CXXXVII. THE POTENTIALS OF ASCORBIC ACID.

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The remarkable reducing properties of ascorbic acid (vitamin C) suggest the possibility that it may function as a reversible oxidation-reduction catalyst in cellular respiration. The reduction potentials have therefore been subjected to experimental analysis.

Szent-Györgyi [1928], who first isolated and identified ascorbic acid, observed that solutions of the acid exerted negative potentials at a gold electrode. Georgescu [1932] titrated ascorbic acid with ferricyanide and obtained the typical S-shaped curve for reversible systems. However, little stress can be laid upon this work in that the author failed to present evidence of (1) the purity of his ascorbic acid preparation, (2) the suitability of his apparatus of unique design and principle for potentiometric measurements, and (3) the reproducibility of the observed potentials. Karrer, Schwarzenbach and Schöpp [1933], working with chemically pure ascorbic acid, reinvestigated the reduction potentials. They failed to obtain an S-shaped titration curve. They concluded that the oxidised form of ascorbic acid did not affect the electrode. They also studied the variation of the potential with change in p_H. Unfortunately the scantiness of experimental details included in their paper does not permit a critical evaluation of the accuracy of their measurements. Their potentials were much more positive than those obtained by the present author in some preliminary experiments. The indications are strong that all traces of oxygen were not completely removed from their titrating vessel.

When this paper was being submitted for publication, a note appeared by Laki [1933] dealing with the potentials of ascorbic acid. He confirmed the inactivity of oxidised acid at the electrode and noted further the variation of potential with change in the concentration of reduced ascorbic acid. Here again, the omission of details of experimental technique makes it difficult to analyse his results. No mention is made whether the observed potential differences refer to the normal hydrogen electrode or to the reference calomel electrode.

Methods.

The gases were freed from all traces of oxygen by being passed through a combustion tube 30 cm. in length, heated electrically to 500–600° and tightly packed with palladised copper turnings. The gases were conducted to the titration vessel through coils of thin, flexible copper tubing. Glass-copper contacts were made with pressure rubber tubing, protected with a mercury seal (see Fig. 1).

Titration cell, reference electrode and a test-tube were all mounted firmly on a board which could be raised or lowered by a pulley arrangement. The cell consisted of a vessel of 50 cc. capacity with a rubber bung to fit tightly. Seven holes in the bung permitted a gas inlet and outlet, an agar bridge, a micro-

burette and three electrodes, platinised platinum, blank platinum and blank gold respectively. A decinormal calomel electrode was used as the reference electrode. It was standardised at frequent intervals against a sodium acetate quinhydrone half-cell.

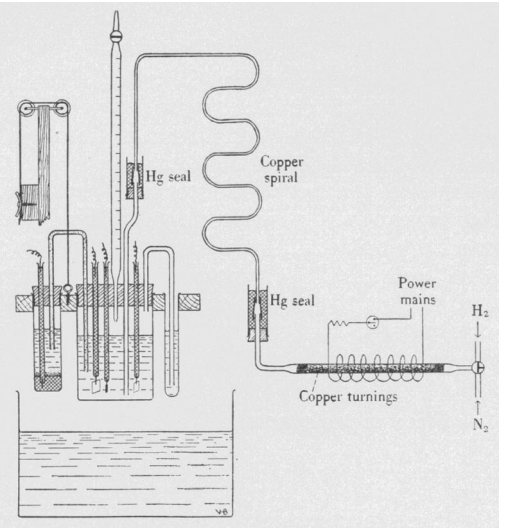

Fig. 1.

The movable board could be lowered into an oil-bath maintained at 30° ± 0·01° by thermostatic control. A motor-driven stirrer ensured thorough mixing of the oil.

A student's potentiometer together with a wall galvanometer were used for measuring potentials. The resistance of the galvanometer was 2000 ohms. Readings could be made accurately to 0·2 mv.

