core and the subsequent symmetrical arrangement of these protein units is currently under investigation in our laboratory.

Acknowledgments. It is a pleasure to thank Mrs. Carolyn Billups and Mr. Clyde Stitt for their valuable technical assistance in various phases of this work. The electron microscope facilities were provided by Dr. Robert Bils. The financial support of the John A. Hartford Foundation, Inc., to the laboratories at the University of Southern California and a U. S. Public Health Service Research Grant (GM 13498) to T. G. S. are gratefully acknowledged.

The Hydrolytic Polymerization of Ferric Citrate. II. The Influence of Excess Citrate

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Abstract: Addition of base to iron(III) solutions containing excess citrate ion leads to the formation at high pH of an anionic chelate which probably contains two citrates, each with its hydroxyl proton removed. This reaction competes kinetically with polymerization of the 1:1 chelate, stable at low pH. For 10^{-3} *M* iron solutions the presence of 0.02 *M* citrate is sufficient to prevent detectable polymerization. Once formed, the polymer is attacked slowly by excess citrate and by EDTA.

In the previous publication,[2] we demonstrated that the addition of base to a solution containing equimolar iron(III) and citrate leads to the formation at low pH of an anionic chelate, probably $FeCit^-$, which then polymerizes at high pH with continued addition of base. The product has a molecular weight of 2×10^5 and is spherical, with a diameter of 72 A. Most of the citrate is dissociated during polymerization and the remainder, ~15%, is probably bound to the surface of a hydroxy-iron polymer, similar to that formed at low pH in chelate-free solutions. We now wish to report on the effect of added citrate on the polymerization process. The finding that excess citrate accelerates the rate of incorporation of iron into transferrin[3] stimulated our interest in this problem. Our conclusion is that there must be a low molecular weight species containing more than one citrate per iron, probably $[Fe(Cit)_2]^{5-}$, whose formation competes with the polymerization reaction.

Experimental Section

Titration Studies at High Citrate Concentration. A series of solutions was prepared containing reagent grade 10^{-3} *M* ferric nitrate, 0.031 *M* sodium citrate, and 0.1 *M* sodium nitrate, with varying amounts of 0.01 *M* CO_2-free sodium hydroxide added last with vigorous stirring. Determination of pH was made with a Heathkit recording pH meter equipped with a Corning combination electrode, which was standardized with 10^{-3} *M* CO_2-free NaOH in 0.1 *M* $NaNO_3$. Visible and ultraviolet spectra of the solutions were recorded with a Cary Model 15 spectrophotometer, using 1-mm light-path cells. The spectra as a function of added base are shown in Figure 1. The pH titration curve for this same series of solutions is presented in Figure 2. The initial pH (no added base) is 7 and is established by citrate buffering. It has previously been shown that protons are released when iron complexes citrate ion. The protons are simply taken up by the excess citrate present. The titration curve then shows a fairly well-defined buffer region ending at 2.0 base equiv per mole of iron. Throughout this region, the spectra show a monotonic variation which ends abruptly at 2.0OH/Fe. An isosbestic point at 315 mμ is preserved throughout the titration, implying that there are only two absorbing species present. The absorbance at 280 mμ is not, however, linear with added base, Figure 2, presumably because of the participation of the excess citrate in the buffering system.

Dialysis as a Function of Excess Citrate. A series of solutions was prepared with 0.001 *M* ferric nitrate, 0.1 *M* sodium nitrate, varying concentrations of sodium citrate, and 0.003 *M* NaOH, added last as 0.01 *M* CO_2-free NaOH, with vigorous stirring. This amount of base corresponds to the end of the polymer buffer region in the 1:1 ferric citrate system, and therefore should yield the maximum polymer fraction possible. Spectra and pH values were determined as above. Duplicate solutions were prepared containing ^{59}Fe tracer, added prior to the addition of base. Dialysis was carried out using the techniques of Lindskog and Malmström[4] as in the preceding study.[2] The dialysis results are shown in Figure 3 as the log of the fraction of iron undialyzed as a function of time. As in the case of 1:1 iron to citrate, the solutions contained both a rapidly and a slowly dialyzing component. The fraction of the latter can be estimated by extrapolation to zero time of the slow phase of the dialysis curves. This fraction decreases with increasing citrate concentration, as shown in Table I, and reaches zero at about 0.02 *M* excess citrate. The rate of dialysis of the slow fraction, however, is about the same for all solutions. At the same time the spectra (Figure 4) show a monotonic variation, with an isosbestic point at 315 mμ, ending in a limiting spectrum at about 0.02 *M* excess citrate. This spectrum is identical with that found at the end of the excess citrate buffer region. The pH rises with increasing citrate concentration (Table I) to a limiting value of 10.8, at about 0.02 *M* excess citrate. This pH corresponds fairly closely to 10^{-3} *M* NaOH, *i.e.*, to the release of about one hydroxide per iron.

