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A Potentiometric, pH and Polarographic Investigation of the Complex Ions of Zinc(II) and Ethylenediamine

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RECEIVED FEBRUARY 26, 1955

The complexity constants of the complex ions formed between zinc(II) ion and ethylenediamine were investigated by the polarographic, *p*H and potentiometric methods. It was found that the *p*H method and the potentiometric method gave complexity constants of the complex ions which were generally in agreement, while the polarographic method indicated somewhat more stable complexes. This discrepancy is probably due to the presence of gelatin required to suppress the maximum occurring in the polarograms of the zinc amine complex ion solutions.

Previous investigations of the formation of zinc–ethylenediamine complexes by Bjerrum and Anderson[4] and by Carlson, McReynolds and Verhoek[5] by the *p*H method of Bjerrum[6] yielded results wherein essential agreement was obtained. The values of the complexity constants obtained by these authors are listed in Table III. A report of a polarographic investigation was made by D. Eyke,[7] wherein the results obtained were in agreement with the polarographic results reported here, in so far as the formation of the $[Zn(en)_2]^{++}$ ion was concerned. However, as seen below, the results of the polarographic investigation gave complexity constants considerably greater than those obtained by the *p*H method. The problem then was to determine whether the polarographic method or the *p*H method gave the more nearly correct values and to determine the reason for the discrepancy.

Experimental

Reagents.—Solutions of ethylenediamine, 2.01 and 3.92 *M*, were prepared by dilution of distilled material (b.p. 114° at 700 mm.) and were standardized by titration with standard hydrochloric acid solution.

A 0.203 *M* and a 0.0213 *M* solution of zinc chloride were prepared and standardized by precipitation of zinc as the double ammonium phosphate and subsequent ignition to the pyrophosphate.

Salt solutions were prepared by dissolving weighed samples of reagent grade material.

Gelatin solutions (0.1%) and methyl cellulose solutions (0.2%) were freshly prepared before use.

Zinc amalgam was prepared by electrolysis of 1.4 *M* zinc chloride between a mercury pool cathode and a zinc anode. The amalgam was stored under oxygen-free distilled water.

Polarographic Measurements.—All polarograms were obtained at 25° with a Fisher Scientific Company Elecdropode. Applied potentials were determined by means of a Leeds and Northrup Student Potentiometer. The cell used in these experiments was an H-cell with an agar salt bridge to a bulb-type saturated calomel electrode. The mass of mercury flowing through the dropping electrode was 2.21 mg./sec., and the drop time was 2.80 seconds at −1.5 volts *vs.* the S.C.E.

The solutions were prepared by dilution of the above stock solutions. All solutions had a zinc concentration of 4.26×10^{-4} mole per liter. Gelatin (0.008%) was employed as a maximum suppressor when the supporting electrolyte was 0.1 or 1.0 *M* potassium chloride or 0.1 *M* sodium perchlorate. In one set of data taken in 0.1 *M* potassium nitrate, 0.02% methylcellulose was employed as a maximum suppressor. The concentration of ethylenediamine was varied from 0.02 to 2.8 *M*.

In an attempt to get a more accurate value of the free ethylenediamine concentration, [en], in these solutions, a correction was made for the amount of ethylenediamine complexed with zinc and for the ionization of ethylenediamine. It was assumed that all the zinc was in the form of the $[Zn(en)_3]^{++}$ complex, and that only the monohydrogen ethylenediamine ion was produced in significant amount by the ionization of ethylenediamine. The constant for the ionization of the monohydrogen ethylenediamine ion is reported in a later section. This approximation is undoubtedly a better one than the assumption that the free ethylenediamine concentration is equal to the stoichiometric amount of ethylenediamine present.

***p*H Measurements.**—The *p*H measurements were made at an ionic strength of 0.5 molar at a temperature of 25°. In this series of measurements, the concentrations of zinc chloride, potassium chloride and hydrochloric acid were kept constant at 0.0203, 0.40 and 0.0390 *M*, respectively, and the total concentration of amine varied from 0.02 to 0.16 *M*. The solutions were placed in the thermostat for at least one hour before the *p*H was measured. A Beckman Model G *p*H meter was employed for determination of the *p*H and was standardized against standard buffers of either *p*H 7.00 or 10.00, depending on the *p*H of the solution being tested.

