1963

Oxidation-reduction couples bearing chelating groups

Anthony David Pietrzykowski
Iowa State University

Recommended Citation

Pietrzykowski, Anthony David, "Oxidation-reduction couples bearing chelating groups " (1963). *Retrospective Theses and Dissertations*. 2551.
https://lib.dr.iastate.edu/rtd/2551

PIETRZYKOWSKI, Anthony David, 1935-
OXIDATION-REDUCTION COUPLES BEARING
CHELATING GROUPS.

Iowa State University of Science and Technology
Ph.D., 1963
Chemistry, analytical

University Microfilms, Inc., Ann Arbor, Michigan

OXIDATION-REDUCTION COUPLES BEARING CHELATING GROUPS

by

Anthony David Pietrzykowski

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Analytical Chemistry

Approved:

In Charge of Major Work

Head of Major Department

Dean of Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa

1963

TABLE OF CONTENTS

I. INTRODUCTION

The important role played by the hydrogen ion in chemical reactions has been understood for many years and various methods have been devised for its measurement. The most precise of the methods which have been developed for the determination of the hydrogen ions are those which depend on electrochemical measurements. Today, the most common method is that using the glass electrode, that is the so-called "pH meter". The glass electrode is compact, precise, largely free from disturbance by other ions, and does not contaminate the solution being examined. As an analytical method it is without equal for specificity, sensitivity, range, and convenience.

The determination of the concentration of ions of the various metals is in a far less happy state. During the past twenty years several attempts have been made to devise membranes sensitive to metal ions in the fashion the glass membrane is sensitive to hydrogen ions. However, the potentials across these membranes are small and must be measured through very high resistances but this problem has been solved by modern electronics. The present problem is one of building specificity into the membrane and no real solution has yet been offered. Marshall (1) has shown that membranes consisting of clays and zeolites give rise to potentials related to the total cationic species in the solutions and he has succeeded in incorporating some degree of specificity in some clay membranes by heat treatment, making them selective to monovalent cations. Ives and Janz (2, p. 429) report that specificity can also be increased by soaking some of the membranes in silicone fluids. Similar work was done by Wyllie (3) using membranes of ion exchange materials, and membranes permeable to anions were devised by Sollner (4) by suitably modifying collodion. A somewhat different approach was adopted by Gregor (5) using a multilayer of an alkaline earth stearate applied to the two broken ends of a glass plate. The plate is then fitted together and the two solutions placed in contact with the opposite faces. Identical reference electrodes inserted into the solutions show between them a potential difference determined by the difference in the alkaline metal ion concentrations. So far no practical use has been made of this electrode although Gregor reports fine results with barium.

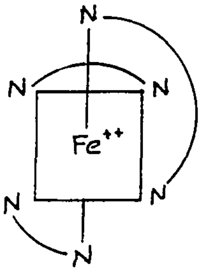
Recently, Diehl and Lohman (6) developed a theory for a metallic ion indicating electrode based on analogy with the quinhydrone electrode but incorporating also chelation, that is, the property of forming metal derivatives of cyclic nature. The theory involved the quinhydrone of 5,8-dihydroxyquinoline and 5,8-dioxoquinoline but the electrode could not be realized because the quinhydrone was not stable.

The present work is a further investigation of organic compounds which have both oxidation-reduction and chelating properties. In particular the investigation concerns the effect that the union with a metal ion has on the oxidation-reduction property of certain organic molecules. The techniques of polarography have been used principally to measure these effects.

II. HISTORICAL REVIEW

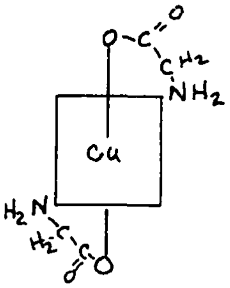
A. Chelate Rings

Compounds such as trisbipyridine iron(II) ion

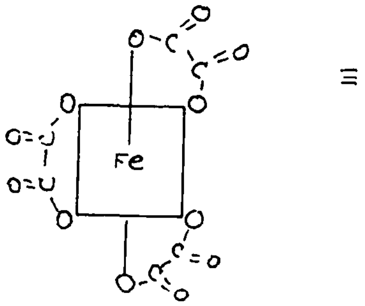


($\overset{\frown}{N\ N}$ represents bipyridine),

copper(II) diglycinate



and trioxalato ferrate(III) ion



in which the organic compounds are joined to the metal to form ring structures are examples of chelate-ring compounds. The term "ligand" is used to designate the organic (or other) radical or compound uniting with the metal atom. The term chelate is derived from a Greek word χελα , claw of the crab, referring to the pincer-like action of the ligand on the metal atom.

In the classification of chelate-ring compounds by Diehl (7), ligands are grouped according to the number of attachments made to the metal. Thus, unidentate, literally one-toothed; bidentate; tridentate; quadridentate; and sexadentate. Ligands are further classified according to the functional groups present. The elements through which attachments are made are commonly nitrogen, oxygen and sulfur. These elements are usually incorporated in some functional group, such as the carboxy, hydroxy, amino and thiol groups. The groups may be acid or basic; thus, the acid groups are -COOH, -OH, =NOH, $-SO_3H$, -SH and the basic groups, $-NH_2$, =N-, =O, =NOH. The acidic groups lose a proton on union with the metal atom while the bases donate a pair of electrons directly to the metal. Chelate-ring formation may take place with ligands bearing any combination of acidic and basic groups. The charge remaining on the metal compound is determined by the original charge on the free metal ion and the number of protons displaced from the ligands which become attached.

Chelate rings form chiefly when the positions of the functional groups in the ligand are such that five-membered or six-membered rings form on union with the metal atom. Four-membered and seven-membered chelate-ring compounds are known but the strains in such rings reduce the stability of such compounds. Additional stability is obtained as the number of attachments per ligand increases. A striking example of this is the calcium derivative of ethylenediaminetetraacetic acid; normally, calcium does not form coordination compounds but with ethylenediaminetetraacetic acid, a sexadentate ligand, with which calcium unites by no less than six bonds, a compound is formed and the stability is very high.

Chelate-ring compounds are important in diverse fields, notably in biochemistry (hemoglobin, chlorophyll, many enzymes) and in analytical chemistry (chelometric titrations, masking agents, precipitating agents).

B. Organic Oxidation-Reduction Couples; Reversible Systems

Many organic oxidation-reduction couples are known and have been thoroughly studied, the prominent early workers in the field being J. B. Conant, L. F. Fieser and L. Michaelis. Couples which exhibit complete reversibility are relatively few. To say that a couple is reversible is to imply that the electrical potential of a cell, made between two reversible couples, is related to the chemical process in that cell. A reversible cell, when connected to some external source of voltage of such magnitude that no current is allowed to flow in the cell, will show no chemical change of the couples involved. However, when the external voltage exceeds the null point (no current flow in the cell) a current will flow and a chemical change proportional to the quantity of electricity passing will take place. When the external voltage is less than the voltage at the null point, current will flow, but in the opposite direction and the chemical reaction will be exactly reversed.

Organic oxidation-reduction systems which are reversible, generally occur in compounds in which the hydrogen atom unites with a sufficiently electronegative atom such as oxygen or nitrogen. The most widely known couples which show reversibility are the aromatic dihydroxy or diamine compounds. The aromatic character of some of these compounds help to stabilize them by distributing the charge of the added or removed electron or proton.

The most extensively studied organic couple has been the quinone-hydroquinone system. This system shows immediate electrochemical response and is the basis of a pH indicating electrode, the quinhydrone electrode. Quinhydrone is a 1:1 compound formed between quinone and hydroquinone.

Haber and Russ (8) were the first to establish that quinone and hydroquinone form an electrochemically reversible oxidation-reduction couple in which the hydrogen ion is involved. It was Biilmann (9), seventeen years later, who realized the potentialities of the discovery and utilized the quinhydrone electrode for the determination of hydrogen ion concentration. The quinhydrone electrode is based on the following sequence of reactions.

$$(2.1) \quad Q + 2H^+ + 2e \rightleftharpoons H_2Q$$

where Q and H_2Q represent quinone and hydroquinone, respectively. By applying the Nernst equation, the potential of the half-cell is

$$(2.2) \quad E = E^o + \frac{0.059}{2} \log (Q)\ (H^+)^2/(H_2Q) \quad \text{at} \quad 25^o.$$

E^o is the standard potential of the quinone-hydroquinone couple and the parentheses represent concentration units. Assuming concentration is equal to activity the above equation holds true. The equation may be rearranged to

$$(2.3) \quad E = E^o + \frac{0.059}{2} \log (Q)/(H_2Q) + 0.059 \log (H^+).$$

Under the conditions where Q and H_2Q form an insoluble molecular complex, quinhydrone, the concentration of Q and H_2Q are equal to each other, and the equation simplifies to

$$(2.4) \quad E = E^o + 0.059 \log (H^+).$$

That is, the potential varies directly with the logarithm of the hydrogen ion concentration. The operation involves simply saturating the solution to be measured with quinhydrone, dipping a platinum electrode into the solution, and measuring the potential of the platinum electrode against a reference electrode.

With the advent of modern electronic equipment making it possible to measure accurately small voltages through very high resistances, the quinhydrone electrode was displaced by the glass electrode for the practical measurement of pH. About the only work with the quinhydrone electrode in very recent times is that of Diehl and Lohman (6) who attempted to prepare an analog which would be responsive to metal ions by combining oxidation-reduction and chelating functions.

In the Diehl and Lohman theory, the equation

$$(2.5)\quad E = E^{o}_{Q,H_2Q} + \frac{0.059}{2}\log\frac{K_2}{K_1} - \frac{0.059}{2}\,\mathrm{pM}$$

is developed, in which E^{o}_{Q,H_2Q} is the standard potential of a couple such as 5,8-dioxoquinoline and 5,8-dihydroxyquinoline, K_2 is the solubility product constant of the quinhydrone of 5,8-dioxoquinoline and 5,8-dihydroxyquinoline, K_1 is the formation constant of the metal with 5,8-dihydroxyquinoline, and pM represents the negative logarithm of metal concentration. By measuring the potential of the cell,

$$\mathrm{Pt};\ M^{++}\left(xM\ \begin{matrix}\text{satd. } M(HQ)_2\\ \text{satd. } H_2Q_2\end{matrix}\right)\ //\ \mathrm{KCl(satd.)};\ \mathrm{Hg_2Cl_2{:}Hg}$$

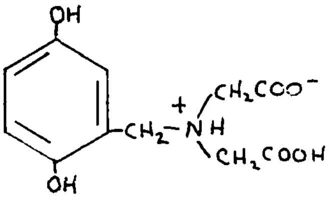
where $M(HQ)_2$ and H_2Q_2 represent the metal derivative of 5,8-dihydroquinoline and the quinhydrone of this particular couple, respectively, it would be possible to determine the metal ion concentration using equation (2.6).

$$(2.6) \quad E = E^{o'} \frac{-0.059}{2} \quad pM$$

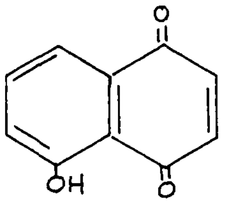
In equation (2.6), $E^{o'}$ is a new constant made up by incorporating the first two terms on the right-hand side of equation (2.5). The cell reaction is represented by

$$H_2Q_2 + M^{++} + 2Cl^- + 2Hg \rightleftharpoons M(HQ)_2 + Hg_2Cl_2 .$$

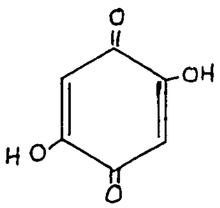
In the present work, compounds are investigated which also contain chelating and oxidation-reduction functions. Specifically the compounds are: 1,4-dihydroxyphenylmethyleneiminodiacetic acid (I), 5-hydroxy-1,4-naphthoquinone (II), and 2,5-dihydroxy-p-benzoquinone (III).



I



II



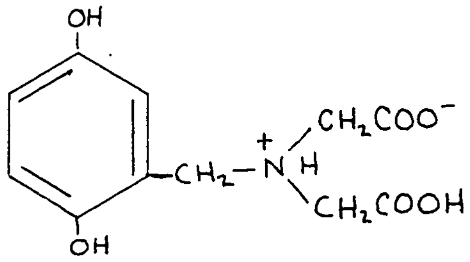
III

The investigation is centered on the combination of these compounds with various metals, the effect of such chelation on the oxidation-reduction characteristics, and the existence of quinhydrones.

III. STUDY OF 1,4-DIHYDROXYPHENYLMETHYLENEIMINODIACETIC ACID

A. Introduction

1,4-Dihydroxyphenylmethyleneiminodiacetic acid



has not been reported previously. The synthesis proposed is the reaction of hydroquinone, formaldehyde, and sodium iminodiacetate in the ratio of one to one to one with glacial acetic acid as solvent. 1,4-Dihydroxyphenylmethyleneiminodiacetic acid would be expected to exist as the zwitter ion as shown in the structural formula above. It would be expected also to be susceptible to oxidation and that with the dioxo, or quinone so produced should constitute a reversible couple. This couple in turn should be influenced by union of the oxidized or reduced forms of the couple with metal ions. The chelating properties of 1,4-dihydroxyphenylmethyleneiminodiacetic acid with alkaline earth metals calcium and magnesium, and some other selected metals, should be of interest also because of the analogy of the molecule to ethylenediaminetetraacetic acid and the possibilities of using it for chelometric titrations.

B. Experimental Work

1. Preparation

One hundred fifty milliliters of glacial acetic acid were placed into a one liter beaker and heated to 65°. Twenty grams of disodium iminodiacetate was dissolved in the glacial acetic acid. Then 11.0 g. of hydroquinone and 10 ml. of 37% formaldehyde were added with stirring. The mixture was stirred until all of the hydroquinone was rendered soluble. The mixture was light yellow in color at this stage of the reaction. The solution was then cooled slowly and allowed to stand for about two hours at room temperature. A white precipitate formed which was then washed several times with water to remove the acetic acid. The product was filtered and washed with three portions of absolute alcohol and with three portions of ether. The ether was evaporated, the product was transferred to a vacuum desiccator containing sodium hydroxide, and allowed to dry for six hours. Finally, the product was dried in an oven for eight hours at 120°. The yield was 24.2 g., 95% of theoretical; melting point with decomposition 235°. No weighable ash remained after ignition in a platinum crucible.

2. Ultimate analysis

Found (Huffman Microanalytical Laboratory, Wheatridge, Colorado): 51.68% C, 5.14% H, 4.77% N; calculated for $C_{11}H_{13}NO_6$, 51.77% C, 5.13% H, 5.49% N.

A nitrogen determination by the Kjeldahl method using mercuric oxide and potassium sulfate as digestion aids was carried out by H. W. Wharton of the Procter and Gamble Company, Cincinnati, Ohio, the value found being 5.39% N.

3. Alkalimetric titration and dissociation constants

A neutralization titration of 1,4-dihydroxyphenylmethyleneiminodiacetic acid was carried out using the Beckman Model G pH meter. The Beckman 40495 blue glass electrode was used in conjunction with a Beckman saturated calomel fiber electrode. The glass electrode was calibrated with Beckman buffer solutions having a pH of 4.00 and 7.01, respectively. 1,4-dihydroxyphenylmethyleneiminodiacetic acid,0.1615 g., was dissolved in 100 ml. of deionized water by heating to 60°. The solution was cooled to 24° and titrated with 0.1581 N sodium hydroxide, Figure 1. Two end points were observed. The respective equivalent weights were 254.8; the theoretical 255.2, and 127.1; theoretical 127.6.

4. Oxidimetric titration

A titration of 1,4-dihydroxyphenylmethyleneiminodiacetic acid with potassium dichromate was carried out potentiometrically using the usual platinum-saturated calomel electrode couple and a Beckman Model G pH meter to measure the potential. The titration was carried out in 1.0 M sulfuric acid. 1,4-dihydroxyphenylmethyleneiminodiacetic acid, 0.4135 g. was placed in 50 ml. of deionized water and 5.5 ml. of sulfuric acid was added. The solution was heated to 60° to dissolve the sample. The solution was diluted to 100 ml. with deionized water, cooled to room temperature, then titrated with 0.2153 N potassium dichromate, 15.00 ml. being required. Equivalent weight found: 128.2; theoretical for a two-electron oxidation: 127.6.

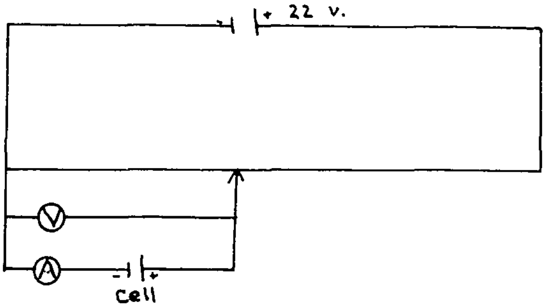
5. Preliminary examination of chelating properties

Qualitative tests made on a few metal ions to see if slightly ionized compounds were formed. 1) To a solution containing a few milligrams of magnesium perchlorate and Calmagite at pH 10 was added 1,4-dihydroxyphenylmethyleneiminodiacetic acid. The color changed from red to blue indicating that the magnesium-Calmagite compound was broken up by the formation of a more stable compound between magnesium and 1,4-dihydroxyphenylmethyleneiminodiacetic acid. 2) The pH of a solution, 0.001 M in 1,4-dihydroxyphenylmethyleneiminodiacetic acid, was adjusted to pH 8 with ammonia, and 0.1 M calcium chloride solution was added very slowly. The pH of the solution dropped to 6.2, indicating that a calcium compound was formed with the displacement of hydrogen ions. 3) To a solution 0.1 M in aluminum at pH 4.0 was added 0.1 M 1,4-dihydroxyphenylmethyleneiminodiacetic acid. The pH dropped to 3.1, indicating that a compound was formed.

6. Polarography; 1,4-dihydroxyphenylmethyleneiminodiacetic acid at pH 6.0 acetate buffer

The polarography of 1,4-dihydroxyphenylmethyleneiminodiacetic acid was investigated in an acetate buffer-supporting electrolyte using a Sargent Model XXI polarograph. The cell consisted of a 4 oz. wide-mouth bottle with a rubber stopper having three holes for insertion of the capillary, salt bridge and the nitrogen inlet tube. A saturated calomel electrode was made according to the recommended procedure in Steinbach and King (10). The solutions were deareated with tank nitrogen. The voltage span was 1.0 volt and the sensitivity 0.006 microamp./mm. The capillary drop time was 5 seconds. When necessary, to avoid contamination with chloride ion, a potassium nitrate salt bridge was employed.

A solution, 0.001 M in 1,4-dihydroxyphenylmethyleneiminodiacetic acid, was prepared by pipetting 1.00 ml. of a 0.010 M solution of the reagent into a 100-ml. volumetric flask, adding 25 ml. of pH 6.0 acetate buffer, and then diluting with deionized water. A 50 ml. portion of the 0.0001 M solution was placed into the polarographic cell, and after purging the solution with nitrogen a polarogram obtained, Figure 3. The cell resistance was measured according to the procedure of Müller (11). The circuit diagram is:



Voltages ranging from 3 to 18 volts were applied to the cell and the current measured with an ammeter. The resistance for each combination of voltage and current was calculated until a limiting value was obtained. The value obtained for the acetate solution was 3000 ohms.

7. Polarograph; 1,4-dihydroxyphenylmethyleneiminodiacetic acid in pH 8.3, borate buffer

The use of borate buffer was called for because it allowed a larger voltage span to be traversed on the polarogram at slightly alkaline pH values. Buffers using ammonia allowed positive potentials of 0.1 volt vs. s.c.e. to be attained, while borate can be used to 0.2 volts. Attainment of positive potentials was required because any shift of the half-wave potential due to compound formation would be in the positive direction for this particular system.

Exactly one milliliter of 0.010 M 1,4-dihydroxyphenylmethyleneiminodiacetic acid was placed into a 100 ml. volumetric flask. Fifty milliliters of pH 8.3 borate buffer were added, and the solution diluted to mark. A 50 ml. portion was placed into the polarograph cell, flushed with nitrogen, and a polarogram obtained. The wave was fairly well defined and was of the same form as in Figure 3, the polarogram of 1,4-dihydroxyphenylmethyleneiminodiacetic acid in pH 6.0 buffer.

8. Polarography; 1,4-dihydroxyphenylmethyleneiminodiacetic acid in the presence of various metals

Studies of the polarography of 1,4-dihydroxyphenylmethyleneiminodiacetic acid in the presence of various metals were carried out with the apparatus described in Section 6.

a. Calcium Calcium nitrate, 0.010 M was prepared by dissolving 0.1001 g. of calcium carbonate in about 5 ml. of dilute nitric acid and diluting to 100.0 ml. with deionized water. Exactly four milliliters of 0.010 M calcium nitrate, 1.00 ml. of 0.010 M 1,4-dihydroxyphenylmethyleneiminodiacetic acid, and 50 ml. of pH 8.3 borate buffer were placed into a 100-ml. volumetric flask and diluted to mark. A 50 ml. portion was placed in the cell and flushed with nitrogen, and a polarogram obtained. The voltage scan was from -0.2 to +0.2 volts vs. s.c.e.

b. Magnesium Magnesium sulfate, 0.010 M, was prepared by dissolving 0.1201 g. of magnesium sulfate in deionized water and diluting to mark in a 100-ml. volumetric flask. Four milliliters of 0.010 M magnesium sulfate, 1.00 ml. of 0.010 M 1,4-dihydroxyphenylmethyleneiminodiacetic acid, and 50 ml. of pH 8.3 borate buffer were placed into a 100-ml. volumetric flask and diluted to mark. A 50 ml. portion was placed in the cell, flushed with nitrogen, and a polarogram obtained. The voltage scan was from 0.2 to 0.2 volts vs. s.c.e.

c. Copper Copper acetate, 0.010 M, was prepared by dissolving 0.2007 g. of cupric acetate monohydrate in deionized water and diluting to 100.0 ml. in a volumetric flask. Exactly one milliliter of 0.010 M copper acetate was pipetted into a 100-ml. volumetric flask, 25 ml. of pH 6.0 acetate buffer was added, and the solution diluted to mark. A portion of the solution was placed in the polarographic cell, flushed with nitrogen, and a polarogram obtained.

Exactly one milliliter of 0.010 M copper acetate, and 4.00 ml. of 0.010 M 1,4-dihydroxyphenylmethyleneiminodiacetic acid, and 25 ml. of pH 6.0 acetate buffer were placed in a 100-ml. volumetric flask, and the solution diluted to mark. A 50 ml. portion was placed in the cell, flushed with nitrogen, and a polarogram obtained. The voltages scanned included the region at which the oxidation of the 1,4-dihydroxyphenylmethyleneiminodiacetic acid and the reduction of the copper compound could be observed.

d. Zinc Zinc acetate, 0.010 M, was prepared by dissolving 0.2188 g. of zinc acetate dihydrate in deionized water and diluting to 100 ml. in a volumetric flask. 1.00 ml. of 0.010 M zinc acetate, 25 ml. of pH 6.0 acetate buffer were placed into a 100-ml. volumetric flask and diluted to mark. A 50 ml. portion of this solution was placed in the polarographic cell, purged with nitrogen, and a polarogram obtained.

Exactly one milliliter of 0.010 M zinc acetate, and 4.00 ml. of 0.010 M 1,4-dihydroxyphenylmethyleneiminodiacetic acid, and 25 ml. of pH 6.0 acetate buffer were placed into a 100-ml. volumetric flask and diluted to mark. A 50 ml. portion was placed into the cell, flushed with nitrogen, and a polarogram obtained. The voltages scanned included the regions at which the oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid and the zinc compound could be observed.

e. Nickel Nickel acetate, 0.010 M, was prepared by dissolving 0.2499 g. of nickel acetate tetrahydrate in deionized water and diluting to mark in a 100-ml. volumetric flask. 1.00 ml. of 0.010 M nickel acetate, 25 ml. of pH 6.0 acetate buffer were placed into a 100-ml. volumetric flask and diluted to volume. A 50 ml. portion was placed into the polarographic cell, flushed with nitrogen, and a polarogram obtained.

Exactly one milliliter of 0.010 M nickel acetate, 4.00 ml. of 1,4-dihydroxyphenylmethyleneiminodiacetic acid and 25 ml. of pH 6.0 acetate buffer were placed into a 100-ml. volumetric flask and diluted to mark with deionized water. A 50 ml. portion was placed into the cell, flushed with nitrogen, and a polarogram obtained.

f. Aluminum Aluminum nitrate, 0.010 M, was prepared by dissolving 0.3751 g. of aluminum nitrate nine hydrate in deionized water and diluting to mark in 100-ml. volumetric flask. Exactly four milliliters of 0.010 M aluminum nitrate, 1.00 ml. of 0.010 M 1,4-dihydroxyphenylmethyleneiminodiacetic acid and 25 ml. of pH 6.0 acetate buffer were placed into a 100-ml. volumetric flask and diluted to mark with deionized water. A 50 ml. portion was placed into the polarographic cell, flushed with nitrogen and a polarogram obtained.

g. Bismuth Bismuth nitrate, 0.010 M, was prepared by dissolving 0.4851 g. of bismuth nitrate pentahydrate in 10 ml. of dilute nitric acid and diluting to mark with deionized water in a 100-ml. volumetric flask. Exactly four milliliters of 0.010 M bismuth nitrate, 1.00 ml. of 1,4-dihydroxyphenylmethyleneiminodiacetic acid and 50 ml. of pH 6.0 acetate buffer were placed into a 100-ml. volumetric flask and diluted to mark with deionized water. The pH of the solution was 6.0. A 50 ml. portion was placed into the polarographic cell and flushed with nitrogen, and a polarogram obtained.