Proton Relaxation as a Function of Excess Citrate. The bulk solvent proton magnetic relaxation times were measured for a series of solutions containing 10^{-3} *M* ferric nitrate in the presence of

(1) (a) Princeton University. (b) Predoctoral fellow supported by Training Grant 5TO1-GM-197-08. (c) Research Career Development Awardee, U. S. Public Health Service.

(2) T. Spiro, L. Pape, and P. Saltman, *J. Am. Chem. Soc.*, **89**, 5555 (1967).

(3) G. W. Bates, C. Billups, and P. Saltman, *J. Biol. Chem.*, **242**, 2810 (1967).

(4) S. Lindskog and B. Malmström, *J. Biol. Chem.*, **237**, 1129 (1962).

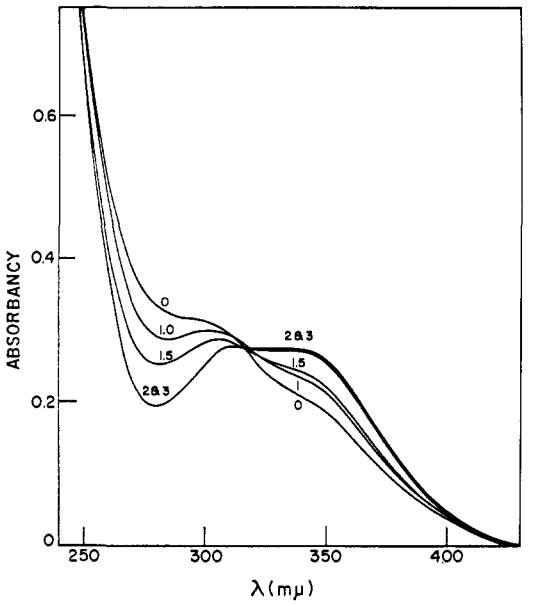

Figure 1. Visible and ultraviolet spectra for solutions containing 0.001 *M* ferric nitrate, 0.031 *M* trisodium citrate, and 0.1 *M* sodium nitrate at various base equivalents per mole of iron as indicated by numbers on curves. The light path is 1 mm.

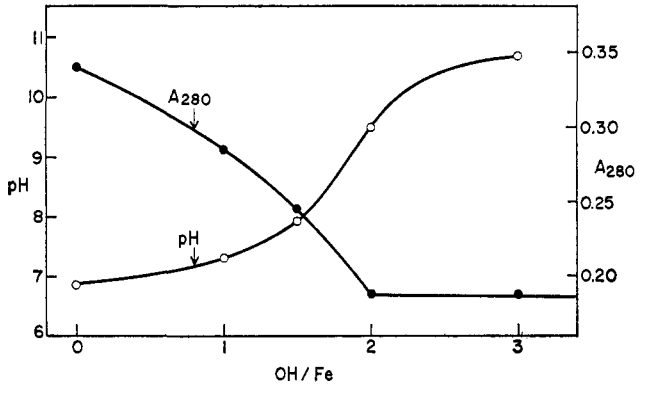

Figure 2. pH titration curve (O) for solutions containing 0.001 *M* ferric nitrate, 0.031 *M* trisodium citrate, and 0.1 *M* sodium nitrate as a function of increasing base equivalents per mole of iron added as 0.01 *M* NaOH. The absorbancies at 280 mμ, 1-mm light path, for the same solutions are also shown (●).

varying concentrations of sodium citrate. All solutions were taken to pH 7.5 with 10^{-2} *M* NaOH. Relaxation times were measured using the spin-echo techniques developed and described by Aisen, *et al.*,[5] using the instrument at the IBM Watson Laboratories. These experiments were carried out in collaboration with Drs. Philip Aisen and Haskell Reisch. The results are presented in

Table I. Fraction of Polymer as a Function of Citrate Iron[a]

Citrate/Fe	% polymer	pH
1	78	9.0
6	50	9.5
11	42	10.4
16	16	10.5
21	0	10.8
26	0	10.8
31	0	10.8

[a] All solutions were 10^{-3} *M* in $Fe(NO_3)_3$, 0.1 *M* sodium nitrate, and contained 3 base equiv per mole of iron.

