Kinetics of Aerobic Oxidation of Ascorbic Acid

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Abstract □ The pH–log rate profile of the rate of disappearance of ascorbic acid from an aqueous solution under aerobic conditions was determined at 67° in a pH range of 3.52 to 7.22, ionic strength 0.4. The profile shows a maximum near pK_{a_1} of ascorbic acid. The aerobic rate shows buffer catalysis in the pH range studied. Since ascorbic acid oxidation is readily catalyzed by metal ions, the oxidation was also followed in the presence of disodium ethylenediaminetetraacetate. The results duplicated those obtained by following the aerobic oxidations of ascorbic acid in the absence of a chelating agent. Thermodynamic parameters were calculated from the Arrhenius plots.

Keyphrases □ Ascorbic acid—aerobic oxidation, pH–log rate profile, effect of disodium ethylenediaminetetraacetate □ Oxidation, aerobic, ascorbic acid—pH–log rate profile, effect of disodium ethylenediaminetetraacetate □ pH–log rate profile—aerobic oxidation of ascorbic acid

The kinetics of autoxidation of ascorbic acid have been studied mainly in the presence of metal ions or some type of catalyst. The degradation products vary according to the catalyst or the metal ion used and to other reaction conditions. Ferrous and ferric ions are less effective than cupric ions for the oxidation in aqueous solutions. However, reactions are similar in that both dehydroascorbic acid, as the first oxidation product, and hydrogen peroxide are produced. The same stepwise degradation of ascorbic acid has been observed to occur as a result of oxidation catalyzed by a wide variety of cations, some antibiotics, inorganic compounds, metal chelates, *etc.*

The rate of aerobic oxidation is pH dependent, exhibiting maxima at pH 5.0 and 11.5 (1). The nature of the oxidation products also exhibits pH dependence.

The photochemical oxidation of ascorbic acid can proceed under either aerobic or anaerobic conditions. X-rays and gamma irradiation are more effective than UV light irradiation, but all produce dehydroascorbic acid and hydrogen peroxide (2). Metal ions and oxygen accelerate oxidation. The reaction is postulated to proceed *via* the hydrogen and hydroxyl radicals produced by photochemical decomposition, and some evidence for the involvement of the perhydroxy radical has also been obtained (3).

A recent kinetic study by Grochmalicka (4) shows that the oxidation of ascorbic acid proceeds in three steps. Reversible reaction produces dehydroascorbic acid which, on hydrolysis, yields 2,3-diketogulonic acid. The latter then oxidizes to produce threonic acid and oxalic acid. All three reactions are found to be pH dependent.

Since ascorbic acid oxidizes much more rapidly in solution than in the solid state, the stability of ascorbic acid solutions has received much more attention. Generally, the following factors (5) are considered in the preparation of a stable solution of ascorbic acid: exclusion of air and oxygen, pH adjustment, metal-ion contamination, protection from light, and the use of additives. Usually, antioxidants and chelating or complexing agents are used as additives.

The stability of ascorbic acid in a variety of pharmaceutical vehicles has been reported. Giral (6) attributed the stability of ascorbic acid in simple syrup to the viscosity of the syrup. The stability of ascorbic acid was reportedly enhanced in aqueous solutions of sucrose, sorbitol, glycerin, and propylene glycol (7–9).

The copper-catalyzed oxidation of ascorbic acid was studied in solutions containing polysorbates 20 and 80 (10, 11). The oxidation rate declined rapidly in solutions containing between 40 and 75% polysorbate 20 (10) and in solutions containing up to 10% polysorbate 80 (11). The rate remained constant at polysorbate 80 concentrations between 10 and 30%.

Ascorbic acid is widely used in vitamin formulations and in other pharmaceutical preparations. Although most of the liquid formulations containing ascorbic acid differ in pH, their pH values generally fall within the range of 3.52–7.22. The purpose of this investigation was to study the aerobic degradation of ascorbic acid in aqueous solutions over this pH range.

EXPERIMENTAL

Materials—All reagents used in this study were of analytically pure grade. These included ascorbic acid, sodium 2,6-dichloroindophenol, sodium bicarbonate, anhydrous sodium acetate, glacial acetic acid, metaphosphoric acid, monobasic potassium phosphate, anhydrous dibasic sodium phosphate, potassium chloride, dehydroascorbic acid, and sodium ascorbate.

Standardization of Ascorbic Acid—The ascorbic acid was standardized against primary standard ascorbic acid USP using the 2,6-dichloroindophenol method of Tillman (12). It was found to contain 99.78% ascorbic acid.

Methods—Acetate and phosphate buffers were prepared at 25°; however, the pH values reported in this study were those observed at the temperature of the study and after addition of ascorbic acid. Buffers free of potassium chloride were used in the comparison of the spectroscopic method with the 2,6-dichloroindophenol method of analysis for ascorbic acid. The remainder of the study was carried out using buffers containing potassium chloride, which was added to adjust the ionic strength of the buffers to 0.4. In studying the primary salt effect, varying amounts of potassium chloride were added to the buffers to adjust them to the desired ionic strength.

In the past, most kinetic studies of ascorbic acid involved the determination of the rate of autoxidation of ascorbic acid in terms of the volume of oxygen absorbed during the reaction. However, there is general agreement that the oxygen absorbed during autoxidation is only partly used for the oxidation of ascorbic acid to dehydroascorbic acid, while the rest is used in the formation of other degradation products. For this study two methods of analysis for ascorbic acid were compared: a spectrophotometric method and the 2,6-dichloroindophenol titration method.

