Polarographic Investigations of Vitamin C. I. On the Oxidation Waves of L-Ascorbic Acid and the Reduction Wave of Dehydro-L-ascorbic Acid

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Although the physiological importance of vitamin C (V. C) has been well known since its discovery, there still remain many problems unsolved concerning this vitamin. Namely, very little knowledge has been obtained even for the mechanism of its antiscorbutic processes[1]. The vitamin has been also discussed on its role as a member of the oxidation-reduction systems in both vegetable and animal bodies, and the measurements of the oxidation-reduction potentials of the system have been exhaustively carried out by several investigators[2~4]. In spite of their efforts, the previous knowledge may not seem to be sufficient to interpret the specificity of V. C. There are considerably many related substances of V. C which also possess close oxidation-reduction potentials to those of V. C and still do not show any physiological similitude[5~7].

On the other hand, as to the polarographic investigations of V. C hitherto reported, L-ascorbic acid (A. A.) has been known to be readily oxidizable at the dropping mercury electrode and the half-wave potential of the first wave (at pH=0) is about 200 mV. more positive than the corresponding standard oxidation-reduction potential measured potentiometrically, while its oxidized form dehydro-L-ascorbic acid (D. A. A.) has been believed to show no reduction wave under the ordinary polarographic conditions[8~12].

In order to interpret these phenomena, many experimental and theoretical studies have been made by several investigators[13~16]. Indeed, the analysis of these disagreements between the polarographic and the potentiometric results would be expected to offer some clue to obtain real knowledge on the specificity of V. C.

It is, however, important to realize that the previous discussions have not been developed on the basis of the sufficient experimental results of both A. A. and D. A. A., and therefore the problem may not be said to have been solved even qualitatively.

In the present experiments, first the reinvestigations of the already known facts on the oxidation waves of A. A. were made and followed by the more careful and detailed experiments in alkaline region than previously known, and further a reduction wave of D. A. A. was obtained.

Although some of the observations concerning this reduction wave of D. A. A. have been already reported by the present authors[17], more detailed results are presented in this paper and discussions are made on the mechanism of the electrode reactions of both A. A. and D. A. A.

Experimental

Materials:—A. A. was a product of Takeda Pharmaceutical Ind. which was used without further purification.

D. A. A. was first prepared by using Pecherer's method[18], but later A. A. in aqueous solution was oxidized to D. A. A. with equimolar amount of iodine or bimolar amount of silver nitrate.

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4) E. G. Ball, *J. Biol. Chem.*, **118**, 219 (1937).

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6) N. Mayer, *J. chim. phys.*, **34**, 107 (1937).

7) R. Wurmser, N. Mayer and O. Crepy, ibid., **33**, 101 (1936).

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10) Y. Okada, *J. Agr. Chem. Soc. Japan*, (*Nippon Nogei Kagaku Kaishi*), **19**, 749, 955 (1943).

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13) Z. Vavrin, *Coll. Czech. Chem. Communs.*, **14**, 367 (1949).

14) R. Brdicka and P. Zuman, ibid., **15**, 766 (1950).

15) D. H. M. Kern, *J. Am. Chem. Soc.*, **76**, 4234 (1954).

16) J. Koutecky, *Coll. Czech. Chem. Communs.*, **20**, 116 (1955).

17) S. Ono, M. Takagi and T. Wasa, *J. Am. Chem. Soc.*, **75**, 4369 (1953).

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In order to obtain D. A. A. solutions of relatively high concentrations as purely as possible, the latter method was used preferably. In the case of the oxidation with iodine, iodine was used in an aqueous solution containing about twice mole of potassium iodide, and after the oxidation in a volumetric flask, the resulting solution was partly neutralized with sodium hydroxide or sodium acetate and then filled up to the mark. The solution was used without removing iodide ions. Silver nitrate as an oxidizing reagent was also used in an aqueous solution. After the oxidation of A. A. in a volumetric flask, some amounts of sodium hydroxide or sodium acetate which had been taken to adjust the pH of the solution to the desired value. The solution was filled up to the mark and then the silver precipitate was filtered off. The filtrate was used as D. A. A. solution.

The aqueous solution of D. A. A. was prepared each time freshly, since D. A. A. is fairly unstable even in acidic solution.

Buffer Solutions:—For the acidic range, McIlvaine's buffer solutions (pH 2.2∼8.0) and acetate buffer solutions (pH 3.6∼5.6) were used. For the alkaline range Kolthoff's buffer solutions (pH 8.0∼12.0) and 0.1N sodium hydroxide (pH 13) were used. In the course of experiments, it was sometimes necessary to avoid the use of borate in the electrolytic solutions as will be seen later and in such cases, pH's were adjusted with sodium hydroxide and disodium hydrogen phosphate.

