[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STANFORD UNIVERSITY]

OXIDATION-REDUCTION POTENTIALS OF THE PENTACYANO-FERROATES

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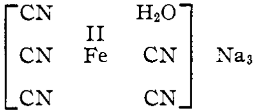
Baudisch and his coworkers[2] have shown that sodium pentacyano-aquo-ferroate[3] (I) and sodium pentacyano-ammine-ferroate[4] (II) may act as catalysts in the oxidation of pyrimidines, purines and hydantoins by means of oxygen or air. The present paper deals with the oxidation-reduction potentials of mixtures of the ferrous and ferric forms of these salts as well as of sodium pentacyano-nitrito-ferroate[3] (III) as compared with the ferrocyanide (IV) electrode.

Previous investigators[5] have found that the potential of the ferrocyanide electrode depends on the concentration of potassium ions. For a given solution of potassium chloride, however, the potential of the electrode followed the equation

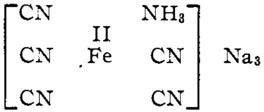
$$\text{E.m.f.} = E_0' - RT/F \ln C_o/C_i$$

C_o and C_i were the total concentrations of the ferrous and ferric salt, respectively. $C_o + C_i$ was small compared with [KCl].



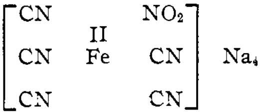
$$\left[\begin{matrix} CN & & H_2O \\ CN & \overset{II}{Fe} & CN \\ CN & & CN \end{matrix}\right] Na_3$$

I



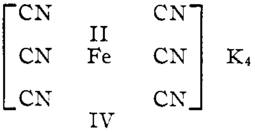
$$\left[\begin{matrix} CN & & NH_3 \\ CN & \overset{II}{Fe} & CN \\ CN & & CN \end{matrix}\right] Na_3$$

II



$$\left[\begin{matrix} CN & & NO_2 \\ CN & \overset{II}{Fe} & CN \\ CN & & CN \end{matrix}\right] Na_4$$

III



$$\left[\begin{matrix} CN & & CN \\ CN & \overset{II}{Fe} & CN \\ CN & & CN \end{matrix}\right] K_4$$

IV

Similar results have been obtained with the molybdo- and tungstocyanides.[6] The preliminary study was therefore limited to solutions of the iron salts in normal potassium chloride.

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[1] Rockefeller Institute Fellow.

[2] (a) Pfaltz and Baudisch, THIS JOURNAL, **45**, 2972 (1923); (b) Pfaltz, *ibid.*, **45**, 2980 (1923); (c) Baudisch and Bass, *ibid.*, **46**, 184 (1924); (d) Baudisch and Davidson, *J. Biol. Chem.*, **71**, 501 (1927); (e) **75**, 247 (1927).

[3] Hofmann, *Ann.*, **312**, 1 (1900).

[4] Manchot, *Ber.*, **45**, 369 (1912).

[5] (a) Lewis and Sargent, THIS JOURNAL, **31**, 357 (1909); (b) *ibid.*, **31**, 363 (1909); (c) Müller, *Z. physik. Chem.*, **88**, 46 (1914); (d) Schoch and Felsing, THIS JOURNAL, **38**, 1928 (1916); (e) Linhart, *ibid.*, **39**, 615 (1917); (f) Butler and Davies, *J. Chem. Soc.*, **125**, 1101 (1924).

[6] (a) Collenberg, *Z. physik. Chem.*, **109**, 353 (1924); (b) Collenberg and Guthe, *Z. anorg. allgem. Chem.*, **136**, 252 (1924).

