OXIDATION-REDUCTION POTENTIAL OF ASCORBIC ACID (VITAMIN C)

BY HENRY BORSOOK AND GEOFFREY KEIGHLEY

WILLIAM G. KERCKHOFF LABORATORIES OF THE BIOLOGICAL SCIENCES, CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA

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The measurement of the oxidation-reduction potential of ascorbic acid (Vitamin C) is desirable in the elucidation of its physiological action. Two attempts have been made to measure this potential. Georgescu[1] concluded that he had obtained a thermodynamically reversible potential at pH 6.9, and 7.0 at 20°C. From the data presented, however, it is not possible to accept this interpretation without reservation. For instance at pH 6.9 the potential difference observed between 5 per cent and 80 per cent oxidation of the vitamin was only 22 millivolts where it should have been 55 millivolts. At pH 7.0 no potential difference was observed between 12.5 per cent and 75 per cent oxidation where the theoretical difference is 39 millivolts. Moreover the values of the mid points of the two curves given at pH 6.9 and pH 7.0 lead to the exceedingly improbable value for $-\frac{dE}{dpH}$ of 240 millivolts.

More recently Laki[2] working at pH 7.33 reported his failure to confirm the findings of Georgescu. Laki's observations indicated that the potential was proportional only to the concentration of the reduced form.

By working through a range of hydrogen-ion concentrations from pH 2.0 to pH 5.75 we have succeeded in measuring thermodynamically reversible potentials with ascorbic acid. The failure of the two previous workers is to be attributed, we believe, chiefly to their having worked for the most part near neutrality where the oxidized form of ascorbic acid decomposes very rapidly.

The measurements were carried out at 35.5°C. with the vacuum technique previously described[3] except that no reversibly oxidizable dye was added. For the preparations of the various mixtures of oxidized and reduced ascorbic acid the reduced form was oxidized with iodine. The ascorbic acid used, kindly given us by Professor von Szent-Györgyi, gave a titration value with iodine of 100% on the basis of 1 molecule of reduced ascorbic acid yielding on oxidation two hydrogen ions.

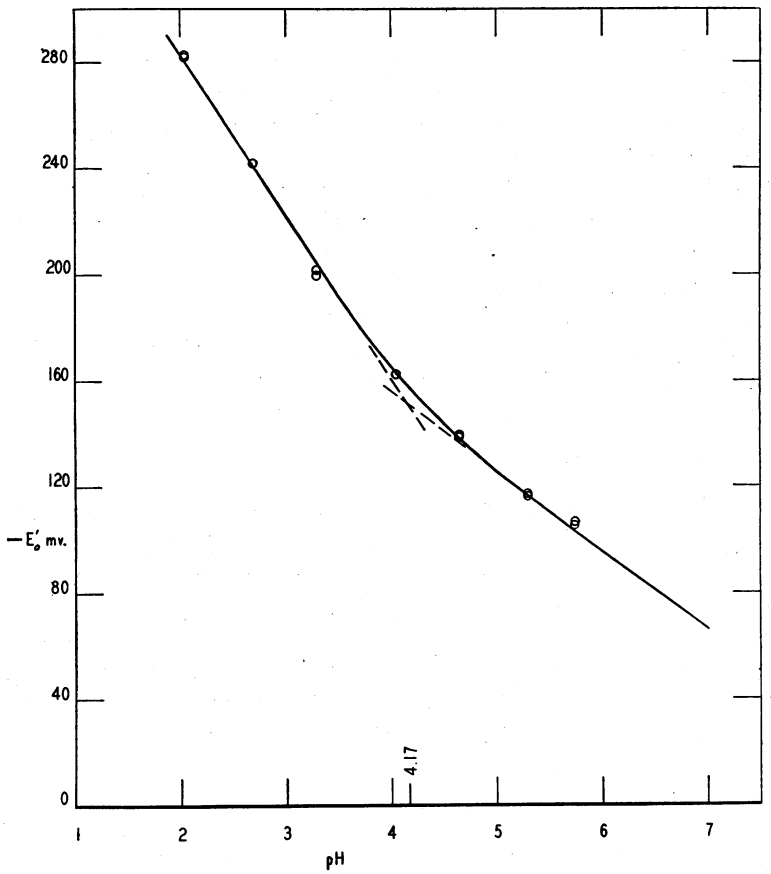
The electrode equation was obtained by the derivation described earlier.[3] For the mechanism

$$\left[\begin{matrix}\text{Reduced}\\ \text{Ascorbic Acid}\end{matrix}\right]^{-} \longrightarrow \left[\begin{matrix}\text{Oxidized}\\ \text{Ascorbic Acid}\end{matrix}\right]^{-} + 2H^{+} + 2(e)$$

the equation is

$$E_{obs} = \tilde{E} + \frac{RT}{F}\,\mathrm{pH} + \frac{RT}{2F}\ln\frac{[\mathrm{Red}]}{[\mathrm{Ox}]} + \frac{RT}{2F}\ln\frac{K_R}{K_o} + \frac{RT}{2F}\ln\frac{K_o + [H^+]}{K_R + [H^+]}$$

where [Red] and [Ox] indicate the total concentration of reduced and oxidized forms, respectively, and K_R and K_o the first dissociation constants of