The first and second acid dissociation constants of the dihydrogen ethylenediamine ion, as determined in a solution of ionic strength 0.5 at 25°, were 3.98×10^{-8} and 7.24×10^{-11}.

Potentiometric Measurements.—In this series of measurements, the solutions prepared for the polarographic and *p*H investigations were used without any change whatsoever.

The cell employed was similar to that made use of in the polarographic investigation with the dropping mercury electrode being replaced by a zinc amalgam electrode. Oxygen was removed from these solutions by passing oxygen-free nitrogen through the solution. The potentials were measured with a Leeds and Northrup student-type potentiometer, and it was found that they could be reproduced within ±1 mv.

Data and Results

Polarographic Investigation.—In the polarographic study, the half-wave potential of zinc ion was determined for various concentrations of ethylenediamine. The data obtained with a 0.1 *M* sodium perchlorate solution as a supporting electrolyte are recorded in Table I and plotted in Fig. 1. Solutions using 0.1 *M* potassium nitrate, 0.1 or 1 *M* potassium chloride as supporting electrolytes gave results which, within experimental error, were the same as those obtained with sodium perchlorate.

The general equation for the difference in half-wave potentials of the simple and complex ions is

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[1] This project supported in part by Office of Ordnance Research, U. S. Army, Project No. DA-04-200-ORD-65.

[2] Taken in part from a thesis by E. W. Murbach, submitted in partial fulfillment of the requirements for the Ph.D. degree, The State College of Washington, 1952.

[3] Taken in part from a thesis by G. B. Millard, submitted in partial fulfillment of the requirements for the M.S. degree, The State College of Washington, 1951.

[4] J. Bjerrum and P. Anderson, *Kgl. Danske Videnskab Selskab, Mat.-fys. Medd*, **22**, No. 7 (1945).

[5] G. A. Carlson, J. P. McReynolds and F. H. Verhoek, THIS JOURNAL, **67**, 1334 (1945).

[6] J. Bjerrum, "Metal Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, 1941.

[7] D. Eyke, Ph.D. Thesis, Univ. of Michigan, 1952, Univ. Microfilms, Ann Arbor, Michigan, Pub. No. 5668, 1953.

TABLE I

VARIATION OF $(E_{1/2})_c$ AS A FUNCTION OF ETHYLENEDIAMINE CONCENTRATION

[en]total, (mole/l.)	[en], (mole/l.)	Reciprocal slope of $E_{d.e.}$ vs. $\log\left(\frac{i}{i_d - i}\right)$ (volts)	i_d (μamp.)	$-(E_{1/2})_c$, volts vs. S.C.E.
0.00	0.00	−0.030	3.02	0.998
.020	1.72×10^{-2}	− .034	2.90	1.299
.040	3.65×10^{-2}	− .032	2.89	1.319
.1005	9.56×10^{-2}	− .032	2.89	1.347
.201	1.95×10^{-1}	− .032	2.81	1.362
.5025	4.93×10^{-1}	− .033	2.89	1.392
1.005	9.92×10^{-1}	− .031	2.76	1.420
1.96	1.94	− .031	2.29	1.450
2.88	2.86	− .031	2.14	1.462

given by equation 1.[8] In this equation, $(E_{1/2})_s$

$$(E_{1/2})_s - (E_{1/2})_c = RT/nF \ln (1 + K_1[\text{en}^0] + K_2[\text{en}^0]^2 + K_3[\text{en}^0]^3) + \frac{RT}{nF} \ln \frac{I_c}{I_s} \quad (1)$$

and $(E_{1/2})_c$ are the half-wave potentials of the simple and complex ions, respectively; I_s and I_c are the diffusion current constants of the simple and complex ions, respectively; K_1, K_2 and K_3 are the equilibrium constants for the reaction of free metal ion with the proper number of molecules of ethylenediamine to form the complex ions $[\text{Zn(en)}]^{++}$, $[\text{Zn(en)}_2]^{++}$ and $[\text{Zn(en)}_3]^{++}$, respectively; and $[\text{en}^0]$ represents the concentration of ethylenediamine at the electrode surface.