9. Polarography; determination of the combining ratio of 1,4-dihydroxyphenylmethyleneiminodiacetic acid and nickel

To each of six 50-ml. volumetric flasks was placed 15 ml. of pH 6.0 acetate buffer and 1.00 ml. of 0.010 M nickel nitrate solution. Into the flasks successively was added 0, 0.50, 1.00, 1.50, 2.00, 3.00 ml. of 0.010 M 1,4-dihydroxyphenylmethyleneiminodiacetic acid solution. The solutions were diluted to volume with deionized water. Each solution was flushed with nitrogen for ten minutes and polarograms obtained. The sensitivity of the polarograph was 0.006 microamp./mm. and the voltage span was 2.00 volts.

10. Determination of the combining ratio of 1,4-dihydroxyphenylmethyleneiminodiacetic acid and copper

To each of five 50-ml. volumetric flasks was added 15 ml. of pH 6.0 acetate buffer and 1.00 ml. of 0.010 M copper nitrate solution. To successive flasks was added 0, 0.25, 0.50, 1.00, 2.00 ml. of 0.010 M 1,4-dihydroxyphenylmethyleneiminodiacetic acid. The solutions were diluted to mark with deionized water. Each solution was flushed with nitrogen for ten minutes and polarograms obtained. The sensitivity of the polarograph was 0.006 microamp./mm. and the voltage span of 1.00 volt.

11. Polarography; determination of the combining ratio of 1,4-dihydroxyphenylmethyleneiminodiacetic acid and aluminum

To each of six 50-ml. volumetric flasks was placed 10 ml. of pH 6.0 buffer and 1.00 ml. of 1,4-dihydroxyphenylmethyleneiminodiacetic acid. Into successive flasks were placed 0, 0.25, 0.50, 0.75, 1.00, and 2.00 ml. of 0.010 aluminum nitrate solution. The solutions were diluted to mark with deionized water. Polarograms were obtained for each solution after flushing with nitrogen for ten minutes. A sensitivity of 0.008 microamp./mm. and a one volt span was used.

12. Determination of the formation constants of calcium, magnesium and copper with 1,4-dihydroxyphenylmethyleneiminodiacetic acid

a. Apparatus and solutions A constant temperature water bath maintained at $30 \pm 0.1^{\circ}$ was used to keep the solutions to be titrated at a constant temperature. The titration vessel was a 200-ml. closed vessel which contained three openings; one each for the glass electrode, calomel electrode, and the buret tip. Along the side of the vessel, near the bottom, was a gas inlet tube for introducing nitrogen into the solution. Nitrogen was used to stir the solution to be titrated and to keep the solution free from carbon dioxide and oxygen. The Beckman Model G pH meter and 40495 blue glass electrode was used to measure the pH. The meter was calibrated with Beckman pH 4.00 and 7.01 pH buffers.

The titrant, 0.07070 N potassium hydroxide, was carbonate-free. Carbonate-free potassium hydroxide was prepared by passing a 4% solution of potassium hydroxide through Amberlite IRA-400 strong base anion exchange resin in the chloride form. One hundred five milliliters of the carbonate free 4% solution was diluted to one liter and standardized against potassium hydrogen phthalate.

<u>b. Procedure</u> One hundred fifty milliliters of 1.030×10^{-3} M 1,4-dihydroxyphenylmethyleneiminodiacetic acid, which was adjusted to an ionic strength of 0.100 with potassium chloride, was placed into the titration vessel. The vessel containing the acid was then placed into the constant temperature bath which was maintained at $30\pm 0.1^{\circ}$. After allowing thirty minutes for the solution to equilibrate, the solution was titrated by the addition of small increments of 0.07060 N potassium hydroxide, and the pH was measured.

Solutions containing 1.030×10^{-3} M 1,4-dihydroxyphenylmethyleneiminodiacetic acid and 1.030×10^{-3} M metal ion were titrated in the same manner as above. The metal ions investigated included calcium, magnesium and copper. The source of these ions were from anhydrous calcium chloride, anhydrous magnesium perchlorate and reagent grade copper sulfate. The titration curves are shown in Figure 7. The shape of the titration curves of the metal compounds indicated that a third replaceable hydrogen was present. This could not be determined from the shape of the titration curve of the free-acid.

<u>c. Determination of the acid dissociation constant of the ortho hydroxyl group of 1,4-dihydroxyphenylmethyleneiminodiacetic acid</u> A series of twelve solutions, containing 1.00 ml. of 0.010 M 1,4-dihydroxyphenylmethyleneiminodiacetic acid diluted to 50.0 ml. in a volumetric flask with universal buffer of pH values ranging from 8.3 to 11.4, was prepared. The universal

buffer was prepared according to the directions given in Vogel (12). The universal buffer consisted of citric acid, potassium dihydrogen phosphate, boric acid and diethylbarbituric acid which was adjusted to the desired pH with 0.2 N sodium hydroxide. The pH was checked with a Beckman Model G pH meter. Each of the solutions was deaerated with nitrogen and a polarogram obtained.

C. Results and Discussion

1,4-Dihydroxyphenylmethyleneiminodiacetic acid was synthesized by reacting hydroquinone, formaldehyde and disodium iminodiacetate in the ratio of 1:1:1 in glacial acetic acid solvent at 65^{o}. Upon slow cooling, white crystals separated. The reaction went smoothly and the yield of the 1,4-dihydroxyphenylmethyleneiminodiacetic acid was high, 95%.

The purity of 1,4-dihydroxyphenylmethyleneiminodiacetic acid was found by potentiometric pH titration with standard base. The neutralization curve is shown in Figure 1. The curve shows two breaks, indicating that two replaceable hydrogen ions are present. The equivalent weight based on the first break is 254.8; the theoretical value for one replaceable hydrogen is 255.2. The equivalent weight for the second break is 127.7; the theoretical value for two replaceable hydrogen atoms is 127.6. The purity of the 1,4-dihydroxyphenylmethyleneiminodiacetic acid is thus 99.93%.

The oxidimetric equivalent weight was determined by titration with standard potassium dichromate in 1.0 M sulfuric acid. The titration curve is shown in Figure 2. The calculated equivalent weight is 128.2; the theoretical value for a two-electron oxidation is 127.6.

Figure 1. Neutralization of 1,4-dihydroxyphenylmethylenediacetic acid.

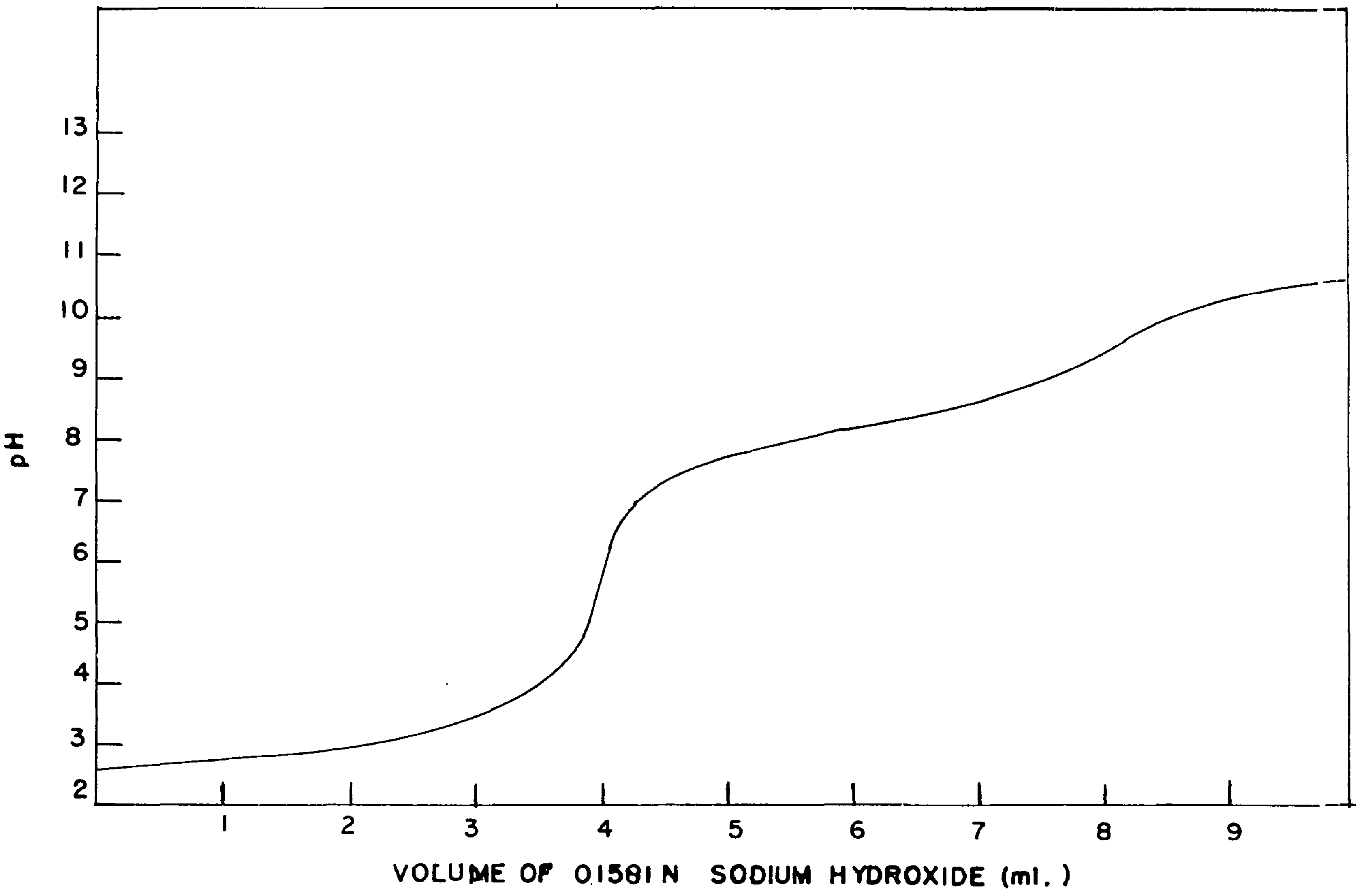
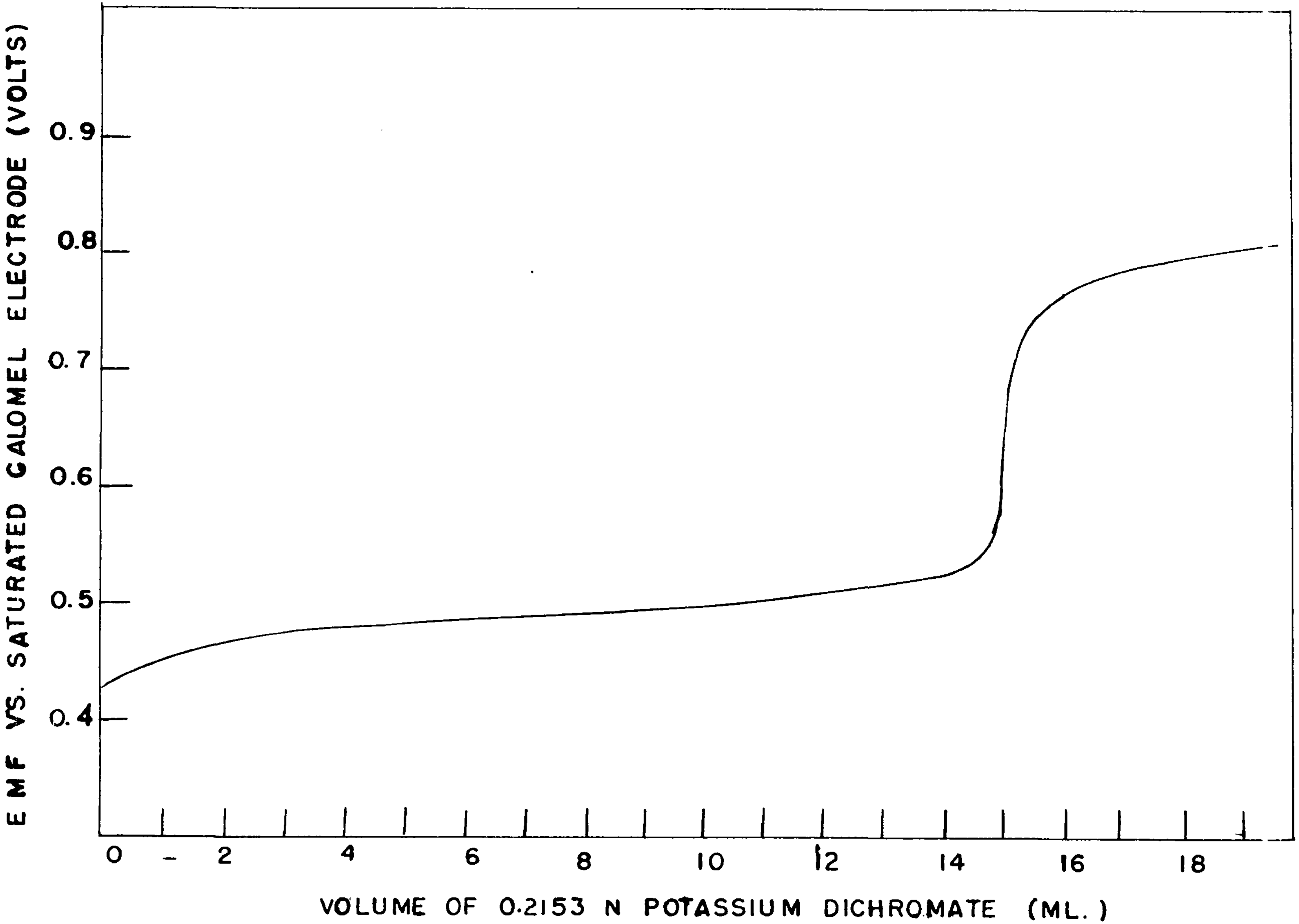
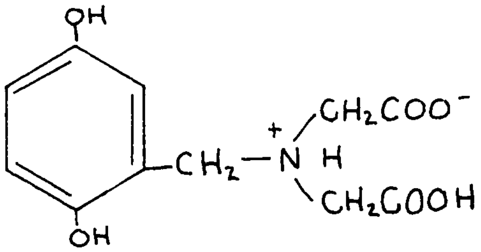

pH
2
3
4
5
6
7
8
9
10
11
12
13
1
2
3
4
5
6
7
8
9
VOLUME OF 0.1581 N SODIUM HYDROXIDE (ml.)

Figure 2. Oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid in 1.0 M sulfuric acid.


EMF VS. SATURATED CALOMEL ELECTRODE (VOLTS)
0.9
0.8
0.7
0.6
0.5
0.4
0
2
4
6
8
10
12
14
16
18
VOLUME OF 0.2153 N POTASSIUM DICHROMATE (ML.)

The acid dissociation constants of 1,4-dihydroxyphenylmethyleneiminodiacetic acid were determined from the midpoints of the neutralization titration; expressed as the negative logarithms the values obtained were $pK_1 = 2.93$ and $pK_2 = 8.13$. The compound probably exists as a zwitter ion;



pK_1 being the value of the dissociation for the carboxylic acid group and pK_2 for the ammonium proton.

The formal reduction potential of 1,4-dihydroxyphenylmethyleneiminodiacetic acid was determined from the oxidimetric titration curve, Figure 2. The potential at 50% oxidation is taken as the value of the formal reduction potential, the value being 0.737 volts vs. N.H.E. in 1.0 M sulfuric acid.

The index potential, as defined by Michaelis (13) is the potential obtained by subtracting the potential at 25% oxidation from the potential at 50% oxidation. The index potential is the measure of the amount of semiquinone formed during an oxidation titration. The semiquinone is a species which is formed by the loss of only one electron in a two-electron oxidative process. The index potential also makes it possible to calculate the formation constant of the semiquinone. The value of the index potential as determined from Figure 2 is 0.014 volts. The formation constant of the semiquinone is $10^{-0.03}$ in 1.0 M sulfuric acid.

The polarographic behavior of 1,4-dihydroxyphenylmethyleneiminodiacetic acid was investigated in acetate supporting electrolyte at pH 6.0. This pH was chosen because at values below six, polarograms could not be obtained. The polarographic oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid lower than pH 6 occurred at potentials where the anodic dissolution of mercury occurs. Any wave in this region is completely obscured. At pH 6.0, the polarogram of 1,4-dihydroxyphenylmethyleneiminodiacetic acid is only fairly well-defined, Figure 3. The diffusion current was obtained graphically. Currents and potentials along the rising portion of the polarogram were measured, and a plot of log I/I_d-I vs. the applied potential was made, Figure 4. The plot is linear and the slope -0.041 volts. The half-wave potential is determined from the point at which the log of I/I_d-I is equal to one, the value being +0.13 volts vs. s.c.e. The slope of -0.041 volts does not agree too well to the expected value of -0.030 volts, the value for a reversible two-electron process.

The polarographic behavior of 1,4-dihydroxyphenylmethyleneiminodiacetic acid at pH 8.3 borate supporting electrolyte was also studied. The use of this alkaline buffer enabled polarograms to be obtained at +0.2 volts vs. s.c.e., while alkaline buffers containing ammonia allowed voltages in the neighborhood of +0.1 volts to be attained. The polarogram of 1,4-dihydroxyphenylmethyleneiminodiacetic acid in pH 8.3 buffer showed the same form as for pH 6.0 buffer. The treatment of the data was done as above and the plot of log I/I_d-I vs. the applied potential yielded a slope of -0.050 volts. The half-wave potential was 0.00 volts vs. s.c.e. The slope indicates that the wave is irreversible in pH 8.3 borate buffer.

Figure 3. Polarogram of 1,4-dihydroxyphenylmethyleneiminodiacetic acid at pH 6.0, acetate buffer.

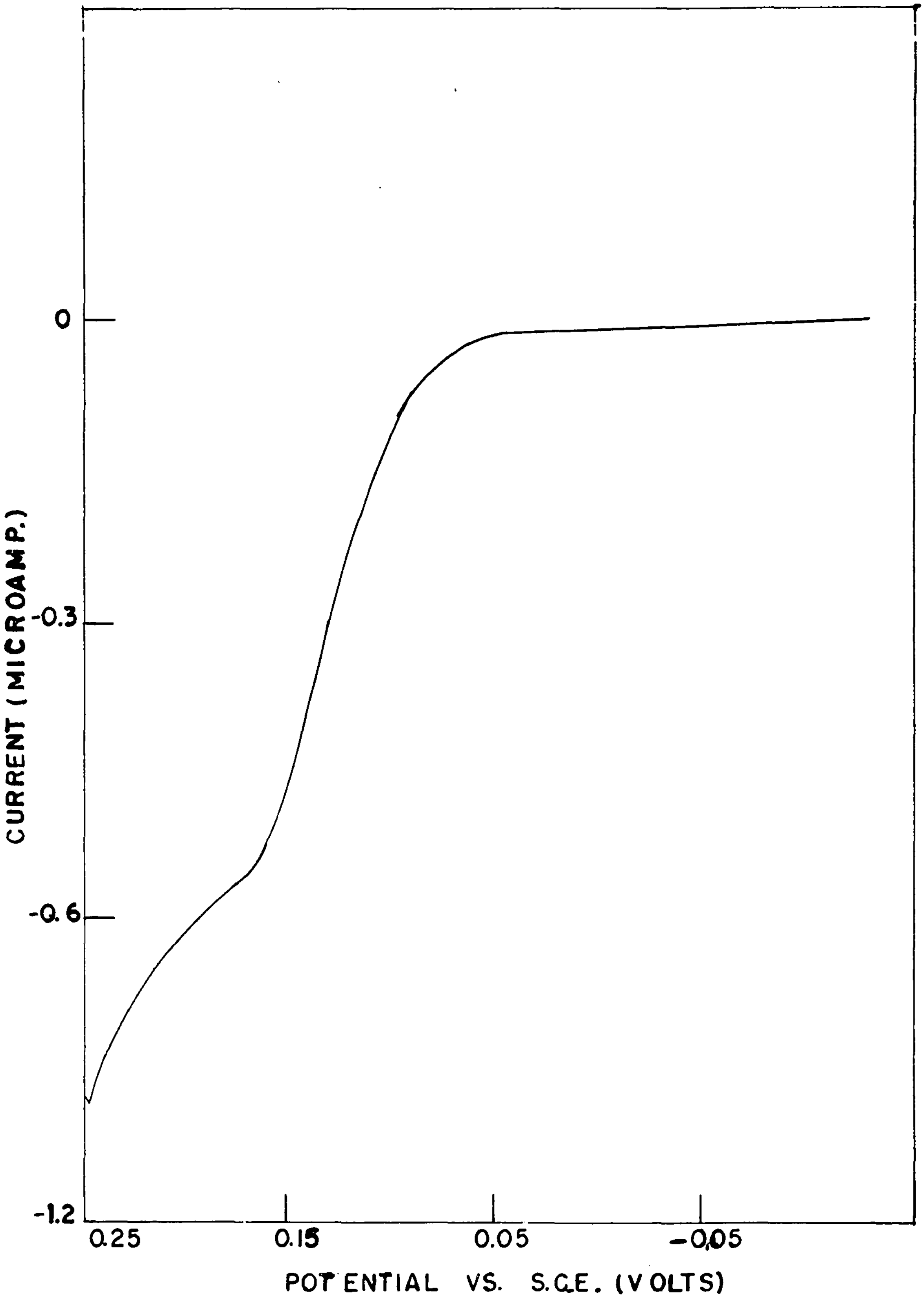
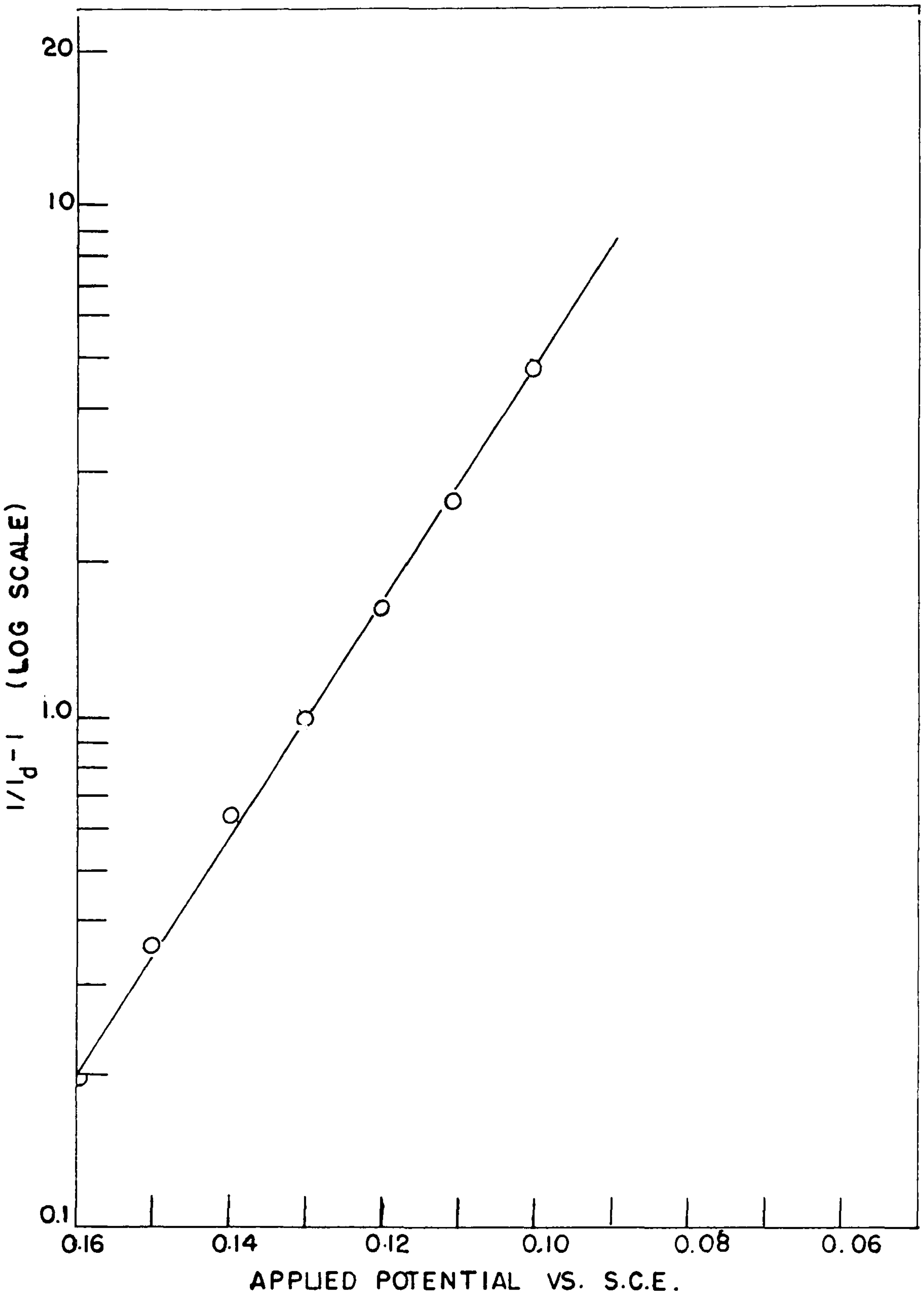

0
-0.3
-0.6
-1.2
CURRENT (MICROAMP.)
0.25
0.15
0.05
-0.05
POTENTIAL VS. S.C.E. (VOLTS)

Figure 4. Plot of I/I_d-I (log scale) vs. applied potential from data of Figure 3.