(5) P. Aisen, A. Leibman, and H. A. Reisch, *J. Biol. Chem.*, **241**, 1666 (1966).

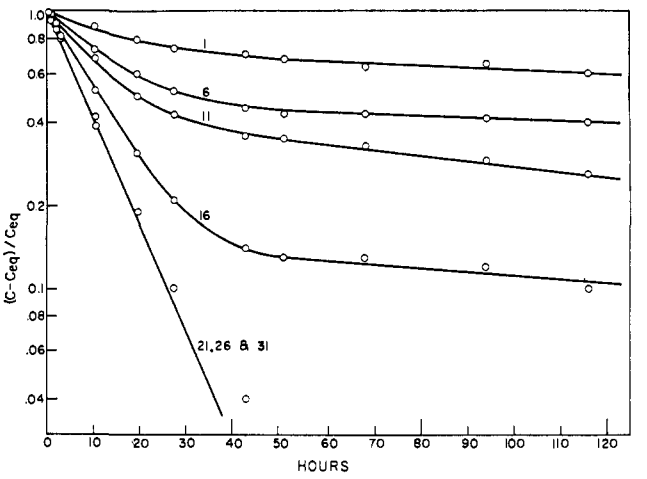

Figure 3. Fractions of ^{59}Fe undialyzed as a function of time for solutions containing 0.001 *M* ferric nitrate, 3 base equiv per mole of iron, 0.1 *M* sodium nitrate, and the various mole ratios of citrate: iron indicated on the curves. The fraction is given as $(C - C_{eq})/C_{eq}$, where C is the concentration of ^{59}Fe in the radioactive compartment at any time and C_{eq} is the equilibrium concentration, *i.e.*, half the initial concentration.

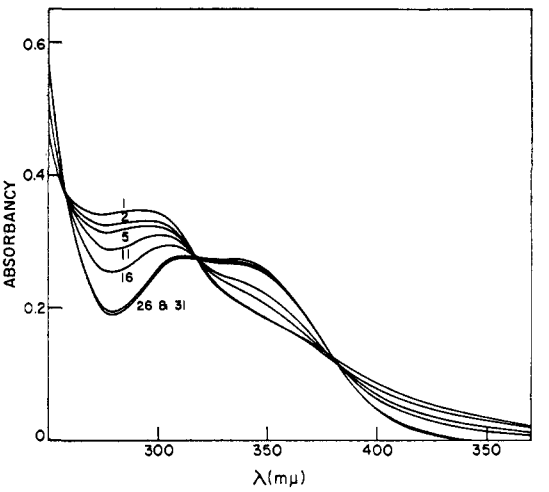

Figure 4. Visible and ultraviolet spectra for solutions containing 0.001 *M* ferric nitrate, 3.0 base equiv per mole of iron, 0.1 *M* sodium nitrate, and the citrate:iron ratios indicated on the curves.

Figure 5. The relaxation rate increases with concentration and attains an asymptotic maximum at 0.05 *M* citrate.

Depolymerization Kinetics. Polymer solutions were prepared as above to contain 2×10^{-3} *M* each of ferric nitrate and sodium citrate, 0.1 *M* sodium nitrate, and 3.0 equiv of NaOH per mole of iron. To these were added rapidly, with vigorous stirring, equal volumes of solutions containing 0.1 *M* $NaNO_3$ and 0.1 *M* of one of three chelating agents: (a) Na_2H_2EDTA (EDTA = ethylenediaminetetraacetate), (b) Na_3HCit (trisodium citrate), and (c) Na_2H_2Cit (prepared from Na_3HCit and HNO_3). The mixtures were placed immediately in 1-cm cuvettes along with a portion of the polymer solution diluted with an equal volume of 0.1 *M* $NaNO_3$ used as a control. The light absorbance at 520 mμ (where absorption by the polymer is higher than by the iron chelates) was followed with a Gilford modification of a Beckman DU. The change in absorbance, plotted on a log scale, as a function of time is shown for the three solutions in Figure 6.