The 2,6-dichloroindophenol titration method (Method A) is precise when used to determine pure ascorbic acid and is not affected by the presence of dehydroascorbic acid (13). The determination was carried out according to the USP XVII method for ascorbic acid injection (14). A standard solution of the dye was

Table I—Analysis of Ascorbic Acid at 25° by 2,6-Dichloroindophenol Titration (Method A) and by Spectroscopy (Method B)[a]

pH 3.52			pH 4.55			pH 5.85		
	Concentration, $M \times 10^3$			Concentration, $M \times 10^3$			Concentration, $M \times 10^3$	
Hours	A	B	Hours	A	B	Hours	A	B
0	9.95	9.70	0	10.35	10.80	0	9.95	9.81
12	9.10	9.15	24	8.15	8.30	6	9.18	9.20
36	8.75	8.65	36	7.12	7.50	18	7.92	8.00
65	7.55	7.90	55.5	5.80	6.30	28	7.24	7.45
96	6.95	7.11	84	4.70	4.80	42	6.16	6.08
137	6.50	6.30	103	—	3.80	66	4.51	4.65
—	—	—	150	2.08	2.48	91	3.31	3.58
—	—	—	180	—	1.84	119	—	2.64
—	—	—	216	—	1.12	—	—	—
K	3.12	3.10		10.56	10.18		12.00	11.11
$T_{1/2}$	2.22×10^{-2}	2.23×10^{-2}		65.6	68.1		56.8	62.4
$-R$	9.81	9.99		9.97	9.99		9.99	10.0
SE	0.16	0.04		0.20	0.19		0.07	0.07

[a] $K = k \times 10^3$ hr.$^{-1}$; $T_{1/2}$ in hours; $R = r \times 10$, where r = correlation coefficient; $SE = SE \times 10$, SE = standard error of estimate.

prepared and standardized daily against an ascorbic acid solution of known strength. All other reagents were freshly prepared.

The spectrophotometric method (Method B) is rapid, but one drawback to its successful application for the determination of ascorbic acid is that beyond specified limits the intensity of the absorption is not directly proportional to the concentration (15). Buffered solutions containing approximately $1-6 \times 10^{-5}$ mole l.$^{-1}$ of ascorbic acid were prepared, and their $\lambda_{max.}$ values were obtained using a spectrophotometer[1]. Beer's law plots also were obtained using a spectrophotometer[2]. Since the absorptivity and $\lambda_{max.}$ of ascorbic acid vary with pH, it was necessary to prepare a calibration curve for each pH. The $\lambda_{max.}$ values at pH 3.52, 4.55, and 5.85 were 245, 264, and 265.5 nm., respectively. Interference from dehydroascorbic acid was negligible at these wavelengths.

An accurately weighed quantity of ascorbic acid (176 mg.) was added to a 250-ml. amber glass volumetric flask and dissolved in 100 ml. of appropriate buffer solution, which had been adjusted to the temperature at which the analysis was to be made. The flask was placed in a constant-temperature bath previously adjusted to 25°. For Method A, adequate sample sizes (1–2 ml.) were withdrawn at appropriate time intervals and analyzed by titration with the standardized dye solution. For Method B, samples were withdrawn (0.25–2.00 ml.) and diluted to 100 ml. with the appropriate buffer. Absorbance was measured at the $\lambda_{max.}$ for ascorbic acid (previously determined at the pH of the buffer). The results obtained from the two methods are shown in Table I, along with the calculated rate constants in hours^{-1}, half-lives in hours, correlation coefficients, and standard errors of estimate. The aerobic degradation of ascorbic acid followed first-order kinetics, as shown in Fig. 1. Rate constants were calculated using the method of least squares.

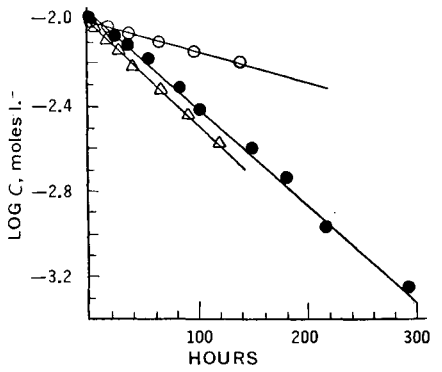

Figure 1—*Plot showing the overall first-order character of the aerobic oxidation of ascorbic acid at different pH values and 25°. Key: △, pH 3.52; ●, pH 4.55; and ○, pH 5.85.*

[1] Beckman DK-2.
[2] Beckman DU.

The 2,6-dichloroindophenol method was used in the kinetic study since it gave results comparable to those obtained by the spectrophotometric method and it required far less buffer solution. The end-point for the dye method was determined visually (colorless to pink). After approximately 80% of the ascorbic acid in solution was degraded, the solution became dark yellow in color. Therefore, most of the ascorbic acid solutions were studied until 60–80% degradation had occurred.

KINETIC STUDY

Method of Analysis—An accurately weighed quantity (approximately 440 mg.) of ascorbic acid was added to a 250-ml. amber-colored volumetric flask and dissolved in the appropriate buffer solution (potassium chloride added to the desired ionic strength), which had been preheated to the temperature at which the study was made.

Seven-dram amber screwcap vials were preheated to the desired temperature and filled with approximately 15 ml. each of the ascorbic acid solution. The vials were tightly sealed and then placed in a constant-temperature circulator bath previously adjusted to the desired temperature (±0.1°). The vials were allowed to equilibrate thermally; at appropriate time intervals, vials were removed from the bath and chilled, and their contents were analyzed for the remaining ascorbic acid using the 2,6-dichloroindophenol method. The pH of each sample was measured to ensure the constancy of pH during the entire procedure.

