[DEPARTMENT OF CHEMISTRY, HARVARD UNIVERSITY]

The Polarographic Oxidation Potential of Ascorbic Acid

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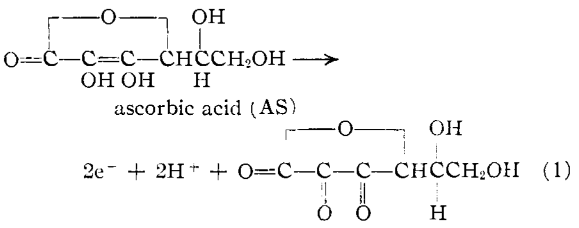
The ascorbic acid oxidation wave is interpreted on the basis of a mechanism involving a reversible electrode reaction followed by the irreversible conversion of an unstable intermediate to stable dehydroascorbic acid. The theoretical relationship between current, potential, conversion rate and drop time is rigorously derived, and found to agree satisfactorily both with earlier approximations and with experimental data. The data do not support a mechanism in which the electron transfer is the rate-determining step.

Ascorbic acid yields a well defined oxidation wave at the dropping mercury electrode (d.m.e.), corresponding to the reaction[1]



$$\mathrm{O{=}C{-}\underset{OH}{C}{=}\underset{OH}{C}{-}CH\underset{H}{\overset{OH}{C}}CH_2OH \longrightarrow}$$

ascorbic acid (AS)

$$\mathrm{2e^- + 2H^+ + O{=}C{-}\underset{O}{\underset{\|}{C}}{-}\underset{O}{\underset{\|}{C}}{-}CH\underset{H}{\overset{OH}{C}}CH_2OH} \quad (1)$$

dehydroascorbic acid (DA)

The pH dependence of the half-wave potential ($E_{1/2}$) is similar to that observed potentiometrically in mixtures of the two compounds.[2] However, the polarographic potentials are several tenths of a volt more positive than the equilibrium values.[3-6] This fact, together with the non-reducibility of dehydroascorbic acid at the d.m.e., indicates an irreversible electrode process. Cattaneo accounted for the pH dependence by suggesting that the irreversible oxidation is preceded by the reversible equilibrium

$$\mathrm{C_6H_8O_6 \rightleftharpoons 2H^+ + C_6H_6O_6^{=}}$$

On the other hand, in an oscillographic study of the analog dioxymaleic acid, Wiesner[7] found evidence for a reversible oxidation. If the potential of the drop was alternated at sufficiently high frequencies, reversible reduction of a transitory oxidation product occurred. Unequivocal evidence for the same phenomenon for ascorbic acid could not be obtained, suggesting that the half-life of the transitory product is extremely short. Vavřin showed that such a product in equilibrium with ascorbic acid at the electrode surface, as in the equations

$$\mathrm{AS \rightleftharpoons 2e^- + 2H^+ + DA^*\ (transitory)} \qquad (2)$$
$$\mathrm{DA^* \xrightarrow{k} DA}$$

could account for the observed pH dependence of $E_{1/2}$, and also the shift to potentials more positive than the potentiometric value.

Lately several other ene-diols have been shown to exhibit the same relationship between the potentiometric and polarographic pH–potential curves.[5,6] The rapid reaction in which the primary oxidation products of these compounds engage is most likely a hydration of the carbonyl groups.[6] Thus equation 1 for ascorbic acid actually represents only the primary electrode process.

In a recent article[8] it was suggested that on the basis of mechanism (2) the rate constant k sec.$^{-1}$ for the disappearance of the transitory state could be related to the drop time t_1 and to $E_{1/2}$ by the equation

$$E_{1/2} = E_0 - \frac{RT}{2F} \ln 0.81\sqrt{\frac{D_{\mathrm{DA}}}{D_{\mathrm{AS}}}} - \frac{RT}{4F} \ln k - \frac{RT}{4F} \ln t_1 \quad (3)$$

in which D_{DA} and D_{AS} are the diffusion coefficients of DA* and ascorbic acid, and E_0 is the formal potential of the DA*, AS couple at the prevailing pH. Since both $E_{1/2}$ and t_1 are easily measured, it was pointed out that mechanism (2) could be tested by experiment.