The water for preparing stock solutions of the sample and the buffer solutions was redistilled 3 or 4 times with a glass apparatus, since even the presence of trace of the copper ion is still unfavorable for the stability of A. A.

As maximum suppressor, aqueous solution of gelatin was used whose concentration was about 0.005% in the final electrolytic solution.

Apparatus and Procedure:—A Yanagimoto Polarograph with photographic recorder was used. The maximum sensitivity of the galvanometer was 5.5×10^{-9} A. The characteristics of the capillary used were $m=1.323$ mg./sec. and $t=2.7$ sec./drop in 0.1N potassium chloride solution at open circuit when the height of the mercury reservoir was 60 cm. A normal calomel electrode (N.C.E.) was used as a reference electrode. For the experiments of the oxidation waves of A. A. and D. A. A., the height of the mercury reservoir was usually 60 cm. The electrolytic cells were the most simple and common ones. Although no thermostat was used, the room temperature was roughly controlled at 15°±1°C during the experiments. For the experiments in acidic range, polarograms were taken from +0.3 V. (vs. N. C. E.).

As it is well known, A. A. in aqueous solution is readily oxidized by the atmospheric oxygen, especially in an alkaline medium, even the stock solution, whose pH was usually adjusted to less than 4 with buffer solution or meta phosphoric acid, was freed from oxygen by bubbling nitrogen gas, while a known volume of buffer solution (usually less than 7 ml.) was taken into an electrolytic cell and it was also bubbled with nitrogen gas for twenty minutes. Thereafter, a knwon volume of the stock solution (usually about 1 ml.) was pipetted out and added into the electrolytic cell, bubbling of nitrogen gas being still continued through the buffer solution. The bubbling was stopped after a few minutes and the polarogram was taken immediately. The concentration of the sample was usually 10^{-4} to 10^{-3} mol./l.

For obtaining the reduction wave of D. A. A. experiments were carried out under the following conditions.

Concentration range of D. A. A.:
0.025∼0.1 mol./l.
pH range: 2∼5
Temperature range:
10∼50°C at 5°C intervals.

For the temperature control, the electrolytic cell fitted with a jacket was used, through which water of a constant temperature was circulated. The accuracy of the temperature control was ±0.05°C. Nitrogen was used for the removal of oxygen from the electrolytic solution.

Controlled Potential Electrolysis:—In order to know whether the electroreduction product of D. A. A. is A. A. or not, a manually controlled potential electrolysis, modifying Lingane's method[19], was employed. To omit the procedure to take up some portion of the electrolytic solution each time after the electro-reduction at the mercury pool, the electrolytic cell as shown in Fig. 1 was used; after the electrolysis for a given time at a fixed potential (a), the capillary is dipped into the same cell and polarograms are taken, using a calomel electrode as the outer reference electrode (b). For the salt bridge potassium nitrate was used in stead of potassium

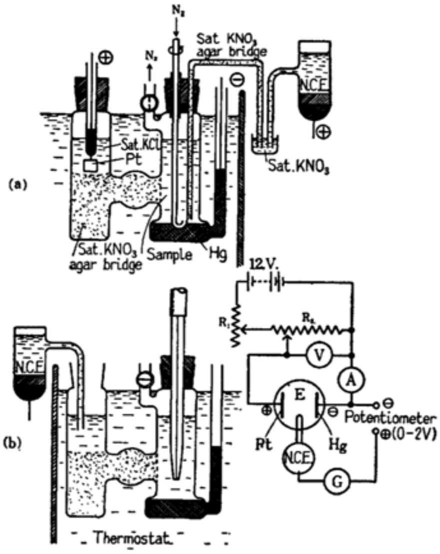

Fig. 1. Controlled potential electrolysis.
V—Voltometer A—Ammeter
E—Electrolytic cell G—Galvanometer

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[19] J. J. Lingane, *Discussions Faraday Soc.*, **1**, 203(1947).

chloride, since the anodic wave of A. A. is masked by that of the chloride ion. The preparation of D. A. A. by oxidation with iodine was not used for this electrolysis, since the presence of the iodide ion is not favorable in the same meaning as above.

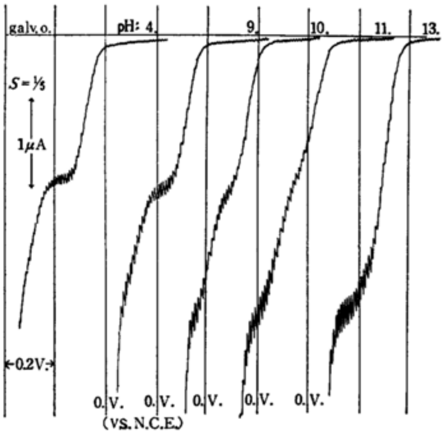

Fig. 2. Polarograms of A. A. in various pH. concn. of A. A. = 2.3×10^{-4} mol./l.