Effect of Ionic Strength—The effect of ionic strength on the rate of oxidation of ascorbic acid was investigated by studying the oxidation rate at 59.5° in acetate buffers (pH 3.52, 4.55, and 5.45) and in phosphate buffer (pH 6.60). The buffers were adjusted to ionic strengths of 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 by the addition of potas-

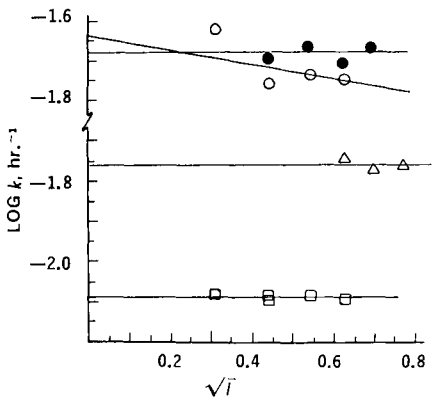

Figure 2—*Effect of ionic strength* (I) *on the oxidation of ascorbic acid at 59.5° at various pH's (acetate buffers, pH 3.52–5.45 and phosphate buffer, pH 6.60). Key: □, pH 3.52; ○, pH 4.55; ●, pH 5.45; and △, pH 6.60.*

Table II—Specific Rate Constants and Half-Life Periods for the Oxidation of Ascorbic Acid at Various Temperatures and pH Values and Ionic Strength 0.4

Temperature	pH[a]	$k \times 10^2$, hr.$^{-1}$	$t_{1/2}$, hr.
67.0°	3.52	1.79	38.6
72.0°		2.43	28.5
77.0°		3.37	20.6
80.0°		3.93	17.6
85.0°		4.25	16.3
85.0°		4.62	15.0
59.5°	4.55	1.78	38.9
67.0°		2.74	25.3
72.0°		3.58	19.5
80.0°		5.50	12.6
85.0°		5.63	12.3
85.0°		5.85	11.8
59.5°	5.45	1.96	35.3
67.0°		2.57	26.9
72.0°		3.36	20.6
80.0°		4.68	14.8
80.0°		4.84	14.3
85.0°		5.59	12.4
59.5°	6.60	1.79	38.7
67.0°		2.20	34.9
72.0°		2.58	26.9
77.0°		3.04	22.8
85.0°		4.15	16.7

[a] Acetate buffers used at pH 3.52–5.45; phosphate buffer, pH 6.60.

sium chloride. The results are shown in Fig. 2.

Effect of pH and Temperature—To study the effect of pH and temperature on the rate of oxidation of ascorbic acid, the reactions were carried out in acetate buffers (pH 3.52, 4.55, and 5.45) and in phosphate buffer (pH 6.60) at 59.5, 67, 72, 77, 80, and 85°. All buffers were adjusted to an ionic strength of 0.4 using potassium chloride. Reactions were carried out for a maximum of 50 hr. and all aliquots were analyzed using the previously described volumetric method. The rate constants and half-life periods are summarized in Table II. A typical log concentration *versus* time plot is shown in Fig. 3. Activation energies, $\Delta H\ddagger$, $\Delta S\ddagger$, and $\Delta F\ddagger$ values were determined from the Arrhenius plots obtained by plotting log k *versus* $1/T$. Results are shown in Table III. Arrhenius plots are shown in Fig. 4.

Effect of Ascorbic Acid Concentration—The rate constants were determined using three different concentrations of ascorbic acid (0.00545, 0.0100, and 0.0200 mole l.$^{-1}$) in acetate buffers (pH 3.52 and 4.55) and in phosphate buffer (pH 6.60) at 67° and ionic strength 0.4. The results are shown in Table IV.

Oxidation in Acetate and Phosphate Buffers—The oxidation of ascorbic acid was studied at 67° in buffer containing varying concentrations of acetate and phosphate at varying pH's and the constant ionic strength of 0.4, using the volumetric procedure previously described. Figures 5 and 6 show the relationship between buffer concentration and the rate of oxidation of ascorbic acid.

Table III—Thermodynamic and Statistical Parameters Obtained from Arrhenius Plots for Ascorbic Acid Oxidation at 67° at Various pH's and Ionic Strength 0.4

Parameter	pH 3.52	pH 4.55	pH 5.45	pH 6.60
Thermodynamic				
Ea, kcal. mole^{-1}	12.2	10.9	10.1	7.8
$\Delta H\ddagger$, kcal. mole^{-1}	11.5	10.3	9.4	7.1
$\Delta S\ddagger$, cal. mole^{-1} deg.$^{-1}$	−49.2	−52.1	−54.5	−61.7
$\Delta F\ddagger$, kcal. mole^{-1}	28.2	28.0	28.0	28.1
Statistical				
r (correlation coefficient)	−0.985	−0.989	−0.997	−0.995
SE (standard error of estimate)	0.031	0.035	0.014	0.161

Table IV—Effect of Ascorbic Acid Concentration on the Oxidation Rate at 67° and Various pH's with Ionic Strength 0.4

pH[a]	Concentration, moles l.$^{-1}$	$k \times 10^2$, hr.$^{-1}$	$t_{1/2}$, hr.
3.52	0.00545	2.03	34.2
	0.0100	1.79	38.6
	0.0200	1.07	64.6
4.55	0.00545	4.52	15.3
	0.0100	2.74	25.3
	0.0200	1.34	51.9
6.60	0.00545	5.27	13.1
	0.0100	2.20	31.6
	0.0200	1.04	66.4

[a] Acetate buffers used at pH 3.52 and 4.55; phosphate buffer, pH 6.60.