Apparatus and Methods

The electrode vessel consisted of a glass cup of 75 cc. capacity, fitted with a rubber stopper which bore a dropping funnel, nitrogen inlet, gold plated platinum electrode, salt bridge containing *N* KCl, 5 cc. buret (when employed) and a mechanical stirrer equipped with mercury seal and driven by an air turbine. The second leg of the salt bridge was placed in a beaker containing *N* KCl, into which a normal calomel electrode dipped. Weighed quantities of the salts were placed in the cup, the stopper was affixed and the air rinsed out with nitrogen. Fifty cc. of *N* KCl was placed in the dropping funnel. By properly regulating the nitrogen pressure and opening the cock of the dropping funnel, the potassium chloride solution could be de-aerated just before use. Finally the solution was allowed to run into the cup, stirring was begun and readings were taken at intervals. The potentiometer used was a Leeds and Northrop Student Type with a needle galvanometer. The measurements were carried out at room temperature (21°).

The solutions studied were prepared by dissolving a total of 0.2 millimole of the two salts in 50 cc. of *N* KCl. Usually three ratios of ferrous to ferric salt were measured: (a) 0.15 ferrous + 0.05 ferric (3:1); (b) 0.10 ferrous + 0.10 ferric (1:1); (c) 0.05 ferrous + 0.15 ferric (1:3).

Potassium Ferrocyanide

The apparatus and method were checked by measuring the ferrocyanide electrode. The potentials given in Table I remained constant during the twenty minutes they were observed.

TABLE I

POTENTIALS OF THE FERROCYANIDE ELECTRODE

Soln.	*N* KCl cc.	Ferrous, millimole	Ferric, millimole	E.m.f.	E.m.f. $- E_0'$ Found	E.m.f. $- E_0'$ Calcd.
(a)	50	0.15	0.05	0.171	−0.029	−0.028
(b)	50	.10	.10	.198	..	..
(c)	50	.05	.15	.225	.027	.028

The value of 0.198 volt is practically identical with that obtained by Butler and Davies,[5f] the difference in temperature being allowed for.

Sodium Pentacyano-Ammine-Ferroate

Solutions containing ferrous and ferric "ammine" salt gave potentials which varied with time as shown in Fig. 1. Moreover, at no time was the difference between the potentials of solutions (b) and (a) or of (b) and (c) equal to the value of 0.028 volt calculated from the electrochemical equation given above. It was constantly greater. Thus, for example, ten minutes after the solutions were prepared, (b) − (a) = 0.040 volt and (c) − (b) = 0.041 volt. Since this anomalous behavior of the electrode could be attributed to the hydrolysis of the "ammine" salts[3]

$$Na_3Fe(CN)_5{\cdot}NH_3 + H_2O \rightleftharpoons Na_3Fe(CN)_5{\cdot}H_2O + NH_3$$
$$Na_2Fe(CN)_5{\cdot}NH_3 + H_2O \rightleftharpoons Na_2Fe(CN)_5{\cdot}H_2O + NH_3$$

a second series of measurements was made in which the solvent contained 0.5 cc. of concentrated ammonia, the total volume and normality in potassium chloride being maintained by using the proper quantities of N and $2N$ potassium chloride.

As is seen from Fig. 2, this had the effect of stabilizing the potentials except in solution (c), in which case, apparently, reduction of the ferric salt by the added ammonia caused a gradual fall in the potential, which fall became uniform after five minutes. The extrapolation indicated in the figure yielded a value which harmonized with those of (a) and (b) which are given in Table II.