Results and Discussion

Oxidation Waves of A. A.—As is seen in Fig. 2, A. A. shows one oxidation wave at pH less than 8 and two at pH more than 8. Both the first and the second waves have almost an equal height. Since two-electron transfer has been known with the first wave[8~16] the second wave should also involve another two-electron transfer. This total four-electron transfer for the oxidation of A. A. in alkaline range coincides with the observation by Euler and Hasselquist[20,21] who studied the oxidation of A. A. by means of the titration with 2, 6-dichlorophenol indophenol. Concerning the presence of the second wave, Müller and Phillips[22] have already stated it briefly, but they did not present its polarograms and, since then, there has been no other report which confirms even the presence of the wave. The second wave should not be overlooked, since there is certain possibility that it might be confused with the first one.

The relation between the half-wave potential of the first wave and pH was not so much different from those reported by other authors[9~14], but it was remarked that above pH 12 the measurement of the potential becomes difficult because of the superimposing of the first wave with the second.

The instability of A. A. should be also taken into consideration to obtain reliable data with A. A., especially in alkaline range. Fig. 3 shows an example of the change of the waves with time. The rate of the decrease of the wave height strongly depends on pH.

In Fig. 3 the first wave seems to decrease more quickly than the second. Considering only this phenomenon, it was first

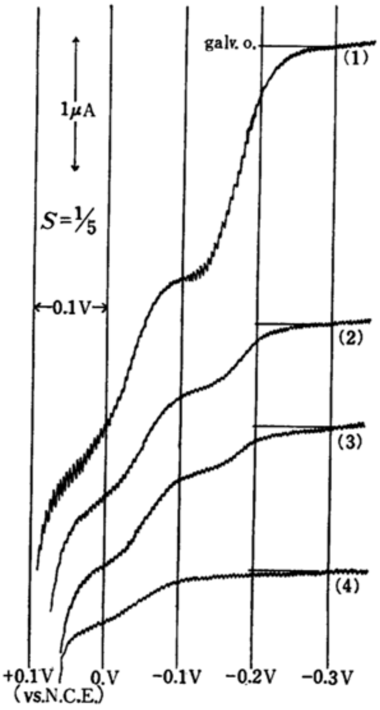

Fig. 3. Decrease of wave heights of A. A. due to the air-oxidation. pH=10.27, borate-NaOH buffer solution.
(1) concn. of A. A.=2.3×10^{-4} mol./l. without airation. (2) after 20 min. airation. (3) after 30 min. airation. (4) after standing for 24 hr. without airation.

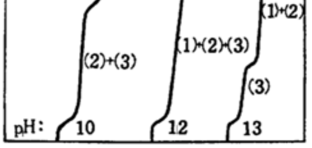

Fig. 4. Schematic polarograms of A. A. containing its decomposition product in alkaline buffer solution. (1) 1st wave (2) 2nd wave (3) 3rd wave

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[20] H. v. Euler and H. Hasselquist, *Arkiv Kemi*, **4**, 169 (1952).

[21] H. Hasselquist, ibid., **4**, 370 (1952).

[22] O. H. Müller and R. A Phillips, *Am. J. Physiol.*, **129**, 426 (1940).

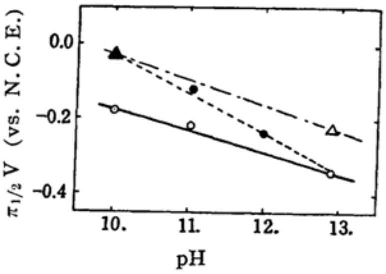

Fig. 5. pH dependence of the three waves of A. A.
○ 1st wave ●, 2nd wave, △ 3rd wave; $\pi_{1/2}$ of the large drawn wave at pH 12 was taken as the value of the 2nd wave at the pH.

supposed that the first oxidation product of A. A. in bulk alkaline solution would be identical with the electro-oxidation product corresponding to the first wave. But it was soon revealed that the above supposition was not adequate; it is readily seen when the same kind of experiment as shown in Fig. 3 is carried out at higher pH's. Fig. 4 schematically shows three different waves in alkaline range after partial oxidation of A. A. Here A. A. was first partially oxidized in alkaline solution and reacidified by metaphosphoric acid to pH 4 and used as stock solution. The experimental values of the half-wave potential of the three waves are presented in Fig. 5. From Figs. 4 and 5, it is apparent that the third wave which appeared after oxidation should be distinguished from the other two which have been initially present. At present it is, however, still difficult to predict to which stage of the oxidation processes of A. A. this third wave corresponds.