Equation 3 was arrived at by a non-rigorous analogy with kinetic and catalytic currents. The argument rested on general considerations concerning the applicability of the reaction volume or heterogeneous rate constant method to the interpretation of kinetic currents. In this article the relationship between $E_{1/2}$, t_1 and k will be derived rigorously, and compared with the earlier expression and with experimental data.

Theoretical

In the following section the concentration of the depolarizer will be denoted A, and that of the transitory product B. The treatment here is for an oxidation process, but the final result will hold for reduction also after a simple reversal of current and potential axes.

It is assumed that the mechanism of the reaction is that of equation 2, a rapid reversible electrode reaction followed by a conversion rate k which is slow compared with the rate of the electrode process.

Under these conditions a reaction rate term must be introduced into the usual diffusion equations that describe the flow of material in the neighborhood of the drop, and the potential must appear in the boundary conditions, since we are considering the rising part of the curve in which the current is potential dependent.

These requirements are fulfilled by the following equations, which hold for a plane electrode of unit area

$$\frac{\partial A}{\partial t} = D_{\mathrm{A}} \frac{\partial^2 A}{\partial x^2} \qquad (4)$$

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(1) I. M. Kolthoff and J. J. Lingane, "Polarography," 2nd ed., Vol. II, Interscience Publishers, Inc., New York, N. Y., 1952, p. 727.

(2) E. G. Ball, *J. Biol. Chem.*, **108**, 219 (1937).

(3) C. Cattaneo and G. Sartori, *Gazz. chim. ital.*, **72**, 351 (1942).

(4) Z. Vavřin, *Collection Czechoslov. Chem. Communs.*, **14**, 367 (1949).

(5) F. Šantavý and B. Bitter, *ibid.*, **15**, 112 (1950).

(6) R. Brdička and P. Zuman, *ibid.*, **15**, 766 (1950).

(7) K. Wiesner, *Chem. Listy*, **38**, 91 (1944), and private communications.

(8) D. M. Kern, THIS JOURNAL, **75**, 2473 (1953).

$$\frac{\partial B}{\partial t} = D_{\mathrm{B}}\frac{\partial^2 B}{\partial x^2} - kB \qquad (5)$$

with the boundary conditions: at $x = \infty$, $A = a$ and $B = 0$; at $t = 0$, $A = a$ and $B = 0$; and at $x = 0$ $(t > 0)$, $A/B = c$ (const.) and $-D_{\mathrm{B}}(\partial B/\partial x) = D_{\mathrm{A}}(\partial A/\partial x)$. The bulk concentration of A is given by a. The constant c is related to the applied potential E by the equation

$$E = E_0 - \frac{RT}{nF}\ln c \qquad (6)$$

If D_{A} and D_{B} can be assumed equal, the solution is greatly simplified. Application of the Laplace transformation leads to the expression

$$L\left(\frac{\partial A}{\partial x}\right)_{x=0} = \frac{a}{\sqrt{D}}\,\frac{1}{\sqrt{p}}\,\frac{\sqrt{p+k}}{c\sqrt{p}+\sqrt{p+k}}$$

The term on the right can be separated and, after passing through the inverse transformation, yields the result

$$\frac{\sqrt{D}}{a}\left(\frac{\partial A}{\partial x}\right)_{x=0} = \frac{c^2\sqrt{k}}{(c^2-1)^{3/2}}\exp\left(\frac{kt}{c^2-1}\right)\left[\operatorname{erf}\sqrt{\frac{kc^2t}{c^2-1}} - \operatorname{erf}\sqrt{\frac{kt}{c^2-1}}\right] + \frac{ce^{-kt}-1}{(c^2-1)\sqrt{\pi t}} \qquad (7)$$

The instantaneous current i is directly proportional to $(\partial A/\partial x)_{x=0}$.

This form of the solution holds for $c > 1$, which is the only case of interest, since the effect of k is to drive $E_{1/2}$ to more negative potentials than E_0 (*i.e.*, into regions where $c > 1$).