Effect of Disodium Ethylenediaminetetraacetate—The effect of disodium ethylenediaminetetraacetate was carried out in acetate buffers (pH 3.52, 4.55, and 5.45) and in phosphate buffer (pH 7.22) at 67° and ionic strength 0.4, using 0.10% disodium ethylenediaminetetraacetate. The results are shown in Figs. 5 and 6.

pH–Log Rate Profile—Table V shows the effect of hydrogen-ion concentration on ascorbic acid oxidation at 67°. The first-order rate constants (k_0) were obtained by extrapolation from Figs. 5 and 6 at zero buffer concentration, and $k_{calc.}$ was obtained using the derived rate equation (Eq. 9). The pH–log rate constant profile is shown in Fig. 7.

Determination of pKa_1 of Ascorbic Acid—The pKa_1 was determined by measuring the pH (using glass and calomel electrodes on a pH meter[3]) at 67° of solutions containing 0.01 mole l.$^{-1}$ each of ascorbic acid and sodium monohydrogen ascorbate and a sufficient amount of potassium chloride to give an ionic strength of 0.4. The average of three readings gave a value of pKa_1 3.98. Using the Henderson–Hasselbach equation, the fraction of ascorbic acid species in the solution, as a function of pH, was calculated. The species profile is shown in Fig. 8.

DISCUSSION

All of the rate constants were calculated from the first-order rate equation. The slopes of the lines were calculated by regression analysis (16, 17) on an electronic calculator[4]. All rate constants were calculated with n (number of points on the line) equal to 5–10. The correlation coefficient in all of the calculations was between −0.950 and −1.000. The thermodynamic parameters were calculated from Arrhenius plots using specially programmed cards.

Figure 2 shows the effect of ionic strength on the oxidation rate of ascorbic acid. At pH 3.52, 5.45, and 6.60 the slope of the line

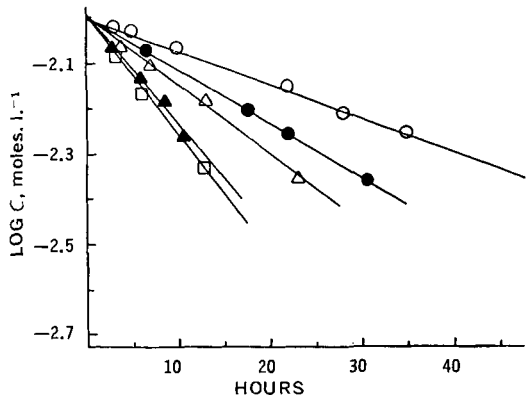

Figure 3—*Pseudo-first-order plots for the oxidation of ascorbic acid in acetate buffer (pH 4.55) at various temperatures and ionic strength 0.4. Key:* ○, *59.5°;* ●, *67°;* △, *72°;* ▲, *80°; and* □, *85°.*

[3] Beckman Zeromatic II.
[4] Wang model 360K/362K. Card programmer CP-1 with specially programmed cards was used.

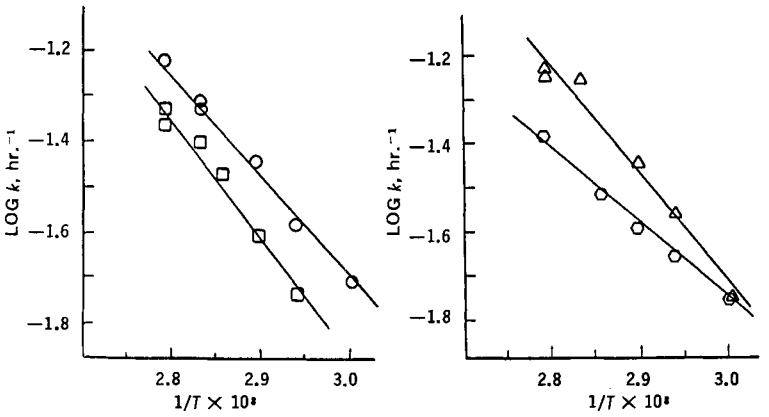

Figure 4—*Arrhenius plots showing temperature dependence of the ascorbic acid oxidation at pH 3.52–5.45 (acetate buffer) and pH 6.60 (phosphate buffer) and ionic strength 0.4. Key: □, pH 3.52; △, pH 4.55; ○, pH 5.45; and ○, pH 6.60.*

is approximately zero. Zero slope indicates the reaction of positive or negative ions with a neutral molecule, or a unimolecular reaction of positive, negative, or neutral molecules. At pH 4.55, the slope of the line is negative but much less than 1. A negative slope of 1 indicates a reaction of ions of unlike sign. A negative slope of less than 1 (approximately 0.33) suggests that the reaction of ions of unlike sign is not a dominating one and that other reactions are occurring in this pH region.

The effect of pH and temperature on the aerobic oxidation of ascorbic acid was studied at several temperatures and pH values. The calculated values of the rate constants and half-lives at different pH values and temperatures are summarized in Table II. The effect of temperature on the reaction rate can be expressed using the Arrhenius equation. As shown in Fig. 4, plots of log k *versus* $1/T$ yielded a straight-line relationship with a negative slope at each of the four pH's studied. Table III shows the statistical and thermodynamic parameters for the straight-line relationships.

Different values of activation energies, Ea, at various pH values indicate the possibility that a different reaction is dominant at different pH values. The large negative values of entropy of activation, $\Delta S\ddagger$, indicates that the degree of disorder in the activated complex and in the reaction is great. A simple atomic reaction can thus be ruled out for the mechanistic pathway of ascorbic acid oxidation. The calculated thermodynamic values compare favorably with those found in the literature (18–20).