The following procedure was employed in a titration experiment. The oxidising agent was suitably diluted with buffer solution, de-aerated with a stream of pure hydrogen and then sucked up into the burette. 20 cc. of the same buffer were placed in the titration vessel and completely de-aerated. A glass disc with curled edges, bearing a few mg. of ascorbic acid, was dropped into the titration vessel and its contents were thereby spilled into the buffer fluid. The titration vessel and reference electrode were lowered into the oil-bath. With hydrogen bubbling through, the initial p_H was recorded. The gas lead was switched to the nitrogen for the rest of the experiment.

The ascorbic acid was kindly supplied by Dr L. J. Harris. The purity of the preparation was attested by the theoretical figures for iodine titration, neutralisation equivalent and carbon-hydrogen analysis.

Experimental.

Potential drifts.

At least 2 hours were required for the potential to reach a steady value after the gas lead was switched from the hydrogen to the nitrogen. The potentials were very weakly maintained and were easily upset by drawing current for a few seconds. The blank gold and platinum electrodes agreed to 1 mv. when the

equilibrium value was reached. Addition of oxidising agent caused an immediate shift of several hundred mv. to more positive potentials. In about 1 hour's time, the potentials returned to the original level. The criterion for equilibrium was the constancy of the potentials to a few mv. over a period of 30 minutes.

Titration curve.

A 0·001 *M* solution of ascorbic acid was titrated with a 0·002 *N* solution of potassium ferricyanide at p_H 7·08. The completely reduced solution gave an E_h of − 0·054 v. Addition of oxidising agent resulted in no change of the potential provided equilibrium was allowed to be reached. When about 95 % of the ascorbic acid was oxidised the potential rose to − 0·020 v.; further addition caused the potential to reach the positive range of the oxidant. The same type of result was obtained at p_H 3, 4, 5, 6 and 8, using a variety of oxidising agents, *viz.* phenolindophenol, quinone, 2:6-dichlorophenolindophenol and *o*-cresolindophenol. It seemed clear that the potentials of ascorbic acid solutions depended uniquely upon the reductant. The oxidant apparently was not in equilibrium with the reductant at the electrode surface.

Effect of the concentration of reductant.

From the individual titration experiment it also followed that the potential of ascorbic acid solutions did not depend upon the absolute concentration of reductant, for if that were so there should have been a gradual falling off of the potential with each addition of oxidising agent. To check this point the following experiment was performed. A 0·001 *M* solution of ascorbic acid at p_H 3·80 recorded an E_h of + 0·163 v. Enough ascorbic acid was added to make the solution 0·01 *M*. The potential remained at exactly the same value when equilibrium was reached.

Reproducibility of potentials.

For a given p_H, the potentials reached in different experiments agreed to within 5 mv. The drifting nature and ease of polarisability of the potentials made it exceedingly difficult to attain greater accuracy.

Variation of potential with p_H.

In Table I are recorded the E_h values for different degrees of acidity and alkalinity. These values represent the means of several experiments. Below p_H 3 and above p_H 8, the variation of potential with p_H is not linear. Within

Table I.

0·001 *M* ascorbic acid at 30°.

p_H	E_h in volts	E_0 in volts	r_H
8·54	−0·122	—	—
7·45	−0·067	+0·380	12·7
7·08	−0·054	+0·371	12·4
6·66	−0·022	+0·378	12·6
5·96	+0·013	+0·371	12·4
4·30	+0·116	+0·374	12·5
3·74	+0·153	+0·377	12·5
2·74	+0·308	—	—
	Mean	E_0 +0·375	r_H 12·5

this restricted range, however, the following equation approximately expresses the relation of the two variables at 30°.

$$E_h = + 0{\cdot}375 - 0{\cdot}060\, p_H.$$

Colorimetric results.

From the potential measurements, it follows that all the indicators of the Clark series down to indigo tetrasulphonate should be reduced by ascorbic acid solutions. The experiment was carried out in Thunberg tubes. To insure the complete removal of all traces of oxygen the tubes were exhausted and filled with purified nitrogen. This process was repeated three times. In Table II are recorded the results for the reduction of 0·0001 *M* solutions of Clark indicators by 0·01 *M* solutions of ascorbic acid at p_H 7·0 and 30°.

Table II.

0·01 *M* ascorbic acid at 30° and p_H 7·0.

