They represent the experimental values of R with an average error of 0.0005 which is less than the experimental error I would assign to R and it therefore follows that Harned's rule is applicable within the experimental error of these measurements.

TABLE IV

α_{12} AND α_{21} FOR CESIUM CHLORIDE–SODIUM CHLORIDE MIXTURES

m	$-\alpha_{12}$	α_{21}	$(\alpha_{12} + \alpha_{21})$
0.5	0.035	0.056	0.021
1.0	.021	.047	.026
1.5	.0123	.0457	.0334
2.0	.0079	.0440	.0361
2.5	.0055	.0432	.0377
3.0	.0048	.0429	.0381
3.5	.0034	.0434	.0400
4.0	.0030	.0436	.0406
5.0	.0028	.0438	.0410
6.0	.0033	.0437	.0404

It has been claimed recently[6] that if Harned's rule does hold, then $(\alpha_{12} + \alpha_{21})$ must be a constant independent of m. It can be seen from Table IV that although $(\alpha_{12} + \alpha_{21})$ does tend toward a constant value of about 0.04 in concentrated solution, there is a marked diminution in the more dilute solution. If $(\alpha_{12} + \alpha_{21})$ is put equal to 0.04 at $m = 0.5$, then the "best" values of α_{12} and α_{21} are -0.0223 and 0.0623, respectively, and these represent the experimental values of R with a mean

(6) E. Glueckauf, H. A. C. McKay and A. R. Mathieson, J. Chem. Soc., S 299 (1949).

deviation of 0.0016 compared with 0.0005 for the values given in Table IV. It must be admitted that measurements at this comparatively low concentration are difficult and it may be surprising to find an agreement with a mean deviation as low as 0.0005 in R. Nevertheless I do not think that the measurements could have been subject to a mean error of as much as 0.0016 in R and I believe, therefore, that these results are not consistent with a constant value of $(\alpha_{12} + \alpha_{21})$. It is also to be noted that this constancy of $(\alpha_{12} + \alpha_{21})$ results from the "cross-differentiation" relation

$$(\partial \ln a_1 / \partial m_2)_{m_1} = (\partial \ln a_2 / \partial m_1)_{m_2}$$

to which any *theoretical* relation in mixed salt solutions must conform. Harned, however, has never claimed that his rule is more than a useful but empirical equation representing certain observations within the limits of experimental error. One has only to add a very small quadratic term to either or both of equations (1) and (2) to destroy the necessity of a constant $(\alpha_{12} + \alpha_{21})$ and yet the contribution of the quadratic term may be well within the experimental error. For this reason I believe that Harned's rule can be used as an empirical equation when supported by experimental data even if $(\alpha_{12} + \alpha_{21})$ is not a constant. In the case of the cesium chloride–sodium chloride salt pair it appears to be valid.

I would like to thank Dr. Henry C. Thomas for his interest in this work and for providing the sample of cesium chloride.

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Irreversible Polarographic Reduction of Simple Metal Ions in the Presence of Acid Serum Albumin

BY CHARLES TANFORD

Shifts in the electrocapillary curve and other evidence suggest that proteins are readily adsorbed on the mercury drop during polarographic measurements. The effect of such adsorption on the reduction of simple metal ions has been studied, using serum albumin at low pH values, where no metal–protein complex formation occurs. It is found that a metal capable of forming a stable neutral or anionic complex with the inert electrolyte (Cd^{++} and Pb^{++} in KCl) gives a polarogram indistinguishable from that obtained in the absence of protein, whereas a metal present largely as uncomplexed cation (Cu^{++}, Cd^{++} and Pb^{++} in KNO_3) produces a highly irreversible wave. This phenomenon is ascribed to electrostatic repulsion between cations and the positively charged adsorbed layer. The irreversible waves were found to obey equations for activation-controlled reduction.

In the polarography of solutions containing serum albumin (in an H-type cell) the mercury drops falling to the bottom of the cathode compartment do not coalesce, presumably because a layer of serum albumin molecules is adsorbed on the drops as they fall through the solution. Since such a layer would be about 30 Å. thick, it prevents the customary formation of a mercury pool. This phenomenon appears to be independent of the pH, *i.e.*, of the charge on the protein molecules.

It can be concluded from this observation that serum albumin will also be adsorbed on the mercury drop during electrolysis, unless prevented by electrostatic repulsion. That this is indeed so is indicated by shifts in the electrocapillary curve caused by the presence of serum albumin.

