CH_2OH$.

Alternatively, the stoichiometry of Eq. 17 may be applied.

$$H_2A+O_2 \rightarrow DA+H_2O_2 \tag{17}$$

Although most kinetic studies have estimated [H_2A] by indophenol method, the present experiments, were measured [H_2A] by UV spectrophotometry[49]. It was observed that Eq. 16 is not applicable at conversion above *ca.* 50% or for a rapid reaction in the presence of a considerable amount of Cu(II) or in an alkaline solution. The formed H_2O_2 reacts with ascorbic acid at lower conversion at least up to 30% and hence the stoichiometry of Eq. 16 is operating. A certain amount of H_2O_2 is consumed with ascorbic acid independent of H_2O_2 concentration[50]. The UV method[49] was advantageous not only for the proof of the H_2O_2 problem but

also for the kinetics at very low concentration and of very slow reaction, where the manometric method was unsuitable.

3.4. Summary

Kinetics of Cu(II)-catalysed autoxidation of ascorbic acid in aqueous solutions has been studied in the presence of halides, thiocyanate and cyanide ions. The effect of these additives on the rate can be explained in terms of "ligand effect" which includes coordination ability (the order of spectrochemical series[38]), trans effect[39] and instability effect (or leaving ability[39]) of the anion of additive. Fast consumption of ascorbic acid even in the absence of Cu(II) occurs on addition of KNO_2 or KF. Virtually no effect is observed with KNO_3 probably because NO_3^- is a good leaving ligand and its ionic strength does not affect the autoxidation. The acceleration with KCl is maximum, when $[Cl^-]$ is nearly equal to initial concentration of ascorbic acid. Effect of oxygen pressure (p_0) on the rate in the presence of Cl^- suggests the intermediary metallic complex composed of O_2, Cl^- and ascorbate ion. Complex formation and ligand effect are further confirmed both in the autoxidation of ascorbic acid catalysed by Ni(II), Co(II) and Fe(III) and also in the autoxidations of catechol and phenol catalysed by these matallic ions.

4. Spectrophotometric Determination of L-Ascorbic Acid[51]

4.1. Introduction

A considerable amount of L-ascorbic acid (Vitamin C) is now consumed as a medicine and also as ana ntioxidant and an additive in drinks and foods. A number of estimations of ascorbic acid assay have been developed, *i.e.*, visual titration[52], spectrophotometry (or colourimetry)[52], polarography[53], gas chromatography[54], gravimetry[55], bioassay method[56], *etc.* have been postulated. Among them, titration and spectrophotometry are prevailing. The colour change of indicators such as Tillman's reagent (2, 6-dichlorophenolindophenol), Folin's reagent (phosphomolybdate), methylene blue and iodine are often employed for titration and colourimetry. Although some modifications of these methods have been reported[57], they have shortcommings; *i.e.*, they are difficult to carry out, when a sample is either coloured or contaminated with acidic or reducing substances which may react with the indicators. Fujita *et al.*[58] used a metaphosphoric acid solution and Robertson[59] added KCN to the sample solution, and they determined ascorbic acid content by the estimation of difference of absorbances before and after disappearance of ascorbic acid by oxidase or cupric ion. Reducible substances, however, often react with other reducing agents as well as ascorbic acid. In view of a large absorbance of ascorbic acid in UV region, the spectrophotometry was examined in detail. The present paper is a summary of our data concerning the suitable conditions of this very simple procedure for the spectrophotometric analysis in the presence of a number of additives.

Ascorbic acid (H_2A) is unstable in aqueous solutions, especially in the presence of heavy metal ions such as cupric and ferric ions. Though the rate of autoxidation to dehydroascorbic acid (DA) decreases in less polar organic solvents[60], the solubility of the acid is generally small in these solvents[60], but the common organic compounds are very often soluble and their UV absorption bands overlap with that of ascorbic acid.