D. A. A. was also examined, in order to know its behavior in alkaline solution, in comparison with the above described three waves of A. A.; D. A. A. does not show any oxidation wave in the buffer solutions which contain more or less borate, though the three waves of A. A. were observed in the same buffer solutions. D. A. A. or 2, 3-diketogulonic acid (D. G. A.) seems to combine with the borate ion to a product which may be no more oxidizable at the dropping mercury electrode. This coincides with Militzer's observation[23] that the oxidation of D. A. A. or D. G. A. with methylene blue or the copper ion was completely hindered by the borate ion in alkaline solution. The present results may be also referred to Pasternak's report[24] that the disappearance of the polarographic reduction wave of benzil was caused by the reaction of its carbonyl group with the borate ion.

However, when buffer solutions were prepared only with sodium hydroxide and disodium hydrogen phosphate, D. A. A. showed very unstable and complicated oxidation waves. They were much less stable than those of A. A. and under the present experimental conditions they decreased quickly with time, even if the electrolytic solutions were kept from oxygen as free as possible. Fig. 6 shows an example of the change of the waves with time; starting with D. A. A. the waves were first considerably different from those of A. A., but after a certain time they seemed to show quite a similar character to each other. Above all, when the solution was reacidified, the polarogram of the former showed a wave which had the same $\pi_{1/2}$ as that of A. A. at the given pH. From these observations, though the possibility of reduction of D. A. A. to A. A. in alkaline solutions seems not to be denied, it may be more reasonable to consider the formation of some endiol compound which has a similar $\pi_{1/2}$ to that of A. A., e. g., triose-reductone[14].

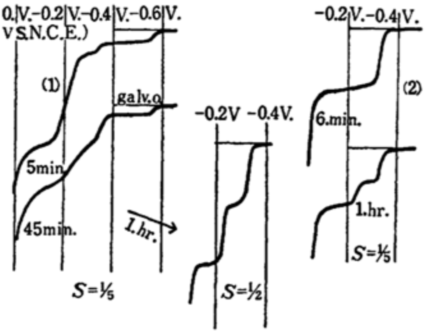

Fig. 6. Change of oxidation waves of A. A. and D. A. A. in alkaline solution (pH=12.9). (1) started with D. A. A. of 4.3×10^{-4} mol./l. (2) started with A. A. of 7.5×10^{-5} mol./l.

The fact that D. A. A. or its decomposition products showed complicated oxidation waves may indicate that there must be several alkaline decomposition products of D. A. A., each of which has an endiol group or some other functional group as readily oxidizable as an endiol, as Petuely and Künssberg[25] have widely investigated with many of the related substances.

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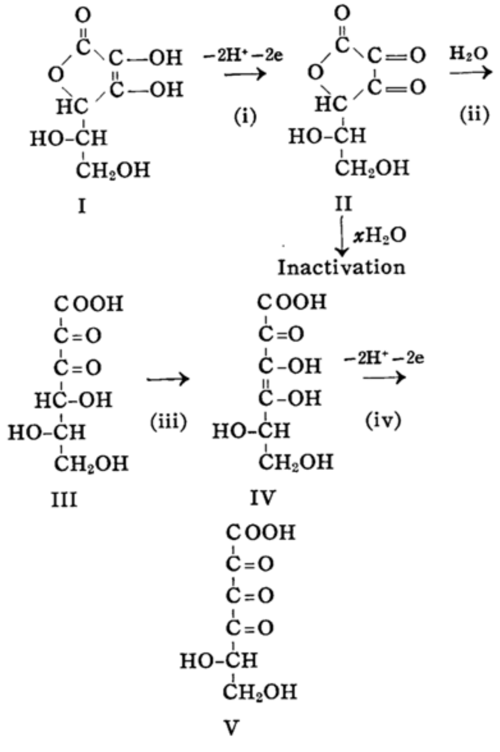
[23] W. E. Militzer, *J. Biol. Chem.*, **158**, 247 (1945).

[24] R. Pasternak, *Helv. Chim. Acta*, **30**, 1984 (1947).

[25] F. Petuely and U. Künssberg, *Monatsh.*, **83**, 80 (1952).