The purpose of the present paper is to investigate whether this adsorbed layer interferes with the polarographic reduction of simple metal ions.

It has been shown in a previous paper that the combination of metals with serum albumin occurs at the same sites as are involved in combination with hydrogen ion.[1] In acid solutions, therefore, where an excess of hydrogen ions is present, no combination with metal ions was found to occur. It is in such solutions, therefore, that the effects of the adsorbed layer can be studied independently of complex formation.

In acid solutions the serum albumin molecule is positively charged. The full effects of adsorption should therefore be observed at applied potentials

(1) C. Tanford, THIS JOURNAL, 74, 211 (1952).

of −0.6 volt or more (vs. S.C.E.), since at more positive potentials the drop is positively charged with respect to the solution, and electrostatic repulsion between it and the protein would be expected to interfere with adsorption.

Experimental

The experimental details are identical with those in the author's previous paper.[1] The same capillary and drop time were used.

Results

The customary test for a thermodynamically reversible reduction is to plot $\log i/(i_l - i)$ versus the applied potential. This plot, for a reversible reduction, is a straight line of slope, $d(-E)/d \log i/(i_l - i) = 0.059/n$ at $25°$,[2] where E is the applied potential, i the current at this potential, i_l the limiting current and n the number of electrons involved in the reduction process. For the divalent metal ions studied in this work the theoretical value of this slope is therefore 0.030.

For an irreversible reduction a similar plot may also yield a straight line, but its slope will generally be very much greater.[3]

Figure 1 shows logarithmic plots of this kind for the reduction of 4×10^{-4} M lead ion in KCl and KNO$_3$ as supporting electrolyte, both in the absence and presence of serum albumin. The slope, $d(-E)/d \log i/(i_l - i)$ in the absence of protein is 0.034, i.e., somewhat higher than the theoretical value. This has been observed in previous studies with lead, Lingane having found a slope of 0.033.[4]
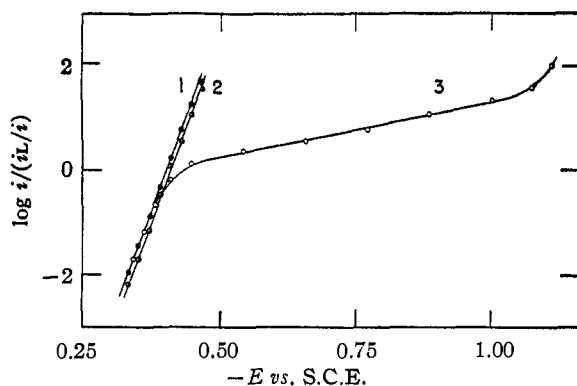

Fig. 1.—Logarithmic plots for the reduction of Pb^{++}: curve 1, in 0.15 M KNO$_3$ at pH 1.80 in the absence of protein; curve 2, in 0.15 M KCl at pH 1.64 in the presence of 1.23% S.A.; curve 3, in 0.15 M KNO$_3$ at pH 2.49 in the presence of 1.23% S.A. The curve in 0.15 M KCl in the absence of protein is not shown: it is virtually coincident with curves 1 and 2. These two curves have the same slope; their difference along the potential axis represents roughly the limit of accuracy of our absolute potential measurements (~0.01 volt).

The remarkable thing about Fig. 1 is that the presence of protein does not interfere at all in the reduction of lead in KCl solution. The slope of the log plot is again 0.034; the half-wave potential is

(2) I. M. Kolthoff and J. J. Lingane, "Polarography," Interscience Publishers, Inc., New York, N. Y., 1941, Ch. IX.

(3) C. Tanford and S. Wawzonek, Ann. Rev. of Phys. Chem., 3, 247 (1952).

(4) Reference 2, p. 145–146.

unchanged within the accuracy with which potential measurements were made (0.01 volt). On the other hand, if KNO$_3$ is used as supporting electrolyte in place of KCl, an entirely different kind of curve is obtained in the presence of protein. The reduction is clearly irreversible. Actual polarograms are shown in Fig. 2.

Similar results have been obtained with cadmium and are also shown in Fig. 2. Again the reduction appears to be close to reversible in KCl, but highly irreversible in KNO$_3$.