The authors studied kinetics of the copper-salt catalysed autoxidation of ascorbic acid (H_2A) to dehydroascorbic acid (DA) (Eqs. 3-7 in § 1)[61].

$$H_2A + \frac{1}{2}O_2 \rightarrow DA + H_2O \quad (1)$$

According to our mechanism, ascorbic acid should be more stable in more acidic media. However, the cuprous ion formed by the reaction of cupric ion with ascorbic acid may easily be oxidized in acidic media to cupric ion (Eq. 2), a catalyst for the autoxidation.

$$Cu(I) + \frac{1}{4}O_2 + H^+ \rightarrow Cu(II) + \frac{1}{2}H_2O \quad (2)$$

It is known[62] that halide ion, *e.g.*, chloride ion, retards reaction (2) and also inhibits the formation of complex of ascorbic acid and cupric ion. These mechanisms suggest that hydrochloric acid free of metal ion is effective to prevent autoxidation. Even a trace of cupric ion, *e.g.*, 1.5×10^{-9} M, however, acts as an effective catalyst[63] and the complete removal of these metallic ions are often difficult. Furthermore, the very low concentration of chloride ion accelerates the autoxidation[62], but its high concentration retards the overall reaction. Thus, as a source of chloride ion a large amount of alkali chlorides besides HCl should be advantageous for the stabilisation of ascorbic acid.

4.2. Results and Discussion

Absorption spectrum and molar extinction coefficient. Ultraviolet absorption peak (λ_{max}) of ascorbic acid varies with solvent, *p*H and temperature. The variation of λ_{max} may be caused by the change of the degree of solvation, ionization and/or association of ascorbic acid. The peak (λ_{max}) was 244 mμ in these acidic solutions in the present experiment. In a solution of 0.4 N HCl and 0.4 M KCl, the absorbance (E_{244}) at 244 mμ of various concentrations of ascorbic acid had the relation $E_{244} = 10^4[H_2A] - 0.035$ with the concentration ($[H_2A]$), at least, at $2 \times 10^{-5} - 9 \times 10^{-5}$ M. Molar extinction coefficient (ε_{244} or ε_{max}) was found to be 10000.

Acidic solutions. Acids can suppress the dissociation of ascorbic acid. The stability of ascorbic acid was examined under various conditions in a mixture of 0.4 N HCl and 0.4 M KCl, 0.1 N HCl, 0.4 N HCl, 0.1 N H_3PO_4 or *ca.* 2% metaphosphoric acid.

In all of these solutions, λ_{max} and ε_{max} were the same, *i.e.*, 244 mμ and 10000, respectively. Metaphosphoric acid may be advantageous in the presence of cupric chloride of *ca.* 15 ppm in a dilute solution. However, polymerisation degree (n) of commercial metaphosphoric acid, $(HPO_3)_n$, and hence the degree of dissociation in solution is not measurable by means of the usual titration method. Further, the solution of metaphosphoric acid for UV measurements should be filtered to remove the turbidity and stored in a refrigerator to prevent depolymerisation. Because of this troublesome treatment as well as instability of the solution (safe storage is within a week), metaphosphoric acid was less suitable as a stabiliser of ascorbic acid. A mixture of aqueous HCl and KCl (a modified Clark-Lubs buffer solution) was found to be the most advantageous solution. All experiments described below were carried out in this buffer solution of 0.4 N HCl and 0.4 M KCl.

Effects of other factors. Our kinetic study[61] shows that the rate of autoxidation of ascorbic acid decreased at high concentration of ascorbic acid and at lower partial pressure of oxygen. These facts imply that ascorbic acid is more stabilized at its high concentration and in the absence of oxygen. These effects are shown in Table 10 combined with the result in Table 9.