Following an initial rapid drop for each solution there is a first-order decrease in absorbance. The fraction of the total absorbance due to the initial rapid process can be estimated by extrapolating the later portion of the curves back to zero time. It is 37, 21, and 26% for the solutions containing H_2EDTA^{2-}, $HCit^{3-}$, and H_2Cit^{2-}, respectively. The apparent first-order rate constants for the slow process are 0.114, 0.071, and 0.172 hr^{-1} for the three anions, respectively.

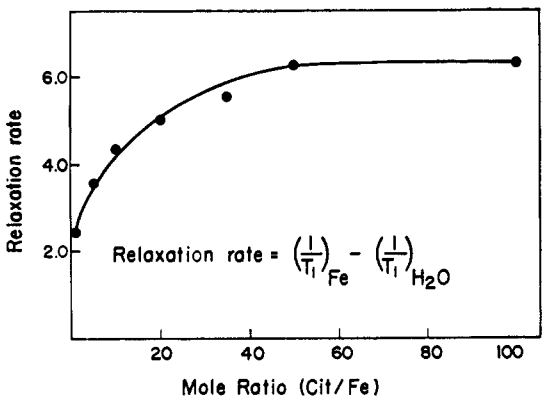

Figure 5. Proton magnetic relaxation rates as a function of increasing ratios of citrate:iron. Solutions contained 0.001 *M* ferric nitrate and were taken to pH 7.5 with 0.01 *M* NaOH.

Discussion

The pH titration curve and spectra in 0.03 *M* excess citrate clearly show that an iron citrate complex is formed with the release of two protons. Since no polymer is found in these solutions, the complex must contain more than one citrate. The most probable species is one containing two citrates, each with a hydroxyl proton ionized, *i.e.*, $[Fe(Cit)_2]^{5-}$. Previous studies[6] have indicated that complexes with more than one citrate are not found in dilute acid solutions. It is unclear whether partially protonated dicitrate species might not be formed at higher pH's, but it is consistent with our results that a deprotonated dicitrate complex might be formed directly from $FeCit^-$. The spectrum of the solution at the beginning of the NaOH titration in excess citrate could be synthesized from the spectra of these two species.

The dialysis results are readily interpreted in terms of a competition between the formation of polymer and of the dicitrate complex, the latter being favored as citrate concentration is increased. The spectra confirm the presence of these two species in varying proportions. The increase in pH with increasing citrate is explained on the basis of the differing stoichiometries. The polymer absorbs three hydroxyls per iron, and the dicitrate complex only two. There is one excess hydroxyl per iron present in the polymer-free solutions.

The proton magnetic relaxation rates are in accord with the other physical and chemical techniques. The paramagnetic iron in the polymer is less effective in relaxing the bulk solvent protons than is the iron in the dicitrate complex. The relaxation rate reaches a limiting value when the fraction of polymer approaches 0 at about 0.05 *M* citrate at pH 7.5. We have also demonstrated that the rate of exchange of iron from ferric citrate into transferrin, a protein which specifically binds ferric iron, reaches a maximum at 0.03 *M* excess citrate.[3]

We postulate, then, two competing reaction paths when hydroxide is added to solutions containing iron and citrate, beyond the point where the 1:1 chelate, $FeCit^-$, is formed.

$$FeCit^- + 1.8OH^- \longrightarrow [(FeO)_{0.95}(OH)_{0.75}(Cit)_{0.15}]_{(polymeric)}^{-0.24} + 0.85(HCit)^{3-} + 0.1H_2O \quad (1)$$