In this case, the data can be reproduced satisfactorily by the equation

$$(E_{1/2})_s - (E_{1/2})_c = \frac{RT}{nF} \ln (K_2[\text{en}^0]^2 + K_3[\text{en}^0]^3) + \frac{RT}{nF} \ln \frac{I_c}{I_s} \quad (2)$$

if K_2 is 4.5×10^{13} and K_3 is 3×10^{14}. This is illustrated in Fig. 1 where the points represent experimental data and the curve is that calculated from equation 2 using the above values of K_2 and K_3 and the experimental values of I_c and I_s, which are directly proportional to the diffusion currents. The term $(1 + K_1[\text{en}^0])$ is not included in equation 2 because it is small compared to the term $(K_2[\text{en}^0]^2 + K_3[\text{en}^0]^3)$.

It should be mentioned at this point that the plots of $(E_{d.e.})$ vs. $\log i/(i_d - i)$ gave reciprocal slopes ranging from 0.034 volt at the lowest amine concentration to 0.031 volt at the highest. The reciprocal slope for a reversible reduction at 25° should be 0.0296 volt for a two-electron reduction.

pH Investigation.—For completeness of this investigation as a whole, a reinvestigation of the system by Bjerrum's pH method[4–6] was carried out. The values obtained for the complexity constants of the three species of complex ions are as follows: $K_1 = 1.00 \times 10^6$, $K_2 = 1.29 \times 10^{11}$ and $K_3 = 1.41 \times 10^{13}$.

Potentiometric Investigation.—A potentiometric investigation was carried out in an effort to corroborate the results of the polarographic investigation. The data are presented in Table II and are plotted in Fig. 1. For the solutions previously used in the pH studies, the concentration of free amine had been calculated, and thus it remained only to determine the electromotive force of the cell.

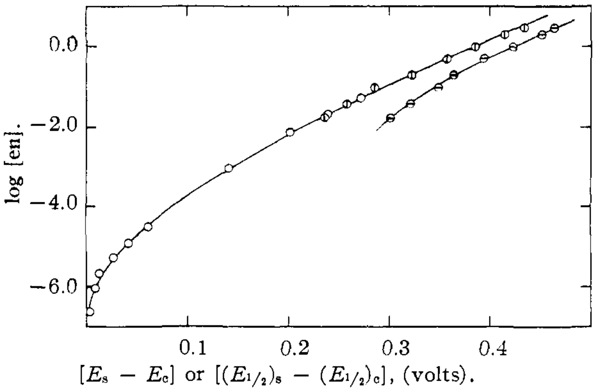

Fig. 1.—Variation of $[E_s - E_c]$, the difference of zinc amalgam electrode potentials in the absence and in the presence of ethylenediamine, and $[(E_{1/2})_s - (E_{1/2})_c]$, the difference in polarographic half-wave potentials in the absence and in the presence of ethylenediamine, as a function of the logarithm of the ethylenediamine concentration: ○, $[E_s - E_c]$ for solutions used in pH measurements; ⦶, $[E_s - E_c]$ for solutions used in the polarographic measurements; ⊖, $[(E_{1/2})_s - (E_{1/2})_c]$. The circles represent experimental data and the curves are calculated from the constants listed in the text.

The equation for the difference between the electrode potentials of the zinc amalgam electrode in the presence and absence of a complexing agent is quite analogous to equation 1 and is given by

$$E_s - E_c = \frac{RT}{nF} \ln (1 + K_1[\text{en}] + K_2[\text{en}]^2 + K_3[\text{en}]^3) \quad (3)$$

It was found that with $K_1 = 1.0 \times 10^6$, $K_2 = 6.5 \times 10^{10}$ and $K_3 = 9.5 \times 10^{12}$, the experimental data could be represented satisfactorily over most of the concentration range, as is shown in Fig. 1, where the points are the experimental values and the solid curve is that calculated from equation 3.