Ascorbic acid is more stable in nitrogen that in air or oxygen, but the effect was too low to necessitate determination under nitrogen atmosphere, except for

TABLE 9. Stability of ascorbic acid in various acidic solutions in the absence or presence of cupric chloride at 20°C

Solution	Added $CuCl_2$ (10^{-4} M)	Concentration of ascorbic acid (10^{-5} M)				
		Initial	1 hr	5 hr	1 day	2 day
0.4 N HCl + 0.4 M KCl	0	3000	2980	2940	2900	2620
		6.01	5.89	5.68	4.49	3.71
	2.3	3000	2940	2930	2500	2330
		6.01	5.84	4.22	2.16	—
0.1 N HCl	0	3040	2930	2890	2740	2550
		6.00	5.97	5.62	5.32	3.55
	2.3	3040	3000	2890	2590	2100
		6.00	4.83	1.22	0.22	—
0.4 N HCl	0	2990	2910	2910	2780	2550
		6.03	5.82	5.61	5.51	4.27
	2.3	2990	2920	2880	2610	2340
		6.03	5.90	3.06	0.48	—
0.1 N H_3PO_4	0	3000	3000	2870	2750	2510
		6.00	5.87	5.78	5.40	4.15
	2.3	3000	3000	2760	2550	1790
		6.00	5.93	5.67	5.21	4.92
ca. 2% $(HPO_3)_n$	0	2950	2920	2860	2770	2770
		5.83	5.80	5.75	5.46	5.19
	2.3	2950	2950	2910	2870	2770
		5.83	5.83	5.58	5.54	4.90
Water	0	3000	2920	2890	2850	2540
	2.3	3.00	2.82	2.80	2.60	2.33

TABLE 10. Effects of concentration of ascorbic acid and nitrogen or oxygen on the stability in a buffer solution of 0.4 NHCl−0.4 M KCl

Atmosphere	Added $CuCl_2$ (10^{-4} M)	Concentration of ascorbic acid (10^{-2} M)				
		Initial	1 hr	5 hr	1 day	2 day
Air	0	12.0	12.0	11.9	11.6	10.8
	2.3	12.0	12.0	11.7	10.7	—
N_2	0	3.00	2.96	2.95	2.94	2.74
	2.3	3.00	2.95	2.84	2.83	2.67
O_2	0	3.00	2.95	2.95	2.70	2.64
	2.3	3.00	2.87	2.74	2.60	2.21

the presence of a large amount of metallic ion.

Ferric ion also catalyses the autoxidation of ascorbic acid, but the examination of the effect of ferric chloride (Table 11) shows that ferric ion of the concentration below *ca.* 13 ppm in a dilute solution does not affect stabilisation.

TABLE 11. Effect of ferric chloride on the stability of ascorbic acid in a buffer solution of 0.4 N HCl−0.4 M KCl

Added $FeCl_3$ (10^{-4} M)	Concentration of ascorbic acid (10^{-2} M)				
	Initial	1 hr	5 hr	1 day	5 day
2.3	3.00	2.99	2.98	2.78	2.47
2300	3.00	1.76	1.69	1.49	1.13

Absorption of organic compounds. For the application of this analytical method to drugs, drinks and foods, it is necessary to know the possibility of the overlap of the absorption band with those of organic compounds which may be present together with ascorbic acid.

Results are shown in Table 12 as ε_{max}, ε_{244} and ε_{275} where ε_{275} of ascorbic acid is only 400.

TABLE 12. Molar extinction coefficients at λ_{244}, λ_{275} and λ_{max} of some organic compounds in a buffer of 0.4 N HCl−0.4 M KCl

Compound	ε_{244}	ε_{275}	λ_{max} (mμ)	ε_{max}
Dehydroascorbic acid	9.4×10	1.1×10^2	302	1.3×10^2
Nicontinamide	2.05×10^3	8.3×10^2	261.5	5.6×10^3
Aspartic acid	0.1	0.1	—	—
Pantothenic acid	1.4×10	4.6	—	—
DL-Methionine	1.7×10	0.1	—	—
Taurine	0.1	0.1	—	—
Glycine	0.1	0.1	—	—
Dextrose	0.1	0.1	—	—
D-Fructose	0.8	2.7	280.5	2.8
Saccharose	1.2	1.1	258	1.3
D-Sorbitol	0.6	0.7	264	0.1
Saccharin	1.8×10^3	7.5×10^2	278.5	7.7×10^2
Sodium cyclohexylsulfamate	3.1	0.1	—	—
Citric acid	4.7	0.1	—	—
DL-Malic acid	1.6×10	1.6	—	—
Tartaric acid	6.5	0.1	—	—
Oxalic acid	3.7×10	1.1×10	244	3.7×10
Succinic acid	6.5	1.2	—	—
Tannic acid	2.5×10^3	6.9×10^3	275	6.9×10^3
Caffeine	2.8×10^3	7.5×10^3	269.5	8.0×10^3
Tartrazine	1.3×10^4	1.3×10^4	(Visible region)	