Also the fact that some endiol compounds are formed during the decomposition process of D. A. A. in alkaline solution has been known by Euler and Hasselquist[26]. On the other hand, Trnka[27] found three different anodic waves during the decomposition process of glucose in alkaline solution from which he predicted three kinds of endiol compound. Taking into account the possibility of some common processes between the decomposition of glucose and that of A. A., it would be expected to find some common endiols, and further investigations of this kind may seem to be interesting to continue. For that purpose, however, not only polarography but some other more direct methods should be used. At present, therefore, no further discussion is made.

In consideration of the present results together with the previous knowledge on endiol compounds obtained by Euler et al.[28,29] and Petuely et al.[25], the stepwise electrode reactions of A. A. in alkaline solution may be expressed in one way as follows.



In the above diagram, i) is the reaction corresponding to the first wave of A. A. and iv) to the second. Here it is supposed that D. A. A. which has been produced by the reaction i) opens its lactone ring (ii) and further it is converted into an endiol by the rearrangement iii). The endiol is further oxidized at the dropping mercury electrode (iv). In this case the rate of reaction of both ii) and iii) must be by all means enough rapid, compared with the diffusion process. Since it has been found that D. A. A. or D. G. A. reacts with the borate ion to an electroinactive substance, the rate of both ii) and iii) must be also sufficiently rapid than that of the above inactivation reaction. Further it may be supposed that the substances IV and V can be decomposed in the bulk solution. Above all, the fact that though D. A. A. or D. G. A. reacts with the borate ion to produce an inactive substance, A. A. showed an oxidation wave of its decomposition product after standing of the solution even in the presence of the borate ion, may suggest the possibility of the direct opening of the lactone ring.

Reduction Wave of D. A. A.—D. A. A. shows a small reduction wave at the potential between −0.3 and −0.5 V. (vs. N. C. E.) in pH 2∼5, when polarograms are taken at room temperature with D. A. A. of relatively high concentrations (0.025 ∼0.1 mol./l.).

The reduction wave of D. A. A. at 25°C corresponds to about 1/1000 of its expected diffusion current which can be approximately estimated from the oxidation wave of A. A. The shape of the wave of D. A. A. and its inclination differ considerably from those of A. A. Also the reduction wave of D. A. A. shows all the typical characteristics of a kinetic current: constancy of the wave height, independency of the height of the mercury reservoir (Fig. 7) and its great temperature coefficient, which was 7∼8%/degree (Fig. 8).

At a given temperature, the wave height

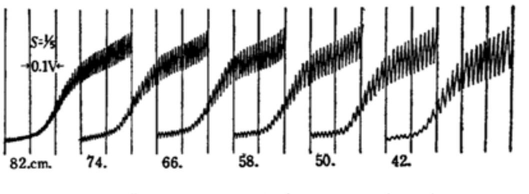


Fig. 7. Constancy of the wave height of D. A. A., independent of the height of the mercury reservoir. Concn. of D. A. A.=0.1 mol./l. in McIlvaine buffer, pH 2.8, 25°C.

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[26] H. v. Euler and H. Hasselquist, *Arkiv Kemi*, **8**, 67 (1955).

[27] J. Trnka, *Proc. I. Intern. Polarogr. Congr. Prag* **III**, 518 (1951).

[28] H. v. Euler and H. Hasselquist, "Die Reduktone", Stuttgart, Enke (1950).

[29] H. v. Euler and H. Hasselquist, *Arkiv Kemi*, **5**, 49 (1952).

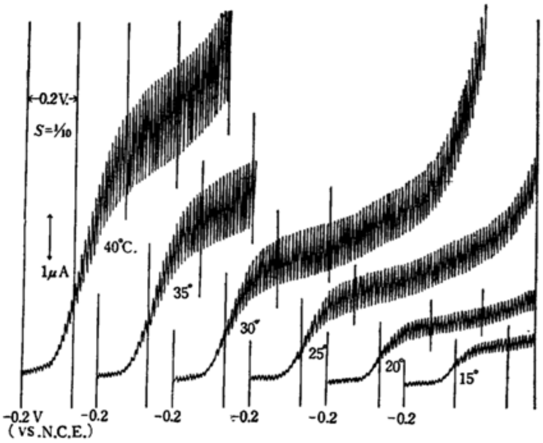


Fig. 8. Increase of wave height of D. A. A. with increasing temperature. Concn. of D. A. A.=0.1 mol./l. in McIlvaine buffer solution, pH 2.8.

of D. A. A. was roughly proportional to its concentration and was not appreciably changed with pH and it was also the case even in the neighborhood of pH 0.

TABLE I

HALF-WAVE POTENTIALS OF D. A. A. IN McILVAINE BUFFER SOLUTION AT 25°C.