TABLE II

VARIATION OF THE POTENTIAL OF A ZINC AMALGAM ELECTRODE AS A FUNCTION OF ETHYLENEDIAMINE CONCENTRATION FOR THE SOLUTIONS USED IN THE POLAROGRAPHIC AND pH INVESTIGATIONS

Polarographic soln. $[\text{Zn}]_{\text{total}} = 4 \times 10^{-4}$ mole/l.; $\mu = 0.1$		pH soln. $[\text{Zn}]_{\text{total}} = 2 \times 10^{-2}$ mole/l.; $\mu = 0.5$	
[en] (mole/l.)	$-E$ (volts vs. S.C.E.)	[en], (mole/l.)	$-E$ (volts vs. S.C.E.)
0.00	1.097	0.00	1.073
1.72×10^{-2}	1.333	2.35×10^{-7}	1.076
3.65×10^{-2}	1.355	9.44×10^{-7}	1.082
9.56×10^{-2}	1.383	2.17×10^{-6}	1.086
1.95×10^{-1}	1.420	5.38×10^{-6}	1.100
4.93×10^{-1}	1.455	1.21×10^{-5}	1.115
9.92×10^{-1}	1.482	3.2×10^{-5}	1.135
1.94	1.512	9.21×10^{-4}	1.214
2.86	1.531	7.49×10^{-3}	1.277
		2.13×10^{-2}	1.312
		5.25×10^{-2}	1.345

Effect of Gelatin on $(E_{1/2})_c$.—The half-wave potential of zinc in a 0.1 M sodium perchlorate and

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[8] D. D. DeFord and D. N. Hume, THIS JOURNAL, **73**, 5321 (1951).

0.0204 *M* ethylenediamine solution was determined as a function of gelatin concentration. It was found that a gelatin concentration of 0.005% was necessary to eliminate the maximum. At gelatin concentrations less than 0.005%, the half-wave potentials taken arbitrarily, because of the maximum, as the potential of the dropping mercury electrode at a current equal to one-half the diffusion current, were essentially constant at -1.280 volts. From 0.005 to 0.02% gelatin, the half-wave potential was a function of gelatin concentration and increased from -1.293 at the former to -1.318 volts at the latter. From 0.02 to 0.05%, the half-wave potentials were again essentially constant at -1.320 volts. Since there is a maximum involved and since the effects of a maximum suppressor are rather unpredictable, a plot of gelatin concentration *vs.* $E_{1/2}$ cannot be extrapolated to zero gelatin concentration in order to obtain the half-wave potential in the absence of gelatin.

Table III

Logarithm of the Complexity Constants of the Zinc–Ethylenediamine Complex Ions

	This investigation			Bjerrum[4]	Carlson[5]
Method	Polarographic	Potentiometric	*p*H	*p*H	*p*H
log K_1	...	6.00	6.00	5.92	5.71
log K_2	13.65	10.81	11.11	11.07	10.37
log K_3	14.48	12.98	13.15	12.93	12.09
Temp., °C.	25	25	25	25	30
μ	0.1, 1.0	0.1, 0.5	0.5	1.0	1.3

Discussion

In Table III are collected the complexity constants for the zinc–ethylenediamine complex ions which were determined in this investigation and those previously reported by other investigators.

The general agreement of the complexity constants, as determined by *p*H measurements and potentiometric measurements, is such that it would be difficult to dispute their validity. The activity of the zinc ion is determined directly in the potentiometric method and indirectly in the *p*H method, and yet these two vastly different procedures yield values in close agreement.

The complexity constants determined polarographically are much greater than any of those determined by other methods. If there were ever to be any agreement between the potentiometrically and polarographically determined values, it should be in this case where portions of the same identical solutions were used in both investigations. In principle, the polarographic method and the potentiometric method are both based on direct measurement of the activity of the zinc ion, and since they require the measurement of the same quantity, the two methods should give identical values for the complexity constants.

It should also be observed that the two sets of data obtained in the potentiometric investigations, where the nature of the solutions and the ionic strengths were different, could be interpreted with the same set of constants.