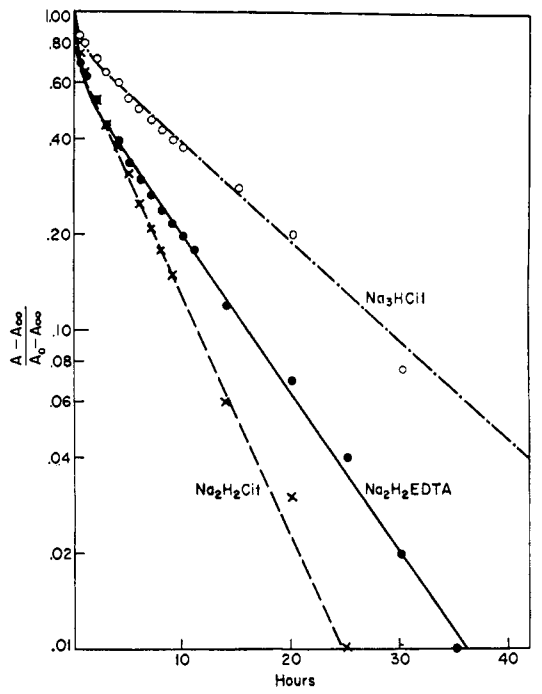

Figure 6. Depolymerization kinetics of 0.002 *M* ferric citrate polymer in 0.1 *M* $NaNO_3$. Three chelating agents were tested: (a) 0.1 *M* Na_2H_2EDTA, (b) Na_3HCit, and (c) Na_2H_2Cit. Absorbancy was measured at 520 mμ. The first-order plot of the log $(A - A_\infty)/(A_0 - A_\infty)$ *vs.* time is shown, where A_0 is the initial absorbancy of the polymer solution, A_∞ the absorbancy value for the completely depolymerized species, and A the absorbancy at the time measured.

and

$$FeCit^- + HCit^{3-} + OH^- \longrightarrow [FeCit_2]^{5-} + H_2O \quad (2)$$

As the concentration of citrate ion, $HCit^{3-}$, is increased the rate of reaction 2 increases at the expense of reaction 1, until, at about 0.02 *M* excess citrate, reaction 1 becomes negligible.

The polymer is attacked slowly by excess citrate or EDTA as shown in Figure 6. It is reasonable to interpret the slow first-order decrease in absorbance at 520 mμ as breakdown of the polymer and formation of iron chelate: $[FeCit_2]^{5-}$ in citrate solutions, and [Fe-EDTA(OH)]$^{2-}$ and its dimer[7] in the EDTA solution. When citrate is present as H_2Cit^{2-} (with, of course, equilibrium amounts of the other protonated forms) attack on the polymer is more than twice as fast as when it is present as $HCit^{3-}$. The extra proton appears to aid in breaking up oxy and hydroxy linkages in the polymer. The rate for attack by diprotonated EDTA is intermediate between the two citrate solutions.

The initial rapid decrease in absorbance is probably related to the low molecular weight iron fraction found in hydrolyzed 1:1 ferric citrate solutions.[2] The visible-ultraviolet spectrum of the fraction was obtained from the ultrafiltrate of a hydrolyzed solution and found to be quite similar to that of the original hydrolyzed solution (spectrum 1 of Figure 4) and distinct from that of $[FeCit_2]^{5-}$ (spectrum 31 of Figure 4). It seems likely therefore that the fraction contains a hydroxy–iron–citrate complex, possibly an oligomer. This complex would probably be susceptible to rapid attack by the chelating agents, and, since its absorbance is similar to

(6) R. C. Warner and I. Weber, *J. Am. Chem. Soc.*, **75**, 5086 (1953).

(7) R. L. Gustafson and A. E. Martell, *J. Phys. Chem.*, 67, 576 (1963).

that of the polymer, the initial drop in absorbance could thereby be explained. The observation from dialysis measurements that the hydrolyzed solution in question (3OH/Fe) contains some 20% of the iron in the low molecular weight form[2] accounts quite nicely for the rapid process in the $HCit^{3-}$ and H_2Cit^{2-} solutions, which are responsible for 21 and 26%, respectively, of the total absorbance change. In the H_2EDTA^{2-} solution the initial fraction is somewhat larger, 37%. It is possible that some of the surface irons in the polymer are also susceptible to rapid attack by EDTA.

Acknowledgments. Mrs. Carolyn Billups provided essential technical assistance in some phases of this investigation. The financial support of the John A. Hartford Foundation, Inc., to the laboratories of the University of Southern California and a U. S. Public Health Service Research Grant (GM 13498) to T. G. S. are gratefully acknowledged.

The Mechanisms of Electrolytic Reduction for Decaborane(14), $B_{10}H_{14}$, in an Aprotic Solvent. I. The First Reduction Step[1a]

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Abstract: This paper presents electrochemical and spectral (esr and visible–ultraviolet) data which are interpretable in terms of a one-electron reduction of decaborane(14) to produce a radical anion. This radical undergoes rapid, first-order decay (possibly terminal–bridge hydrogen interchange) to a somewhat more stable radical anion. The latter appears to disproportionate, producing $B_{10}H_{13}^-$ and $B_{10}H_{15}^-$. A more complex mechanism, which also involves radical anion formation, will also explain the data.