Since the shape of the curve and the position of $E_{1/2}$ are the important features to be investigated, the significant function is the ratio i/i_{d}, i_{d} being the maximum current of the plateau region, in which A is essentially zero at the electrode surface. For a plane electrode[9]

$$\frac{\sqrt{D}}{a}\left(\frac{\partial A}{\partial x}\right)^{\max}_{x=0} = (\pi t)^{-1/2}$$

According to equation 3 $E_{1/2}$ should move to more negative potentials as k increases; therefore if k increases without limit, the plateau will be extended to all values of c. In agreement with this result, equation 7 approaches $(\pi t)^{-1/2}$ as $k \to \infty$.

The expression for the current ratio as a function of c, k and t is much simplified if we apply the restrictions $k > 7$ and $c > 10$, in other words, if we consider only those cases in which $E_{1/2}$ is shifted more than 0.03 v. from E^0 (it can be shown that k is automatically greater than 7 under these circumstances, at normal drop times).

Then the current ratio can be written

$$i/i_{\mathrm{d}} = \sqrt{\pi}\beta e^{\beta^2}(1 - \operatorname{erf}\beta),\ \beta = \frac{\sqrt{kt}}{c} \qquad (8)$$

Each point on the wave has its corresponding value of β. If k or t is changed, an adjustment in c (*i.e.*, a shift along the potential axis) will return all the ratios to their original value; the shape of the wave is not distorted in the process. When $i/i_{\mathrm{d}} = 0.5$, $\beta = 0.432$. Thus at $E_{1/2}$, $\sqrt{kt} = 0.432c_{1/2}$ and combining with equation 6

$$E_{1/2} = E_0 - \frac{RT}{2nF}\ln k - \frac{RT}{2nF}\ln t + \frac{RT}{nF}\ln 0.432 \qquad (9)$$

The result is not altered by transposing to the d.m.e., since according to the normal procedure numerator and denominator of the current ratio are simply multiplied by $8.5 \times 10^{-3} \times \sqrt{7/3} \times m^{2/3}t^{2/3}$.

Equation 8 refers to the ratio of instantaneous currents. However, it is the average currents that are measured at the d.m.e. For comparison with experimental data, the following equation is more appropriate

$$\bar{\imath}/\bar{\imath}_{\mathrm{d}} = \frac{\dfrac{\sqrt{\pi k}}{t_1 c}\displaystyle\int_0^{t_1} t^{2/3}e^{\beta^2}(1 - \operatorname{erf}\beta)\,\mathrm{d}t}{6/7t_1^{1/6}} \qquad (10)$$

The time dependent expression for the electrode area has been retained in this equation, and numerator and denominator integrated over the drop time t_1 to give the average currents.

This equation shows the average ratio to be a function of $\sqrt{k}/c$, *i.e.*, as k varies the wave is again simply shifted along the E axis, without changing its shape. The dependence on t_1 is not immediately apparent. The expression has therefore been integrated numerically in the region $0.1 < \sqrt{k}/c < 1$, which for normal drop times coincides with the rising portion of the wave. The curves of $\bar{\imath}/\bar{\imath}_{\mathrm{d}}$ *vs.* c have been plotted for $t_1 = 1.5$, 3 and 6 sec. in Fig. 1, where they are compared with the curves for a reversible wave and for the ratio of instantaneous currents from equation 8.

It is evident that equations 8 and 10 give very similarly shaped waves. The shift of $E_{1/2}$ with t_1 is indistinguishable from the shift with t derived analytically from equation 8 and incorporated in equation 9, within the limits of precision for measuring $E_{1/2}$ experimentally. The effect of measuring $\bar{\imath}$ instead of i is to move the wave to slightly more positive potentials. The dependence of $E_{1/2}$ on k and t_1 is essentially the same as on k and t, and is given by

$$E_{1/2} = E_0 - \frac{RT}{2nF}\ln kt_1 + \frac{RT}{nF}\ln 0.65 \qquad (11)$$

which at 25° and for a two-electron reaction differs only by the constant term -0.008 v. from equation 3.