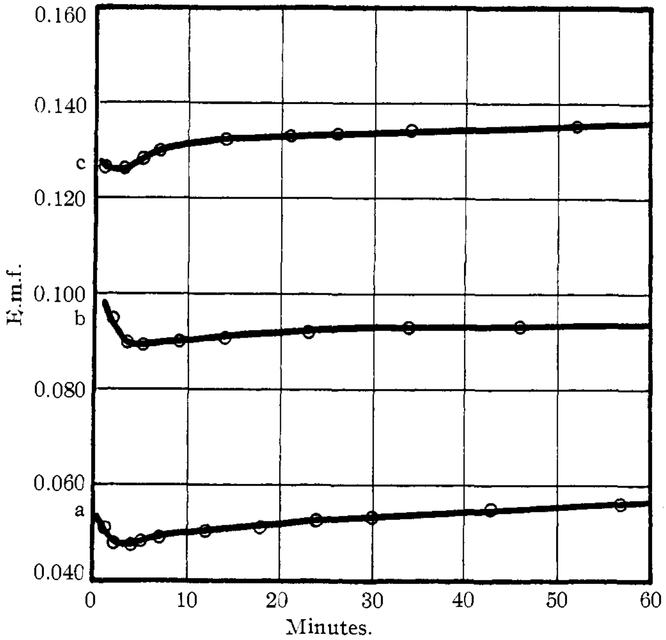

Fig. 1.—Variation of the pentacyano-ammine-ferroate electrode with time. [Ferrous]/[ferric] = a, 3; b, 1; c, 1/3.

TABLE II

POTENTIALS OF THE PENTACYANO-AMMINE-FERROATE ELECTRODE

Soln.	N KCl, cc.	$2N$ KCl, cc.	Concd. NH_3, cc.	Ferrous, milli-mole	Ferric, milli-mole	E.m.f.	E.m.f. − E'_0 Found	Calcd.
(a)	49	0.5	0.5	0.15	0.05	0.064	−0.028	−0.028
(b)	49	.5	.5	.10	.10	.092	..	..
(c)	49	.5	.5	.05	.15	.120	.028	.028

An independent series of measurements was made to determine the effect of ammonia concentration. Fig. 3 and Table III reproduce the results obtained with solvents containing 0.5, 1.0 and 2.0 cc. of concentrated ammonia, respectively.

TABLE III

EFFECT OF AMMONIA CONCENTRATION ON THE POTENTIALS OF THE PENTACYANO-AMMINE-FERROATE ELECTRODE

Soln.	*N* KCl, cc.	2*N* KCl, cc.	Concd. NH_3, cc.	Ferrous, milli-mole	Ferric, milli-mole	E.m.f.	E.m.f. − E_0' Found	E.m.f. − E_0' Calcd.
(a)	49	0.5	0.5	0.15	0.05	0.063	−0.029	−0.028
(b)	49	.5	.5	.10	.10	.092	..	..
(c)	49	.5	.5	.05	.15	.119[a]	.027	.028
(a′)	48	1.0	1.0	.15	.05	.063	−.029	−.028
(b′)	48	1.0	1.0	.10	.10	.092	..	..
(c′)	48	1.0	1.0	.05	.15	.119[a]	.027	.028
(a″)	46	2.0	2.0	.15	.05	.063	−.029	−.028
(b″)	46	2.0	2.0	.10	.10	.092[a]	..	..
(c″)	46	2.0	2.0	.05	.15	?	?	.028

[a] Extrapolated.

As Fig. 3 indicates, the results with different concentrations of ammonia were practically identical as long as the disturbance caused by the oxidation of the ammonia did not occur. Of the (b) solutions, that containing 2.0 cc. of ammonia, (b″), displayed a falling potential. The readily extrapolated value, however, agreed with those obtained in the other (b) solutions. In the (c) solutions the potentials fell increasingly rapidly so that extrapolation was hardly feasible in the last case, (c″). Nevertheless, it is evident that within the limits of ammonia concentrations employed the potential of the electrode is independent of the ammonia concentration,

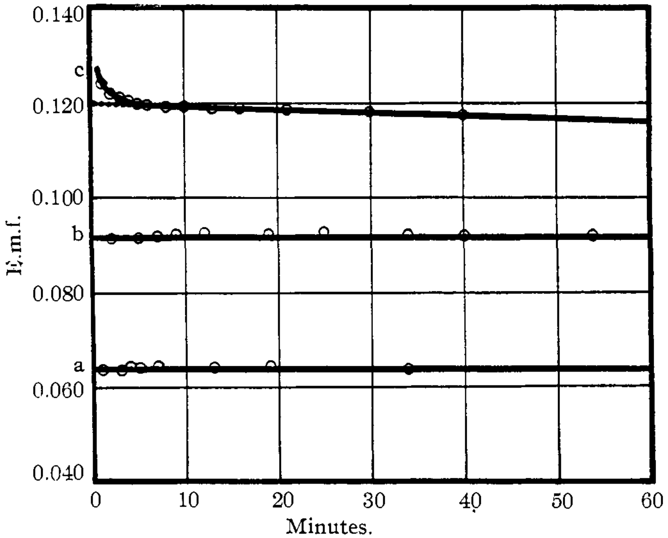

Fig. 2.—Stabilizing effect of ammonia on the pentacyano-ammine-ferroate electrode.

although with increasing ammonia concentration the solutions become increasingly unstable.