20
10
1.0
0.1
I/I_d − I (LOG SCALE)
0.16
0.14
0.12
0.10
0.08
0.06
APPLIED POTENTIAL VS. S.C.E.

Qualitative experiments have shown that calcium and magnesium form soluble compounds with 1,4-dihydroxyphenylmethyleneiminodiacetic acid in alkaline solution of pH 8.0. Polarograms of 1,4-dihydroxyphenylmethyleneiminodiacetic acid were obtained in the presence of calcium and then in the presence of magnesium in pH 8.3 borate buffer. The polarograms appeared to be the same as the polarograms of 1,4-dihydroxyphenylmethyleneiminodiacetic acid with no metallic ions present. The half-wave potentials for the calcium and magnesium compound were found to be 0.00 and +0.01 volts vs. s.c.e., respectively. The half-wave potential of the free 1,4-dihydroxyphenylmethyleneiminodiacetic acid is 0.00 volts. Therefore, there is no shift in the half-wave potential of 1,4-dihydroxyphenylmethyleneiminodiacetic acid as a result of compound formation with calcium and magnesium.

A well-defined reduction wave of copper was obtained in a solution of 1,4-dihydroxyphenylmethyleneiminodiacetic acid at pH 6.0 acetate buffer. The half-wave potential, determined graphically, was -0.050 volts vs. s.c.e. Next, a polarogram of copper in the presence of 1,4-dihydroxyphenylmethyleneiminodiacetic acid was obtained. The half-wave potential of the copper wave is -0.18 volts vs. s.c.e., which is a shift of 0.13 volts negative. The negative shift shows that compound formation had taken place. The effect of copper on the half-wave potential of 1,4-dihydroxyphenylmethyleneiminodiacetic acid was investigated. A polarogram was obtained for a solution containing copper and the ligand. The wave for the oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid was analyzed to see if the presence of copper had any influence on the half-wave potential. The half-wave potential of the copper-1,4-dihydroxyphenylmethyleneiminodiacetic acid compound was not affected, as the value remained at +0.13 volts vs. s.c.e.

The investigation of the formation of a soluble compound between zinc and 1,4-dihydroxyphenylmethyleneiminodiacetic acid was done polarographically. A polarogram of a solution containing zinc in pH 6.0 acetate buffer was obtained. The reduction wave is well-defined and the half-wave potential is -1.05 volts vs. s.c.e. Then, a solution containing both zinc and 1,4-dihydroxyphenylmethyleneiminodiacetic acid was polarized on the polarograph. The half-wave potential is -1.07 volts vs. s.c.e. A shift of 0.02 volts negative represents negligible compound formation, therefore, the pursuit of the influence of the zinc on the half-wave potential of 1,4-dihydroxyphenylmethyleneiminodiacetic acid was dropped.

The formation of a soluble compound between nickel and 1,4-dihydroxyphenylmethyleneiminodiacetic acid was investigated polarographically. First, a polarogram was obtained on a solution containing nickel in pH 6.0 acetate buffer and then on a solution containing nickel and 1,4-dihydroxyphenylmethyleneiminodiacetic acid. The polarogram of the solution containing nickel has a half-wave potential of -1.08 volts vs. s.c.e. and the one containing both the nickel and the ligand is -1.49 volts vs. s.c.e. The shift is 0.041 volts negative and shows that a compound is formed.

To study the effect that nickel has on the half-wave potential of 1,4-dihydroxyphenylmethyleneiminodiacetic acid, a polarogram was obtained on a solution containing both materials. The polarogram appeared to be the same as in the case of 1,4-dihydroxyphenylmethyleneiminodiacetic acid alone and the half-wave potential was found to be +0.13 volts vs. s.c.e., unchanged from that of the ligand alone.

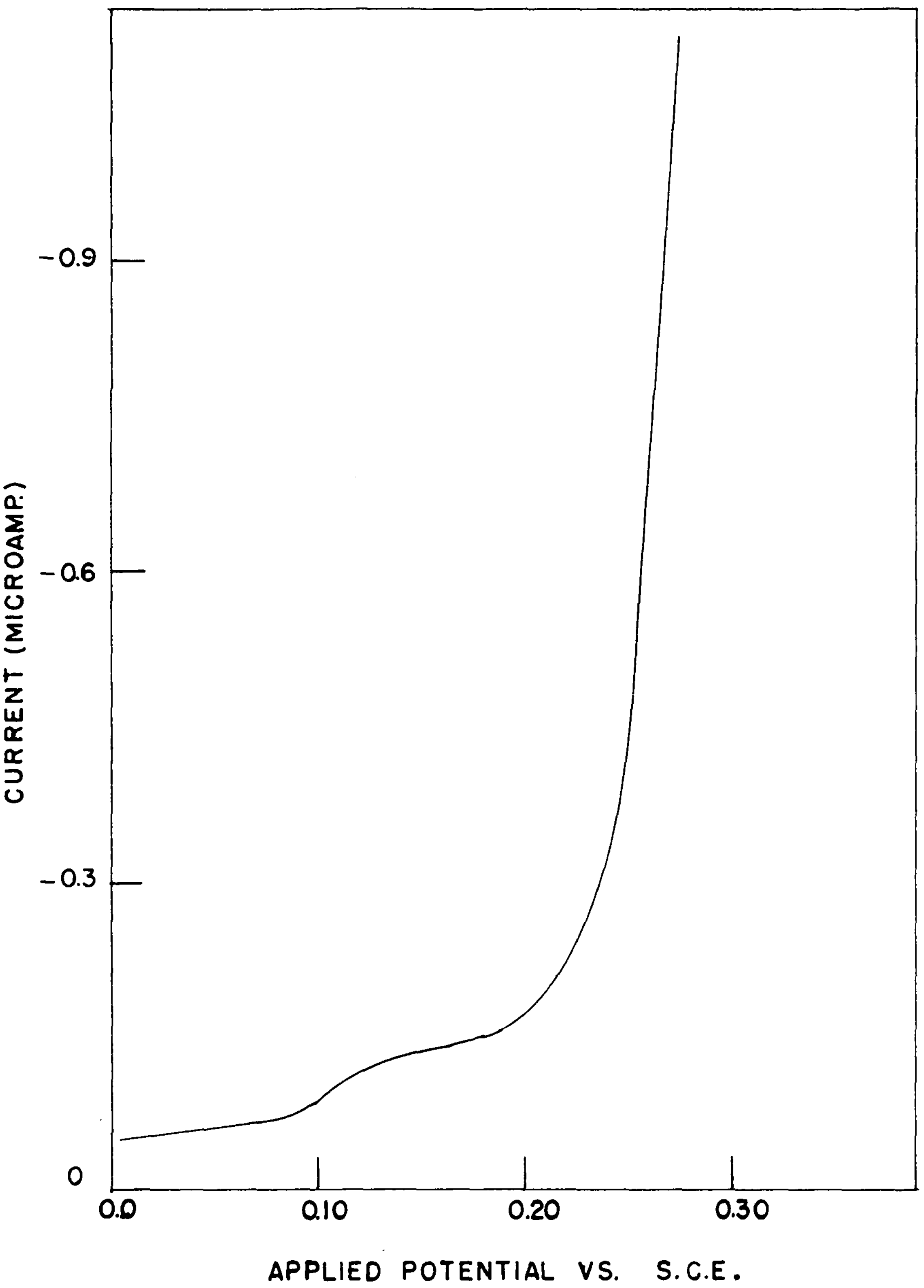
The results were different in the presence of trivalent metals, however.

The polarogram obtained in the presence of aluminum at pH 6.0 is shown in Figure 5. The polarogram shows a very small wave height at about the point where 1,4-dihydroxyphenylmethyleneiminodiacetic acid is oxidized, then it continues to rise rapidly and go off the chart. This steeply rising portion is due to the anodic dissolution of mercury. If any wave due to the oxidation of an aluminum-1,4-dihydroxyphenylmethyleneiminodiacetic acid compound was formed, it would have to occur at more positive values than +0.13 volts vs. s.c.e. Owing to the fact that a mercury is oxidized at a region of +0.20 volts vs. s.c.e., polarograms cannot be obtained beyond this value. Since the wave height of 1,4-dihydroxyphenylmethyleneiminodiacetic acid was lowered to a great extent as a result of the presence of aluminum, compound formation had taken place. Furthermore, it shows that a shift in the half-wave potential had occurred; the shift going to more positive potentials and consequently being obscured by the mercury wave.

A polarogram of 1,4-dihydroxyphenylmethyleneiminodiacetic acid containing bismuth, another trivalent metal, was identical with that of polarogram of 1,4-dihydroxyphenylmethyleneiminodiacetic acid containing aluminum. Bismuth also affects the half-wave potential of 1,4-dihydroxyphenylmethyleneiminodiacetic acid.

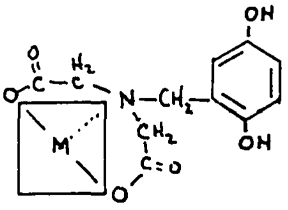
The fact that some metallic ions affect the oxidation-reduction properties of 1,4-dihydroxyphenylmethyleneiminodiacetic acid and others do not must be explained by the method in which bonding takes place in the metal derivative. In order to exert an appreciable influence on the oxidation-reduction properties of 1,4-dihydroxyphenylmethyleneiminodiacetic acid, one or both of the phenolic groups must be utilized in bonding. The phenolic

Figure 5. Polarogram of 1,4-dihydroxyphenylmethyleneiminodiacetic acid in the presence of aluminum.


CURRENT (MICROAMP.)
-0.9
-0.6
-0.3
0
0.0
0.10
0.20
0.30
APPLIED POTENTIAL VS. S.C.E.

group is directly involved in the oxidation process and if the phenolic group is tied up in the bonding to the metal atom an affect on the reduction potential would be expected.

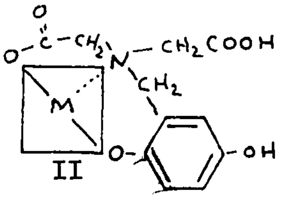
Compounds of the iminodicarboxylic acid class utilize the carboxyl hydroxyl groups and the nitrogen in bonding with metals (14). This is what must occur in the union of 1,4-dihydroxyphenylmethyleneiminodiacetic acid with derivatives of calcium, magnesium at pH 8.3 and with copper and nickel at pH 6.0. The structure is



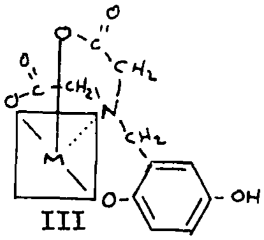
I

The remaining positions can be occupied by coordinated water or by another molecule of 1,4-dihydroxyphenylmethyleneiminodiacetic acid attaching as a tridentate molecule to the remaining positions. Bonding of this sort does not utilize the phenolic groups; consequently, there is no influence on the oxidation-reduction properties of the ligand. The structure above is not unreasonable, because the formation of five-membered rings is one of the requirements of stable chelate systems (7).

Structures II and III are also reasonable.



II



III

In this case the phenolic group occupies one of the coordinating positions, and some different oxidation-reduction properties might be expected.

On the basis of the evidence, 1,4-dihydroxyphenylmethyleneiminodiacetic acid forms two kinds of metallic derivatives. One utilizing (II and III) the phenolic group in coordinating with aluminum and bismuth, and the other (I) using the carboxy and the nitrogen groups as in compounds with calcium, magnesium, copper and nickel.

The results of the experiment for the determination of the combining ratio 1,4-dihydroxyphenylmethyleneiminodiacetic acid and nickel are shown in Table 1. Wave I is due to the reduction of the aquated nickel and wave II to the reduction of nickel-1,4-dihydroxyphenylmethyleneiminodiacetic acid compound. Wave I shows a rapid decrease in current with increasing mole ratio, while wave II shows a corresponding increase. A plot of I_d (mm.) vs. milliliters of added 1,4-dihydroxyphenylmethyleneiminodiacetic acid is shown in Figure 6. The two lines intersect at a value of 1.02 ml. Since equal concentrations of the ligand and nickel were used, 1.02 represents the number of molecules chelated to the metal. The mole ratio of 1,4-dihydroxyphenylmethyleneiminodiacetic acid to nickel is, therefore, 1:1.

In Table 2 are shown the results of the experiment for the determination of the mole ratio of 1,4-dihydroxyphenylmethyleneiminodiacetic acid to copper. Wave I is the wave due to the reduction of aquated copper and wave II to the copper-1,4-dihydroxyphenylmethyleneiminodiacetic acid compound. At a ratio of 1:1 the copper wave has completely vanished and only the wave for the chelated copper is present. The mole ratio of 1,4-dihydroxyphenylmethyleneiminodiacetic acid to copper is, therefore, 1:1.

In Table 3 are shown the results for the determination of the combining ratio of aluminum to 1,4-dihydroxyphenylmethyleneiminodiacetic acid at pH 6.0. The diffusion current is that for the wave of the oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid. The current shows a decrease with increasing amounts of aluminum, and at ratio of 1:1 the current attains a constant value. Thus, the combining ratio of the aluminum to the ligand is 1:1.

Table 1. Data for the determination of the combining ratio of nickel and 1,4 -dihydroxyphenylmethyleneiminodiacetic acid; pH 6.0

Mole Ratio of 1,4-DHP[c] to Nickel	Wave I		Wave II	
	$-E_{\frac{1}{2}}$[a]	I_d[b]	$-E_{\frac{1}{2}}$	I_d
0:1	1.09	122	-	-
0.5:1	1.09	73	1.44	84
1.0:1	1.09	27	1.44	100
1.5:1	1.09	20	1.44	120
2.0:1	1.09	18	1.46	130
3.0:1	1.09	14	1.46	135

[a] diffusion current expressed in mm.

[b] half-wave potential in volts vs. s.c.e.

[c] abbreviation for 1,4-dihydroxyphenylmethyleneiminodiacetic acid

Table 2. Data for the determination of the combining ratio of copper and 1,4-dihydroxyphenylmethyleneiminodiacetic acid: pH 6.0

Mole Ratio of 1,4-DHP[c] to Copper	Wave I		Wave II	
	$-E_{\frac{1}{2}}$[a]	I_d[b]	$-E_{\frac{1}{2}}$	I_d
0:1	0.055	179	-	-
0.25:1	0.050	143	0.17	35
0.50:1	0.050	89	0.16	76
1.0:1	- -	- -	0.145	171

[a] diffusion current in mm.

[b] half-wave potential in volts vs. s.c.e.

[c] abbreviation for 1,4-dihydroxyphenylmethyleneiminodiacetic acid

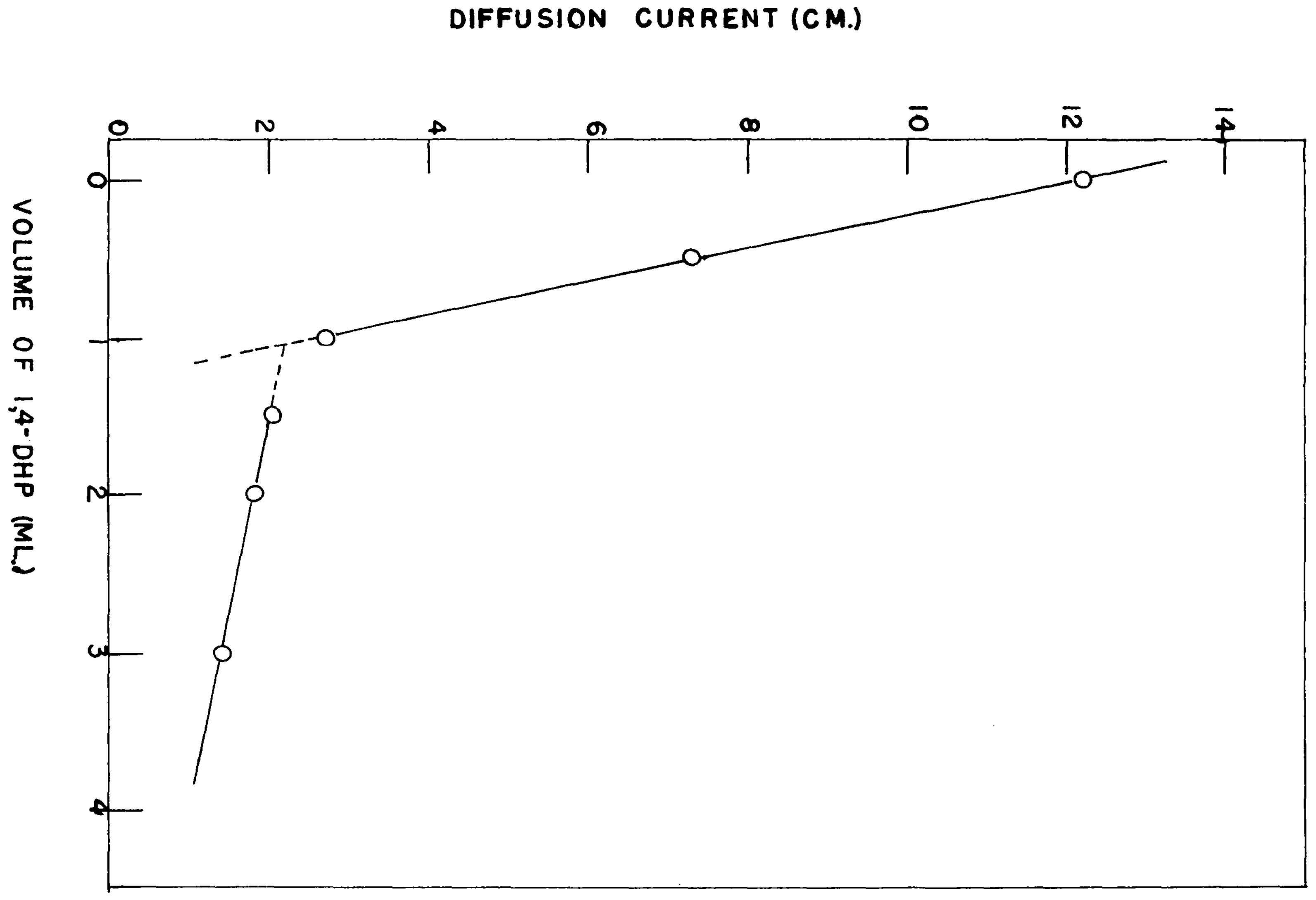
Table 3. Data for the determination of the combining ratio of aluminum and 1,4-dihydroxyphenylmethyleneiminodiacetic acid; pH 6.0

Mole Ratio of Aluminum to 1,4-DHP[b]	I_d (mm.)[a]
0:1	149
0.25:1	116
0.50:1	74
0.75:1	49
1.00:1	20
2.00:1	20

[a] diffusion current expressed in millimeters

[b] abbreviation for 1,4-dihydroxyphenylmethyleneiminodiacetic acid

Figure 6. Plot of diffusion current (I_d) against milliliters of 0.010 M 1,4-dihydroxyphenylmethyleneiminodiacetic acid (1,4-DHP). Concentration of nickel: 0.010 M.


DIFFUSION CURRENT (CM.)
0
2
4
6
8
10
12
14
VOLUME OF 1,4-DHP (ML.)
0
1
2
3
4

In Figure 7 are shown the titration curves of 1,4-dihydroxyphenylmethyleneiminodiacetic acid (A) and the acid in the presence of equimolar amounts of calcium (B), magnesium (C) and copper (D). The first two dissociation constants of the acid were evaluated by the method of Chaberek and Martell (15). The method consists of applying the algebraic expression for K_1 and K_2, where K_1 is the acid dissociation constant for the first replaceable proton and K_2 the acid dissociation constant of the second proton. The expressions involved are:

$$K_1 = (H^+)\ (HA^-)\ /\ (H_2A)$$

$$K_2 = (H^+)\ (A^=)\ /\ (HA^-)$$

The parentheses represent concentration in moles per liter. From the stoichiometry of the reactions, the following expressions yield the acid dissociation constants.

$$K_1 = (H^+)\ aC_A + (H^+)\ /\ C_A - aC_A + (H^+)$$

$$K_2 = (H^+)\ (a-1)\ C_A - (OH^-)\ /\ C_A - (a-1)\ C_A - (OH^-)$$

a = ratio of moles of base per mole of acid

C_A = stoichiometric concentration of acid added

The negative logarithms of the acid dissociation constants found are 2.96 and 8.01, respectively for pK_1 and pK_2. These values are in good agreement with the values obtained by the potentiometric pH titration in Section III B.

The titration curve of 1,4-dihydroxyphenylmethyleneiminodiacetic acid is lowered as a result of the presence of calcium and magnesium. There appears to be a break at $a = 2.5$, Figure 7. The only plausible explanation of this is that part of the metal compound liberates some additional protons to the base. This proton comes from the hydroxyl group ortho to the methyleneiminodiacetic acid group. The driving force for the enhanced strength of acidity may be due to the formation of an additional bond with the chelated metal atom. In the case of copper two breaks appear; at a = 2.0 and 3.0, Figure 7. This clearly indicates that 1,4-dihydroxyphenylmethyleneiminodiacetic acid loses two protons; the carboxyl proton and the ammonium proton forming a strong tridentate compound. Upon further addition of base, another proton is liberated, as the hydroxyl oxygen forms an additional, fourth bond with the metal.

The calculation of the stability constant for the formation of metal chelates was made by the method of Chaberek and Martell (15). A simple algebraic method using the stability constant expression, the concentration of the 1,4-dihydroxyphenyliminodiacetic acid (C_A), the concentration of the added base (C_B), the ratio of moles of base per mole of acid, the hydrogen ion concentration and the expression for the acid dissociation constants for the chelating agent, 1,4-dihydroxyphenylmethyleneiminodiacetic acid, was applied. In these calculations it was assumed that the chelating agent was tribasic. At values of $a<2$ the third dissociation constant was neglected in the calculations. The principal equilibrium considered for the formation of the metal derivative is

$$M^{++} + HA^{=} \rightleftharpoons MHA.$$

The logarithm of the formation constant is designated as log K^{M}_{MHA}. With copper

$$MHA \rightleftharpoons H^{+} + MA^{-} ,$$

and the reaction seems to be stoichiometric at a = 3.0. One may also consider the possibility that MA^- can be derived from

$$M^{++} + A^{\equiv} \rightleftharpoons MA^- ,$$

and the formation constant is designated as K^M_{MA}. In Table 4 is shown the formation constants of the calcium, magnesium and copper compounds.

Table 4. Log formation constants of 1,4-dihydroxyphenylmethyleneiminodiacetic acid with calcium, magnesium, and copper

	Calcium	Magnesium	Copper
Log K^M_{MHA}	3.71	3.87	8.83
Log K^M_{MA}	--	--	11.8

For the determination of K^M_{MA} consider the following equations:

Let the chelating agent be a tribasic acid H_3A

(3.1) $H_3A \rightleftharpoons H^+ + H_2A^-$ (3.2) $K_1 = (H^+)(H_2A^-)/(H_3A)$

(3.3) $H_2A^- \rightleftharpoons H^+ + HA^=$ (3.4) $K_2 = (H^+)(HA^=)/(H_2A^-)$

(3.5) $A^{\equiv} + H^+ \rightleftharpoons HA^=$ (3.6) $K_3 = (HA^=)/(A^{\equiv})\ (H^+)$

In forming a compound assume that at a<2

(3.7) $H_3A + M^{++} \rightleftharpoons MHA + 2\ H^+$.

M^{++} represents the metal ion. This last equation may be broken down into

$$(3.8)\quad M^{++} + HA^{=} \rightleftharpoons MHA \quad ,$$

where

$$(3.9)\quad K^{M}_{MHA} = (MHA) \,/\, (M^{++})\,(HA^{=}) \quad .$$

This constant is readily obtained from the titration curve at $a<2$ and the first two dissociation constants using the method of Chaberek and Martell. An additional equilibrium may be assumed, that of the acid dissociation of MHA.

$$(3.10)\quad MHA \rightleftharpoons H^{+} + MA^{-} \quad .$$

This is assumed to be occurring at $a = 3$ on the titration curve of copper plus the chelating agent. The equilibrium constant is

$$(3.11)\quad K^{H}_{MHA} = (MHA) \,/\, (H^{+})\,(MA^{-}) \quad .$$

This constant may be evaluated by taking the pH at the midpoint of the titration, e. i. $a = 2.5$. At this point the pH is equal to the logarithm of K^{H}_{MHA}. Using (3.9) and substituting for (MHA) in (3.11) there is obtained

$$(3.12)\quad K^{H}_{MHA} = K^{M}_{MHA} \cdot \frac{(M^{++})\,(HA^{=})}{(H^{+})\,(MA^{-})} \; .$$

If $(3.13)\quad A^{\equiv} + M^{++} \rightleftharpoons MA^{-}$, and

$(3.14)\quad K^{M}_{MA} = MA^{-}) \,/\, (M^{++})(A^{\equiv})$, then

$(3.15)\quad (MA^{-}) \,/\, (M^{++}) = K^{M}_{MA}\,(A^{\equiv})$.

By making the appropriate substitutions there is obtained

(3.16)

$$K^{H}_{MHA} = \frac{K^{M}_{MHA}}{K^{M}_{MA}} \cdot \frac{(HA^{=})}{(H^{+})\,(A^{\equiv})}$$

Substituting equation (3.6) into (3.16), there is obtained

(3.17)

$$K^{H}_{MHA} = \frac{K^{M}_{MHA} \cdot K_{3}}{K^{M}_{MA}}$$

Knowing K^{H}_{MHA}, K^{M}_{MHA}, and K_3 the value of K^{M}_{MA} can be calculated.