Experimental

The $E_{1/2}$ of ascorbic acid oxidation waves was measured in citric–monohydrogen phosphate buffers of *p*H 3.6 and 6.7 as a function of drop time, temperature and gelatin concentration.

Eastman Kodak *l*-ascorbic acid was used without further purification. The gelatin was Baker and Adamson gelatin powder.

The buffers had the following compositions: *p*H 3.58, 0.1 *M* citric and 0.09 *M* phosphate; *p*H 6.67, 0.04 *M* citric and 0.2 *M* phosphate. The *p*H was measured with a glass electrode.

The buffers were used as supporting electrolytes. The addition of the ascorbic acid did not change the buffer concentrations sensibly. After thorough deaeration with nitrogen gas (which may not have been necessary for the more acid buffer),[10] residual currents were measured and the ascorbic acid added to give a 0.5–1 m*M* solution. As aqueous ascorbic acid solutions decompose in a few days, fresh stock solutions were prepared frequently. The acid did not decompose noticeably during current measurements in the polarographic cell. In agreement with Cattaneo[3] ascorbic acid in the more basic buffer was found to give a maximum.

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(9) Reference 1, Vol. 1, p. 24.

(10) W. S. Gilliam, *Ind. Eng. Chem., Anal. Ed.*, **17**, 217 (1945).

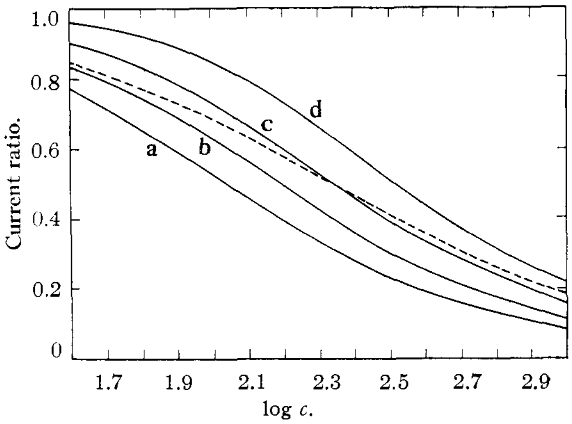

Fig. 1.—Plot of $\bar{i}/\bar{i}_d$ as a function of log c, k = 3.33 × 10^3 sec.$^{-1}$: a, t_1 = 1.5 sec.; b, t_1 = 3.0 sec.; c, t_1 = 6.0 sec.; at $\bar{i}/\bar{i}_d$ = 0.5, ($E_{1/2}$), the shift in log c is 0.150 unit for each doubling of the drop time. Curve d is a plot of the *instantaneous* current ratio when t = 6.0 sec.

It had only a small effect on the limiting current, but caused a marked steepening of the wave in its upper half. 0.0002% methyl red was added to all solutions as a suppressor except where noted. At this low concentration it did not cause a shift in $E_{1/2}$.

Current measurements were made manually with the aid of a Leeds and Northrup type K potentiometer and a Rubicon direct reading galvanometer. The cell was thermostated to ±0.1°. Except where otherwise noted, measurements were at 25°.

A saturated calomel electrode was used as an external anode. Resistance measurements were made with a Serfass conductivity bridge, and IR corrections made at all potentials. Since R varied from 1000 to 2000 Ω, the correction was considerable, but occasional checks with an internal anode and an external reference electrode showed excellent agreement. The latter set-up was not convenient because of rapid drifting of the anode potential.

Shape of the Experimental Curves.—The $E_{1/2}$ was determined with the aid of conventional E *vs.* log $(\bar{i}_d - \bar{i})/\bar{i}$ plots. These were very straight, and in the solution of pH 3.6 had precisely the reversible slope RT/nF, reflecting accurately the dependence of the slope on T. The slopes in the solution of pH 6.7 tended to be a few per cent. high, owing perhaps to incomplete suppression of the maximum, but still fairly straight. In order to determine to what extent theory predicts a straight slope, E *vs.* log $(\bar{i}_d - \bar{i})/\bar{i}$ curves were plotted for the curves of Fig. 1, and are reproduced in Fig. 2. While closely approximating straight lines, the curves evidently do not have the reversible slope and in this respect differ from the experimental results. It is interesting to note that the elementary theory given by Vavřin[4] and Kern[8] predicts a reversible slope.