Sodium Pentacyano-Aquo-Ferroate

Application of the method described above to sodium pentacyano-aquo-ferroate gave the following results.

Table IV

Potentials of the Pentacyano-Aquo-Ferroate Electrode

Soln.	*N* KCl, cc.	Ferrous, millimole	Ferric, millimole	E.m.f.	E.m.f. − E_0' Found	Calcd.
(a)	50	0.15	0.05	0.100	−0.069	−0.028
(b)	50	.10	.10	.169	..	..
(c)	50	.05	.15	.294	.125	.028

The color of the solutions indicated, however, that compound formation was occurring between the ferrous and ferric salts, since the deep blue color of the ferric salt was only apparent in (c), while solutions (a) and (b) were only slightly darker than solutions of the ferrous salt alone. That compound formation actually occurs was demonstrated by titrating the ferrous salt with a solution of the ferric compound.

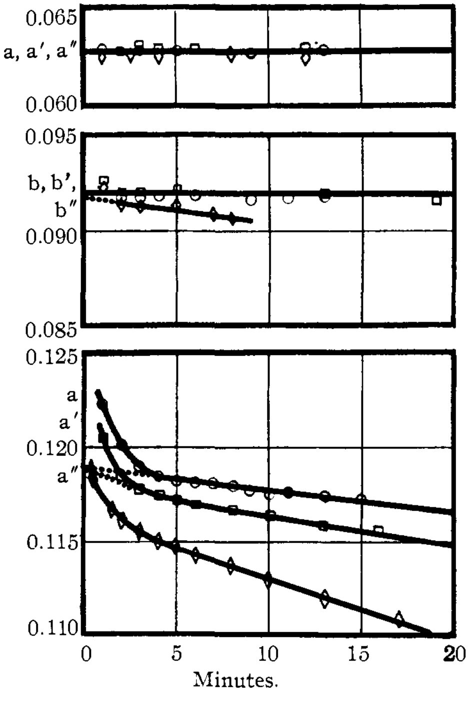

Fig. 3.—Effect of [NH_3] on the pentacyano-ammine-ferroate electrode. a, b, c contain 0.5 cc. of concd. NH_3 in 50 cc. of solution; a′, b′, c′, 1.0 cc.; a″, b″, c″, 2.0 cc.

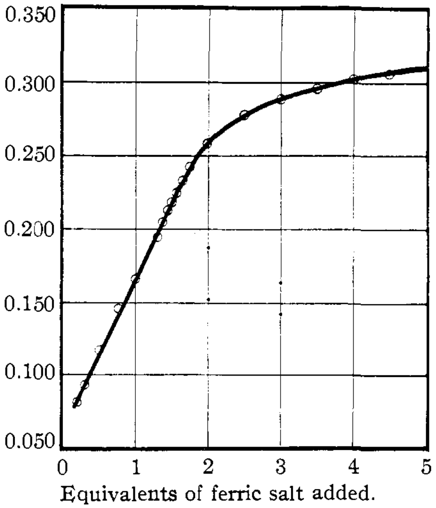

Fig. 4.—Titration of $Na_3Fe(CN)_5{\cdot}H_2O$ with $Na_2Fe(CN)_5{\cdot}H_2O$.