Common vitamins are usually slightly soluble in the buffer solution and hence they are not examined. Extraction of ascorbic acid from solid samples with a small amount of the buffer solution can avoid contamination of such less soluble

substances. If a sample is liquid, extraction with diethyl ether is recommended to remove vitamins A, D, E, *etc.*, and other many organic compounds.

A given amount of ascorbic acid was mixed with organic compounds whose ε's at suitable wave lengths were known (*e.g.*, see Table 12) and analysed spectrophotometrically.

The results in Table 13 indicate that this method is satisfactory, when two absorbances at 244 mμ and at an appropriate wavelength of additives are known.

Even when their compositions are unknown, the following circumstances may take place: (i) some components of drugs, drinks or foods have only small absorbance at 244 mμ on high dilution of the sample, (ii) some components of the sample are slightly soluble in a buffer solution consisting of 0.4 N HCl and 0.4 M KCl, and (iii) the total absorbance of a sample is known, as is often the case, before adding ascorbic aicd.

TABLE 13. Models of application

Sample	Composition	Concentration prepared (10^{-5} M)	$E_{244}^{calc.}$	$E_{244}^{obs.}$	$E_{275}^{calc.}$	$E_{275}^{obs.}$
A	ascorbic acid	6.435	0.608	—	0.026	—
	nicotinamide	5.07	0.117	—	0.045	—
	total absorption		0.725	0.730	0.071	0.073
B	ascorbic acid	4.52	0.417	—	0.018	—
	tannic acid	5.64	0.141	—	0.378	—
	caffeine	3.61	0.101	—	0.274	—
	total absorption		0.659	0.671	0.670	0.669

Applications. A commercial vitamin pill (Sample I) contains many organic compounds. For example, the indicated composition was as follows: vitamin A palmitate 2500 I.U., calciferol 250 I.U., thiamine tetrahydrofurfuryl disulfite 2 mg, vitamin B_2 2.5 mg, nicotinamide 10 mg, vitamin B_6 2.5 mg, folic acid 0.25 mg, calcium pantothenate 5 mg, vitamin B_{12} 0.001 mg, vitamin C 37.5 mg, DL-α-tocopherol acetate 1 mg in one tablet. The pill (0.5506 g) was powdered and extracted with the KCl-HCl buffer solution of less than 20 m*l*. The filtrate was further extracted twice with diethyl ether (each *ca.* 50 m*l*). The residual aqueous solution was diluted to 1 tablet/6250 m*l* buffer solution and its absorbance at 244 mμ was measured to be 0.357. Accordingly, the content of ascorbic acid in the pill was calculated to be 37.7 mg/tablet using a calibration curve. The observed content agree with the indicated content (37.5 mg/tablet) of ascorbic acid and also with the content estimated by indophenol method (37.0 mg/tablet).

Besides ascorbic acid, tannic acid and caffeine are main components of green tea (Sample II). The sum of these two components was estimated to be 2.68×10^{-4} mol/g using $E_{275}=0.495$, assuming that they have the same ε_{275} and ε_{244} values of 7.2×10^3 and 2.65×10^3, respectively (*cf.* Table 12). Thus the ascorbic acid content was calculated from $E_{244}-0.316$ and dilution degree of 1 g/4 *l*.

$$E_{244}(\text{obs.})-E_{244}\ (\text{tannic acid}+\text{caffeine})=0.316-0.177=0.139$$

This value of 0.139 corresponds to 1.67×10^{-5} M ascorbic acid solution and the concentration gives an approximate content of the acid in Sample II to be *ca.*

11.8 mg/g. The sample was diluted to 1 g/100 m*l* and titrated with indophenol method. The content of ascorbic acid was determined to be 9.6–13.1 mg/g. This uncertainty may be due to the colouration of the sample.