The effect of ascorbic acid concentration on its rate of oxidation was carried out at pH 3.52, 4.55, and 6.60. Data obtained from log concentration *versus* time plots indicate a first-order rate of oxidation. However, from the data in Table IV, the order of reaction with respect to ascorbic acid does not appear to be first order. Other workers (21) showed that the rate of aerobic oxidation of ascorbic acid is dependent upon the concentration of oxygen present in the reaction system. Since in the present study the concentration of ascorbic acid varied while the amount of oxygen remained constant, *i.e.*, the ascorbic acid–oxygen ratio varied, the results in Table IV do not show the order of reaction to be first order with respect to ascorbic acid concentration.

The effect of total acetate and phosphate concentration on the rate of oxidation of ascorbic acid is shown in Figs. 5 and 6. The total acetate and total phosphate concentrations are:

$$(Ac)_T = (HOAc) + (OAc^-) \qquad \text{(Eq. 1)}$$

$$(\text{phosphate})_T = (H_2PO_4^-) + (HPO_4^{-2}) \qquad \text{(Eq. 2)}$$

The acetate and phosphate concentrations are shown in Table V for pH 3.52–7.22. The rate constants shown in Table V were obtained from log concentration *versus* time plots for ascorbic acid at each buffer concentration. Each plot yielded a straight-line relationship, indicating a first-order rate of oxidation.

From Figs. 5 and 6 it can be seen that increasing the buffer concentration increases the rate of oxidation of ascorbic acid. It is necessary to know the pKa's of acetic acid, phosphoric acid, and ascorbic acid in order to determine which species are catalytic to ascorbic acid. Since the study was carried out at 67°, it is necessary to know the pKa's at 67°. By using the Harned and Owen equation (22), the pKa's were calculated as 4.83 for acetic acid and 7.22 for the second dissociation constant of phosphoric acid. The pKa_1 for ascorbic acid, determined experimentally at 67°, was 3.98.

In the pH region below 3.75, the HOAc species (undissociated acetic acid) is catalytic to H_2A (undissociated ascorbic acid) and HA^- species (monodissociated ascorbic acid). In the pH region of 3.75–5.00, both acetate species (HOAc and OAc^-) are catalytic to both ascorbic acid species (H_2A and HA^-). Above pH 5.00, both acetate species (HOAc and OAc^-) are catalytic to HA^- in acetate buffers.

In phosphate buffers, in the pH region of 5.85–7.22, ascorbic acid exists mainly as HA^- (monodissociated ascorbic acid) and the buffer species present are $H_2PO_4^-$ and HPO_4^{-2}. Figure 6 shows the increase in the rate of oxidation of ascorbic acid with in-

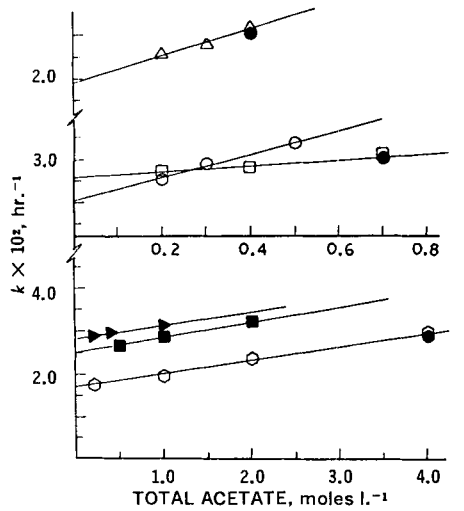

Figure 5—*Effect of acetate concentration on the rate of oxidation of ascorbic acid at 67° at various pH's and ionic strength 0.4. Key: ○, pH 3.52; ■, pH 3.88; ▲, pH 4.25; □, pH 4.55; ○, pH 5.05; △, pH 5.45; and ●, with 0.1% disodium ethylenediaminetetraacetate.*

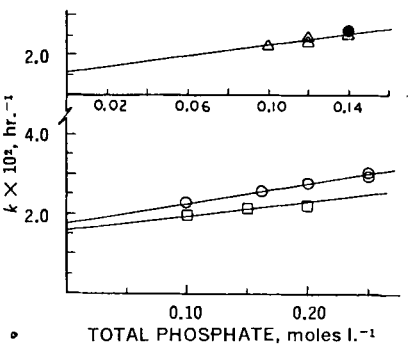

Figure 6—*Effect of phosphate concentration on the rate of oxidation of ascorbic acid at 67° at various pH's and ionic strength 0.4. Key: ○, pH 5.85; □, pH 6.60; △, pH 7.22; and ●, with 0.1% disodium ethylenediaminetetraacetate.*

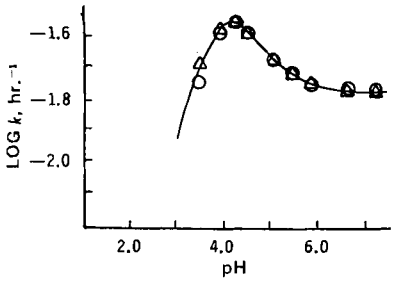

Figure 7—*The pH–log rate constant profile for ascorbic acid oxidation in aqueous solution at 67° and ionic strength 0.4. Key: ○, extrapolated rate constants; and △, calculated rate constants.*

creasing phosphate concentration; both phosphate species present appear to be catalytic to HA^-.

Thus, ascorbic acid is subject to general-acid and general-base catalysis in the acetate and phosphate buffers.