The third dissociation constant was evaluated from the polarographic study at different pH values. The polarograms of 1,4-dihydroxyphenylmethyleneiminodiacetic acid in the universal buffer gave a wave having a maximum. The maximum could not be supressed with solutions of gelatin or Triton X-100. Therefore, the potential at the maximum was measured instead of the half-wave potential, and a plot of potential vs. pH was obtained, Figure 8. The plot is nonlinear. Tangents to the curve were drawn, and the point of intersection of the two tangents was taken as the pK of the ortho hydroxyl group. The value is 9.75. This value agrees quite well with the value of the dissociation constants of the parent hydroquinone as determined by Abichandi and Jatkar (16). The value is pK = 9.92. The pK of the hydroxyl group was converted to K_3 of equation (3.6), and K^{M}_{MA} was calculated for the copper chelate using equation (3.17) the value being 11.8 expressed as the logarithm of the formation constant.

Figure 7. Titration of 1,4-dihydroxyphenylmethyleneiminodiacetic acid with 0.07060 N potassium hydroxide. The abscissa, a, is the ratio of moles of base added to moles of acid.

Curve A. Titration of free acid, 1.030×10^{-3} M.

Curve B. Titration of acid, 1.030×10^{-3} M, in the presence of 1.030×10^{-3} M calcium.

Curve C. Titration of acid, 1.030×10^{-3} M, in the presence of 1.030×10^{-3} M magnesium.

Curve D. Titration of acid, 1.030×10^{-3} M, in the presence of 1.030×10^{-3} M copper.

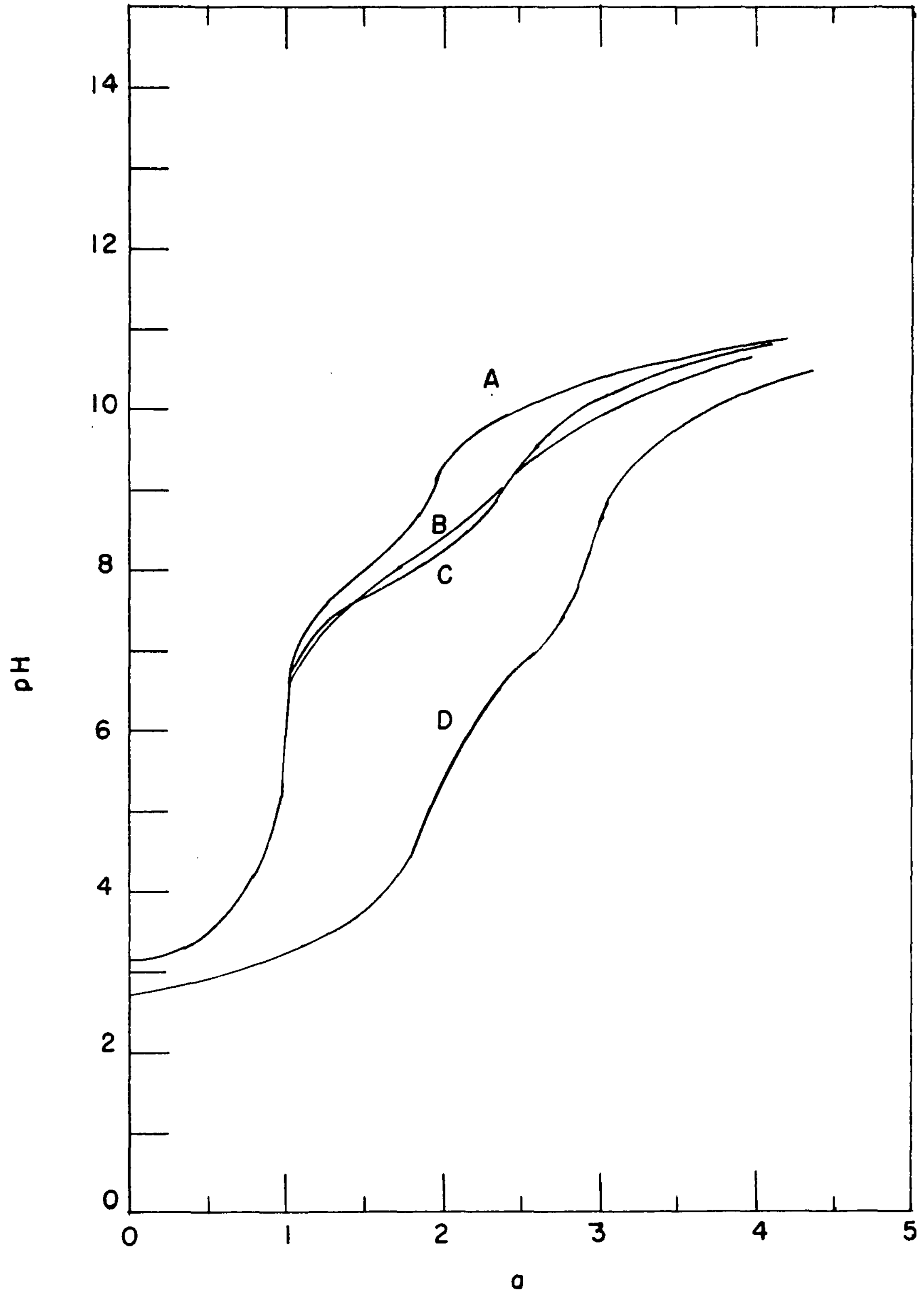
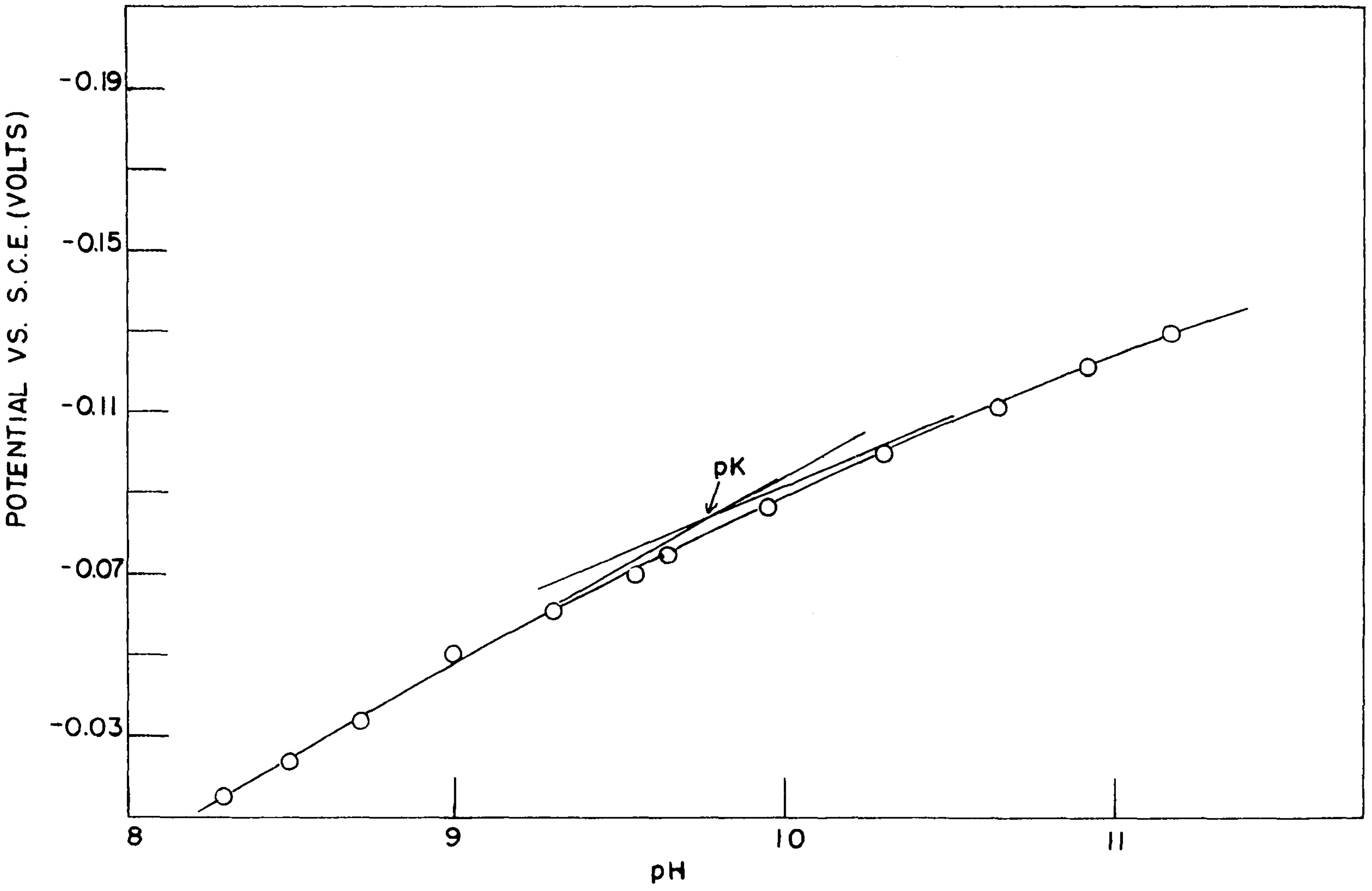

pH
a
0
2
4
6
8
10
12
14
0
1
2
3
4
5
A
B
C
D

Figure 8. Plot of potential of the polarographic maximum against pH to obtain the acid dissociation constant of the ortho hydroxyl group.


POTENTIAL VS. S.C.E.(VOLTS)
-0.19
-0.15
-0.11
-0.07
-0.03
8
9
10
11
pH
pK

The log K^{M}_{MHA} value of the calcium and magnesium derivatives of 1,4-dihydroxyphenylmethyleneiminodiacetic acid are the same order of magnitude as the log K values of the corresponding derivatives of iminodiacetic acid as determined by Schwarzenbach (17). The log K for calcium (IMDA) and magnesium (IMDA), where IMDA is the abbreviation for iminodiacetic acid, is 3.42 and 3.66. The corresponding values of the calcium and magnesium derivatives of 1,4-dihydroxyphenylmethyleneiminodiacetic acid are 3.71 and 3.87. In both series of compounds the formation constants of the magnesium derivatives are slightly larger than those of the calcium derivatives.

The log K^{M}_{MA} for the copper derivative of 1,4-dihydroxyphenylmethyleneiminodiacetic acid is 11.8 . This is the same order of magnitude as that of the copper derivative of nitrilotriacetic acid, the value reported by Schwarzenbach (18) being 12.7. This is expected since nitrilotriacetic acid is a tetradentate ligand, and 1,4-dihydroxyphenylmethyleneiminodiacetic acid becomes a tetradentate ligand after losing a third proton, that of the ortho hydroxyl group.

IV. ATTEMPTED PREPARATION OF 1,4-DIOXOPHENYLMETHYLENEIMINODIACETIC ACID

A. Introduction

Benzoquinones are generally prepared by the oxidation of disubstituted aromatic hydrocarbons having hydroxyl or amino groups para to each other. Thus, hydroquinone, p-aminophenol or p-phenylenediamine would be suitable starting materials for the preparation of p-benzoquinone by oxidation.

The nature of the oxidizing agent, the experimental conditions and the character of the side chains influence the side-reactions which may occur during the preparation of the quinone (19). Often, these side-reactions are a result of the formation of free-radicals. The free-radicals have a tendency to form high molecular weight polymers, giving poor yields of the quinone, and making purification difficult.

The choice of the oxidizing agent is largely arbitrary. Sometimes a given oxidizing agent works for particular compound, but may not work on a compound of similar nature. For this reason many different oxidizing agents are used. Some of the most frequently used oxidizing agents are: sodium dichromate, chromic acid, lead dioxide, ferric chloride, air, hydrogen peroxide, potassium bromate and sodium chlorate.

For the conversion of hydroquinone to quinone, sodium dichromate and sodium chlorate are the most popular reagents. Yields as high as 95% of the quinone have been reported using sodium chlorate and yields of 92% with sodium dichromate. Purification using these reagents is simple. The product precipitates from cold acid solution, and can be purified further by steam distillation.

Some of the above techniques were used to effect the oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid in the work being here reported. Owing to the presence of a chelating group on the molecule, special isolation procedures were used, especially when the oxidizing agent contained a metal capable of combining with 1,4-dihydroxyphenylmethyleneiminodiacetic acid.

B. Experimental Work

1. Apparatus and solutions

A Sargent Model XXI recording polarograph was used in these experiments. The cell consisted of a 4 oz. wide-mouth bottle and a rubber stopper containing three holes, one each for the dropping mercury electrode, salt bridge and the nitrogen inlet tube. A potassium nitrate salt bridge was used to complete the circuit.

Ammonium perchlorate-ammonia buffer of pH 8.0 was prepared by dissolving 188 g. of ammonium perchlorate in 500 ml. of deionized water, adding 6.8 ml. of ammonium hydroxide, and diluting to one liter.

Sodium acetate-acetic acid buffer of pH 6.0 was prepared by dissolving 145 g. of sodium acetate in 800 ml. of deionized water, adding 5.7 ml. of glacial acetic acid, and diluting to one liter.

Potassium persulfate, 0.010 M, was prepared by dissolving 0.2706 g. of the material in water and diluting to 100.0 ml. in a volumetric flask.

A solution, 0.010 M in 1,4-dihydroxyphenylmethyleneiminodiacetic acid was made by making a water slurry of 0.2555 g. of the compound, then dissolving it by the dropwise addition of saturated solution of sodium carbonate, and finally diluting to 100.0 ml.

2. Oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid with potassium dichromate

Two grams of 1,4-dihydroxyphenylmethyleneiminodiacetic acid ware suspended in 75 ml. of 1.0 M sulfuric acid. This was placed in an ice bath and to it was added dropwise a solution of 1.0 gram of potassium dichromate in 20 ml. of 1.0 M sulfuric acid. The temperature of the reaction was held below 5°. After complete addition of the potassium dichromate the solution was allowed to warm to room temperature. The dark, brown-green solution was passed through Amberlite IR-120 cation exchange resin in the hydrogen form and eluted with water. Several passes were made through the column to remove most of the green coloration caused by the presence of the aquated chromous ion. The resulting solution was dark yellow in color. Benzene, chloroform, ether and carbon tetrachloride were used to extract the oxidized product. None of these solvents effected extraction. The reaction mixture was vacuum distilled to remove most of the water. Acetone was then added until a turbidity appeared. Upon standing in a refrigerator for 8 hours a dark brown gummy material separated. The gummy material was dissolved in water and subjected to several treatments of decolorizing charcoal and water. Upon reprecipitation with acetone, the gummy material was again obtained. Sublimation of the gummy material under reduced pressure was also tried without success.

3. Oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid with hydrogen peroxide

Three grams of 1,4-dihydroxyphenylmethyleneiminodiacetic acid were suspended in 100 ml. of distilled water and 10% potassium hydroxide was added dropwise until the acid dissolved. A little ferric chloride was added, producing a purple solution. To this was added dropwise 2.0 ml. of 30% hydrogen peroxide. After several minutes of vigorous stirring a reaction began with the evolution of heat. The solution became rusty-brown in color. The beaker was placed into an ice bath for 2 hours. A light brown precipitate was observed. This was filtered, and a portion was treated with a solution of sodium bisulfite on a spot plate. There was no bleaching of the redish solution. This indicated that the compound was not the expected 1,4-dioxophenylmethyleneiminodiacetic acid. The filtrate was treated with acetone causing a tan, gummy material to separate. This gummy material was not soluble in benzene, ether, chloroform, petroleum ether, nor carbon tetrachloride. It was soluble in water. Recrystallization from water, however, did not yield a pure product, for the product obtained was only partially bleached by sodium bisulfite.

4. Oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid with potassium bromate

Five grams of 1,4-dihydroxyphenylmethyleneiminodiacetic acid was treated with 0.98 g. of potassium bromate in 1.0 M sulfuric acid. One gram of mercuric nitrate was added to form the undissociated mercuric bromide, as bromide is formed during the oxidation. The reaction was allowed to proceed to completion as determined by periodically testing a few drops of the reaction solution on a spot plate. To the test solution was added potassium bromide which generates free bromine as it reacts with potassium bromate. The generated bromine decolorized methyl red when placed into the mixture. The reacted solution consisted of a dark brown solution and a pink precipitate. The precipitate was filtered and washed with water to remove bromide. A sample of the precipitate was ashed. No visible residue remained. The crucible was washed with dilute nitric acid and tested with silver nitrate. No bromide was present as shown by the absence of a precipitate with silver nitrate. Another sample of the pink precipitate was dissolved by the slow addition of saturated sodium carbonate solution. The solution was yellow-green in color. A little sodium bisulfite was added. The solution became darker green, finally colorless. On acidifying, a grey turbidity was produced. Another portion of the pink precipitate was dissolved and treated with stannous chloride solution. The solution of the pink material turned black and finally a black precipitate settled out. Still another portion of the pink precipitate was dissolved in a solution of sodium bicarbonate and extracted with a solution of dithizone and chloroform. The aqueous phase became red in color. When this layer was acidified the solution turned purple. All these tests confirmed that the purple material is some kind of mercury compound. A polarogram of the pink precipitate could not be obtained. The behavior of the instrument was abnormal in that the current could not be adjusted to remain on the recorder chart. The same kind of pink precipitate is obtained when mercuric nitrate was added to a solution of 1,4-dihydroxyphenylmethyleneiminodiacetic acid. Thus, the pink material is a derivative of 1,4-dihydroxyphenylmethyleneiminodiacetic acid.

The filtrate from the above reaction was worked up in the usual way and did not yield the desired product.

5. Oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid with sodium chlorate

Five grams of 1,4-dihydroxyphenylmethyleneiminodiacetic acid was suspended in 200 ml. of water and dissolved by the dropwise addition of saturated sodium carbonate solution. A little vanadium pentoxide in 1.0 M sulfuric acid was added as a catalyst. The pH of the solution was adjusted to 6.0 with dilute sulfuric acid, and finally 0.70 g. of sodium chlorate was added. The solution was warmed to 35^{o} and held at that temperature for four hours. The solution became dark red after this period. It was extracted with xylene. Upon evaporation of the xylene layer, a yellow oil was recovered. Sublimation of this yellow oil did not yield a solid material. The oil was taken up in water and the pH adjusted to 6.0 with acetic acid-sodium acetate buffer. A polarogram was obtained. The polarogram which resulted had no indication of a reduceable or an oxidizable species in the solution. The raffinate layer did not change color as a result of the extraction. Thus, the xylene only removed a minor impurity. Absolute alcohol was added to the raffinate. A mass of black material was obtained. The black material was taken up in water and treated with decolorizing charcoal. Upon filtration of the charcoal mixture, a colorless solution was obtained. The solution was evaporated to dryness on a steam plate; no residue was left. A portion of the filtrate from the alcohol precipitation was extracted with ethyl acetate, chloroform, carbon tetrachloride, benzene, ether, ethylenechloride and butyl alcohol. These solvents did not appear to extract anything.

A portion of the solution from the oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid was placed on a chromatographic column packed with silica gel. A light brown band separated with an eluent consisting of

10% butanol and 90% methanol. A tan solution was obtained by eluting with 60% butanol and 40% methanol. Water also forced down a band which was tan in color. Ten per cent acetic acid and 90% water stripped off a sharp purple band. Finally the column was stripped of material with 0.1 M sulfuric acid. This gave a green solution. The five fractions were evaporated to dryness on a steam plate. The tan fraction gave a black residue while the purple and green ones yielded slightly crystalline purple and green material. All of these residues were taken up in water, the pH adjusted to 6.0 with acetate buffer, and the solution examined polarographically. No wave was present, indicating that the desired product was not obtained.

6. Oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid with air, the reaction followed polarographically

Following the failure to produce 1,4-dioxophenylmethyleneiminodiacetic acid by the oxidation with strong oxidizing agents, attempts were made to follow the course of the oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid polarographically.

Air was used in weakly basic solution inasmuch as it is a weak oxidant in such a media. The air was filtered and then introduced into a solution containing 1,4-dihydroxyphenylmethyleneiminodiacetic acid buffered to pH 8.0 with ammonium perchlorate-ammonia buffer. Aliquots were then removed at various stages of the reaction and examined on the polarograph to observe if any oxidation had occurred.

A weighed amount of 1,4-dihydroxyphenylmethyleneiminodiacetic acid, 5.0 g., was placed into a conical flask containing 200 ml. of water. A saturated solution of sodium carbonate was added slowly until the compound dissolved.

The pH of this solution was 6 as determined by pH paper. To this was added 100 ml. of pH 8.0 buffer, bringing the pH to 8. Filtered air was introduced into the flask in a fairly rapid stream. A polarogram was taken on a 5.0 ml. aliquot buffered with 10 ml. of pH 8.0 buffer and diluted to 70 ml. in the cell. The voltage span was one volt and the sensitivity at 0.2 microamp./mm. A poorly defined anodic wave due to the oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid was produced. After twenty hours of oxidation, another 5.0 ml. aliquot was withdrawn and treated as above. A polarogram similar to the one above was obtained; the two indicated that no oxidized species was present.

a. Oxidation of quinhydrone in alkaline and acidic media A saturated solution of quinhydrone was made by equilibrating the solid quinhydrone with water. The aqueous layer, separated by filtration, was buffered with 50 ml. of pH 8.0 buffer. A polarogram was obtained on a 70 ml. portion. The regular sigmoid shaped curve with a cathodic and anodic portion was obtained. Air was then introduced into the saturated solution for 15 minutes. A five milliliter aliquot was removed, diluted to 50 ml., and a polarogram was obtained after removing the residual air by purging with nitrogen. The symmetrical shape of the wave was lost, the cathodic portion being decreased. Air was introduced for an extra hour, and another 5.0 ml. aliquot removed. The aliquot was treated as above, and a polarogram obtained. The polarogram showed that the cathodic portion had decreased to a larger extent.

A saturated solution of quinhydrone was made as described above and buffered with pH 6.0 acetate buffer. Air was introduced for 15 minutes, and a 5.0 ml. aliquot removed. This was diluted to 50 ml. with water and deaerated for ten minutes with nitrogen. A polarogram was obtained, and it indicated that the cathodic portion decreased as a result of adding air.

7. Oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid with potassium persulfate, the reaction followed polarographically

Exactly one milliliter of 0.010 M 1,4-dihydroxyphenylmethyleneiminodiacetic acid was introduced into a polarographic cell. To this was added 38 ml. of deionized water and 40 ml. of pH 6.0 acetate buffer. The solution was deareated with nitrogen for ten minutes, and a polarogram obtained. The anodic wave was fairly well-defined with the half-wave potential of +0.13 volts vs. s.c.e.

To the above solution was added 1.00 ml. of 0.010 M potassium persulfate. Nitrogen was allowed to flow through the solution to stir and to keep the solution free from air. After a 5 minute reaction time, a polarogram was obtained, Figure 9. A cathodic wave due to the sought-for quinone was shown.

To the above solution was added 1.00 ml. of 0.010 M 1,4-dihydroxyphenylmethyleneiminodiacetic acid. The solution was permitted to react for 5 minutes with nitrogen flowing through the system. A polarogram was obtained, Figure 9. It showed both a cathodic and an anodic wave.

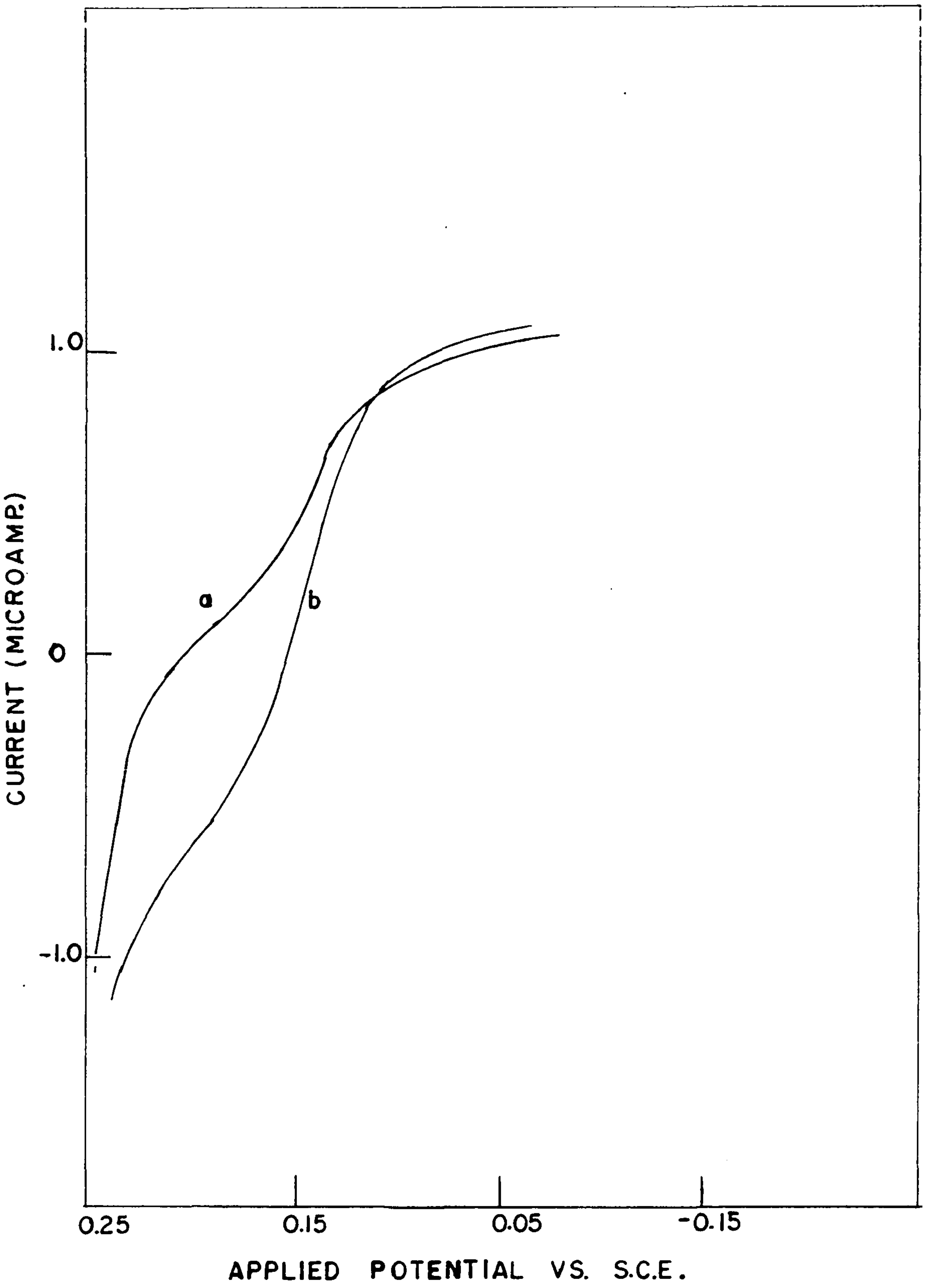
8. Oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid with potassium persulfate on a large scale

Five grams of 1,4-dihydroxyphenylmethyleneiminodiacetic acid was dissolved in 250 ml. of water. Saturated sodium carbonate solution was added slowly until the material dissolved. One hundred milliliters of pH 6.0 acetate buffer were added, and the solution diluted to 500 ml. Finally, 5.3 g.