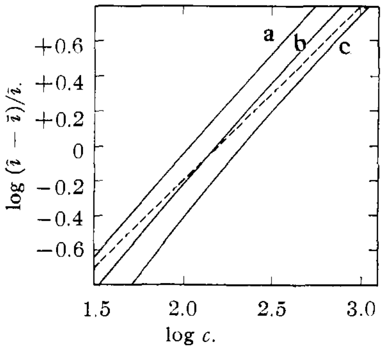

Fig. 2.—log c *vs.* log $(\bar{i}_d - \bar{i})/\bar{i}$ for curves a, b and c of Fig. 1. The dotted line represents a reversible wave.

Relation between $E_{1/2}$ and pH.—The pH dependence of $E_{1/2}$ has been measured by several workers.[3-6] They are in good agreement except that Cattaneo's values are consistently about 20 mv. more negative. The $E_{1/2}$ for the two pH's used in this paper agreed with Cattaneo. However, as pointed out by Brdička and Zuman, the disagreement among the different workers is not significant.

An interesting observation was made during this work concerning the large influence of junction potentials on the value of $E_{1/2}$. Agar salt bridges containing 2 M KNO_3 were used in order to avoid contamination of the cell solution with chloride. When these bridges were replaced by bridges containing cell solutions in one half and saturated KCl in the other, the potential corresponding to a given point in the wave was found to have shifted −5.5 mv., regardless of pH or temperature. Thus the junction potentials of the systems cell solution ‖ KNO_3 ‖ KCl sat. and cell solution ‖KCl sat. differed by 5.5 mv. Since the latter system is the one normally used, and is supposed to reduce the junction to a minimum, the potentials reported in this paper should be changed by −5.5 mv. if absolute values are desired. This correction has been applied to the potentials given in Table I.

Relation between $E_{1/2}$ and t_1.—Careful $E_{1/2}$ measurements were made in the acid buffer at three temperatures and three drop times. The results are presented graphically in Fig. 3. In view of the small changes in potential involved, the observed relation between t_1 and $E_{1/2}$ is in excellent

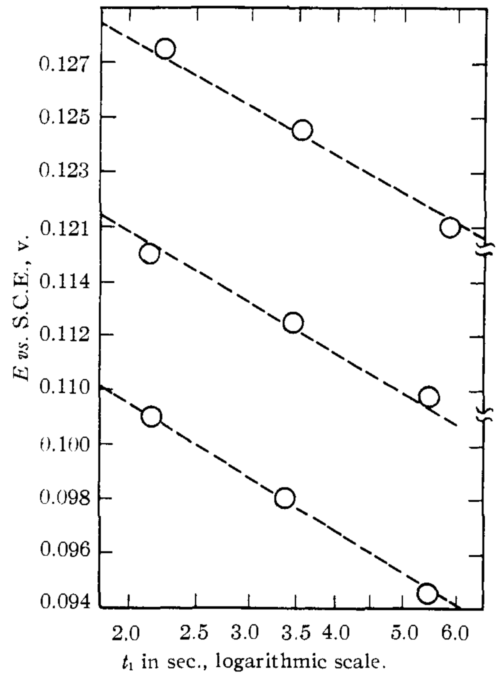

Fig. 3.—$E_{1/2}$ as a function of t_1 at various temperatures. The dotted lines have the theoretical slopes. Top curve, at 40°: center curve, 25°; bottom curve, 10°.

agreement with that predicted by equations 3 and 11.