Indicator	E_0 at p_H 7·0 in volts	Result
(1) 2: 6-Dibromophenolindophenol	+0·217	Completely reduced
(2) Cresyl blue	+0·032	,,
(3) Methylene blue	+0·011	,,
(4) Indigo tetrasulphonate	−0·046	,,
(5) Indigo trisulphonate	−0·081	50 % reduced
(6) Indigo carmine	−0·125	No reduction
(7) Nile blue	−0·142	,,
(8) Cresyl violet	−0·167	,,
(9) Janus green (pink to colourless)	−0·258	,,
Colorimetric r_H 11·3.	Potentiometric r_H 12·5.	

It is indeed very surprising to note that the colorimetric potential is about 40 mv. more negative than the potentiometric value. This discrepancy between the results of the two methods is not unusual for irreversible systems, like glutathione for example. From the potential measurements, indigo tetrasulphonate should be half reduced and indigo trisulphonate not reduced at all. Actually indigo tetrasulphonate was completely reduced and indigo trisulphonate reduced to the extent of 50 %.

Potentials of lemon juice.

The relatively high concentration of ascorbic acid in lemon juice and the relatively low concentration of interfering substances such as phenols, sugars, *etc.*, permit the study of ascorbic acid in a natural biological medium. 5 cc. decitrated lemon juice were added to 15 cc. of *M*/5 phosphate buffer. The initial p_H was 5·96. From the data of Table I it followed that the E_h should be + 0·013 v. ± 0·003. Actually the equilibrium potential was + 0·010 v.

Discussion.

It is becoming abundantly clear that the reducing substances of biological importance rarely answer the description of reversible systems. Cysteine and glutathione were shown by Dixon and Quastel [1923] to form irreversible systems. The haemin compounds investigated by Conant and his co-workers [1925; 1928] likewise exhibit anomalous behaviour and are in no way comparable with systems of the type methylene blue-methylene white. Whether the so-called irreversible systems may lend themselves to precise formulation

is still uncertain. At any rate, the classic equations do not permit any extension to these cases.

A rather interesting biological problem develops from the reducing properties of ascorbic acid. The colorimetric studies presented in this paper show that ascorbic acid can reduce indicators which lie below the aerobic reducing level of cells [approximately r_H 14, see Chambers, Beck and Green, 1933]. Yet ascorbic acid is always found in the reduced state in tissues and plant fluids. Further, ascorbic acid is very rapidly oxidised by atmospheric oxygen in an irreversible way. Its persistence in the reduced state proves very puzzling indeed. Three possibilities present themselves: (1) there is present some stabiliser which cuts down the autoxidation of ascorbic acid to a negligible rate; (2) some dehydrogenase system can reduce oxidised ascorbie acid more rapidly than reduced ascorbic acid can autoxidise; and (3) the production of ascorbic acid goes on continuously at a rate comparable with the rate of irreversible oxidation. There is some evidence that the first explanation is correct.

A comparison of the potentials of glutathione and ascorbic acid brings out interesting differences in the type of irreversibility. The potentials of glutathione, like those of ascorbic acid, do not depend upon the concentration of oxidant. Addition of oxidant to a solution of reductant is without effect on the final potential. However, the potentials of glutathione, unlike those of ascorbic acid, depend upon the absolute concentration of reductant. A tenfold increase in concentration makes the potential more negative by 60 mv. for a constant p_H value. In the case of ascorbic acid, the potentials are independent of the absolute concentration of reductant. Lastly, the colorimetric measure of glutathione potentials is more positive than the potentiometric measure. The reverse is true for ascorbic acid.

Summary.

The potentials of ascorbic acid have been shown to be drifting and easily polarisable. The potential for a given p_H is a function only of the reductant and not of the oxidant. The molar strength of reductant is likewise not a determining factor. The same potential for a given p_H is reached regardless of the absolute concentration of reductant. The variation of E_h with p_H has been studied, and an empirical equation assigned to express approximately the relation of the variables between p_H 3 and 8. The presence of ascorbic acid in lemon juice was confirmed by potential measurements.

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