Trace quantities of metals catalyze the oxidation of ascorbic acid. Since buffers usually contain trace quantities of metal, the results shown in Figs. 5 and 6 and in Table V could be due to metal-ion contamination. The oxidation of ascorbic acid was followed at the highest buffer concentration shown in Table V at pH 3.52, 4.55, 5.45, and 7.22 in the presence of 0.1% disodium ethylenediaminetetraacetate. It is apparent that the catalytic effect of trace metal ions in the buffers was negligible. However, the buffers may contain traces of metal ions which have no effect on the oxidation rate of ascorbic acid or, if catalytic, their concentration may be too low to affect the oxidation rate of ascorbic acid.

The pH–log rate or pH–log k profile is a valuable way of presenting kinetic data for reactions that are pH sensitive, *i.e.*, the oxidation of ascorbic acid. To study only the effect of hydrogen ion and/or hydroxyl ion, the rate constants at zero buffer concentration are necessary. These were obtained by extrapolation from Figs. 5 and 6 and by calculation using Eq. 9. Results are shown in Fig. 7 and Table VI.

The pH–log rate profile shown in Fig. 7 suggests that the overall oxidation rate represents a summation of several separate reactions. It is not possible, from the experimental data, to establish the correct reactions with certainty. However, the following proposed reactions would give a pH–log rate profile that appears to fit the experimental points well:

Table V—Effect of Buffer Concentration on the Rate of Oxidation of Ascorbic Acid at 67° and Ionic Strength 0.4

pH[a]	Buffer Concentration, moles l.$^{-1}$	$k \times 10^2$, hr.$^{-1}$	$t_{1/2}$, hr.
3.52	0.20	1.79	38.6
	1.00	1.94	35.7
	2.00	2.34	29.6
	4.00	2.91 (2.78)[b]	23.8 (24.9)
3.88	0.50	2.65	26.2
	1.00	2.78	25.0
	2.00	3.20	21.7
4.25	0.20	2.84	24.4
	0.40	2.95	23.5
	1.00	3.10	22.2
4.55	0.20	2.74	25.3
	0.40	2.77	25.0
	0.70	3.01 (3.05)	23.0 (22.7)
5.05	0.20	2.55	27.0
	0.30	2.88	24.0
	0.50	3.38	20.5
5.45	0.20	2.57	26.9
	0.30	2.88	24.0
	0.40	3.11 (2.96)	22.3 (23.4)
5.85	0.10	2.30	30.0
	0.175	2.60	26.7
	0.20	2.77	25.0
	0.25	2.99	23.0
6.60	0.10	2.01	34.5
	0.15	2.14	32.4
	0.20	2.20	31.5
7.22	0.10	2.23	31.0
	0.12	2.32	30.0
	0.14	2.57 (2.60)	27.0 (26.7)

[a] Acetate buffers, pH 3.52–5.45; phosphate buffers, pH 5.87–7.22. [b] In the presence of 0.1% disodium ethylenediaminetetraacetate.

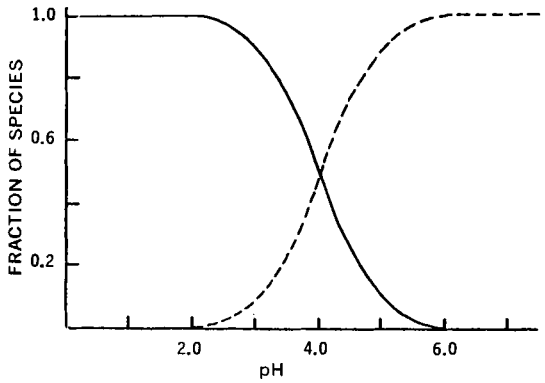

Figure 8—*Fraction of ascorbic acid species in solution as a function of pH at 67° and ionic strength 0.4. Plot calculated from the experimentally determined value of pKa_1 3.98. Key:——, undissociated ascorbic acid; and ---, monohydrogen ascorbate ion.*

$$HA^- \xrightarrow{k_1} \text{products} \qquad \text{(Reaction 1)}$$

$$H_2A \cdot HA^- \xrightarrow{k_2} \text{products} \qquad \text{(Reaction 2)}$$

$$H_2A \xrightarrow{k_3} \text{products} \qquad \text{(Reaction 3)}$$

In these reactions, H_2A and HA^- are undissociated and monodissociated ascorbic acid, and $H_2A \cdot HA^-$ is a complex of undissociated ascorbic acid and monohydrogen ascorbate ion.

The overall velocity of the reaction is equal to the sum of the rates of these reactions:

$$-\frac{d[A_T]}{dt} = k_1[HA^-] + k_2[H_2A \cdot HA^-] + k_3[H_2A] \qquad \text{(Eq. 3)}$$

$$[A_T] = [HA^-] + [H_2A] \qquad \text{(Eq. 4)}$$

If the complex has a formation constant, K_f:

$$K_f = \frac{[H_2A \cdot HA^-]}{[H_2A][HA^-]} \qquad \text{(Eq. 5)}$$

and if $K_f k_2 = k_2'$, then substituting these into Eq. 3 gives:

$$-\frac{d[A_T]}{dt} = k_1[HA^-] + k_2'[H_2A][HA^-] + k_3[H_2A] \qquad \text{(Eq. 6)}$$

Because of the overall first-order character of the reaction:

$$-\frac{d[A_T]}{dt} = k[A_T] \qquad \text{(Eq. 7)}$$

Combining Eq. 7 and:

$$Ka_1 = \frac{[H^+][HA^-]}{[H_2A]} \qquad \text{(Eq. 8)}$$

gives:

$$k = \frac{k_1 Ka_1 + k_3[H^+]}{Ka_1 + [H^+]} + \frac{k_2' Ka_1 A_T[H^+]}{(Ka_1 + [H^+])^2} \qquad \text{(Eq. 9)}$$

where $k = k_0$, and k_0 is the rate constant at zero buffer concentration obtained by extrapolation from Figs. 5 and 6. If Eq. 9 holds true, it should be possible to calculate the value of k at different pH's; the calculated values should agree with those determined experimentally.