A mechanism involving an ordinary irreversible oxidation with the electron as the rate determining step would also predict a negative shift of $E_{1/2}$ with increasing t_1. Kivalo, *et al.*,[11] recently have published an equation for the time shift of an irreversible wave which at 25° and $n = 2$ reduces to $\Delta(-E_{1/2}) = 14.8\ \alpha^{-1}\Delta\log t_1$ mv. Since α generally = *ca.* 0.5, the calculated shift becomes 29.6 $\Delta\log t_1$ mv. The observed shifts corresponding to a change in t_1 from 2.15 to 3.5 and 3.5 to 5.5 sec. were 2.7 and 2.5 mv., respectively. The shifts of an irreversible wave according to the above equation would be 6.3 and 5.8. Those calculated from equation 9 would be 3.1 and 2.9. It is apparent that the agreement of experiment with equation 9 is much better. Even better agreement was found at the two other temperatures.

Relation between $E_{1/2}$ and the Gelatin Concentration.—In the process of choosing a maximum suppressor, the effect of gelatin on $E_{1/2}$ was studied in both buffers in the absence of methyl red. There was no maximum at *p*H 3.6, but at *p*H 6.6, 0.005% gelatin was required to give a symmetrical curve, a concentration which caused a shift of about 6 mv. in the positive direction and a 6% decrease in the diffusion current. Part of this decrease was probably caused by the elimination of the maximum, which was low and broad and not readily recognizable. By plotting i_d against the gelatin concentration and extrapolating to zero gelatin (the points for concentrations less than 0.005% being disregarded), the "true" i_d could be estimated. On this basis 0.005% gelatin caused only a 2% decrease in the true diffusion current.

A plot of $E_{1/2}$ against the gelatin concentration for both buffers at 25° is given in Fig. 4. To what extent the larger shift in the neutral solution was due to the more negative charge on the gelatin and its greater adsorbability on the positively charged mercury surface was not investigated. One observation at *p*H 6.6 at 40° showed an over threefold greater shift than at 25°.

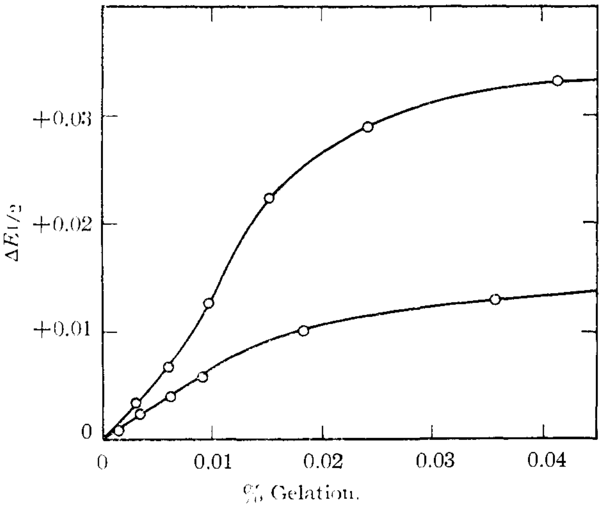

Fig. 4.—$E_{1/2}$ as a function of % gelation at 25°: upper curve, *p*H 6.7; lower curve, *p*H 3.6, t_1 = 3.5 sec.

Even at the highest gelatin concentrations there was no perceptible distortion of the wave.

If the shift in $E_{1/2}$ is interpreted as the result of a change in k at the mercury surface, it follows that there must be a roughly exponential decrease in k with increasing gelatin concentration. It is likely, however, that the reversibility of the DA*, AS couple is also affected.

Relation between $E_{1/2}$ and the Ascorbic Acid Concentration.—Measurements in the buffer of *p*H 3.6 showed a very slight shift to more positive potentials with increasing concentration, amounting to about 1.5 mv. in a tenfold concentration change. Such a shift is not predicted by theory, but is too small to be considered a serious inconsistency.

Relation between $E_{1/2}$ and Temperature.—The data in Fig. 3 are not a reliable measure of the temperature effect, since different calomel cells were used at the various temperatures. A special set of experiments, with a carefully prepared calomel cell which was allowed to come to equilibrium for several days at each temperature before use, yielded the data in Table I.

TABLE I

$E_{1/2}$ AS A FUNCTION OF T; t_1 = 3.5 SEC.

*p*H	10.2°	25°	40°
3.58	0.1276 v.	0.1125 v.	0.0963 v.
6.67	−0.0110 v.	−0.0335 v.	−0.0544 v.