The reduction of copper in both KCl and KNO$_3$, in the presence of serum albumin, begins at zero applied potential, much as if no protein were present. The current passes through a maximum, drops virtually to zero, and finally settles down in both cases to a highly irreversible wave. The observed wave in KNO$_3$ is shown in Fig. 2.
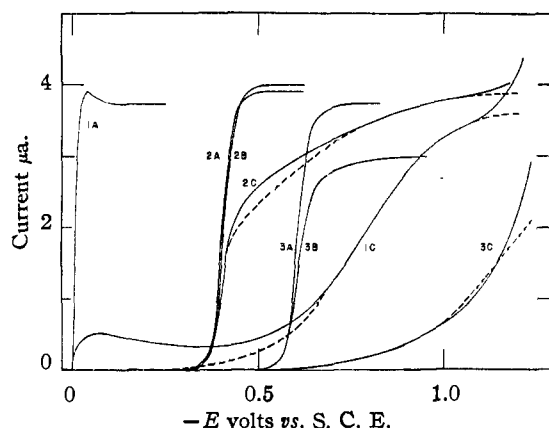

Fig. 2.—Polarograms for the reduction of roughly 4×10^{-4} M metal at pH 2.5 or below in 0.15 M KNO$_3$ in the absence of protein (curves A); in 0.15 M KCl in the presence of 1.23% S.A. (curves B); and in 0.15 M KNO$_3$ in the presence of 1.23% S.A. (curves C). The numbers 1, 2 and 3 refer to Cu^{++}, Pb^{++} and Cd^{++}, respectively. Curve 3B contained about 20% lower concentration of Cd^{++} than curves 3A and 3C. The dashed lines are computed curves corresponding to polarograms 1C, 2C and 3C. The values of α and $k_1\gamma_x/k_{Dx}$ used for the computed curves are: Pb^{++}, 0.070 and 0.090; Cu^{++}, 0.120 and 0.00069; Cd^{++}, 0.096 and 0.00013. The deviation of the computed curves from the experimental ones above −1.2 volts is due to the onset of the discharge of H$^+$; the deviations below −0.6 volt are explained in the text.

The effect of pH on these irreversible waves is shown for cadmium in Fig. 3. The lower the pH, i.e., the greater the positive charge on the protein molecule, the greater the irreversibility. At pH 4.5, where serum albumin in 0.15 M KNO$_3$ is very close to having an over-all zero charge, the observed wave is virtually identical with that obtained in the absence of protein. The effect of pH on the irreversible reduction of the other metals studied is essentially similar.

A change in pH has no influence whatever on the reversible waves obtained with Pb^{++} and Cd^{++} in KCl, except for the lowering of the current, due to complex formation, at higher pH values.[1]

For the sake of comparison, the experiments with copper in KNO$_3$ were repeated, using two other
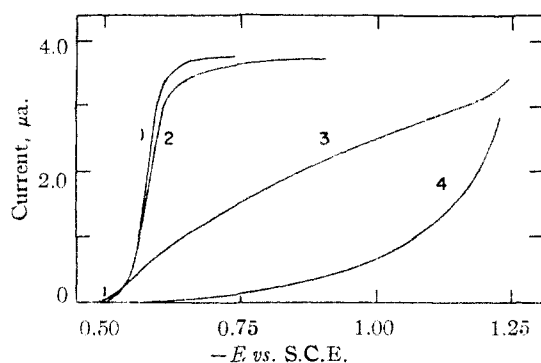
Fig. 3.—The effect of pH on the polarograms of 4×10^{-4} M Cd^{++} in 0.15 M KNO$_3$: curve 1, in the absence of protein; curve 2, in 1.23% S.A. at pH 4.5; curve 3 in 1.23% S.A. at pH 3.45; curve 4, in 1.23% S.A. at pH 2.5.

proteins, egg albumin and pepsin. In the case of egg albumin, which, like serum albumin, bears a positive charge in the acid pH range, a polarogram almost identical with curve 1c in Fig. 2 was obtained. In the case of pepsin, which has its isoelectric point below pH 2,[5] and, therefore, bears a negative charge throughout the pH range studied, the effects found with serum albumin were *not* observed.