One possible explanation of why the potentiometric method and the polarographic method do not give the same result might lie in the fact that, in the polarographic method, ethylenediamine is liberated at the surface of the dropping mercury electrode as the zinc complex ion is reduced. In order for the reciprocal slope of the reversibility plot to be 0.0296 volt, the concentration of free amine must be so large that the amount of amine generated at the electrode surface by reduction of the zinc complex ion is negligible. If the concentration of the amine is not great enough for the increase at the electrode surface to be negligible, the polarographic wave will be more drawn out, and the half-wave reduction potential will be more negative than if the increase is negligible. Overlooking this increase results in the complexity constants evaluated from polarographic data being greater than those evaluated from potentiometric data. Ringbom and Eriksson[9] have outlined a method of treatment of data to take into account such an increase. The increase in concentration of amine at the electrode surface can also be calculated approximately in a manner indicated by Kolthoff and Lingane.[10] It would be expected that any influence of the increase of amine concentration would be greatest at the lowest concentration of ethylenediamine, because the increase is independent of the total amine concentration. For 1.72×10^{-2} *M* ethylenediamine, the increase is calculated to be 0.6×10^{-3} mole/l., and its effect on the half-wave potential is calculated to be of the order of 1 mv. An effect of the order of 65 mv. is necessary to account for the difference between the polarographically and potentiometrically determined constants. Thus the discrepancy between the constants cannot be attributed to the increase in amine concentration at the electrode surface.

It is usually assumed, when the reciprocal slope of the reversibility plot is between 0.030 and 0.035, that the reduction process is reversible. However, Randles[11] and Somerton[12] have found that the rate constant for the exchange of zinc ion between a zinc salt solution and a dropping zinc amalgam electrode is somewhat smaller than the rate constants for the corresponding process for thallous, plumbous, cadmium and alkali metal ions. Consequently the zinc electrode system would not be as reversible as the others. In addition, these authors also found that the presence of gelatin retarded the exchange reaction. Thus the presence of gelatin in the solutions under investigation here would tend to make the reduction process still more irreversible.

If the system were not a reversible one, the effect of an overvoltage on a cathodic process would be to make the half-wave potential more negative and thus increase the apparent formation constant, which is in agreement with the observed derivations. It was found in this investigation that a maximum variation in the half-wave potential of approximately 40 mv. could be achieved by changing the gelatin concentration. It is entirely possible that gelatin would have sufficient effect on the

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(9) A. Ringbom and L. Eriksson, *Acta Chim. Scand.*, **7**, 1105 (1953).

(10) I. M. Kolthoff and J. J. Lingane, "Polarography," Interscience Publishers, Inc., New York, N. Y., 1952, p. 213.

(11) J. E. B. Randles, *Discs. Faraday Soc.*, **1**, 11 (1947).

(12) J. E. B. Randles and K. W. Somerton, *Trans. Faraday Soc.*, **48**, 951 (1952).

polarographic half-wave potential, while not having a corresponding effect on the potential of a stationary electrode, to cause the difference observed between the polarographically and potentiometrically determined values of the complexity constants.

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[CONTRIBUTION FROM THE STUDY GROUP ON RHEUMATIC DISEASES, AND THE DEPARTMENTS OF CHEMISTRY AND MEDICINE, NEW YORK UNIVERSITY COLLEGE OF MEDICINE]

A Qualitative Theory of Metachromasy in Solution[1]

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RECEIVED FEBRUARY 1, 1955

A theory is proposed to account for the production of metachromatic colors in solutions of anionic mucopolysaccharides or anionic detergents. It is based on four points: (1) that chromotropes exist in solution as anionic clusters or micelles, (2) that cations are reversibly bound within such micelles, and are bound more firmly the higher the cationic charge, (3) that metachromatic dyes exist in solution in a set of equilibria among polymeric dye ions with different charges, (4) that the chromotropic micelle selectively and reversibly binds the dye ion of highest charge and so causes a shift in the equilibrium of dye ions in the intermicellar space. Some new experimental data in support of this theory are found in a detailed examination of the deviations from Beer's law of several dyes. The theory is able to make some predictions on whose verification its validity will depend.