Conc. of D. A. A.=0.025 mol./l.

pH	2.20	2.66	2.96	3.48
$\pi_{1/2}$(vs. N.C.E.)	−0.350	−0.372	−0.392	−0.410
pH	3.80	4.22	4.63	5.04
$\pi_{1/2}$(vs. N.C.E.)	−0.432	−0.450	−0.462	−0.480

The half-wave potential of the reduction wave is presented in Table I. Whether the half-wave potential, the wave height or the shape of the wave can be influenced by the components of the buffer or the manner of preparation of D. A. A. was examined and no appreciable change has been observed except for the D. A. A. prepared by the oxidation with iodine in aqueous solution, in which the presence of the iodide ion caused the shift of the half-wave potential of D. A. A. toward a little more negative value.

On plot of the logarithms of the wave heights of D. A. A. against reciprocal absolute temperature, a linear relationship has been obtained and its inclination was found to be independent of pH and the concentration of D. A. A. as seen in Fig. 9.

Above pH 5 the rate of decomposition of D. A. A. increases abruptly and besides the reduction wave of D. A. A., another reduction wave begins to grow up which may be due to one of the decomposition products of D. A. A. whose $\pi_{1/2}$ is slightly more negative than that of D. A. A. Consequently it becomes difficult to take reproducible polarograms of D. A. A. and in the present studies further experiments in pH more than 5 have not been made.

As stated in the experimental part, gelatin, an ordinary maximum suppressor, was not used for obtaining well defined polarograms, since gelatin markedly influenced the shape of the reduction wave of D. A. A. Fig. 10 shows the effect of gelatin on the reduction wave of D. A. A. On addition of only 1 drop of 0.5% gelatin

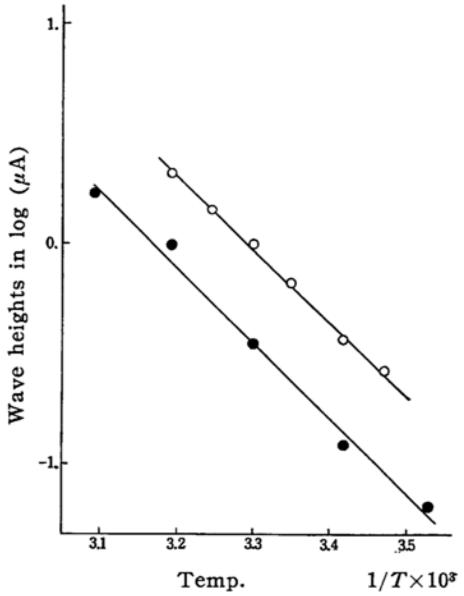


Fig. 9. Relationship between the logarithm of the height of the reduction wave of D. A. A. and reciprocal absolute temperature.

○ Concn. of D.A.A.=0.1 mol./l. at pH 2.8

● Concn. of D.A.A.=0.05 mol./l. at pH 2.7

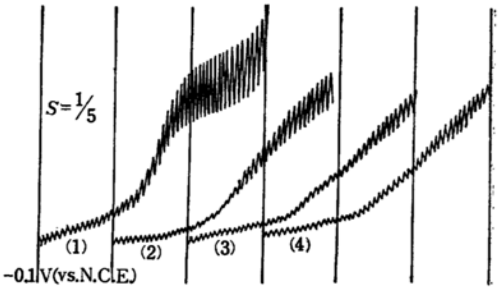


Fig. 10. Influence of gelatin on the reduction wave of D. A. A. Concn. of D. A. A.=0.1 mol./l., pH 2.8, 30°C.

(1) without gelatin

(2) 1 drop of 0.5% gelatin to 10 ml. of the soln. (1)

(3) 2 drops of 0.5% gelatin

(4) 3 drops of 0.5% gelatin

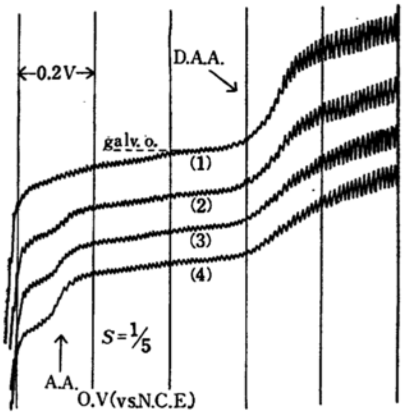


Fig. 11. Controlled potential electrolysis of D. A. A. at −0.7 V. (vs. N. C. E.), Concn. of D. A. A.=0.1 mol./l., pH 3.5, 25°C
(1) before electrolysis (2) after 20 min. (3) 40 min. (4) 60 min.

aqueous solution into 10 ml. of the electrolytic solution, the wave was strongly deformed; with three drops of the same gelatin solution, the wave became completely drawn out. Though no further experiments have been carried out concerning this problem, the above phenomena may indicate that a reaction which proceeds before the electrode reaction is retarded by the presence of the surface active substance such as gelatin.