Discussion

Qualitative Explanation.—These phenomena can be explained qualitatively as follows. (1) The adsorbed layer of protein is not a physical barrier to the reduction of metal ions. A very soluble protein like serum albumin has a loose structure, with pores filled with solvent, through which small ions can pass without hindrance. (2) In 0.15 M KCl both lead and cadmium exist in large part as the complexes MCl$_2$ and MCl$_3^-$.[6,7] These forms pass through the protein layer as if it were not there. (3) On the other hand, lead and cadmium form only weak complexes with nitrate ion and copper forms only weak complexes with both nitrate and chloride. In the solutions in which irreversible reduction was observed, therefore, the metals were present largely as the uncomplexed ions, M^{++}. These ions, while physically able to pass through the protein layer, are electrostatically repelled by its positive charge, *i.e.*, it is necessary for them to cross a potential energy barrier before they can be reduced. (4) At zero applied potential the mercury drop has a positive potential of about 0.6 volt with respect to the solution, so that the adsorption of the positively charged serum albumin is considerably decreased. The reduction of copper at zero applied potential therefore begins as though no protein were present, but, with the increased adsorption of protein on the drop at more negative potentials, the effects of the potential energy barrier are soon observed.

The results with egg albumin and pepsin confirm the qualitative explanation offered here. The former, which is also positively charged in

(5) J. H. Northrop, M. Kunitz and R. M. Herriott, "Crystalline Enzymes," 2nd ed., Columbia University Press, New York, N. Y., 1948, p. 52.
(6) I. Leden, Z. physik. Chem., **A188**, 160 (1941).
(7) M. V. Noble and A. B. Garrett, THIS JOURNAL, **66**, 231 (1944).

acid solution, behaves like serum albumin. In the presence of pepsin, which is negatively charged, the reduction of metal cations appears to be close to reversible at low pH.

A more quantitative discussion of some of the curves is given in the following paragraphs.

The Theory of Irreversible Waves.—Activation-controlled polarographic reduction has been discussed recently by a number of authors, notably by Eyring and co-workers[8] and by Tanaka and Tamamushi.[9] Designating the oxidized form of the substance being reduced by X, and the reduction product by Y, the equation for the current, on the assumption of a single potential energy barrier, is

$$i = nFA(a_x k_1 e^{-\alpha nFE/RT} - a_y k_2 e^{(1-\alpha)nFE/RT}) \quad (1)$$

where nF is the number of faradays corresponding to the reduction of one mole of X, A is the area of the mercury drop,[10] a_x and a_y are the surface activities of X and Y (the latter in the amalgam if Y is soluble in mercury), k_1 and k_2 are the rate constants for reduction and oxidation, respectively, in the absence of an applied potential, and α is a constant representing the fraction of the applied potential which is effective in lowering the potential energy barrier for reduction. Both X and Y are assumed to reach steady state concentrations at the electrode surface, so that the net rate given by (1) must be exactly balanced by the rate of diffusion of X to the surface and of Y away from it

$$i = nFA k_{DX}(c_x^0 - c_x) = nFA k_{DY} c_y \quad (2)$$

where k_{DX} and k_{DY} are constants obtained from the solution of the diffusion equations,[11–13] c_x and c_y are surface concentrations, and c_x^0 is the concentration of X in the bulk of the solution. The concentration of Y in the solution into which it is diffusing is zero in the present experiments, as in most polarographic reductions not involving amalgam electrodes.

Eliminating c_x and c_y from equations (1) and (2) with the aid of the substitution $a = \gamma c$ (γ being an activity coefficient), and taking into account the interdependence of k_1 and k_2

$$k_1/k_2 = e^{nFE^0/RT} \quad (3)$$

where E^0 is the standard potential of the X–Y half-cell, referred to the same reference electrode as the applied potential E, one obtains the following equation for an irreversible polarographic wave

$$i = \frac{nFA k_{DX} c_x^0}{1 + (k_{DX}/k_1\gamma_x)e^{\alpha nFE/RT} + (\gamma_y k_{DX}/\gamma_x k_{DY})e^{nF(E-E^0)/RT}} \quad (4)$$

It is seen at once that at sufficiently high negative

(8) H. Eyring, L. Marker and T. C. Kwoh, J. Phys. Colloid Chem., **53**, 1453 (1949).
(9) N. Tanaka and R. Tamamushi, Bull. Chem. Soc. Japan, **22**, 227 (1949); Proc. I. Pol. Congr., **1**, 486 (1951).
(10) This can be considered either the maximum or the average area during the time of a drop, depending on whether maximum or average currents are measured.
(11) D. MacGillavray and E. K. Rideal, Rec. trav. chim., **56**, 1013 (1937).
(12) J. J. Lingane and B. A. Loveridge, THIS JOURNAL, **72**, 438 (1950).
(13) H. V. Strehlow and M. von Stackelberg, Z. Elektrochem., **54**, 51 (1950).

potentials the second and third terms in the denominator of equation (4) must vanish, so that a limiting current is obtained, $i_l = nFAk_{DX}c_x^0$. The numerator of equation (4) can therefore be replaced by i_l.