According to the absolute rate theory

$$k = \frac{\mathbf{k}T}{h}\exp\frac{(-\Delta H^{\mp} + T\Delta S^{\mp})}{RT} \qquad (12)$$

where **k** and h are the Boltzmann and Planck constants, and $\Delta H^{\mp}$ and $\Delta S^{\mp}$ are the heat and entropy of activation.

Combination of equations 12 and 11 leads to the equation

$$E_{1/2} = E_0 + \frac{\Delta H^{\mp} - T\Delta S^{\mp}}{2nF} - \frac{RT}{2nF}\ln\frac{0.423\ \mathbf{k}t_1}{h} - \frac{RT}{2nF}\ln T \qquad (13)$$

which on differentiation with respect to T becomes

$$\frac{\mathrm{d}E_{1/2}}{\mathrm{d}T} = \frac{\mathrm{d}E_0}{\mathrm{d}T} - \frac{\Delta S^{\mp}}{2nF} - \frac{R}{2nF}\left[\ln\frac{0.423\ \mathbf{k}t_1T}{h} + 1\right] \qquad (14)$$

At 25° and for t_1 = 3.5 sec. the last term equals 0.66 mv.

If equation 11 is correct, it leads to the peculiar result that the heat of activation of k does not affect the temperature dependence of $E_{1/2}$, though $E_{1/2}$ is dependent on k.

Although several unknown entropies are involved, it is worthwhile to determine whether the observed temperature dependence is consistent with equation 11 and the absolute rate theory.

For the half-cell

$$2e^- + \mathrm{DA}^* + 2\mathrm{H}^+\,(p\mathrm{H}\ 3.58) = \mathrm{AS}$$
$$\Delta S = (S_{\mathrm{AS}} - S_{\mathrm{DA}^*}) - 1.99 \times 2 \times 2.3 \times 3.58 - S_{\mathrm{H}_2}$$

The *p*H of a citric buffer is essentially temperature independent.[12] Using Latimer's value[13] for S_{H_2},

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[11] P. Kivalo, K. Oldham and H. Laitinen, THIS JOURNAL, **75**, 4149 (1953).

[12] H. Britton, "Hydrogen Ions," 3rd Ed., Chapman and Hall, Ltd., London, 1942, p. 318.

[13] W. M. Latimer, "Oxidation Potentials," 2nd Ed., Prentice-Hall, Inc., New York, N. Y., 1952, p. 30.

we find

$$\Delta S = \Delta S_A - 64 \text{ cal./deg.}$$

where ΔS_A is the bracketed term above. Thus $dE_0/dT = \Delta S/nF = \Delta S_A/nF - 1.39$ mv./degree. The reference cell (S.C.E.) itself has a temperature coefficient of −0.76 mv./degree. Hence, with S.C.E. as the reference electrode

$$\frac{E_{1/2}}{dT} = \frac{\Delta S_A}{nF} - \frac{\Delta S^\ddagger}{2nF} - 0.63 \text{ mv.} - 0.66 \text{ mv.}$$

$$= \frac{1}{nF}\left[\Delta S_A - {}^1/_2\Delta S^\ddagger\right] - 1.29 \text{ mv.} \qquad (15)$$

The observed temperature coefficient of $E_{1/2}$ is −1.03 mv. This leaves for the bracketed term a value of *ca.* 12 e.u., a small value considering the size of the molecules involved, and in keeping with the small difference in entropy between hydrogenated and olefinic hydrocarbons, and the small entropy of activation to be expected for the simple transition (probably involving a hydration) to stable DA.

The coefficient at *p*H 6.67 differs from that at *p*H 3.58 because of the more negative S_{H^+} term and also because the active hydrogen atom in the center of the molecule is dissociated at this *p*H,[2] so that ΔS must also include the entropy of dissociation. The first effect adds an additional −0.62 mv. to equation 15, the second a small positive increment, in view of the small positive entropy of dissociation of most acids. The observed increment in $dE_{1/2}/dT$ is −0.4 mv.