A quantity of the ferric salt (approximately 0.25 millimole) was weighed into a 5cc. volumetric flask, while a weight of ferrous salt equivalent to one-fifth of the ferric salt (approximately 0.05 millimole) was weighed into the cup. Fifty cc. of N KCl was introduced into the cup in the usual way. The ferric salt was made up to 5 cc. with N KCl, the solution introduced into the buret and the titration begun. Fig. 4 is a plot of this titration. More information is obtained from a plot of e.m.f. against log C_0/Ci (Fig. 5). This curve shows an inflection point at e.m.f. = 0.219 and log $C_0/Ci = -0.176$. This point corresponds to the composition of the compound.[7] Hence, the ratio of ferrous to ferric salt in the com-

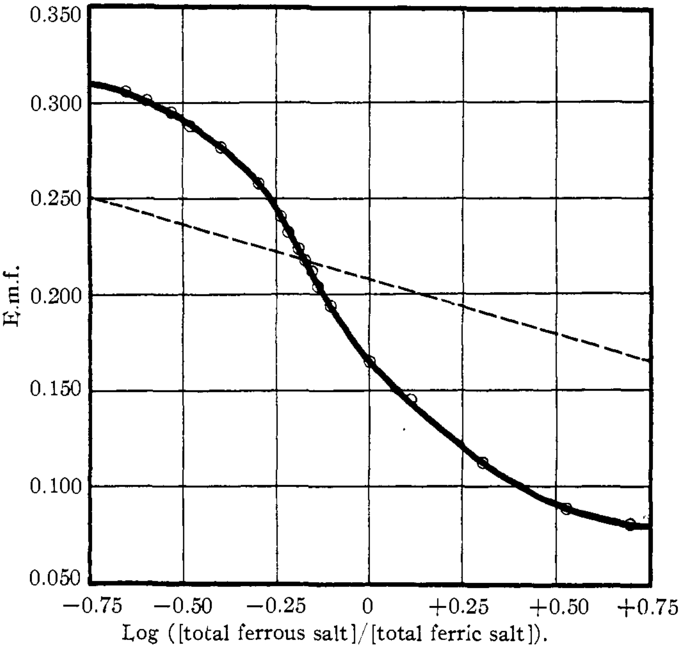

Fig. 5.—Titration of $Na_3Fe(CN)_5 \cdot H_2O$ with $Na_2Fe(CN)_5 \cdot H_2O$. The dotted line is the curve of e.m.f. = $E_0' - 0.058 \log([\text{ferrous}]/[\text{ferric}])$.

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[7] This becomes more evident by considering the simpler case of a system in which the reductant, *red.*, and the oxidant, *ox.*, form a compound, *red.ox.* A solution of the "pure" compound will contain *red.ox.*, as well as the two components, *red.* and *ox.* arising from its dissociation.

Let [*red.*] = concentration of uncombined reductant
[*ox.*] = concentration of uncombined oxidant
[*Red.*] = concentration of total reductant
[*Ox.*] = concentration of total oxidant
concentration = activity.

If one adds an excess of *red.* to a solution of the compound its dissociation is depressed; that is to say, a small proportion of this excess combines with a large proportion of the free *ox.* present. In other words, [*red.*] is slightly less than the sum of that originally present and that added, while [*ox.*] has been greatly decreased. Therefore, [*red.*]/[*ox.*] >[*Red.*]/[*Ox.*], and the e.m.f.-log [*Red.*]/[*Ox.*] curve lies below the normal curve;

that is, below that of Equation 1. Moreover, since equal increments in log *Red.* cause diminishing decrements in log [*ox.*], (d log [*red.*]/[*ox.*])/(d log [*Red.*]/[*Ox.*]) diminishes as log [*Red.*]/[*Ox.*] increases. Similar reasoning applies to additions of excess of *ox.* Hence, the resulting curve, Fig. 6, has an inflection point at log [*Red.*]/[*Ox.*] corresponding to the compound. This point also lies on the straight line which normally obtains in the absence of compound formation. In general, for a compound *m·red.·n·ox.*, the inflection point will occur at log [*Red.*]/[*Ox.*] = log *m*/*n*. Fig. 7 represents the curves calculated for a titration, during which, of course, the quantity of the compound varies widely.