At potentials only a few hundredths of a volt more negative than E^0 the last term in the denominator of (4) vanishes. For reversible waves, for which k_{DX}/k_1 is small the second term in the denominator also vanishes under such conditions, but for irreversible waves it does not, as is witnessed by the fact that i does not become equal to i_l until the potential is several tenths of a volt more negative than E^0. For irreversible waves, then, at least over the final portion of the wave

$$i = i_l/[1 + (k_{DX}/k_1\gamma_x)e^{\alpha nFE/RT} \qquad (5)$$

or, rearranging, and substituting appropriate values for the constants at 25°

$$\log i/(i_l - i) = \log k_1\gamma_x/k_{DX} - (\alpha n/0.059)E \qquad (6)$$

Thus at least the final part of an irreversible wave of this type should give a straight line plot of log $i/(i_l - i)$ versus E.

The curves reported in this paper provide an excellent opportunity for testing equation (4), and, hence, the theory of activation-controlled waves underlying it. All the constants appearing in the equation can be computed experimentally. The numerator is equal to i_l, the value of α is obtained from the straight-line portion of a logarithmic plot of the kind shown in Fig. 1 (cf. equation (6)), and the value of $k_{DX}/k_1\gamma_x$ is obtained from an extrapolation of this straight-line plot to the point $E = 0$. Finally $(\gamma_y k_{DX}/\gamma_x k_{DY})$ exp $(-nFE^0/RT)$ must have the same value as in the reversible reduction of a metal in the absence of protein. Moreover, in a reversible reduction this factor is equal to exp. $(-nFE_{1/2}/RT)$, and can therefore be measured directly from the curves given here.[14]

The small changes in these factors due to changes in viscosity and drop time are not significant within the accuracy of the present work.

(14) Cf. ref. 2. The procedure used here is possible only if the same reduction process can be studied both reversibly and irreversibly at identical temperature, ionic strength, etc. If this favorable situation does not exist, then E^0 would have to be obtained from standard electrode potentials or free energies of formation, and $\gamma_y k_{DX}/\gamma_x k_{DY}$ would have to be estimated as best as possible.

Curves computed in this manner for each of the metals are shown as dashed curves in Fig. 2. They show very satisfactory agreement with the corresponding experimental curves at potentials more negative than −0.6 volt. At potentials more positive than −0.6 volt the experimental waves are higher than the computed ones, approaching the latter asymptotically. This was to have been expected, as the adsorption of protein is probably incomplete at these potentials, and the potential energy barrier therefore reduced. A constant potential barrier is, of course, assumed if k_1 of equation (1) is taken as a constant.

It is particularly gratifying that equation (4) can be used to fit an unusual curve like that for Pb^{++}, which is virtually "reversible" over the first half, and then becomes highly irreversible; especially as the constants used in the equation come from the irreversible portion of the wave. The values of these constants are given in Fig. 2. The value of k_1 is much higher for Pb^{++} than for Cd^{++} and Cu^{++}. This may be due to the fact that Pb^{++} is a smaller ion than the other two. It is also of interest that when curves are computed for the Cd^{++} waves at various pH values (Fig. 3), the values of α are affected relatively little, but the value of $k_1\gamma_x/k_{DX}$ increases rapidly with increasing pH, from 1.3 × 10^{-4} at pH 2.5 to 0.98 at pH 4.48. This is consistent with the view that the charge on the protein layer is the factor responsible for the potential energy barrier, so that k_1 would be expected to vary exponentially with the charge.

One other prediction which can be made from equation (4) is that the limiting current obtained in an irreversible wave should be identical with the diffusion current obtained in a reversible wave, except in so far as the drop time may differ at the respective potentials at which the limiting current is reached. This prediction has been confirmed in every case here reported. For 4 × 10^{-4} M copper in 0.15 M KNO$_3$, for example, a diffusion current of 3.74 μa. was observed (read at −0.1 volt); in the presence of protein the limiting current (read at −1.3 volts) was found to be 3.7 ± 0.1 μa., the inaccuracy being due to interference by the hydrogen wave (cf. Fig. 2).