We have carried out electrochemical investigations on the reduction of decaborane(14) in an inert solvent (1,2-dimethoxyethane) in an effort to study the reduction mechanism. The reaction of decaborane with alkali metals has been reported on several occasions.[2,3] These studies have afforded some knowledge of the products of reduction and possible mechanisms. However, most aspects of the mechanisms are either unclear or without substantial proof. It seemed to us that the great selectivity of reduction potential which is available in electrochemical experiments and their analytical aspects might provide a clearer picture of the reduction process. Another aspect of the application of electrochemistry to this problem is that, in principle, appropriate electrochemical experiments can provide information on a much shorter time scale than was possible in the previous chemical investigations.[4]

Hough and Edwards have shown that the reaction of sodium amalgam with $B_{10}H_{14}$ is dependent upon reaction time[3]

$$B_{10}H_{14} + 2Na(Hg) \xrightarrow[Et_2O]{5\ hr} Na_2B_{10}H_{14}$$

$$2B_{10}H_{14} + 2Na(Hg) \xrightarrow[Et_2O]{3\ days} 2NaB_{10}H_{13} + H_2$$

Furthermore, Toeniskoetter has shown that the nature of the product is strongly dependent upon the solvent which is employed, as well as reaction time.[2]

$$2Na + B_{10}H_{14} \xrightarrow[NH_3(l)]{} Na_2B_{10}H_{14}$$

$$Na + B_{10}H_{14} \xrightarrow[Et_2O]{} NaB_{10}H_{13} + Na_2B_{10}H_{14} +$$

other products with longer reaction times (proposed $Na_2B_{10}H_{13}$)

$$Na + B_{10}H_{14} \xrightarrow[THF]{} NaB_{10}H_{13} + Na_2B_{10}H_{14}$$

In ether solutions the reaction of sodium with decaborane produces a red transient species which Toeniskoetter proposed might be a free radical, $B_{10}H_{14}^-$. This color faded slowly to a yellow-green. Upon standing, the product of 1 g-atom of hydrogen per mole of original decaborane was noted and explained in terms of the following over-all reaction

$$Na + B_{10}H_{14} \longrightarrow NaB_{10}H_{13} + 0.5H_2$$

A secondary reaction was also noted.

$$2Na + B_{10}H_{14} \longrightarrow Na_2B_{10}H_{14}$$

When the solvent was removed from the initial red solution, a white solid remained which gave back the red color when redissolved. Toeniskoetter proposed that the production of $Na_2B_{10}H_{14}$ is the result of two successive one-electron reductions

$$B_{10}H_{14} \xrightarrow{Na} NaB_{10}H_{14} \xrightarrow{Na} Na_2B_{10}H_{14}$$

(1) (a) This work was supported by grants from the National Science Foundation and is taken in part from the Ph.D. Dissertation of E. B. Rupp, Northwestern University, Evanston, Ill., 1967; (b) to whom reprint inquiries should be addressed; (c) Alfred P. Sloan Fellow, 1967–1969.

(2) (a) R. H. Toeniskoetter, Ph.D. Thesis, St. Louis University, 1958; R. H. Toeniskoetter, G. W. Schaeffer, E. C. Evers, R. E. Hughes, and G. E. Bagley, Abstracts of Papers, 134th National Meeting of the American Chemical Society, Chicago, Ill., 1958, p 23.

(3) (a) W. V. Hough and L. J. Edwards, Abstracts of Papers, 133rd National Meeting of the American Chemical Society, San Francisco, Calif., 1958, p 28L; (b) W. V. Hough and L. J. Edwards, Advances in Chemistry Series, No. 32, American Chemical Society, Washington, D. C., 1961, p 184.

(4) (a) P. Delahay, "New Instrumental Methods in Electrochemistry," Interscience Publishers, Inc., New York, N. Y., 1954; (b) P. Delahay, in "Advances in Electrochemistry and Electrochemical Engineering," Vol. 1, P. Delahay, Ed., Interscience Publishers, Inc., New York, N. Y., Chapter 5.