Figure 9. Polarograms of oxidation products of 1,4-dihydroxyphenylmethyleneiminodiacetic acid.

a) 1,4-Dioxophynylmethyleneiminodiacetic acid.

b) Quinhydrone analog of 1,4-dihydroxyphenylmethyleneiminodiacetic acid and its corresponding quinone.


CURRENT (MICROAMP.)
1.0
0
-1.0
a
b
0.25
0.15
0.05
-0.15
APPLIED POTENTIAL VS. S.C.E.

of potassium persulfate was added. The mixture was allowed to react for 15 minutes and was then extracted with several portions of ether. The ether was evaporated, and a purple crystalline material, 0.2 grams, was obtained.

The melting point was taken on a melting point block and the phase transitions observed through a microscope. At 82° the material sublimed and condensed on the microscope slide. The sublimate appeared as golden crystals. At 140° the golden crystals changed to a pure white. These white needles melted sharply at 180°.

After the ether extraction a 4 ml. portion of the raffinate was diluted to 50 ml. with water, and a polarogram obtained. The polarogram indicated that the ether did not extract all of the oxidized product since a cathodic wave was present. After 24 hours the solution in the polarographic cell was observed to be deep red. Another polarogram showed that the cathodic wave had decreased somewhat. After 32 hours the solution turned a deep brown.

The raffinate from the above ether extraction was treated with alcohol. A precipitate was formed which was collected by filtration. This was washed several times with alcohol and dried. Upon drying the material turned to a black slime which could not be purified. The material was not decolorized by sodium bisulfite. A polarogram of the material also indicated that none of the desired product was present.

The purple crystalline material was sublimed under reduced pressure, and the various crystals which were observed during the melting point experiment were collected. An infrared spectrum was obtained for each of the purple, gold and white crystals.

C. Results and Discussion

1,4-Dihydroxyphenylmethyleneiminodiacetic acid reacts with potassium dichromate in 1.0 M sulfuric acid, but the product could not be purified and identified conclusively. The product which was obtained did not show a positive reaction with sodium bisulfite and did not contain oxidizable or reducible groups which could be observed polarographically. Consequently, the product was not the sought-for 1,4-dioxophenylmethyleneiminodiacetic acid. The product was apparently a polymer.

Hydrogen peroxide in the presence of ferric chloride reacts with 1,4-dihydroxyphenylmethyleneiminodiacetic acid to produce a light-brown, insoluble material. The test with sodium bisulfite was negative and showed that the compound was not the 1,4-dioxophenylmethyleneiminodiacetic acid. Further treatment of the filtrate with acetone gave a brown colored gummy material which could not be purified. This particular material did not react with sodium bisulfite, nor did it give a polarogram showing a cathodic wave.

The reaction of 1,4-dihydroxyphenylmethyleneiminodiacetic acid with potassium bromate gave a pink, insoluble material. Mercuric nitrate was added to this reaction mixture because it provided a means to force the oxidation reaction to completion by forming with the bromide liberated a slightly dissociated bromomercury ion. The pink compound was not sought-for compound, but a mercury compound of 1,4-dihydroxyphenylmethyleneiminodiacetic acid. Mercury was present in the product, and a polarogram of the compound could not be obtained. Owing to the presence of mercury, the polarograph could not be adjusted properly to obtain a polarogram. The conclusive evidence comes from the experiment in which a precipitate, pink in color, forms when

1,4-dihydroxyphenylmethyleneiminodiacetic acid is added to a solution of mercuric nitrate.

The filtrate from the potassium bromate reaction did not contain the desired product. A gummy material is obtained by the addition of alcohol to the filtrate. Several recrystallizations from water and alcohol did not give a product which was pure. Identification, by reacting with sodium bisulfite, was shown to be negative.

1,4-Dihydroxyphenylmethyleneiminodiacetic acid reacts with sodium chlorate in the presence of vanadium pentoxide and in dilute sulfuric acid to give a deep red solution. A yellow oil was recovered by extraction with xylene. The yellow oil was not the desired product, the expected reduction wave of 1,4-dioxophenylmethyleneiminodiacetic acid being absent in the polarogram. A black mass of material was obtained by adding alcohol to the raffinate layer. Treatment of this material with decolorizing charcoal and warm water did not effect purification, for all of the material was absorbed on charcoal. Extraction with organic solvents failed.

Chromotographic separation on silica of the solution obtained from the sodium chlorate oxidation gave five fractions. Each fraction was examined polarographically. None of the polarograms gives an indication of a cathodic wave characteristic of the sought-for quinone.

Oxidation with air in basic solution failed also. The polarograms taken during the oxidation failed to show any evidence that a quinone was formed although a reaction was occurring as indicated by a striking color change. Even after twenty hours of air oxidation the polarogram still showed no reduction wave. It is believed that the presence of oxygen destroyed or catalyzed a side-reaction as soon as 1,4-dioxophenylmethyleneiminodiacetic acid was formed.

The idea that oxygen may catalyze some side-reaction was shown to be correct in the case of an auxiliary study made on quinhydrone with air. Quinhydrone was treated with air in acidic and basic solutions. The polarograms of resulting solutions showed that the cathodic wave was decreased as a result of introducing oxygen into the system. Apparently the benzoquinone was being destroyed in some way. These results indicate that oxygen would be a poor reagent for the oxidations of hydroquinones in general.

The oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid was carried out using potassium persulfate. The choice of potassium persulfate seemed drastic, but logically it would appear to be the best reagent if the oxidation was to be followed polarographically: 1) Persulfate does not introduce anions which affect the anodic dissolution of mercury as do the halides. 2) Persulfate has moderate oxidizing power when not heated. 3) The oxidizing power of persulfate is low in the absence of a catalyst such as silver ion. 4) Persulfate does not introduce metallic ions which might complex with 1,4-dihydroxyphenylmethyleneiminodiacetic acid.

In Figure 9 is shown a polarogram, (a), made by reacting equivalent amounts of 1,4-dihydroxyphenylmethyleneiminodiacetic acid and potassium persulfate. The anodic wave is almost completely transformed into a cathodic wave. Thus, it shows that persulfate has affected the oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid. In Figure 9 is also shown a polarogram, (b), which was made on a solution containing 1,4-dihydroxyphenylmethyleneiminodiacetic acid and potassium persulfate in the ratio of 2:1.

Both cathodic and anodic waves are present, and the polarogram is completely analogous to that of a quinhydrone system. These experiments show that persulfate would be the reagent of choice to effect the very difficult oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid.

The oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid was carried out on a large scale. A reaction time of 15 minutes was allowed, at which time the reaction took on a yellow color. The mixture was extracted with diethyl ether. Evaporation of the ether phase gives a purple crystalline compound. The melting point behavior as observed by a microscope was unusual. At 82° the material sublimed and condensed on the slide. The sublimate appeared as golden crystals. At 140° the golden crystals changed over to pure white which melted sharply at 180°. The purple crystals were sublimed under vacuum, the various crystalline states were collected, and infrared spectra were obtained. The spectrum of the golden crystals was shown to be identical to the spectrum of benzoquinone. The white crystals were hydroquinone as the spectrum was identical with the spectrum of hydroquinone, and the melting point of the two were identical corroborates this. The polarogram of a solution of purple crystals at pH 6.0 shows a rather unsymmetrical curve. Part of the wave is anodic and part cathodic. The material is probably very impure 1,4-dioxophenylmethyleneiminodiacetic acid, possibly a mixture of the reduced and oxidized species, that is, an analog of quinhydrone. The cathodic portion of the polarographic wave decreased with time. The infrared spectrum of the purple material showed only broad bands of low absorbance. Recrystallization from ether gave a product which was still impure.

Polarograms of the raffinate layer from the reaction of persulfate and 1,4-dihydroxyphenylmethyleneiminodiacetic acid showed that not all of the quinone was isolated by the extraction procedure. This wave also decreased with time.

The preparation of 1,4-dioxophenylmethyleneiminodiacetic acid by the oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid was not accomplished. The dioxo compound is formed as shown polarographically but separation of the quinone by various methods proved futile. 1,4-Dioxophenylmethyleneiminodiacetic acid decomposes very rapidly, probably by the formation of a polymer.

V. STUDY OF THE JUGLONE SYSTEM

A. Introduction

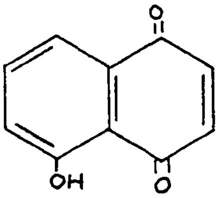
Juglone, a naturally occurring quinone found in the husk of the walnut, is chemically 5-hydroxy-1,4-naphthoquinone. It is made synthetically by the oxidation of 1,5-dihydroxynaphthalene. The yield is low when the oxidation is effected with potassium dichromate in sulfuric acid (20), the juglone being separated by steam distillation. Oxidation with potassium nitrosodisulfonate gives a much better yield, 50% being reported (21). A by-product, 5-hydroxy-1,2-naphthoquinone, is also formed, the juglone being isolated by utilizing its solubility in light petroleum ether.

The oxidation-reduction properties of juglone in alcohol solution were studied by Feiser (22). The reduction potential was given as 0.451 volts vs. N.H.E. in 50% ethyl alcohol. The reduction potential and the acid dissociation constants in aqueous, buffered solution were determined by Friedheim (23), the value obtained being 0.429 volts vs. N.H.E. and 10^{-8} at 20°, respectively. The dissociation constants for the corresponding hydroquinone were found to be 2.5×10^{-7} and 2.5×10^{-11}.

Juglone forms compounds with metal ions. The most widely known of these is formed with nickel, and the reaction with nickel has been used for the detection of nickel. As little as 6 mg./l. can be detected with juglone (24).

The polarographic behavior of juglone has been studied by Zuman (25) who found that juglone was reduced reversibly at the dropping mercury electrode by a two-electron reaction, the half-wave potential being 0.440 volts vs. N.H.E. Zuman found also an anomolous prewave. The acid dissociation constants were also determined by Zuman: $10^{-8.7}$ for the phenolic group, and 10^{-7} and 10^{-11}, respectively for the hydroquinone.

Juglone is of particular interest, for the functional groups which can



form chelate ring compounds are also involved in its oxidation-reduction function. In the present study of juglone the polarographic method has been used to determine the influence of metal ions on the half-wave potential. A relationship has been found relating the reduction to the concentration of the metallic ion. Nickel, magnesium and manganese were studied because these ions form soluble compounds with juglone in slightly alkaline media.

The stability constant of the juglone-nickel compound was also measured. The method of continuous variations was used to determine this constant and to learn the combining ratio of metal and juglone. The combining ratio the nickel-juglone compound and the manganese-juglone compound were also determined polarographically.

B. Experimental Work

1. Apparatus and solutions

The polarograph and accessories are the same as described in Section III B.

A 0.010 M solution of juglone was prepared by dissolving 0.1741 grams of juglone in ethyl alcohol and diluting to 100.0 ml. in a volumetric flask.

A buffer solution, pH 8.48, was prepared by dissolving 16.9 grams of sodium barbital in deionized water, and adding 1.5 ml. of concentrated hydrochloric acid, and diluting to one liter in a volumetric flask.

2. Preparation of 5-hydroxy-1,4-naphthoquinone (juglone)

Juglone was prepared by the method of Teuber and Götz (21) with some modifications. 1,5-Dihydroxynaphthalene, 5.0 g., was placed into a 2-liter beaker and dissolved in 500 ml. of methyl alcohol. To this solution was added a mixture of 75 g. of potassium nitrosodisulfonate, 850 ml. of water, and 250 ml. of 0.1 M potassium dihydrogen phosphate. This mixture was stirred for 2 hours. A dark precipitate formed which was filtered. The filtrate was then extracted with five 200 ml. portions of chloroform. Three-fourths of the chloroform was removed by distillation and the remainder evaporated at room temperature. The residue, together with the dark precipitate from the original reaction mixture, was extracted in a Soxlet apparatus with petroleum ether for 24 hours. The volume was reduced by evaporation, and the remaining solution cooled in dry ice and acetone mixture. Orange crystals formed. The yield of juglone was 3.7 g., melting point: $163\text{-}4^{o}$. The infrared spectrum showed the characteristic carbonyl bands at 5.9 and 6.1 microns.

3. Polarography of juglone

A polarogram of 5.0 ml. of 0.010 M juglone, diluted to 100.0 ml. with pH 8.48 buffer was obtained by spanning voltages from -0.1 to -0.4 volts vs. s.c.e. The sensitivity was set at 0.015 microamp./mm. A potassium nitrate salt bridge was used to complete the circuit. The solution was deaerated with nitrogen for ten minutes. The resistance of the cell was determined by the method of Müller, and the value was 3500 ohms. The polarogram, Figure 10, shows a prewave as reported by Zuman.

In order to verify the prewave was some other phenomena other than diffusion controlled, the following experiment was carried out. Polarograms of juglone were obtained at different pressures of the mercury column. This was done by moving the mercury reservoir to different levels. The heights of the prewave and the diffusion wave were measured as well as the level of the mercury column. Figures 11 and 12 show the results.

4. Polarography of juglone in the presence of varying amounts of nickel

Solutions containing 1.00, 2.02, 3.00, 4.03 millimoles of nickel were made by weighing out the correct amount of nickel nitrate hexahydrate. Five milliliters of 0.010 M juglone was added and finally the solutions were diluted to volume in a 100-ml. volumetric flask with pH 8.48 buffer. A 50 ml. portion of each of these solutions were taken and deaerated with nitrogen. A polarogram was then obtained for each.

5. Polarography of juglone with varying amounts of magnesium

Solutions containing 1.00, 1.50, 2.06, 2.53, 3.03, and 4.11 moles of magnesium were prepared by weighing out the required amount of reagent grade magnesium sulfate and dissolving in the smallest volume of deionized water. Five milliliters of 0.010 M juglone was then added, and finally the solutions diluted to 100.0 ml. with pH 8.48 buffer. About 50 ml. aliquots were taken, and polarograms obtained after deaerating for ten minutes with nitrogen.

6. Polarography of juglone in the presence of varying amounts of manganese

Manganese and juglone react at pH 8.48 to give a violet-purple solution. Solutions containing 1.10, 2.41, 3.40, and 4.20 moles of manganese were prepared by adding the correct amount of manganous chloride tetrahydrate and dissolving in a small amount of deionized water. Five milliliters of 0.010 M juglone were added, and the solutions diluted to mark in 100-ml. volumetric flasks with pH 8.48 buffer. Polarograms were obtained for each of these solutions after deaerating for ten minutes with nitrogen.

7. Polarographic determination of the combining ratio of juglone and nickel

The polarographic method was used to determine the combining ratio of nickel and juglone. The nickel ion gives a well defined polarographic wave, and the decrease of the wave height can be established as increasing amounts of juglone are added. In each of eight 50-ml. flasks was placed 1.00 ml. of 0.010 M nickel nitrate. In each successive flask was placed 0, 0.25, 0.50, 1.00, 1.50, 2.00, 2.5, and 3.00 ml. of 0.010 M juglone. The solutions were buffered with 15 ml. of pH 8.48 buffer and diluted to mark. A polarogram was obtained of each solution after deaerating with nitrogen for ten minutes. Both the wave of juglone and nickel were obtained. Table 8 shows the results.

8. Polarographic determination of the combining ratio of juglone and manganese

The same technique as above was used to determine the combining ratio of juglone and manganese. In each of seven 50-ml. volumetric flasks was placed 1.00 ml. of 0.010 M manganous chloride. In each successive flask was placed 0, 0.50, 1.00, 1.50, 2.00, 3.00 ml. of 0.010 M juglone. The solutions were buffered with 15 ml. of pH 8.48 buffer and diluted to mark. After flushing with nitrogen, polarograms of each solution were obtained. The results are shown in Table 9.

9. Spectrophotometric study of the juglone-nickel compound

Nickel and juglone react at pH 8 to form a soluble purple colored compound. The method of continuous variations (26) will be used to determine the combining ratio, and the formation constant will be estimated.

a. Solutions Nickel Nitrate, 0.00103 M was prepared by dissolving 0.1509 g. of nickel nitrate hexahydrate in a small amount of deionized water. 3.74 g. of potassium chloride was added, and the solution adjusted to 500.0 ml. with deionized water. The ionic strength of the solution was 0.10.

Nickel nitrate, 0.00981 M, was made up by weighing out 0.2854 g. of nickel nitrate hexahydrate and diluting to 100.0 ml. with 0.10 M potassium chloride.

Juglone, 9.98×10^{-4} M, was prepared by dissolving 86.91 mg. of juglone in 95% ethyl alcohol and diluting to 500.0 ml.

Juglone, 1.02×10^{-2} M, was prepared by dissolving 0.1709 g.of juglone in 100.0 ml. of 95% ethyl alcohol.

Michaelis buffer of pH 8 was made by adding 284 ml. of 0.10 N hydrochloric acid to 716 ml. of 0.10 N sodium barbital.

b. Absorption spectrum of juglone and juglone-nickel compound Four milliliters of 1.02×10^{-2} M juglone, 10 ml. of pH 8 buffer were diluted to 100.0 ml. with 0.10 N potassium chloride. A spectrum of this solution was obtained on a Cary Model 12 recording spectrophotometer, Figure 18. The cell blank consisted of 4.00 ml. of ethyl alcohol, 10 ml. of pH 8 buffer diluted to 100.0 ml. with 0.10 N potassium chloride.

Four milliliters of 1.02×10^{-2} M juglone, 8.00 ml. of 9.81×10^{-3} M nickel nitrate, 10 ml. of pH 8 buffer was diluted to 100.0 ml. with 0.10 N potassium chloride. A spectrum was obtained for this solution on the Cary Model 12 spectrophotometer, Figure 18.

c. Procedure for the method of continuous variations To each of eleven 100-ml. volumetric flasks was added 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 ml. of 1.03×10^{-3} M nickel nitrate and 50, 45, 40, 35, 30, 25, 20, 15, 10, 5, 0 ml. of 9.98×10^{-4} M juglone. To each was added 10 ml. of pH 8 buffer. The solutions were adjusted to ionic strength of 0.10 by the addition of a calculated amount of 0.50 M potassium chloride. Each was diluted to 100.0 ml. with deionized water. The pH was measured on a Beckman Model G pH meter confirming that each had a pH of 8.03. The absorbance was measured after five minutes at a wavelength of 530 mu using a Beckman DU spectrophotometer.

10. Preparation of α-hydrojuglone (1,4,5-trihydroxynaphthalene)

The method of Willstätter (27) was used to prepare α-hydrojuglone. One gram of juglone was dissolved in ether (50 ml.) and placed into a separatory funnel containing 100 ml. of 2 N sulfuric acid. Zinc dust was added, and the mixture was shaken until the disappearance of the yellow color of juglone. The ether layer was removed and washed with three portions of distilled water. After evaporation of the ether solution under an atmosphere of nitrogen, white crystals were obtained. Melting point: 151° with decomposition. The solution of α-hydrojuglone at pH 7.29 was deep purple in color. Polarograms of the solution were obtained.

a. Preparation of the quinhydrone of juglone and α-hydrojuglone

Juglone, 0.10 g., and α-hydrojuglone, 0.10 g., were refluxed in ether for one hour. The ether solution was evaporated to about 5 ml. under an atmosphere of nitrogen, after which an olive-green material precipitated. The melting point, observed with a microscope, showed that sublimation of an orange crystalline material had occurred. These orange crystals melted at 165°, which is very close to the melting point of juglone. The bulk of the material melted in a range of 152-163°. A polarogram of a dilute solution of the olive-green material showed only a cathodic wave. The wave was identical to the wave of juglone. Recrystallization of the olive-green material from ether gave a product which was orange, and the melting point, 164°, confirmed that the product isolated from the reaction of juglone and α-hydrojuglone is juglone.

C. Results and Discussion

The preparation of juglone using potassium nitrosodisulfonate and 1,5-dihydroxynaphthalene went smoothly and with good yields. The nature of the reaction is such that dilute solutions of the reacting reagents must be used; consequently, only small amounts of the juglone can be prepared at one time. The juglone is pure as noted by its sharp melting point of 164-5°. The infrared spectrum corroborates the identification and the purity. The infrared spectrum shows two carbonyl groups at 5.9 and 6.1 microns, respectively. The presence of two carbonyl frequencies is a result of hydrogen bonding of the carbonyl oxygen in the 4 position with the phenolic hydrogen on the 5 position of juglone.

The solution containing juglone at pH 8.48 gives a polarogram having two waves, Figure 10. The first wave is of anomalous character as was described by Zuman (25). The anomalous character of the prewave was verified by obtaining polarograms of juglone and at the same time changing the mercury pressure. The height of the prewave and the main wave were measured. A plot of pressure, expressed in centimeters of mercury, against the height of the prewave, Figure 11, gives a straight line. The direct proportionality of the two variables shows that the prewave is not diffusion controlled. A plot of the square root of the pressure head, expressed in centimeters of mercury, against the height of the main wave gives a straight line, Figure 12. This proportionality, square root of pressure vs. current, is followed when the wave is diffusion controlled.

The plot of I/I_d-I vs. applied potential, Figure 13, gives a straight line. The slope is -0.033 volts which agrees very well to the predicted value of -0.030 volts for a two-electron reduction. The half-wave potential is -0.279 volts vs. s.c.e.

The polarogram of juglone in the presence of nickel ion shows a shift in the half-wave potential, the shift being -0.051 volts. The results are shown in Table 5. The concentration of juglone is 0.050 mmoles/100 ml.

A typical polarogram of the nickel-juglone system is shown in Figure 14. The wave now appears as one because the prewave appears to blend into the diffusion wave. The wave height has increased by 30% of the wave of the same concentration of uncomplexed juglone. This increase can be rationalized as being due to more than one molecule of juglone per nickel-juglone molecule being reduced at the mercury drop.

Figure 10. Polarogram of juglone at pH 8.48. Concentration of juglone is 0.050 mmoles/100 ml.

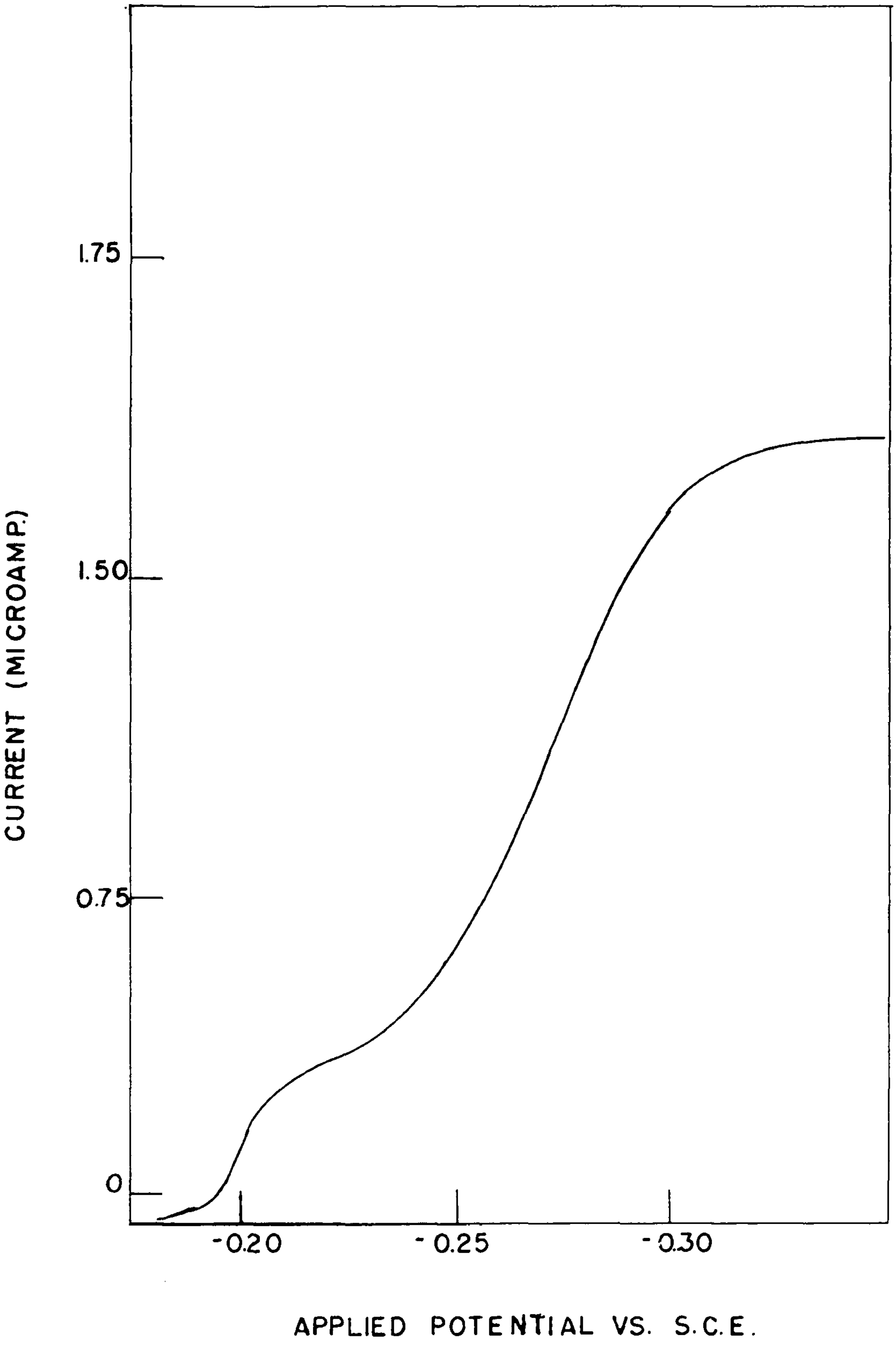

CURRENT (MICROAMP.)
1.75
1.50
0.75
0
-0.20
- 0.25
- 0.30
APPLIED POTENTIAL VS. S.C.E.