Curve is theoretical for $pK_R = 4.17$, $pK_o = 9.0$
O are experimental values

the reduced and oxidized forms considered as monovalent acids. For simplicity we have ignored the second dissociation constants. Birch and Harris[4] found the value of pK_{R_1} to be about 4.17. By a colorimetric titration procedure we have found the value of pK_o to be approximately 9.0. The value of n was taken as 2, which was indicated by the results of the iodine titration, and corroborated by the agreement between the observed

and calculated results in table 1. With these values of the dissociation constants and of n, and a mean value of $\tilde{E}$ of -0.553 volt the graph presented was constructed.

Table 1 shows that at any given pH between 2.0 and 5.75 the potential is proportional to the ratio of the concentrations of reduced and oxidized forms. The observed and calculated potential differences agree whether the concentraton of reduced form is held constant and the concentration of the oxidized form changed, or the reverse, or whether both are varied.

TABLE 1

REDUCTION POTENTIALS AT 35.5°C. OF DIFFERENT MIXTURES OF REDUCED AND OXIDIZED ASCORBIC ACID AT DIFFERENT HYDROGEN-ION CONCENTRATIONS

pH	CONCENTRATION OF REDUCED FORM $\times 10^3$ MOLS	CONCENTRATION OF OXIDIZED FORM $\times 10^3$ MOLS	RATIO $\frac{[RED]}{[ox]}$	CALCULATED P.D. $= 0.0306 \log \frac{[RED]}{[ox]}$ MV.	P.D. OBSERVED MV.	E'_o OBSERVED MV.	E'_o CALCULATED MV.
2.04	2.43	0.504	4.82	18	17	−0.283	−0.281
2.04	2.43	2.12	1.20			−0.281	
2.68	0.74	1.38	0.54	18	18	−0.242	−0.242
2.68	2.96	1.38	2.14			−0.242	
3.30	0.72	1.41	0.51	18	16	−0.202	−0.204
3.30	2.87	1.41	2.03			−0.200	
4.06	0.73	1.40	0.52	18	19	−0.163	−0.164
4.06	2.93	1.40	2.07			−0.163	
4.65	0.75	1.38	0.55	18	17	−0.139	−0.139
4.65	3.01	1.38	2.18			−0.140	
5.31	1.91	1.82	1.05	18	17	−0.117	−0.117
5.31	1.91	0.45	4.2			−0.118	
5.75	4.55	0.56	8.18	37	38	−0.106	−0.104
5.75	1.14	2.23	0.51			−0.107	

In table 2 the values given were taken from the graph shown. Our last reliable experimental determination was at pH 5.75. At more alkaline

TABLE 2

ASCORBIC ACID. RELATION BETWEEN E'_o $\left(\frac{[Red]}{[Ox]} = 1\right)$ AND pH

pH	E'_o
2.0	−0.283
2.5	−0.252
3.0	−0.223
3.5	−0.193
4.0	−0.166
4.5	−0.145
5.0	−0.127
6.0	−0.096
7.0	−0.066

hydrogen-ion concentrations the rate of decomposition of the oxidized form was too rapid for any reliable measurements to be made. The curve and

the figures in table 2 beyond pH 5.75 therefore are extrapolations unsupported at present by any direct experimental data except the measurement of the dissociation constant of the oxidized form.

The potentials obtained elucidate the earlier observations on the stability of Vitamin C, its behavior toward various oxidizing and reducing agents and its physiological action. These considerations will be presented in detail later.

We wish to acknowledge our indebtedness to Professor A. von Szent-Györgyi for his gift of the specimen of pure ascorbic acid used in this investigation, and to thank him heartily for his generosity.

Summary.—1. Data regarding the thermodynamically reversible reduction potentials of ascorbic acid (Vitamin C) are presented.

[1] Georgescu, I. D., *J. Chimie physique*, **29**, 217 (1932).
[2] Laki, K., *Zeit. physiol. Chem.*, **217**, 54 (1933).
[3] Borsook, H., and Schott, H. F., *J. Biol. Chem.*, **92**, 535 (1931).
[4] Birch, T. W., and Harris, L. J., *Biochem. J.*, **27**, 595 (1933).