Previous studies from this Laboratory[2-4] as well as from others[5-7] provide a collection of data which with further data here presented makes possible a theory of metachromasy limited to aqueous solutions. The theory is therefore not directly applicable to the conditions under which tissues are stained for histological purposes. Though it is only qualitative, it should be possible to develop it quantitatively when further information is available.

The theory consists of four postulates, parts of which have been proposed by earlier workers. (1) Chromotropes exist in solution as anionic globular clusters or micelles whose charge density depends on the nature of the chromotrope and the solution environment. These micelles are separated by intermicellar spaces of small anion density. (2) Cations are reversibly, and more or less firmly, bound within such anionic clusters or micelles. The relative firmness of binding of different cations increases with their charge. (3) In solutions of metachromatic dyes there is a set of equilibria among several polymers of the cation represented by the equations,

$$D^{+} \rightleftarrows D_2^{++} \rightleftarrows D_3^{+++} \rightleftarrows D_4^{++++} \rightleftarrows \qquad \text{(I)}$$

The proportions of the several molecular species will depend on the dye concentration. Each molecular species has its own characteristic absorption spectrum. (4) The metachromatic color is produced as a result of the selective and reversible binding within the anionic cluster, of the polymeric dye cation of highest charge available. Binding of the dye cation of high charge causes a shift in the equilibria (I) with a drop in the concentration of ions of lower charge. The effect is that even in dilute dye solutions the concentration of dye cations of high charge is increased in the total micellar and intermicellar space. Experimental data in support of each of these postulates will now be discussed.

Chromotropes are of two kinds: anionic polyelectrolytes of high molecular weight such as chondroitin sulfate, heparin, hyaluronate, alginate, silicates and metaphosphates and anionic detergents. Work of Ogston and Stanier[8] and of Blumberg and Oster[9] shows that hyaluronate exists in solution as spherical particles of molecular weight of the order of 10^7. The same particle shape is assumed by Fuoss and Strauss[10] to account for the viscosity properties of synthetic linear polyelectrolytes. Detergents have been assumed to exist in solutions as micelles.[11] Thus the first postulate extends to all chromotropes a property which has been found for some and is generally assumed for others.

That cations become reversibly bound within charged globular micelles is supported by experimental data on the properties of Fuoss clusters[12] and Hartley micelles.[13] That the firmness of binding of cations within such micelles should increase with the cationic charge is an extension of this idea. The dependence of firmness of binding on cationic charge is a phenomenon analogous to the rule of Schulze and Hardy in the flocculation of hydrophobic colloids[14] and to the reversal of charge produced in anionic polyelectrolytes by cations of different valence.[15] The second postulate is merely a statement of the behavior that would be expected of cations in a solution containing anionic micelles. Such binding can lead to precipitation particularly with cations of high charge. This occurs when lysozyme or protamine is added to solutions of chondroitin sulfate, heparin or hyaluronate, or when dye

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[1] This investigation was supported (in part) by research grant A28 (C) from the National Institute of Arthritis and Metabolic Diseases, of the National Institutes of Health, U. S. Public Health Service, and (in part) by the Masonic Foundation for Medical Research and Human Welfare.

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[3] A. Levine and M. Schubert, *ibid.*, **74**, 5702 (1952).

[4] M. Schubert and A. Levine, *ibid.*, **75**, 5842 (1953).

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[7] L. Michaelis and S. Granick, THIS JOURNAL, **67**, 1212 (1945).

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[10] R. M. Fuoss and U. P. Strauss, *Ann. N. Y. Acad. Sci.*, **51**, 836 (1949).

[11] A. W. Ralston, *ibid.*, **46**, 351 (1946).

[12] R. M. Fuoss, *Science*, **108**, 545 (1948).

[13] H. L. Booij, in "Colloid Science," ed. H. R. Kruyt, Vol. 2, Elsevier Publishing Co., New York, N. Y., 1949, p. 681.

[14] J. T. G. Overbeek, ref. 13, Vol. 1, 1952, p. 81.

[15] H. G. Bungenberg de Jong, ref. 13, Vol. 2, 1949, p. 259.