Figure 11. Plot of wave height of juglone prewave, expressed in cm. against the pressure of the mercury column in terms of cm. of mercury.

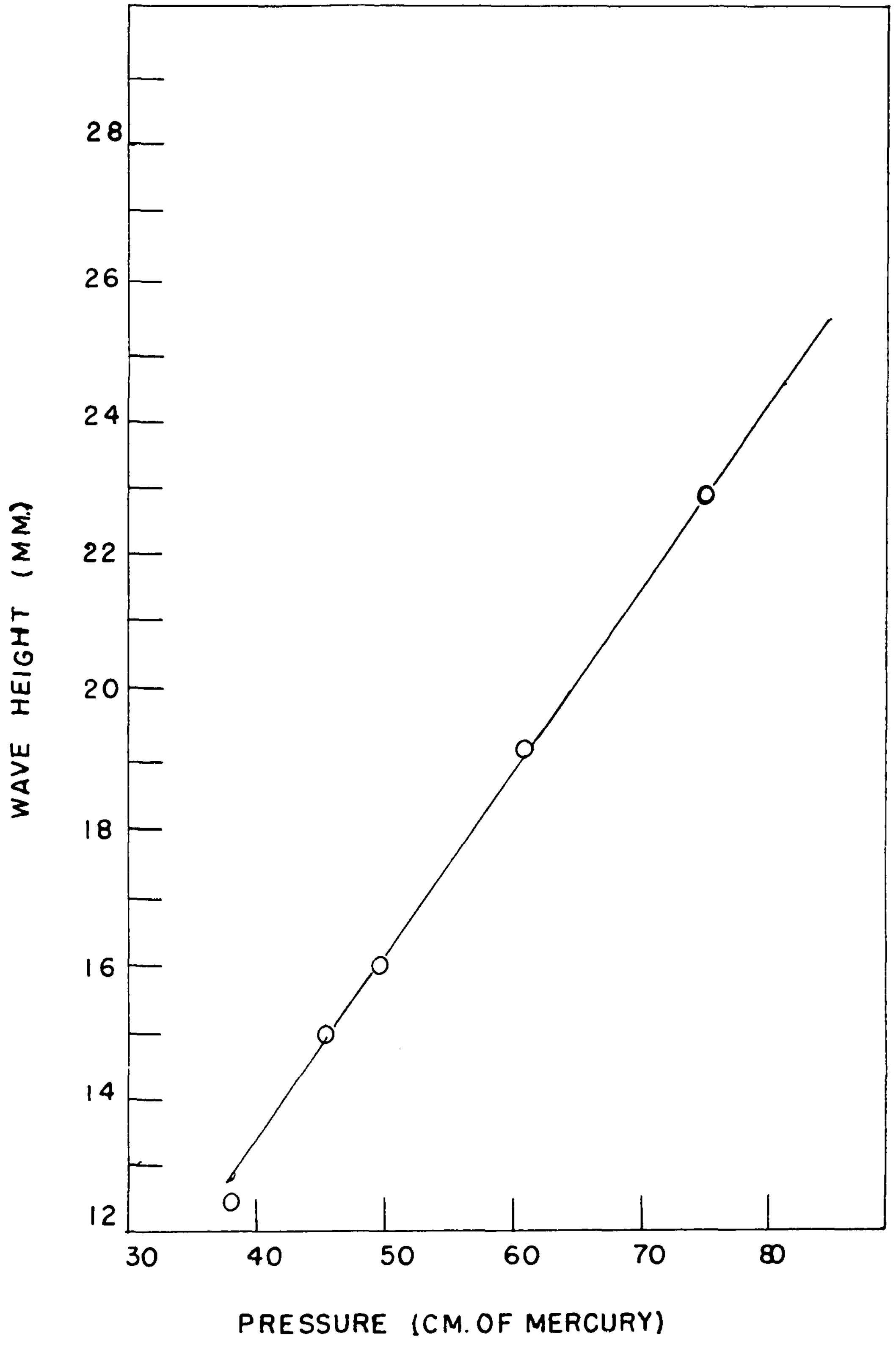

WAVE HEIGHT (MM.)
28
26
24
22
20
18
16
14
12
30
40
50
60
70
80
PRESSURE (CM. OF MERCURY)

Figure 12. Plot of the wave height of juglone, expressed in cm., against the $(\text{pressure})^{\frac{1}{2}}$. The pressure is expressed in cm. of mercury.

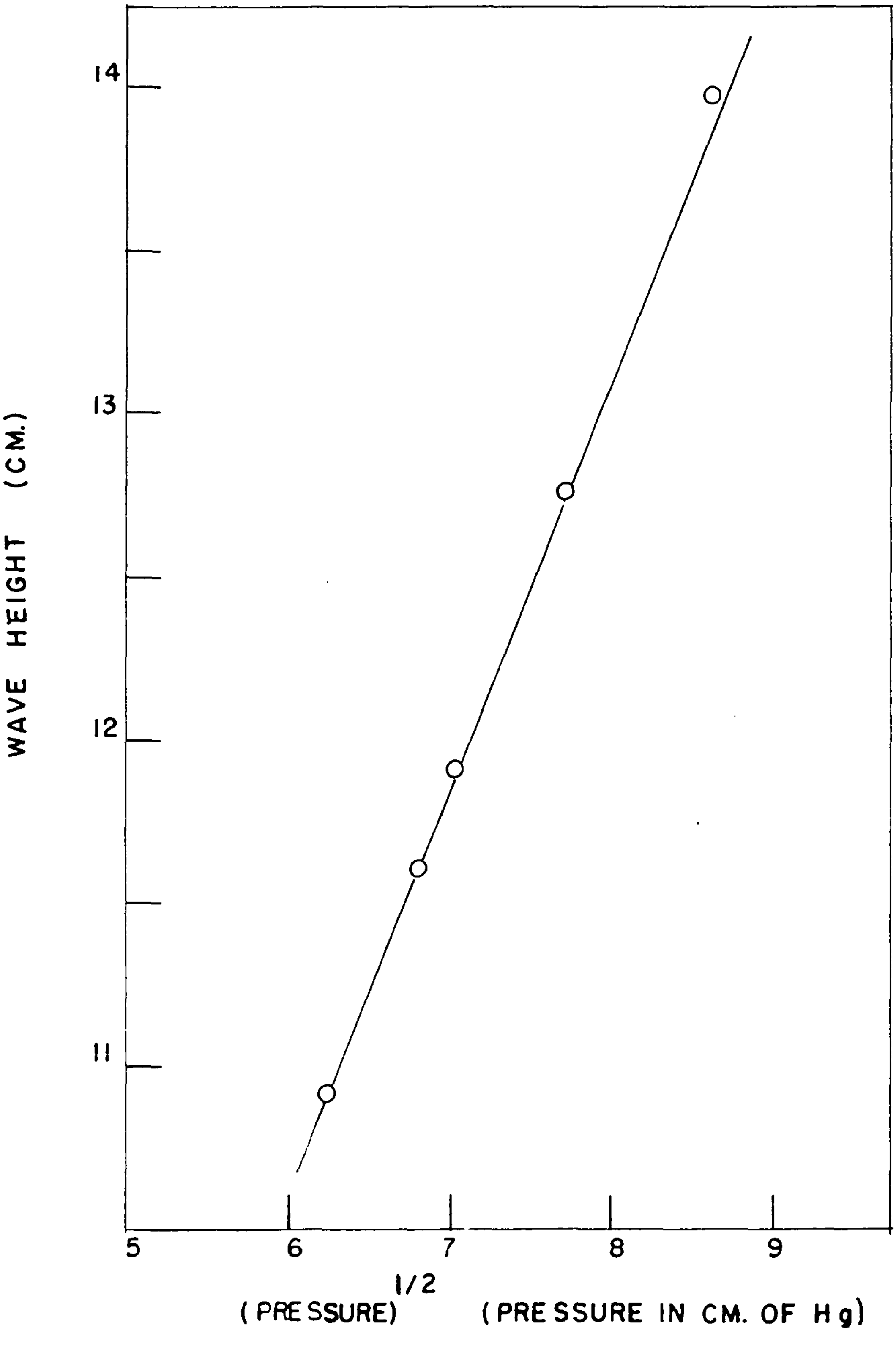

WAVE HEIGHT (CM.)
14
13
12
11
5
6
7
8
9
(PRESSURE)$^{1/2}$ (PRESSURE IN CM. OF Hg)

Table 5. Half-wave potential of juglone as a function of nickel concentration; pH 8.48

C_{nickel}[a]	Half-wave Potential (volts vs. s.c.e.)
0.0	-0.279
1.00	-0.330
3.02	-0.330
4.03	-0.328

[a]concentration in mmoles/100 ml.

A plot of I/I_d-I vs. applied potential, Figure 15, gives a straight line. The slope is -0.056 volts. Thus, it appears that the system is reversible with a one-electron reduction. However, no other wave was observed at more negative potentials. This, plus the fact the prewave blended into the diffusion wave, points to the possibility that the process is actually a two-electron reduction and irreversible.

The data in Table 4 shows that there is no net increase of the shift in the half-wave potential with increasing nickel ion concentration.

The polarogram of juglone in the presence of magnesium shows a maximum shift of -0.010 volts. The results are shown in Table 6. The concentration of juglone is 0.05 mmoles/100 ml.

Figure 13. Plot of I/I_d-I (log scale) vs. the applied potential for the polarogram of juglone. Data obtained from Figure 10.

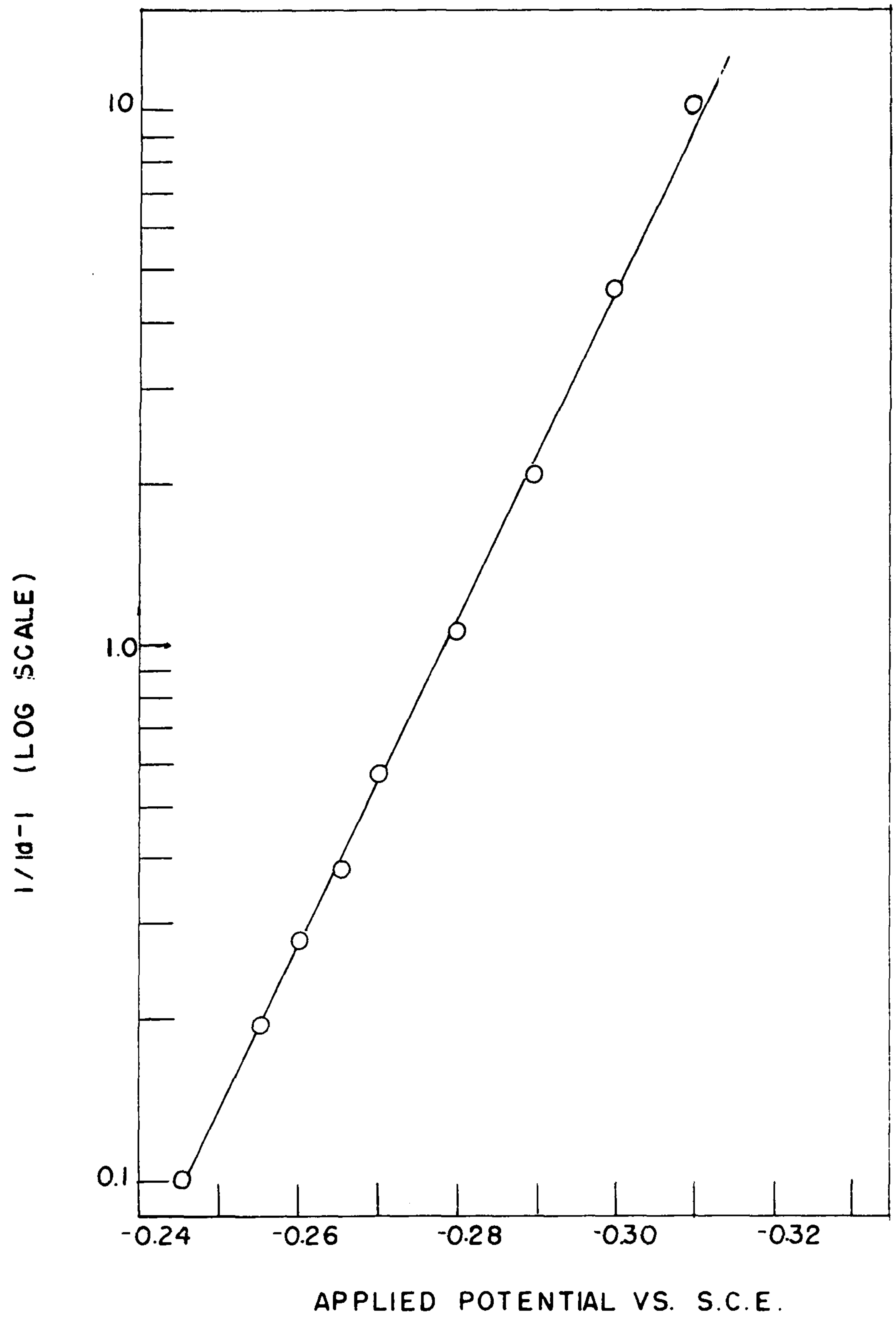

10
1.0
0.1
1/Id-1 (LOG SCALE)
-0.24
-0.26
-0.28
-0.30
-0.32
APPLIED POTENTIAL VS. S.C.E.

Figure 14. Polarogram of 0.05 mmoles of juglone in the presence of 1.00 mmoles of nickel nitrate; pH 8.48.

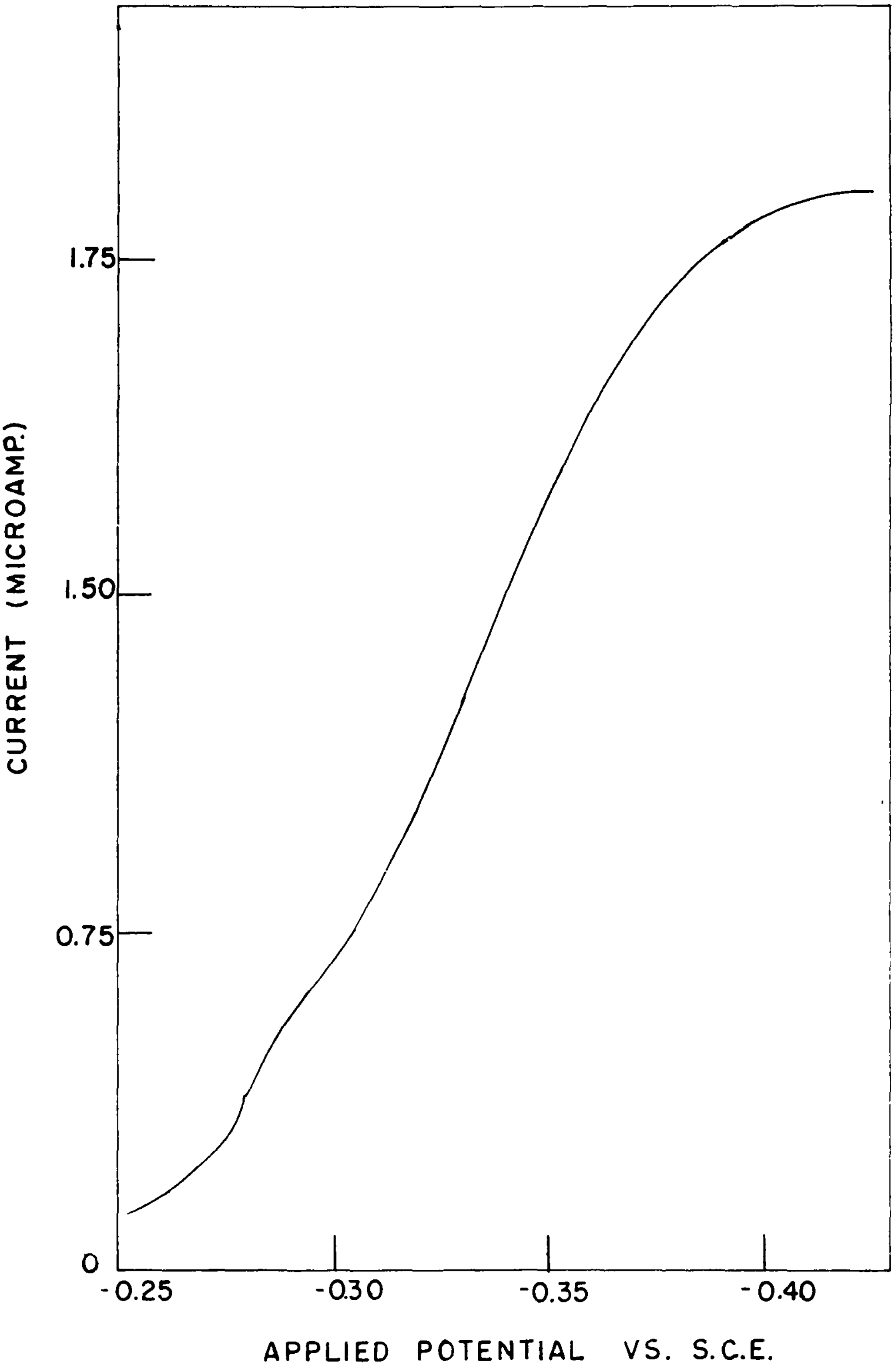

CURRENT (MICROAMP.)
1.75
1.50
0.75
0
-0.25
-0.30
-0.35
-0.40
APPLIED POTENTIAL VS. S.C.E.

Figure 15. Plot of I/I_d-I (log scale) against applied potential for the polarogram of juglone in the presence of nickel. Data taken from Figure 14.

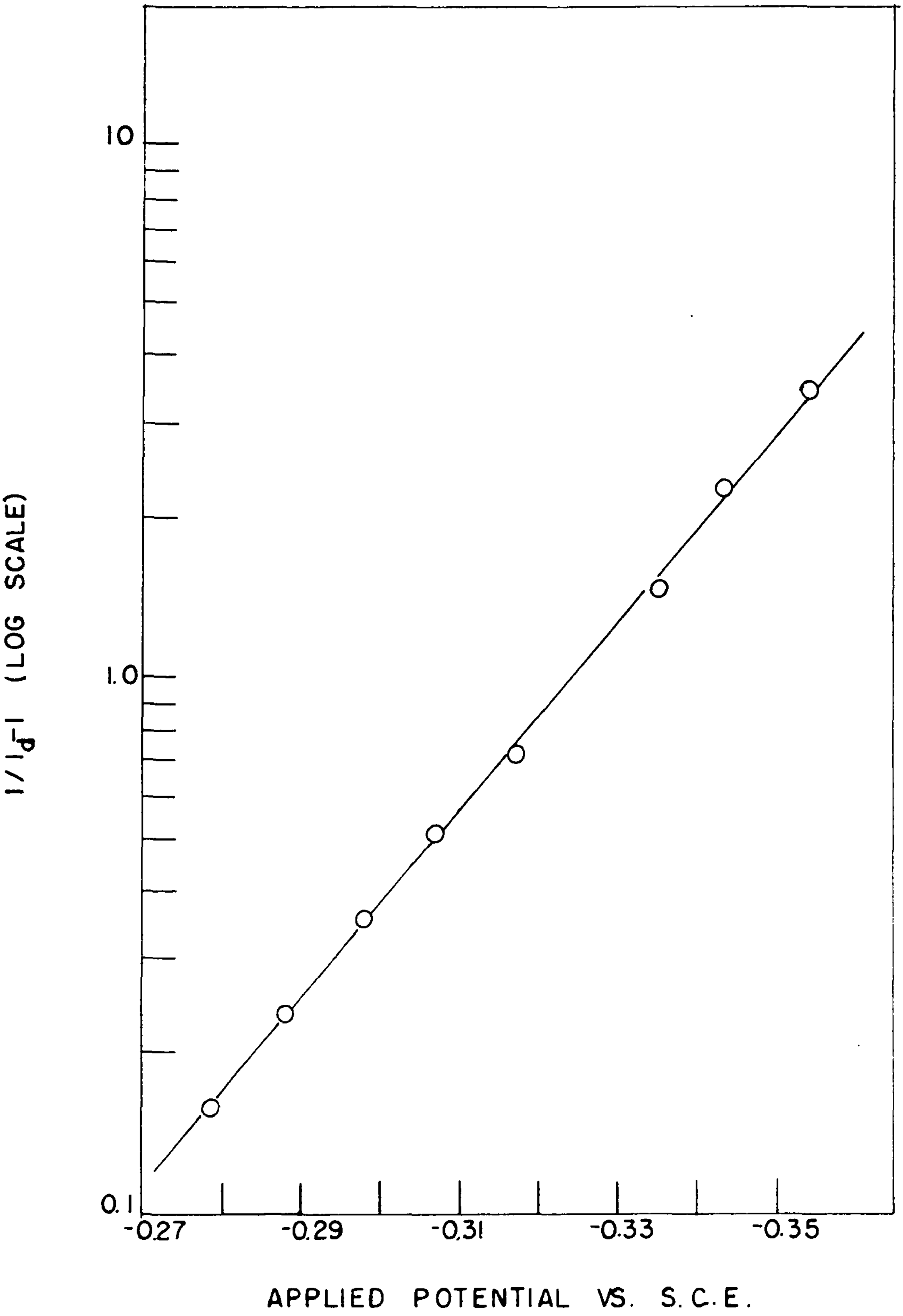

10
1.0
0.1
-0.27
-0.29
-0.31
-0.33
-0.35
$i / i_d - i$ (LOG SCALE)
APPLIED POTENTIAL VS. S.C.E.

Table 6. Half-wave potential of juglone as a function of magnesium concentration; pH 8.48

$C_{magnesium}$[a]	Half-wave Potential (volts vs. s.c.e.)
0	-0.280
1.00	-0.283
1.50	-0.286
2.06	-0.290
2.53	-0.290
4.11	-0.286

[a] concentration in mmoles/100 ml.

A typical polarogram of the magnesium-juglone system is shown in Figure 16. A prewave is present as in the case of the polarogram of free juglone. The wave height has increased by about 25% of the wave of the same concentration of free juglone. Again the same rationalization as in the case of the nickel-juglone case is applied. A plot of I/I_d-I vs. applied potential gives a straight line with a slope of -0.035 volts. This corresponds very closely to the expected value of -0.030 volts for a two-electron reduction.

The data in Table 6 shows that no increase of the half-wave potential results as the magnesium ion concentration is increased.

The polarogram of juglone in the presence of manganese shows a maximum shift in the half-wave potential of about -0.010 volts. The results are shown in Table 7. The concentration of juglone is 0.05 mmoles/100 ml.