In acidic solutions, where $H^+ \gg Ka_1$, Eq. 9 reduces to:

$$k = k_3 + \frac{k_1 Ka_1 + k_2' Ka_1 A_T}{[H^+]} \qquad \text{(Eq. 10)}$$

Table VI—Catalytic Effect of Hydrogen Ion on the Oxidation of Ascorbic Acid at 67° and Ionic Strength 0.4[a]

pH	$k_0 \times 10^2$, hr.$^{-1}$	$k_{calc.} \times 10^2$, hr.$^{-1}$
3.52	1.75	2.06
3.88	2.50	2.68
4.25	2.80	2.80
4.55	2.60	2.55
5.05	2.03	2.06
5.45	1.90	1.83
5.85	1.85	1.72
6.60	1.70	1.66
7.22	1.70	1.65

[a] k_0 values were obtained by extrapolation from Figs. 5 and 6, and $k_{calc.}$ values were obtained using Eq. 9.

From Eq. 10, a plot of k *versus* $1/[H^+]$ at pH values much less than pKa_1 gives a straight line. The intercept is k_3 and the slope of the line divided by Ka_1 is $k_1 + k_2'A_T$. The following results were obtained: k_3 = intercept = 0.004 hr.$^{-1}$ and the slope/Ka_1 = 0.0865 hr.$^{-1}$.

For solutions where $H^+ \ll Ka_1$, Eq. 9 reduces to:

$$k = k_1 + \frac{k_3[H^+] + k_2'A_T[H^+]}{Ka_1} \qquad \text{(Eq. 11)}$$

From Eq. 11, the plot of k *versus* H^+ gives a straight line whose intercept = k_1 = 0.0165 hr.$^{-1}$ and whose slope × $Ka_1 = k_3 + k_2'A_T$ = 0.074 hr.$^{-1}$. By using the value of 0.01 mole/l. for A_T and the values obtained for k_1 and k_3, the value of k_2', 7.00 hr.$^{-1}$ mole^{-1} l., was obtained.

On the basis of the species profile for ascorbic acid shown in Fig. 8, the overall rate profile was calculated using Eq. 10 at pH < pKa_1 and Eq. 11 at pH > pKa_1; the result is shown in Fig. 7 as a solid line.

Since the oxygen concentration was constant in this study, it does not appear in the rate law. It is known from the literature that the rate of oxidation of ascorbic acid is dependent upon the oxygen concentration; hence, the proposed rate law may or may not hold at different oxygen concentrations. The proposed rate law also predicts an increase in the oxidation rate with increasing ascorbic acid concentration. However, because of the dependence on oxygen concentration, a decrease in the rate of ascorbic acid oxidation was observed with increasing ascorbic acid concentration (Table IV).

The relatively good agreement of the experimental data and the theoretical profile does not prove that the proposed reactions, 1–3, are the correct ones. However, the reasoning for them justifies their proposal.

In considering the effect of pH on the oxidation rate of ascorbic acid in aqueous solution, the following reactions are possible.

$$HA^- \xrightarrow{k_1} \text{products} \qquad \text{(Reaction 1)}$$

$$H_2A \cdot HA^- \xrightarrow{k_2} \text{products} \qquad \text{(Reaction 2)}$$

$$H_2A \xrightarrow{k_3} \text{products} \qquad \text{(Reaction 3)}$$

$$HA^- + H^+ \xrightarrow{k_4} \text{products} \qquad \text{(Reaction 4)}$$

$$HA^- + OH^- \xrightarrow{k_5} \text{products} \qquad \text{(Reaction 5)}$$

$$H_2A + H^+ \xrightarrow{k_6} \text{products} \qquad \text{(Reaction 6)}$$

$$H_2A + OH^- \xrightarrow{k_7} \text{products} \qquad \text{(Reaction 7)}$$

On the basis of the experimental pH–log rate profile, Reactions 5 and 6 cannot be considered. If Reaction 5 holds, then the experimental rate should increase with pH in the pH range above pKa_1. For Reaction 6, the experimental rate should decrease with increasing pH in the pH range below pKa_1. Also, kinetically, Reactions 1 and 3 are the same as 7 and 4, respectively, and cannot be distinguished from them. Thus, Reactions 1 and 3 are proposed.

The pH–log rate profile in Fig. 7 shows a bell-shaped curve with maxima near pH = pKa_1 of ascorbic acid. The bell shape is generated by the fact that the monoacid form of a diacid is the reactive species (23). Reaction 2 presupposes the existence of a complex between ascorbic acid and the monohydrogen ascorbate ion. From a kinetic point of view, a reaction that presupposes the existence of an ascorbic acid–ascorbate complex can explain the observed pH–log rate profile. It is well known that organic acids are able to form complexes with their anions. Complex formations were reported between: mandelic acid and metal mandelate (24), salicylic acid and sodium salicylate; hippuric acid and potassium hippurate (25); succinic acid and potassium succinate (26); benzoic acid and sodium (27) and ammonium benzoates (28); and *p*-hydroxybenzoic acid, dihydroxybenzoic acid, phenylacetic acid, adipic acid, saccharin, barbituric acid, barbital, phenobarbital, and their sodium salts (29).