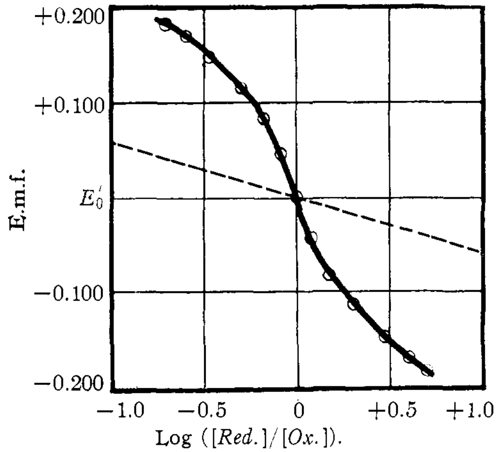

Fig. 6.—Calculated curve for the effect of excess of *red.* or *ox.* on the potential of *red.ox.*, whose dissociation constant = 0.011. [*red.ox.*] = 1. The dotted line is the curve of e.m.f. = E_0' − 0.058 log ([*Red.*]/[*Ox.*]).

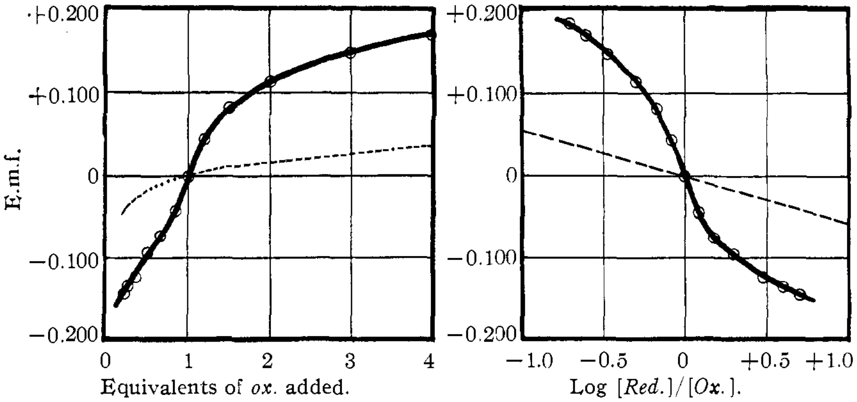

Fig. 7.—Calculated titration curves of one mole of *red.* in one liter of solution titrated with *ox.* *Ox.* and *red.* form a compound, *red.ox*, whose dissociation constant is 0.011. The volume of the solution is not appreciably altered during the titration. The dotted curves represent the equation e.m.f. = E_0' − 0.058 log ([*Red.*]/[*Ox*]).

plex is antilog $-0.176 = 0.67$, or 2:3, and the formula of the compound[8] is $2Na_3Fe(CN)_5H_2O{\cdot}3Na_2Fe(CN)_5H_2O$. The value of E'_0 is obtained from the coördinates of the inflection point since this is the only point at which the ratio of uncombined ferrous salt to uncombined ferric salt is equal to the ratio of the total ferrous salt to the total ferric salt. Thus

$$0.219 = E'_0 - (0.058)(-0.176);\ E'_0 = 0.209 \text{ volt}$$

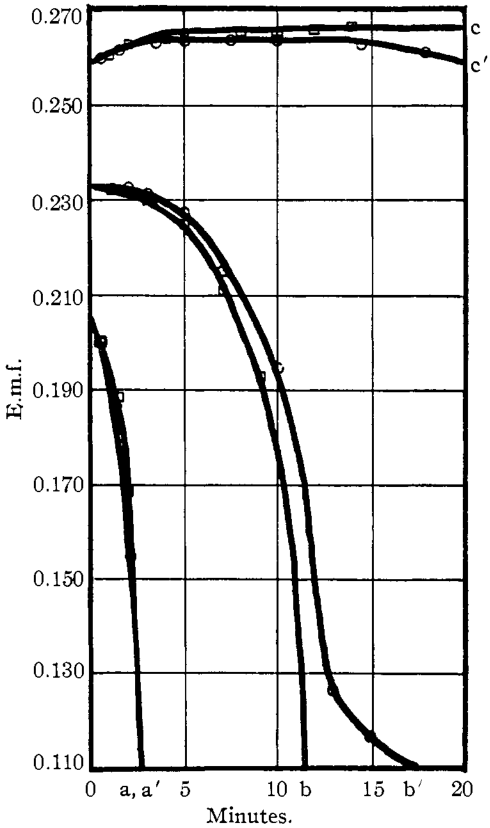

Fig. 8.—Variation of the pentacyano-nitrito-ferroate electrode with time. a′, b′, c′ contain added nitrite.