Table 7. Half-wave potential of juglone as a function of manganese concentration; pH 8.48

$C_{\text{manganese}}$[a]	Half-wave Potential (volts vs. s.c.e.)
0	-0.280
1.00	-0.283
2.41	-0.285
3.40	-0.290
4.20	-0.288

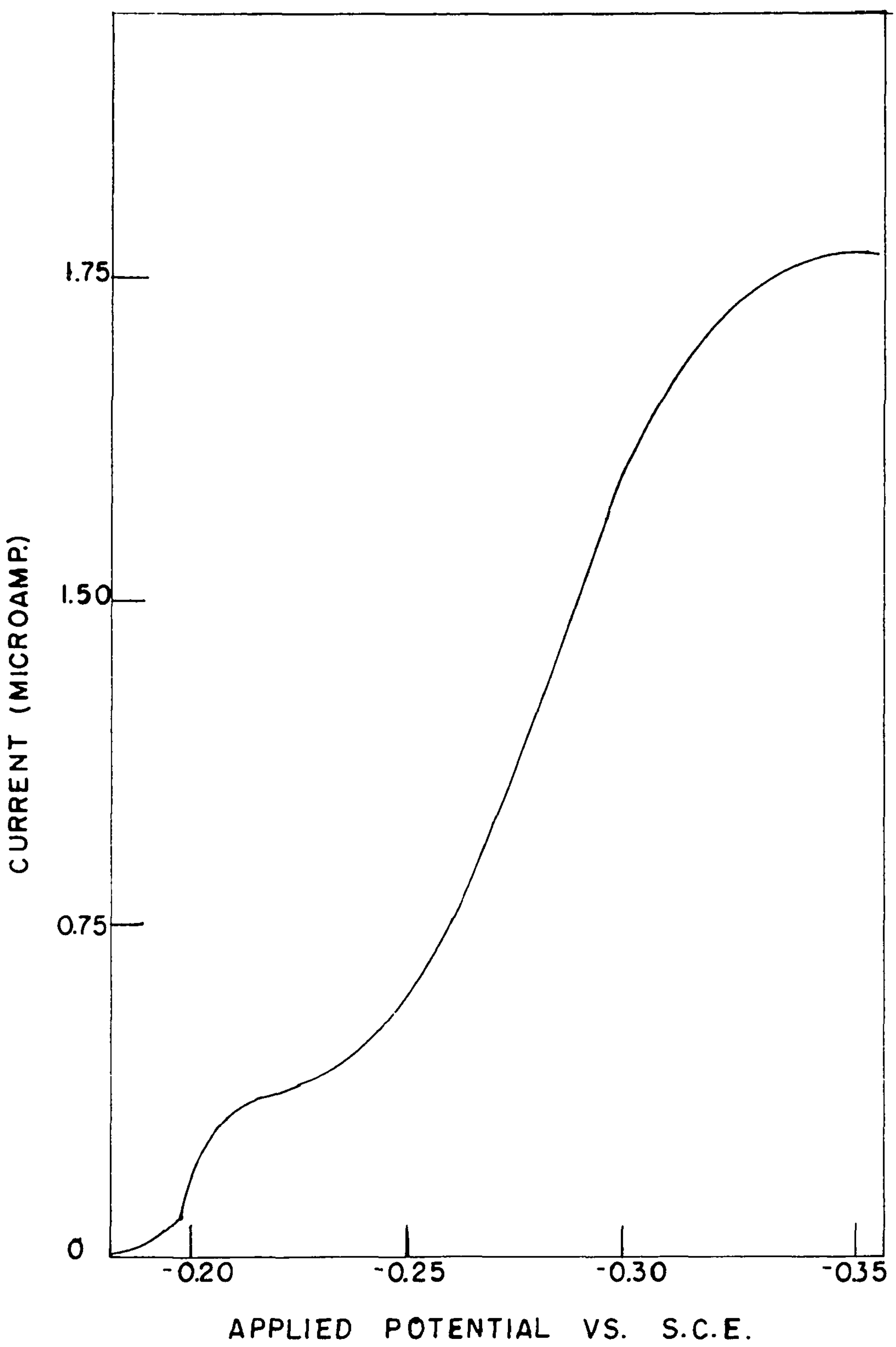
[a]concentration in mmoles/100 ml.

A typical polarogram of juglone in the presence of manganese is shown in Figure 17. The prewave is present as in the case of free juglone. The height of the diffusion wave is twice as great as the wave for the same concentration of free juglone. Apparently more than one juglone molecule is being reduced at the mercury drop. A plot of I/I_d-I vs. applied potential gives a straight line with a slope of -0.038 volts. This value agrees with the expected value of -0.030 volts for a two-electron reduction.

The data of Table 7 shows that no significant shift in the half-wave potential occurs with increasing concentration of manganese.

In the main, the results of the previous experiments show that by incorporating a metal ion into a molecule bearing oxidation-reduction group and if the bonding directly gets involved with these groups, the potential at which the electrochemical reaction can take place is influenced.

Figure 16. Polarogram of 0.05 mmoles of juglone in the presence of 1.50 mmoles of magnesium sulfate; pH 8.48.


CURRENT (MICROAMP.)
1.75
1.50
0.75
0
-0.20
-0.25
-0.30
-0.35
APPLIED POTENTIAL VS. S.C.E.

The combining ratio of juglone and nickel was found to be two molecules of the quinone to one of nickel. The polarographic method was used to determine this inasmuch as nickel alone shows a well defined wave. By holding the concentration of the nickel constant and increasing the concentration of juglone, the polarograms indicated the combining ratio by a decrease in the diffusion current. In Table 8 are shown the results.

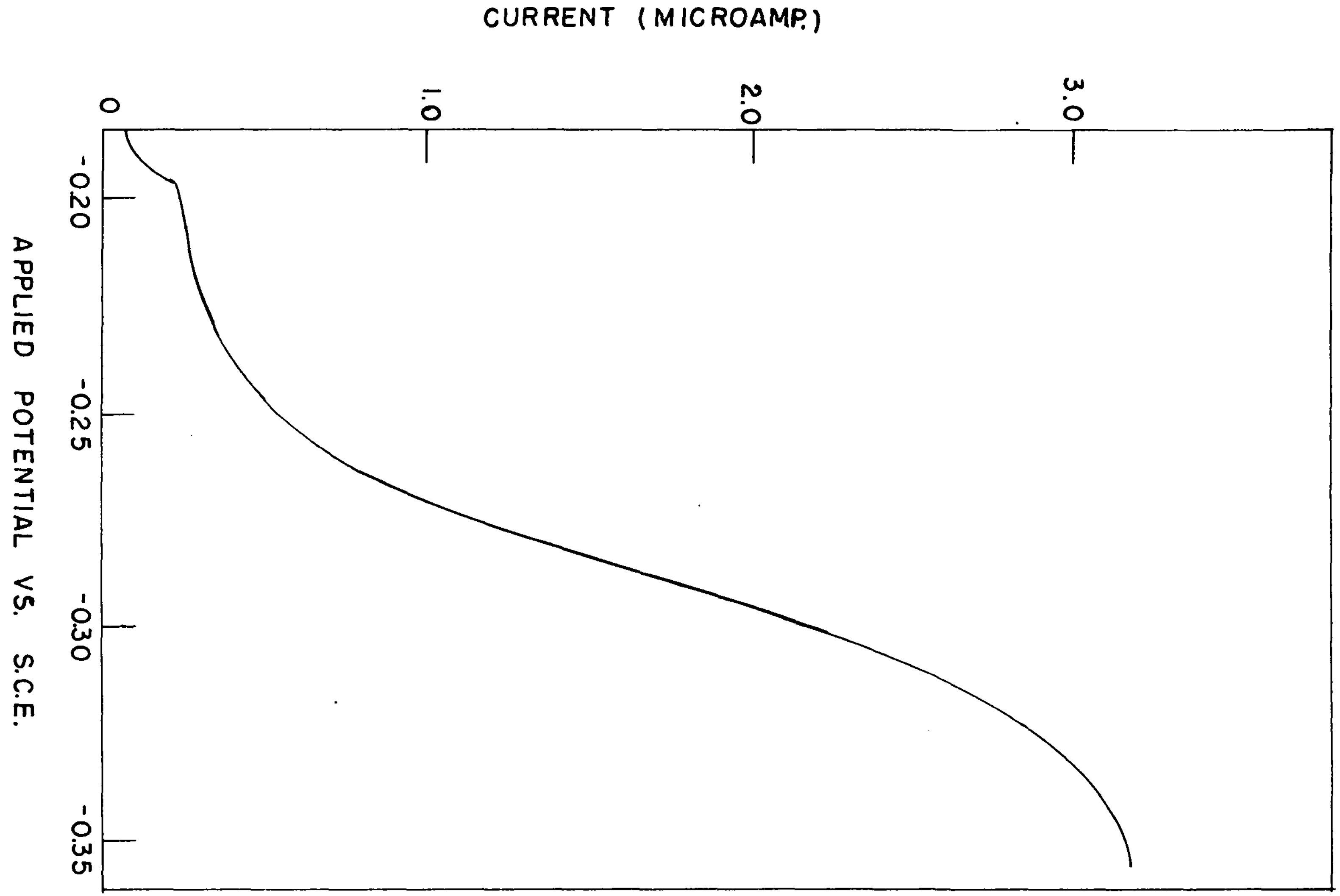
Table 8. Data for the determination of the combining ratio nickel and juglone; pH 8.48

Mole Ratio of Juglone to Nickel	Wave I I_d (microamp.)[a]	Wave II I_d (microamp.)
0:1		2.10
0.25:1	0.38	2.00
0.50:1	0.76	2.05
1:1	0.76	1.70
1.5:1	0.54	0.82
2:1	0.64	0.48
2.5:1	1.02	0.48
3:1	1.64	0.48

[a] diffusion current

Wave I is the wave for the reduction of juglone and wave II for the reduction of nickel. Two additional waves should have been present; one for the reduction of the juglone in the juglone-nickel compound and the other

Figure 17. Polarogram of 0.05 mmoles of juglone in the presence of 2.41 mmoles of manganous chloride; pH 8.48.


CURRENT (MICROAMP.)
0
1.0
2.0
3.0
-0.20
-0.25
-0.30
-0.35
APPLIED POTENTIAL VS. S.C.E.

for the reduction of nickel in the juglone-nickel compound. The absence, however, is due to the precipitation of the juglone-nickel complex. It was noted that a purple solid remained in the polarographic cell.

The data of Table 8 show that the diffusion current for wave II reaches a constant value at a ratio of 2:1. Rationalization of wave I is difficult since no trend appears. Beyond the ratio of 2:1, however, the current increases considerably, due possibly to juglone in excess of the amount required to form the compound.

The same technique as above was used to elucidate the combining ratio of the juglone-manganese compound. In Table 9 are shown the results.

Table 9. Data for the determination of the combining ratio manganese and juglone; pH 8.48

Mole Ratio of Juglone to Manganese	Wave I I_d (microamp.)[a]	Wave II I_d (microamp.)
0:1	-	1.06
0.5:1	0.47	1.00
1.0:1	1.17	1.00
1.5:1	1.34	0.96
2.0:1	1.46	0.85
3.0:1	2.00	0.68
4.0:1	2.38	0.66

[a] diffusion current

Wave I is due to the reduction of juglone and wave II to the reduction of manganese. As can be seen the diffusion current of wave II reaches a constant value at a ratio of 3:1. Therefore, the combining ratio of juglone to the manganese is 3:1. As in the preceeding case, two other waves should be present; one for the reduction of the juglone in juglone-manganese compound and the other for the reduction of the manganese in the complex compound. The insolubility of the compound explains this effect. The diffusion current of the juglone wave is difficult to interpret since the trend is erratic.

The absorption spectrum of juglone and the compound formed between juglone and nickel was obtained using the recording spectrophotometer, Figure 18. The wavelength of maximum absorbance for the juglone is 425 mμ and for the nickel-juglone compound, 530 mμ. The two maxima are widely separated, but a small absorbance due to juglone persists at the maximum for the nickel-juglone compound.

The combining ratio of the nickel-juglone compound was determined by the method of continuous variations. The absorbance of eleven solutions containing different ratios of nickel to juglone, however, with the total number of moles of the two remaining constant, was measured. A plot of absorbance versus mole fraction nickel was prepared, Figure 19. Owing to some absorbance of the juglone, the absorbance at zero mole fraction nickel was quite large. A line from zero mole fraction to 1.0 mole fraction was drawn. This line represents the absorbance at each mole fraction which is due to juglone. Absorbances along the line were subtracted from the corresponding points along the curve to obtain the true absorbance due to the nickel-juglone compound. These values are plotted in Figure 20. The combining ratio, n, was calculated using the relation $n = x/1-x$, where x is the mole fraction of nickel at the maximum of the curve of Figure 20. The value of n is 1.93. This is in good agreement with the expected value of 2 since nickel usually has a coordination number of four.

The apparent formation constant may be calculated from the above data. The equilibrium involved in the reaction is

$$M + 2X = MX_2$$

Let $\underline{C}$ = limiting concentration of the complex. This is obtained by taking the product of the total molarity of the solution and x, the mole fraction of the metal at the maximum of the curve in Figure 3. (5.08×10^{-4} times 0.34)

$= 1.73\times10^{-4}$ M.

Cm = total analytical concentration of the metal

$= \underline{C} = 1.73\times10^{-4}$ M

Cx = total analytical concentration of juglone. This is equal to $2\underline{C}$

$= 3.46\times10^{-4}$ M

(MX_2) = equilibrium concentration of the compound. This is equal to $A/A_{extrp.}$ times $\underline{C}$, where A is the absorbance at the maximum of the curve of Figure 20 and $A_{extrp.}$ is the extrapolated maximum absorbance.

$$= 1.32\times10^{-4}\ M$$

$$(M) = \underline{C} - (MX_2) = 0.41\times10^{-4}\ M$$

$$(X) = 2\underline{C} - (MX_2) = 2.14\times10^{-4}\ M$$

The equilibrium constant expression is

$$K = \frac{(MX_2)}{(M)\ (X)^2}$$

Substituting the values yields a formation constant of 7.15×10^{7} or a log K of 7.85 at a pH of 8.03.

The nickel-juglone compound is not very stable at pH 8.03 as indicated by its absorbancy decreasing after 30 minutes and a precipitate appearing after one hour.

α-Hydrojuglone (1,4,5-Trihydroxynaphthalene) was prepared by reducing juglone with zinc dust in 2 N sulfuric acid. The reaction went smoothly, and the separation of the product was easy. The product was white in color and highly crystalline. α-Hydrojuglone upon being exposed to air turns green. This is apparently cuased by the formation of the β-isomers of α-hydrojuglone (28). A solution of α-hydrojuglone at pH 7.29 is deep purple in color and after four hours decomposes with precipitation of a dark brown material. Polarograms of a solution of α-hydrojuglone at pH 7.29 show only an anodic diffusion wave. The prewave, which was present in the polarogram of juglone, appears as a postwave in the polarogram of α-hydrojuglone. The polarogram, after four hours, showed that the α-hydrojuglone decomposes with time, the anodic wave having decreased to 50% of the original wave.

Figure 18. Absorption spectra of:
A. Spectrum of juglone
B. Spectrum of nickel-juglone compound

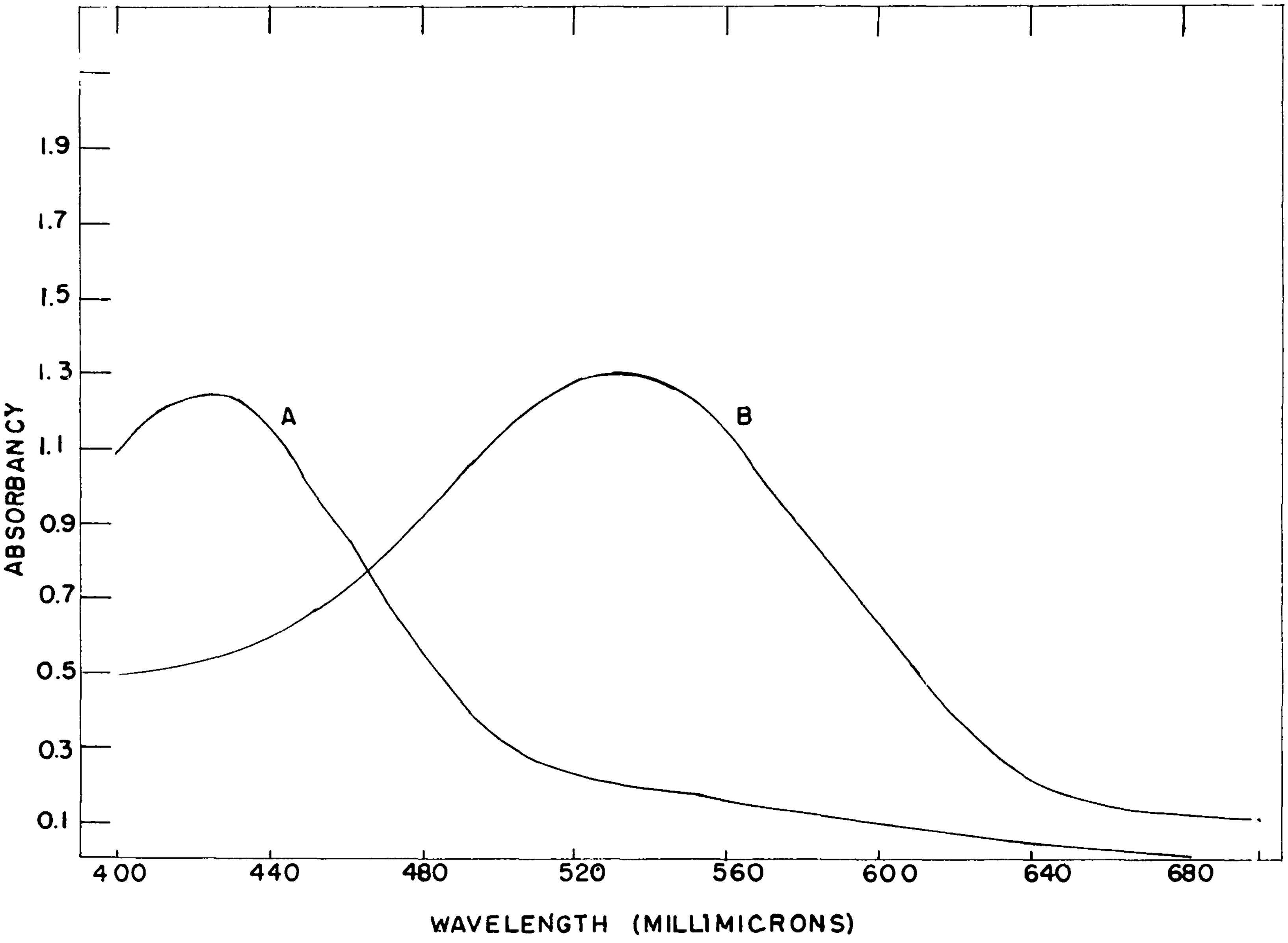

ABSORBANCY
1.9
1.7
1.5
1.3
1.1
0.9
0.7
0.5
0.3
0.1
A
B
400
440
480
520
560
600
640
680
WAVELENGTH (MILLIMICRONS)

Figure 19. Continuous variations plot for the nickel-juglone compound (uncorrected).

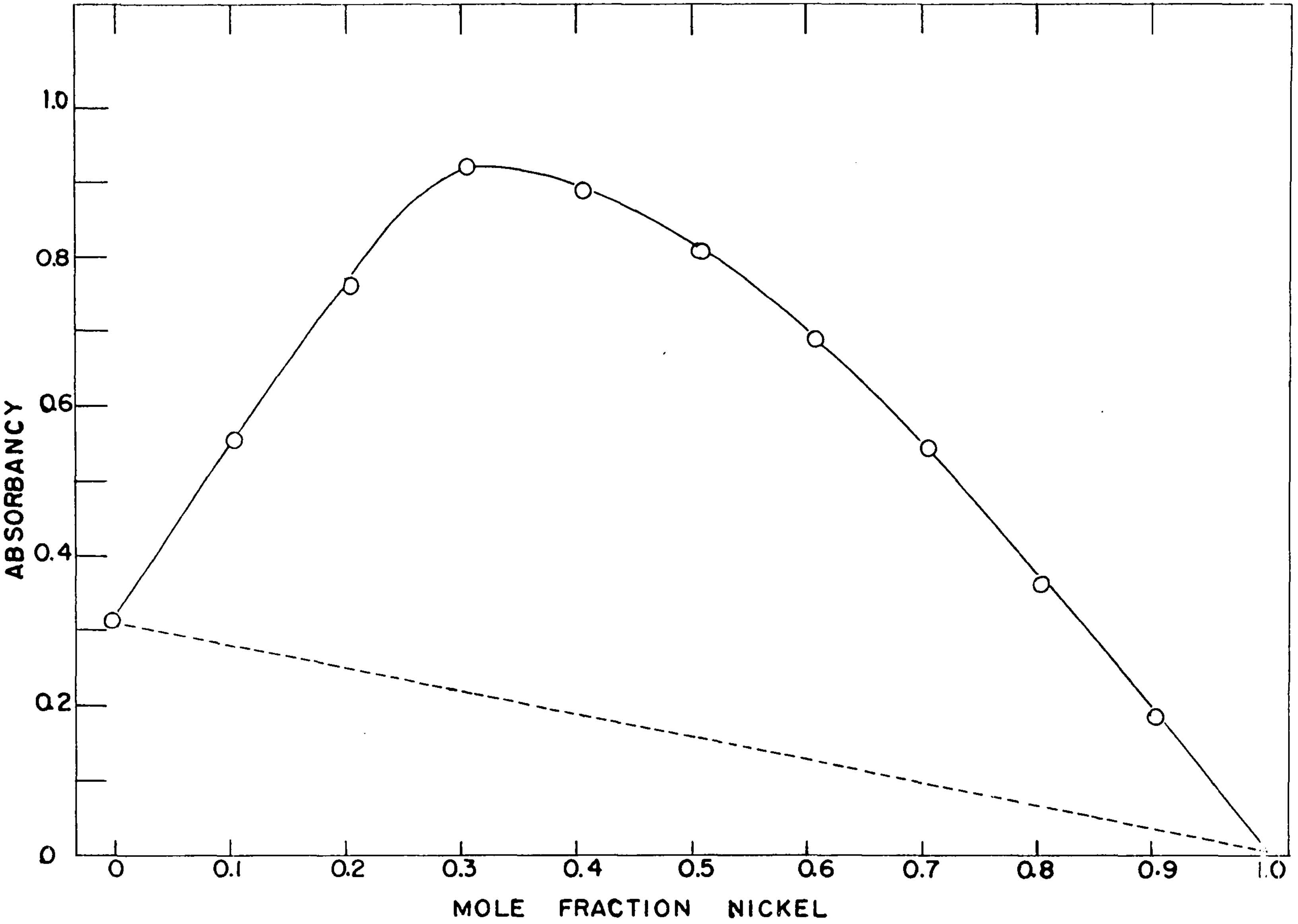
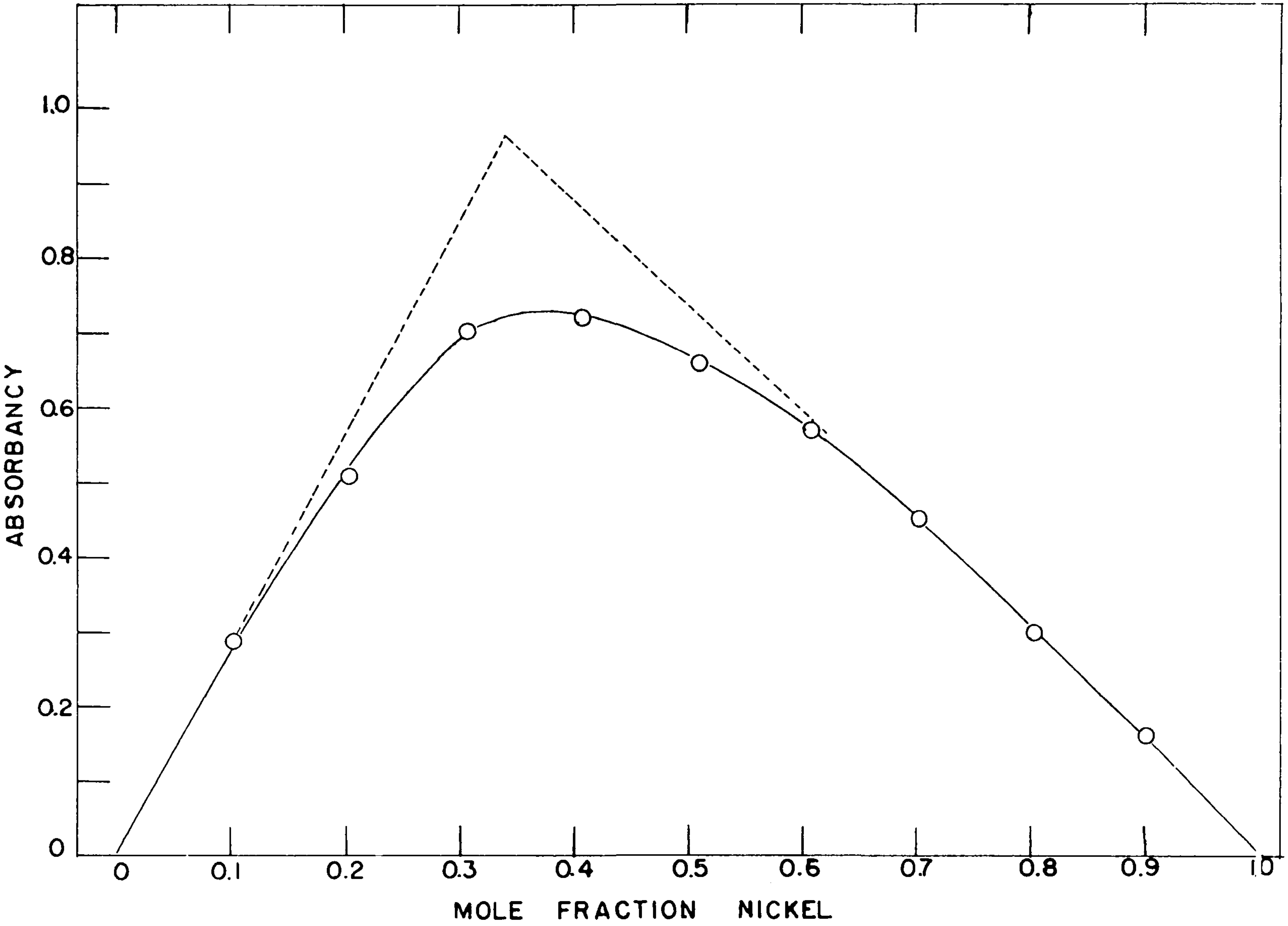

ABSORBANCY
1.0
0.8
0.6
0.4
0.2
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
MOLE FRACTION NICKEL

Figure 20. Continuous variations plot for the nickel-juglone compound (corrected).