The possible complex formation between ascorbic acid and monohydrogen ascorbate was investigated by Finholt *et al.* (30), using freezing-point depression and boiling-point elevation methods and solubility analysis. These methods failed to prove the existence of such a complex; however, kinetic evidence for its presence was given.

SUMMARY AND CONCLUSION

The pH–log profile of the rate of disappearance of ascorbic acid from aqueous solution under aerobic conditions was determined at 67° in the pH range of 3.52–7.22 and the ionic strength of 0.4. The rate of disappearance of ascorbic acid was obtained by determining the concentration of unreacted ascorbic acid *versus* time using the 2,6-dichloroindophenol volumetric method. An apparent first-order rate of degradation was observed.

The primary salt effect was almost zero at pH 3.52, 4.55, 5.45, and 6.60. Acetate ions, monodissociated and undissociated, were catalytic to undissociated and monodissociated ascorbate ions in the pH range of 3.52–5.45. In the pH range 5.85–7.22, mono- and didissociated phosphate ions were catalytic to monodissociated ascorbate ions. The pH–log rate profile was obtained from extrapolated rate constants at zero buffer concentration. Since ascorbic acid oxidation is readily catalyzed by trace amounts of metal ions, the oxidation was also followed in the presence of 0.1% disodium ethylenediaminetetraacetate at the highest buffer concentrations used in the study. The results duplicated those obtained by following the aerobic oxidation of ascorbic acid in the absence of a chelating agent.

The pH–log rate profile shows a maximum near the pKa_1 of ascorbic acid. Complex formation between undissociated ascorbic acid and monodissociated ascorbate ion is proposed. From a kinetic point of view, a reaction that presupposes the existence of an ascorbic acid–ascorbate complex can explain the observed pH–log rate profile. The following rate law is proposed:

$$k = \frac{k_1Ka_1 + k_3[H^+]}{Ka_1 + [H^+]} + \frac{k_2'Ka_1A_T[H^+]}{(Ka_1 + [H^+])^2}$$

where:

$$HA^- \xrightarrow{k_1} \text{products}$$

$$H_2A \cdot HA^- \xrightarrow{k_2} \text{products}$$

$$H_2A \xrightarrow{k_3} \text{products}$$

Ka_1 = first dissociation constant of ascorbic acid

K_f = formation constant of ascorbic acid–ascorbate complex

$k_2' = k_2K_f$

Since oxygen concentration was constant in this study, it does not appear in the rate law. However, it is known from the literature that the rate of oxidation of ascorbic acid is dependent on the oxygen concentration; hence, it was not possible to obtain the increase in oxidation rate with increasing ascorbic acid concentration under the experimental conditions of this study. The proposed rate law does predict the increase in rate with increasing ascorbic acid concentration. A decrease in the rate of ascorbic acid oxida-

tion was observed with increasing ascorbic acid concentration. Thermodynamic parameters were calculated from Arrhenius plots at pH 3.52, 4.55, 5.45, and 6.60.

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ACKNOWLEDGMENTS AND ADDRESSES

Received August 23, 1971, from the *College of Pharmacy, University of Iowa, Iowa City, IA 52240*

Accepted for publication January 5, 1972.

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Pharmacological Aspects of Concurrent Administration of Furosemide and Skeletal Muscle Relaxants

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Abstract □ The pharmacological aspects of concurrent administration of furosemide and the skeletal muscle relaxants, *d*-tubocurarine, succinylcholine, and chlordiazepoxide, were investigated with respect to the effects on diuresis and skeletal muscle relaxation in female albino rats. Administration of *d*-tubocurarine and chlordiazepoxide concurrently with furosemide produced no significant effects on furosemide-induced diuresis. Succinylcholine, when administered concurrently with furosemide, caused a decrease in the volume of urine excreted; other parameters of renal function were not affected. The skeletal muscle relaxant properties of *d*-tubocurarine, succinylcholine, and chlordiazepoxide were investigated upon concurrent administration of the muscle relaxants with furosemide by an *in vivo* study and an *in situ* study. The skeletal muscle relaxant action of succinylcholine was significantly potentiated by pretreatment of the animals with furosemide, both in the exercise wheel study and in the diaphragm–phrenic nerve preparation. A tendency toward an antagonism between *d*-tubocurarine and furosemide was observed on skeletal muscle relaxation both *in vivo* and *in situ*. The effect of *d*-tubocurarine on blood pressure was also antagonized by treatment of the animal with furosemide. Preliminary spectrophotometric evidence suggests that a complex between furosemide and *d*-tubocurarine exists, which may possibly explain an antagonism between the two drugs.

Keyphrases □ Furosemide and concurrent administration of *d*-tubocurarine, succinylcholine, or chlordiazepoxide—effects on diuresis and skeletal muscle relaxation, rats □ *d*-Tubocurarine administered concurrently with furosemide—effect on diuresis and skeletal muscle relaxation, rats □ Succinylcholine administered concurrently with furosemide—effect on diuresis and skeletal muscle relaxation, rats □ Chlordiazepoxide administered concurrently with furosemide—effect on diuresis and skeletal muscle relaxation, rats □ Skeletal muscle relaxants—pharmacology of concurrent administration of furosemide, rats

It has been observed that the effects of *d*-tubocurarine are sensitive to electrolyte disturbances. Ferrari *et al.* (1) reported that the paralytic activity of *d*-tubocurarine was potentiated in rabbits by pretreatment with chlorothiazide, an electrolyte-depleting diuretic (2). A pharmacological interaction between *d*-tubocurarine and