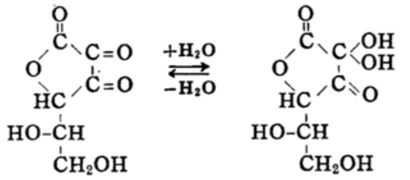
Fig. 11 shows that the growth of the anodic wave of A. A. can be observed as the electrolysis proceeds. In this case, it may be easily understood that the anodic wave of A. A. increases with increasing time, while the cathodic wave of D. A. A. remains almost unchanged, since the former has the character of a normal diffusion current and the latter that of a typical kinetic one. During the electrolysis it was found that the reduction wave of D. A. A. was gradually deformed with time. It may be considered to owe to a surface active property of the agar-agar which was dissolved from the salt bridge into the electrolytic solution.

From the above series of experiments on D. A. A., it has been seen that the reduction wave of D. A. A. is a typical kinetic current and its reduction product is A. A. Then, the attention should be turned to the inactive form of D. A. A. in the follow ingsystem in which the inactiavtion reaction of D. A. A. must be reversible.

$$\begin{array}{l}\text{Inactive form}\\ \text{of D. A. A.}\end{array} \rightleftarrows \text{D. A. A.} \underset{-2\text{H}^+-2e}{\overset{+2\text{H}^++2e}{\rightleftarrows}} \text{A. A.} \quad (1)$$

The inactive form of D. A. A. may be most probably its hydrated form. What D. A. A. may react with water to produce a hydrated product, has long since been suggested by Herbert et al.[30], and also Vavrin[13] supposed the hydration when he interpreted the discrepancy between the $\pi_{1/2}$ of the anodic wave of A. A. and its potentiometrically obtained π_0. However, any detail of the hydration itself has not been discussed. It may be partly because no reduction wave of D. A. A. has been obtained.

The hydrated form of D. A. A. which is proposed as the most probable one by the present authors, is II′ of the following two configurations.



D. A. A.
II

Hydrated D. A. A.
II′

The high reducibility of D. A. A. (II) at the dropping mercury electrode is readily understood since three carbonyl groups are conjugated, while the hydrate (II′) seems difficult to be reduced since the conjugation of II is here completely hindered by the hydration at the central carbonyl group of the three. The most feasible hydration at the central carbonyl group among the three may be inferred from the electronic effect caused with two other carbonyl groups on the central.

As for the similar type of hydration of carbonyl group, it is seen in the investigations of some aldehydes, especially of formaldehyde[31~34] and acetaldehyde[35~37].

On the other hand, there must be certain possibility of hydration not only at the central carbonyl group but also at either one of the other two or both. Let us, however, assume, for convenience'

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[30] R. W. Herbert, E. L. Hirst, E. G. V. Parcival, R., J. W. Reynolds and F. Smith, *J. Chem. Soc.*, **1933**, 1270.

[31] K. Vesely and R. Brdicka, *Coll. Czech. Chem. Communs.*, **12**, 313 (1947).

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[33] R. Brdicka, *Coll. Czech. Chem. Communs.*, **20**, 387 (1955).

[34] J. Koutecky, ibid., **21**, 652 (1956).

[35] R. Bieber and G. Trümpler, *Helv. Chim. Acta*, **30**, 2000 (1947).

[36] R. P. Bell and J. C. Clunie, *Trans. Faraday Soc.*, **48**, 439 (1952).

[37] R. P. Bell and J. C. Clunie, *Proc. Roy. Soc.*, *(London)*, **212A**, 33 (1952).

sake, only one hydrated form of D. A. A., namely II′. Since the reduction wave of D. A. A. is much smaller than its expected diffusion current and possesses all the typical characteristics of the kinetic current, the equation derived by Koutecky[16,38,39] may be applied to the present problem.

$$\text{Hydrated D. A. A.} \underset{k_2}{\overset{k_1}{\rightleftarrows}} \text{D. A. A.} \underset{\substack{\text{Electrode}\\ \text{reaction}}}{\rightleftarrows} \text{A. A.} \qquad (1')$$

$$k_1 = [i_k/(i_d - i_k)]^2 1.31 K_h/t \qquad (2)$$

where k_1 and k_2 are the rate constants of the dehydration and hydration, respectively, and i_d and i_k are the expected diffusion current and actual kinetic current, respectively. K_h is the hydration constant and equal to k_2/k_1 and t, the drop time. If the diffusion constant of D. A. A. is assumed to be approximately equal to that of A. A., i_d may be calculated with the latter which is readily known from the anodic diffusion current of A. A. However, in order to obtain k_1 and k_2 from the kinetic current of D. A. A. by using the above equation, the hydration constant K_h should be known. It seems scarcely possible to obtain its reliable value even by the spectrophotometric method as it was applied by Bieber and Trümpler[32] to measure the hydration constant of formaldehyde, since the equilibrium between D. A. A. and its hydrated form seems much shifted toward the hydrated form and also D. A. A. is unstable in an aqueous solution.