It should be noted that the difference in temperature coefficient at the two acidities cannot be accounted for by a mechanism in which an irreversible oxidation is the rate-determining step. To explain the observed relation between *p*H and $E_{1/2}$ the hydrogen ions must be supposed to take part in a reversible equilibrium preceding the rate determining step. Thus the hydrogen ions are not involved in the formation of the activated complex, and cannot affect the activation energy, which would be the same at both acidities.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

On the Mode of Interaction of Surface Active Cations with Ovalbumin and Bovine Plasma Albumin[1]

BY JOSEPH F. FOSTER AND JEN TSI YANG

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The combination of a cationic detergent, dodecyldimethylbenzylammonium chloride, with ovalbumin (O) and bovine plasma albumin (A) has been studied by electrophoresis, equilibrium dialysis, viscosity and optical rotation. O reacts in an all-or-none manner accompanied by denaturation of the protein and a consequent increase in viscosity and optical rotation. Determination of the number of ions bound is rendered difficult by the tendency toward gelation and precipitation of a part of the protein. Heat-denatured O, and both A and heat-denatured A, fail to show this all-or-none type of reaction, only a single electrophoretic component being observed. At the *p*H of the experiments (2.5-3) A is denatured, there being a nearly tenfold increase in specific viscosity (at low ionic strength) and a 30% increase in optical rotation even in the absence of the detergent. In view of the absence of streaming birefringence and of the instantaneous character of the changes in viscosity and rotation it is concluded that this denaturation is essentially an isotropic expansion of the molecule.

The combination of proteins with surface active anions has been the object of much study during the past decade. It has been amply demonstrated that in the case of native ovalbumin (O) and horse and bovine serum albumin (A) an all-or-none type of combination occurs, a large number of ions being bound essentially as a unit.[2-4] In the case of heat-denatured proteins, on the other hand, the binding is stepwise[2,4] suggesting that the all-or-none reaction is associated with a configurational change in the protein (denaturation).

Studies with surface active cations are much more limited, in spite of the considerable biochemical interest in such compounds arising out of their use as bactericidal agents. Precipitation of proteins on the alkaline side of their isoelectric points by such compounds has been reported by several workers.[5-8] In a more detailed study of the action of dodecylamine hydrochloride on O, Timasheff and Nord[9] concluded that two stages of binding exist, corresponding to 30 and approximately 100 ions per mole of protein, respectively.

In another study from this Laboratory it has been shown recently that several surface active cations denature O in acid solution.[10] Studies by streaming birefringence and light scattering were in accord with the view that the reaction involves an unfolding of the O molecule to rodlets of length about 600 Å. which then aggregate laterally.

In the present study the nature of the interaction of alkyldimethylbenzylammonium chloride with both O and A is examined in more detail by electrophoresis, equilibrium dialysis, viscosity and optical rotation methods.

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[1] Journal Paper Number J-1787 of the Iowa Agricultural Experiment Station, Ames, Iowa, Proj. 1223. Presented in part before the Division of Biological Chemistry of the American Chemical Society, March, 1953. This work was carried out under contract Nonr-803(00) of the Office of Naval Research.

[2] H. P. Lundgren, D. W. Elam and R. A. O'Connell, *J. Biol. Chem.*, **149**, 183 (1943).

[3] F. W. Putnam and H. Neurath, *ibid.*, **159**, 195 (1945).

[4] J. T. Yang and J. F. Foster, THIS JOURNAL, **75**, 5560 (1953).

[5] R. Kuhn, H. J. Bielig and O. Dann, *Ber.*, **73B**, 1080 (1940).

[6] W. G. Jaffe, *J. Biol. Chem.*, **148**, 185 (1943).

[7] K. H. Schmidt, *Z. physiol. Chem.*, **277**, 117 (1943).

[8] H. N. Glassman and D. M. Molnar, *Arch. Biochem. Biophys.*, **32**, 170 (1951).

[9] S. N. Timasheff and F. F. Nord, *ibid.*, **31**, 309 (1951).

[10] G. F. Hanna and J. F. Foster, *J. Phys. Chem.*, **57**, 614 (1953).