Sodium Pentacyano-Nitrito-Ferroate

Solutions of this system gave potentials which varied rapidly with time (Fig. 8), parallel with a change in color from red (the color of the ferric salt) to yellow. Addition of 1 cc. or more of *N* $NaNO_2$ made little difference in the behavior of the electrode. Nevertheless, if the curves (except

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[8] Complex formation between the iron cyanides has also been noted by Briggs, *J. Chem. Soc.*, **117**, 1026 (1915).

the (c) curves, which were constant for several minutes) are extrapolated to zero time, values are obtained which differ from each other in agreement with Equation 1.

TABLE V

POTENTIALS OF THE PENTACYANO-NITRITO-FERROATE ELECTRODE

Soln.	N KCl, cc.	N $NaNO_2$, cc.	Ferrous, millimole	Ferric, millimole	E.m.f.	E.m.f. $- E_0'$	
						Found	Calcd.
(a)	50	0	0.15	0.05	0.207[a]	−0.026	−0.028
(b)	50	0	.10	.10	.233[a]	..	..
(c)	50	0	.05	.15	.266	.033	.028
(a′)	50	1	.15	.05	.208[a]	−.027	−.028
(b′)	50	1	.10	.10	.235[a]	..	..
(c′)	50	1	.05	.15	.264	.029	.028
(b″)	50	10	.10	.10	.232[a]	..	..

Mean $E_0' = 0.234$

[a] Extrapolated.

Summary

Under comparable conditions, the oxidation-reduction potentials of the following complex iron cyanides against the normal calomel electrode were: $K_4Fe(CN)_6$–$K_3Fe(CN)_6$, 0.198 volt; $Na_3Fe(CN)_5{\cdot}NH_3$–$Na_2Fe(CN)_5{\cdot}NH_3$, 0.092 volt; $Na_3Fe(CN)_5{\cdot}H_2O$–$Na_2Fe(CN)_5{\cdot}H_2O$, 0.209 volt; $Na_4Fe(CN)_5NO_2$–$Na_3Fe(CN)_5NO_2$, 0.234 volt.

Indication was obtained of the formation of the compound, $2Na_3Fe(CN)_5{\cdot}H_2O{\cdot}3Na_2Fe(CN)_5{\cdot}H_2O$.

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[CONTRIBUTION FROM THE MAYO CLINIC]

THE USE OF GELATIN OLEATE MIXTURES FOR THE DEMONSTRATION OF SMALL AMOUNTS OF CALCIUM[1]

BY SAMUEL AMBERG,[2] JOHN LANDSBURY[3] AND FRANCES SAWYER[4]

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In a study of the penetrability of gelatin by dyes added to blood serum, Bennhold[5] found that it was impossible to use oxalated plasma because the calcium in the gelatin formed a precipitate of calcium oxalate which interfered with the experiments. Although this difficulty can be overcome by the use of ash-free or isoelectric gelatin,[6] it seemed probable that the formation of a stabilized ring reaction might furnish a method for the

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[1] Read before the American Society of Biological Chemists, Ann Arbor, Michigan, April 12 to 14, 1928.

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[3] Fellow in Medicine, The Mayo Foundation.

[4] Fellow in Chemistry, The Mayo Foundation.

[5] H. Bennhold, "Ueber die Adsorptionsfähigkeit der Serumkolloide tubulär Nierenkranker gegenüber Farbstoffen," *Z. ges. exptl. Med.*, **49**, 71–94 (1928).

[6] Obtained from the Eastman Kodak Company.