Reversibility of the A. A. and D. A. A. System :— Aside from the question how the problem is related to the biological meaning, it must have some value to consider the reversibility of the system from the electrochemical standpoint.

Compared with the fact that an impression as if the reduction wave of D. A. A. were in any way unobtainable has been hitherto maintained among polarographists, the results of the present investigations may be said to have ascertained at least that D. A. A. can be reduced to A. A. at the dropping mercury electrode at a relatively positive potential. But it does not mean that the present data would directly give an evidence of the reversibility of the system in the sense of thermodynamics.

In order to consider the reversibility of the system, both the reduction wave of D. A. A. and the oxidation wave of A. A. should be studied not only with their limiting current but also with the whole current-voltage curve of the system.

Concerning the problem of this kind, after the previous paper[17] had been published by the present authors, Koutecky[16] reported his mathematically rigorous studies on the system which may coincide with the present one (1) in the case where the latter electrode reaction itself is reversible. He also discussed the character of both the reduction wave of D. A. A. and the oxidation wave of A. A. as an application of his theory.

According to Koutecky, if his theory is valid for the case of the A. A. and D. A. A. system, the height of the reduction wave should take the value to satisfy the relation

$$i_0/i_\infty = 10^{-6.9} \qquad (3)$$

where i_0 and i_∞ correspond to the limiting currents of D. A. A. and A. A. of the same concentration, respectively. The above relation may be derived by substituting the experimentally obtained potential shift of the $\pi_{1/2}$ of the oxidation wave from the π_0 of the system, into his equation; the potential shift is usually observed to be about 200 mV. Also the half-wave potential of the reduction wave should be identical with that of the oxidation wave.

In spite of his above conclusion, the discrepancies between the present experimental results and the above theoretically deduced value are quite large; the actual reduction wave is greater by the order of 10^4 and its half-wave potential is about 0.5 V. more negative than that theoretically expected.

Therefore Koutecky's theory may not be said to be applicable to the present problem as he already pointed out. Nevertheless, it seems also interesting to consider the causes from which the above disagreements should arise. In this respect, the assumption of the reversibility of the electrode reaction should be first reconsidered.

If the electrode reaction itself is more or less irreversible, the above phenomena will be more readily understood, though their quantitative analysis may hardly be possible. Also the fact that many investigators remarked the slow establishment of the equilibrium potential of the A. A.

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38) J. Koutecky and R. Brdicka, *Coll. Czech. Chem. Communs.*, **12**, 337 (1947).

39) J. Koutecky, ibid., **18**, 597 (1953).

and D. A. A. system⁽40⁾, when they investigated it potentiometrically, may remind us of the possibility of the polarographically irreversible electrode reaction.

Consequently the reversible character of the oxidation wave should be reinvestigated. Even the experimental results of the half-wave potential dependence on drop time of mercury obtained by Kern⁽15⁾ do not seem to be in good agreement with his own theory in which he also derived an equation for the oxidation wave which differs from Koutecky's only in the numerical factors.

Summary

L-Ascorbic acid (A. A.) shows an oxidation wave at pH less than 8 and two in a more alkaline range. After standing of A. A. in alkaline solution, the third oxidation wave has been observed.

Dehydro-L-ascorbic acid (D. A. A.) has been also tested in alkaline solution. The mechanism of the electrode oxidation of A. A. and its decomposition in alkaline solution are discussed. The reduction wave of D. A. A. has been obtained at pH 2 to 5, in which the half-wave potential is about −0.3 to −0.5 V. (vs. N. C. E.).

The wave height is about 1/1000 of its expected diffusion current at room temperature. The wave has all the typical characteristics of the kinetic current. The wave height is roughly proportional to the concentration and is not appreciably influenced by pH and the components of the buffer solution, but the shape of the wave is strongly deformed by adding small amounts of surface active substance such as gelatin.

The reduction product of D. A. A. at the dropping mercury electrode has been polarographically proved to be A. A.

Discussions are made on the configuration of the inactive form of D. A. A. and the reversibility of the A. A. and D. A. A. system.

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40) See for example E. G. Ball's report⁽⁴⁾.