Ascorbic acid content in an orange juice (Sample III) or a purified drink (Sample IV) was also difficult to be estimated exactly by the indophenol method even by employing the xylene extraction technique[62]. But the UV method is not interfered by colouring materials; moreover the sample is so diluted (*ca.* 5×10^{-5} M of ascorbic acid) that it is almost colourless. In addition, the additive property of the UV absorbance enables the accurate determination of the content. In the present experiments, a known amount of ascorbic acid (0, 1.25×10^{-5}, 2.5×10^{-5}, 2.5×10^{-5} or 3.5×10^{-5} M) was added to a sample and a straight line, obtained from plots of E *vs.* concentration, afforded an exact value of the acid content.

In these two cases of drinks (Samples III and IV), the values found were higher than the labeled values. This may be caused by the fact that the makers added the acid in a greater amount than that indicated on the label taking the consumption of ascorbic acid during storage into consideration.

In conclusion, it will be safe to say as follows: The strong UV absorption band of ascorbic acid (λ_{max} 244 mμ, ε_{max} 10000 in an acidic solution) enables its facile quantitative determination with a very simple procedure, even if, a sample contains a small amount of the acid (10^{-4} M). Oxidizing agents (*e.g.*, some metallic ions) and reducing agents (*e.g.*, oxalic acid and dextrose) which may react with ordinary titration indicators do not interfere with this direct spectrophotometry. Also, coloured materials which may sometimes be contained, do not interfere with it.

4.3. Experimental Section

Ultraviolet spectroscopy. An automatic recording spectrophotometer (Type Simadzu SV-50 A) equipped with quartz cells (light path of 10 mm) was used.

Materials. Ascorbic acid was of 99.5% pure with decomposition point of 190.9°C (lit.[64] 190–192°C (dec.)) and optical rotation of $[\alpha]_C^{25}+20.94°$ (lit.[64] 20.5–21.5°). Dehydroascorbic acid was prepared from ascorbic acid by the method of Fujimura *et al.*[65], and crystallised from acetic acid[66]: decomposition point 218–222°C (lit. 196°C (dec.)[65], 225°C (dec.)[67], 237–240°C (dec.)[66]). Potassium chloride and the following organic compounds were of commercial guaranteed reagent grade; saccharose, oxalic acid, nicotinamide, sodium cyclohexylsulfamate, D-fructose, DL-methionine. Hydrochloric, phosphoric, metaphosphoric acids and other organic compounds were of commercial extra pure grade and were used without further purification. Oxygen and nitrogen gases were of over 99.7% and 99.95% pure, respectively. Distilled water was used. Cupric and ferric chlorides were estimated by iodometry and chelation with EDTA, respectively.

Procednre. Sample solutions of given concentrations in stoppered volumetric flasks were kept standing at constant temperature (20°C) for a known period. There was no appreciable difference in the decreasing rate of ascorbic acid concentration between 2–3 day's storage of samples in a glass flask and that in a polyethylene bottle. After being kept for a certain period, ascorbic acid was diluted to *ca.* 5×10^{-5} M and the absorption curve ranging 238–255 mμ was drawn. The ascorbic acid concentrations in the dilute solutions were obtained by means

of a calibration curve.

Titration with the indophenol method[1]. Samples were diluted to the concentration of *ca.* 10^{-1} M ascorbic acid and titrated with a 0.012% 2,6-dichlorophenol-indophenol solution.

4.4. Summary

A novel quantitative determination of L-ascorbic acid by UV spectrophotometry in a HCl-KCl buffer has been developed. Ascorbic acid (λ_{max} 244 mμ) of over 2×10^{-5} M can quantitatively be determined by this method. Several oxidizing, reducing, acidic or basic reagents and other organic compounds which may be present together with ascorbic acid in ordinary drugs and drinks do not interfere with the determination.

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