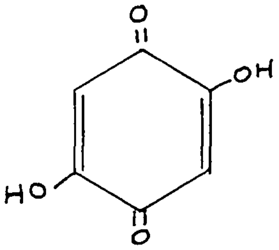
1.0
0.8
0.6
0.4
0.2
0
ABSORBANCY
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
MOLE FRACTION NICKEL

The preparation of the quinhydrone of juglone and α-hydrojuglone was attempted by reacting equimolar quantities of juglone and α-hydrojuglone in ether at reflux temperatures. An olive-green material was separated which was subsequently identified to be impure juglone.

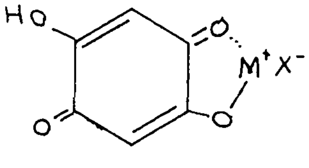
VI. STUDY OF 2,5-DIHYDROXY-P-BENZOQUINONE

A. Introduction

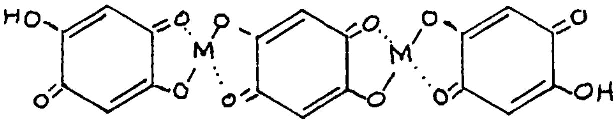
Two kinds of functional groups are present in 2,5-dihydroxy-p-benzoquinone.



The molecule has oxidation-reduction and also chelating properties, for the proximity of the hydroxyl and carbonyl groups makes ring formation with metal atoms possible. 2,5-Dihydroxy-p-benzoquinone may be considered as a stabilized enol of a 1,2-diketone. Although 1,2-diketones do not generally form chelate ring compounds, the enol tautomer, if formed, is capable of forming a five-membered ring by attachment to a metal atom. Thus, 2,5-dihydroxy-p-benzoquinone could form a 1:1 compound with divalent metallic ion



or possibly polymeric chains as in



Some work along this line has already been done. Frank, Clark, and Coker (29) studied the metallic compounds of 2,5-dihydroxy-p-benzoquinone and reported that although polymers are not generally formed, a number of metals do form compounds: silver and mercury form insoluble compounds which contain two atoms of metal to one of the quinone; magnesium, calcium, strontium, copper, cadmium, lead and ferric iron form insoluble compounds combining only one atom of metal to two of the quinone. Soluble compounds are also formed with aluminum, chromium, arsenic and stannic tin. The compounds of aluminum and stannic tin are colorless, but arsenic and chromium form yellow and brown colored solutions, respectively. Frank, Clark, and Coker further reported that the aluminum, arsenic, chromium or stannic tin prevents the precipitation of the insoluble metal compounds of 2,5-dihydroxy-p-benzoquinone.

2,5-Dihydroxy-p-benzoquinone has been recommended for the determination of thorium and zirconium (30). The nature of the thorium compound was established by Moeller (31) using the method of continuous variations; one atom of thorium combined with two molecules of the quinone.

Certain physical properties of 2,5-dihydroxy-p-benzoquinone were measured by Schwarzenbach and Suter (32): the oxidation-reduction equilibrium, the behavior as an acid, and the absorption spectrum. The reduction potential was found to be 0.430 volts vs. the normal hydrogen electrode. The acid dissociation constants, expressed as the negative logarithms, were found to be 5.18 and 2.73 (pK_1 and pK_2 respectively). The absorption spectrum at pH 3.96 showed two peaks, one at 300 millimicrons with a smaller peak at 510 millimicrons.

In the current work the acid dissociation constants have been re-evaluated, using data from a neutralization reaction, in contrast to the method of Schwarzenbach and Suter in which the acid dissociation constant was obtained from reductimetric titrations at different pH values.

A survey of the literature showed that 2,5-dihydroxy-p-benzoquinone had not been studied polarographically and such a study was undertaken, particular attention being devoted to the influence of chelate ring formation with metal ions on the half-wave potential in line with the general subject of the thesis.

B. Experimental Work

1. Purification of the Reagent

Technical grade 2,5-dihydroxy-p-benzoquinone was obtained from Eastman Organic Chemical Company. The crude compound was dissolved in concentrated sodium hydroxide and precipitated as the sodium salt by the addition of absolute alcohol. The sodium salt was redissolved in a little water, and decolorizing charcoal was added. The mixture was heated to 60° and filtered. Concentrated hydrochloric acid was added to an icy slurry of the filtrate to precipitate the free-acid (33). The above procedure was repeated once more. Poor yield of the compound was obtained. The product was only 80% pure, as determined by neutralization titration. Recrystallization from ethyl acetate worked well, however. Crude 2,5-dihydroxy-p-benzoquinone was dissolved in hot ethyl acetate, and insoluble material was removed by filtration. Decolorizing charcoal was added, and the solution was boiled for ten minutes. The mixture was filtered, and the resulting solution was reduced to one-half the volume by evaporation at the boiling point. On cooling to room temperature light-orange crystals appeared, and the mixture was cooled in a dry ice-acetone mixture and then filtered. The product was first air dried and then further dried at 50°. A melting point could not be determined inasmuch as the compound sublimed at about 135°.

2. Titration with alkali; determination of the dissociation constants

2,5-dihydroxy-p-benzoquinone, 0.2651 g., was titrated with 0.1558 N sodium hydroxide, 24.60 ml. being required. The titration was carried out potentiometrically using a Beckman Expanded Scale pH meter. The electrodes were calibrated with Beckman pH 4.00 and 7.01 buffers; the buffers were made according to NBS specifications.

3. Polarography; half-wave potential as a function of pH

A Sargent Model XXI polarograph was used for the polarographic study. The cell and accessories are described in Section IV B-1 of this thesis. A span of one volt and a sensitivity of 0.010 microamp./mm. were used.

A solution of 2,5-dihydroxy-p-benzoquinone, 0.010 M was prepared by dissolving 70.40 mg. of the material in ethyl alcohol and diluting to mark with ethyl alcohol in a 50-ml. volumetric flask. To each of a series of seventeen 50-ml. volumetric flasks was added 2.00 ml. of 0.010 M 2,5-dihydroxy-p-benzoquinone, 10 ml. of buffer and enough 0.50 M potassium nitrate to adjust the ionic strength of the solution to 0.10. Four different buffer systems were used to obtain the pH range of 2.10 to 9.10 pH units. They were: McIlvaine citric acid-disodium phosphate system, the Walpole acetate system, Sorensen sodium acid phosphate-disodium phosphate system, and the Michaelis sodium diethylbarbiturate-hydrochloric acid system. About 35 ml.

of the various solutions were placed successively into the polarograph cell, the solution flushed with nitrogen for ten minutes, and the polarogram taken. The pH was checked with the Beckman Expanded Scale pH meter. The resistance of the cell was measured for one of the solutions by the method described in Section III B-1. The resistance found was 4000 ohms.

4. Polarography; 2,5-dihydroxy-p-benzoquinone in the presence of varying amounts of aluminum

Aluminum forms a stable soluble compound with 2,5-dihydroxy-p-benzoquinone. Because of the solubility of the compound, it is particularly amenable to polarographic investigation.

a. Solutions and apparatus 2,5-Dihydroxy-p-benzoquinone, 0.010 M, was prepared in ethyl alcohol as described in the previous Section, VI B-3.

An acetate buffer solution of pH 3.75 was made by diluting 9.0 ml. of 0.2 N acetic acid with 1.0 ml. of 0.2 N sodium acetate.

A polarograph which was described in Section IV B-1 was used in this work.

b. Procedure Solutions containing various ratios of aluminum to 2,5-dihydroxy-p-benzoquinone were prepared. The solutions were buffered with 10 ml. of pH 3.75 acetate buffer and adjusted to an ionic strength of 0.10 with 0.50 M potassium nitrate. The ratio of 2,5-dihydroxy-p-benzoquinone to aluminum were: 3:0, 3:1, 3:2, 3:3, 3:5, 3:10, 3:20, and 3:40. Polarograms were obtained for each of these solutions after purging them with nitrogen. The pH of each solution was 3.75 as measured with the Beckman Expanded Scale pH meter.

5. Polarography; determination of the combining ratio of 2,5-dihydroxy-p-benzoquinone with aluminum

To each of seven 50-ml. volumetric flasks was added 10 ml. of pH 3.75 acetate buffer and 2.00 ml. of 0.010 M aluminum nitrate solution. Into successive flasks was placed 0, 1.00, 2.00, 3.00, 4.00, 5.00, and 6.00 ml. of 0.010 M 2,5-dihydroxy-p-benzoquinone solution. The ionic strength was adjusted to 0.10 with 0.5 N potassium nitrate, and the solutions diluted to volume with deionized water. The pH of each solution was measured and found to be 3.65. Each solution was flushed with nitrogen, and a polarogram obtained.

6. Preparation of 2,5-dihydroxyhydroquinone

Two grams of 2,5-dihydroxy-p-benzoquinone were dissolved in 100 ml. of diethyl ether in a 500-ml. separatory funnel. One hundred milliliters of 2 N sulfuric acid and 1.0 gram of zinc dust were added, and the mixture shaken vigorously. More zinc was added, and the mixture shaken until the yellow color of 2,5-dihydroxy-p-benzoquinone disappeared. The colorless ether layer was drawn off and washed with three 50 ml. portions of distilled water. The ether solution was then placed under a nitrogen atmosphere and allowed to evaporate at room temperature. A white crystalline powder was obtained which sublimed at 165°. The melting point: 240°. The white crystals gave a solution which was pink when dissolved in water. A polarogram of a solution of the white crystals at pH 3.75 was obtained. The polarogram showed only an anodic wave.

a. Preparation of the quinhydrone of 2,5-dihydroxy-p-benzoquinone and 2,5-dihydroxyhydroquinone A mixture of 0.10 grams of 2,5-dihydroxyhydroquinone and 0.10 grams of 2,5-dihydroxy-p-benzoquinone was refluxed in an ether solution for 30 minutes. The ether solution was evaporated under an atmosphere of nitrogen. Black crystalline material was recovered. While taking a melting point, it was observed that white crystals sublimed from the dark crystals at 160°. The bulk of the black material had not melted at 300°, the upper limit of the thermometer. A solution of the black crystalline material in water was pink in color. A polarogram of the material was obtained at pH 3.75.

C. Results and Discussion

The purification of 2,5-dihydroxy-p-benzoquinone by recrystallization from ethyl acetate proved relatively simple and effective; very little product was lost. The results of the potentiometric pH titration are shown in Figure 21. Two breaks appear in the neutralization curve. The purity, using the second equivalence point, is 99.89%.

The acid dissociation constants of 2,5-dihydroxy-p-benzoquinone, expressed as the negative logarithm, are 3.50 and 5.50, for pK_1 and pK_2 respectively. These values were determined by taking the pH at the midpoints of the neutralization curve.

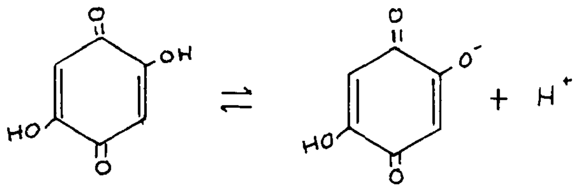
The polarogram of 2,5-dihydroxy-p-benzoquinone shows a well-defined wave at pH 4.32 acetate and pH 6.98 phosphate supporting electrolytes, Figures 22 and 23. That the system is reversible was established by plotting the logarithm of I/I_d-I against the applied potential, Figure 24. The slopes found: -0.036 and -0.037 volts respectively, for the acetate and phosphate supporting electrolytes. These slopes are in good agreement with the value of

-0.030 volts expected for a two-electron reduction.

The effect of pH on the half-wave potential of 2,5-dihydroxy-p-benzoquinone is shown in Figure 25. The relationship is nonlinear. The data for the curve is shown in Table 10. The solutions were buffered at different hydrogen ion concentrations and at constant ionic strength. The half-wave potentials were determined graphically. The intersection of the tangents to the curved portion of Figure 25 represents the negative logarithm of the acid dissociation constants of 2,5-dihydroxy-p-benzoquinone, the values being 3.35 and 5.25 for pK_1 and pK_2, respectively. The formal reduction potential, obtained by extrapolating the curve to zero pH, is 0.420 volts on the hydrogen scale. This is in good agreement with the value of Schwarzenbach which is 0.430 volts.

The acid dissociation constants, expressed as the negative logarithm, are shown in Table 11. Equations I and II represent the dissociation of 2,5-dihydroxy-p-benzoquinone.

I



II

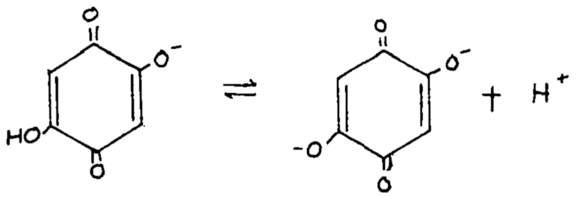


Figure 21. Neutralization curve of 2,5-dihydroxy-p-benzoquinone.

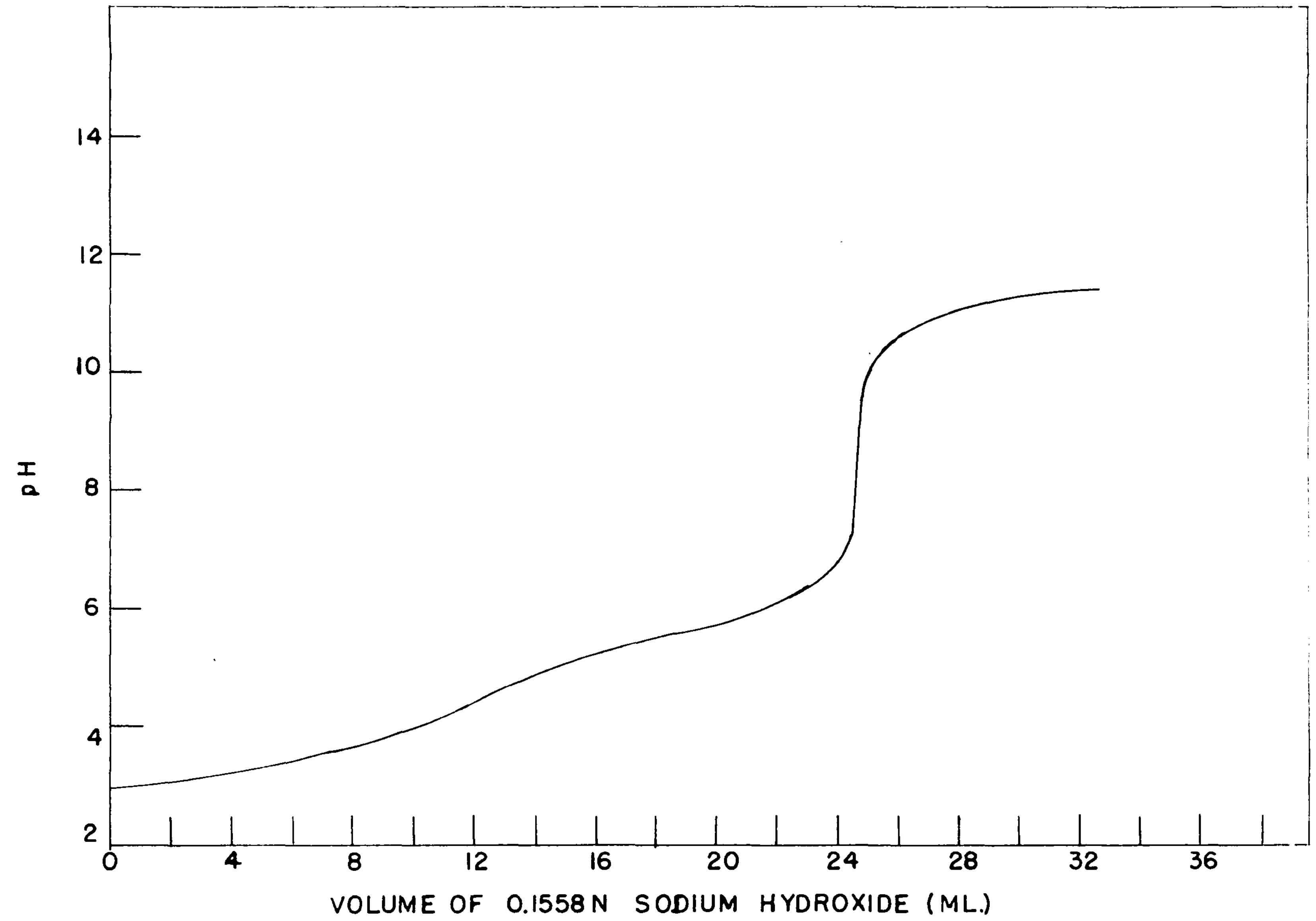

14
12
10
8
6
4
2
pH
0
4
8
12
16
20
24
28
32
36
VOLUME OF 0.1558 N SODIUM HYDROXIDE (ML.)

Figure 22. Polarogram of
2,5-dihydroxy-p-benzoquinone
in pH 4.32, acetate buffer.

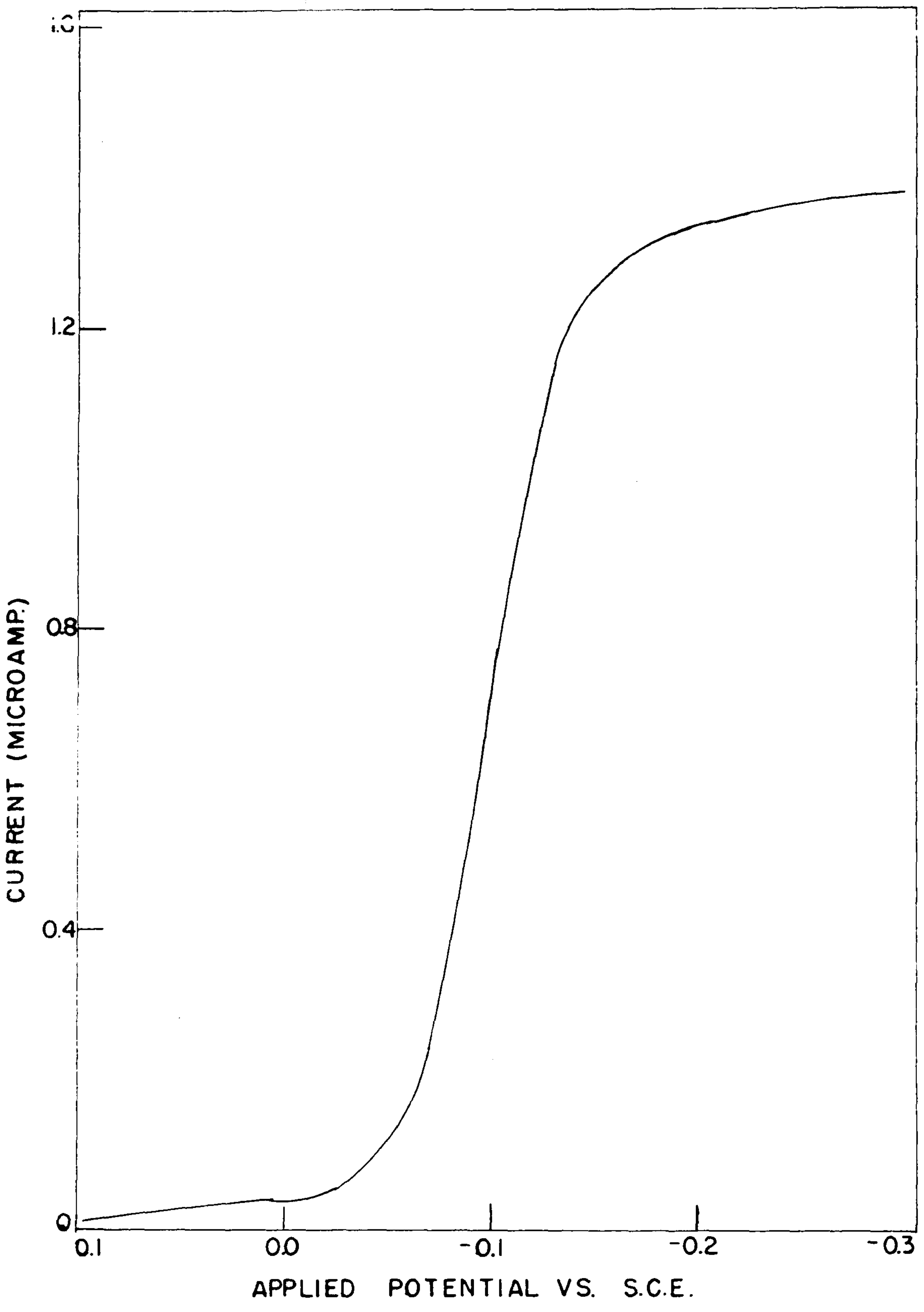

CURRENT (MICROAMP.)
1.2
0.8
0.4
0
0.1
0.0
-0.1
-0.2
-0.3
APPLIED POTENTIAL VS. S.C.E.

Figure 23. Polarogram of 2,5-Dihydroxy-p-benzoquinone in pH 6.98, phosphate buffer.

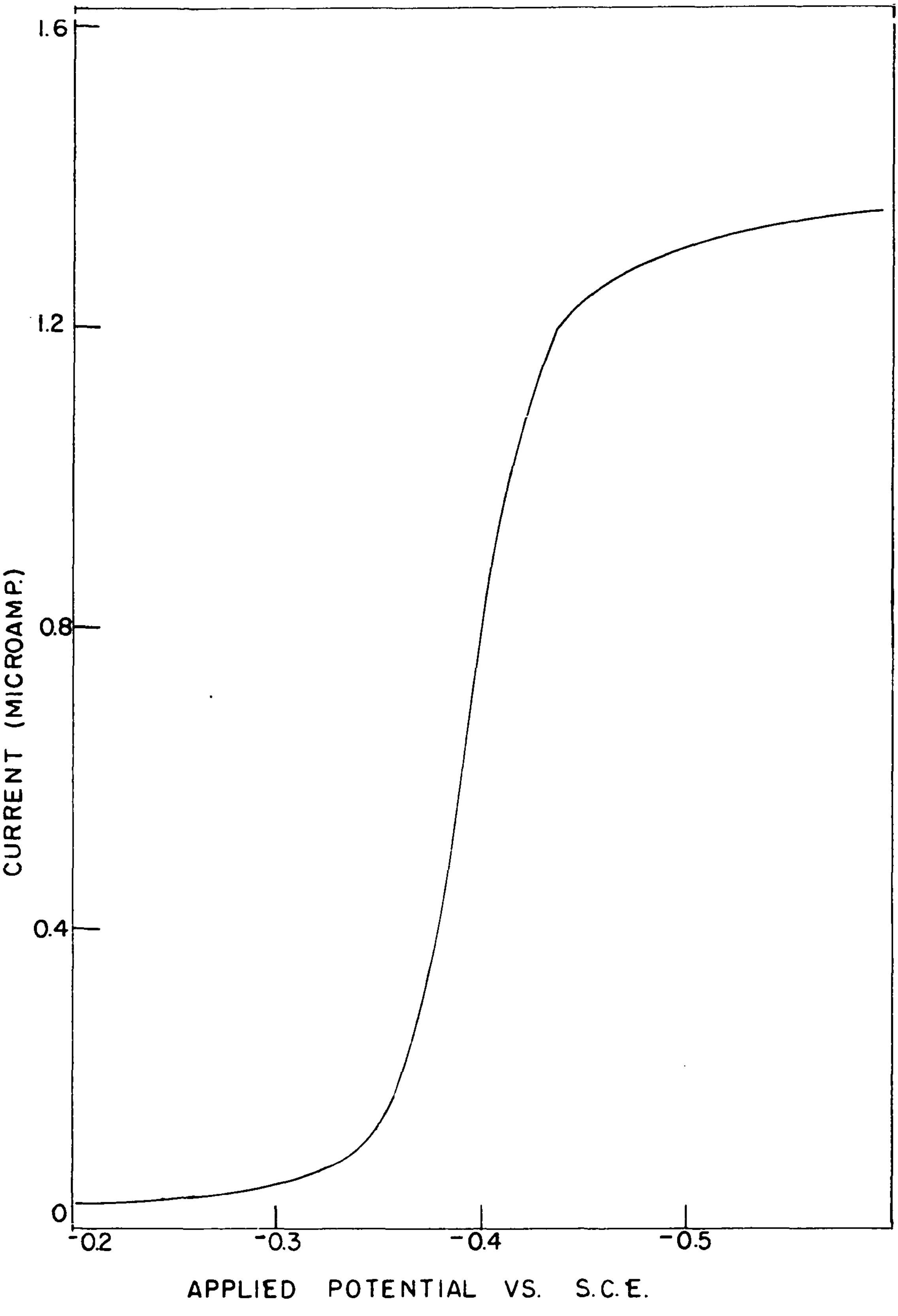

1.6
1.2
0.8
0.4
0
CURRENT (MICROAMP.)
-0.2
-0.3
-0.4
-0.5
APPLIED POTENTIAL VS. S.C.E.

Figure 24. Plot of I/I_d-I (log scale) vs. applied potential from data of Figures 22 and 23. Line A corresponds to abscissa A which is taken from the data of Figure 22. Line B corresponds to abscissa B which is taken from the data of Figure 23.

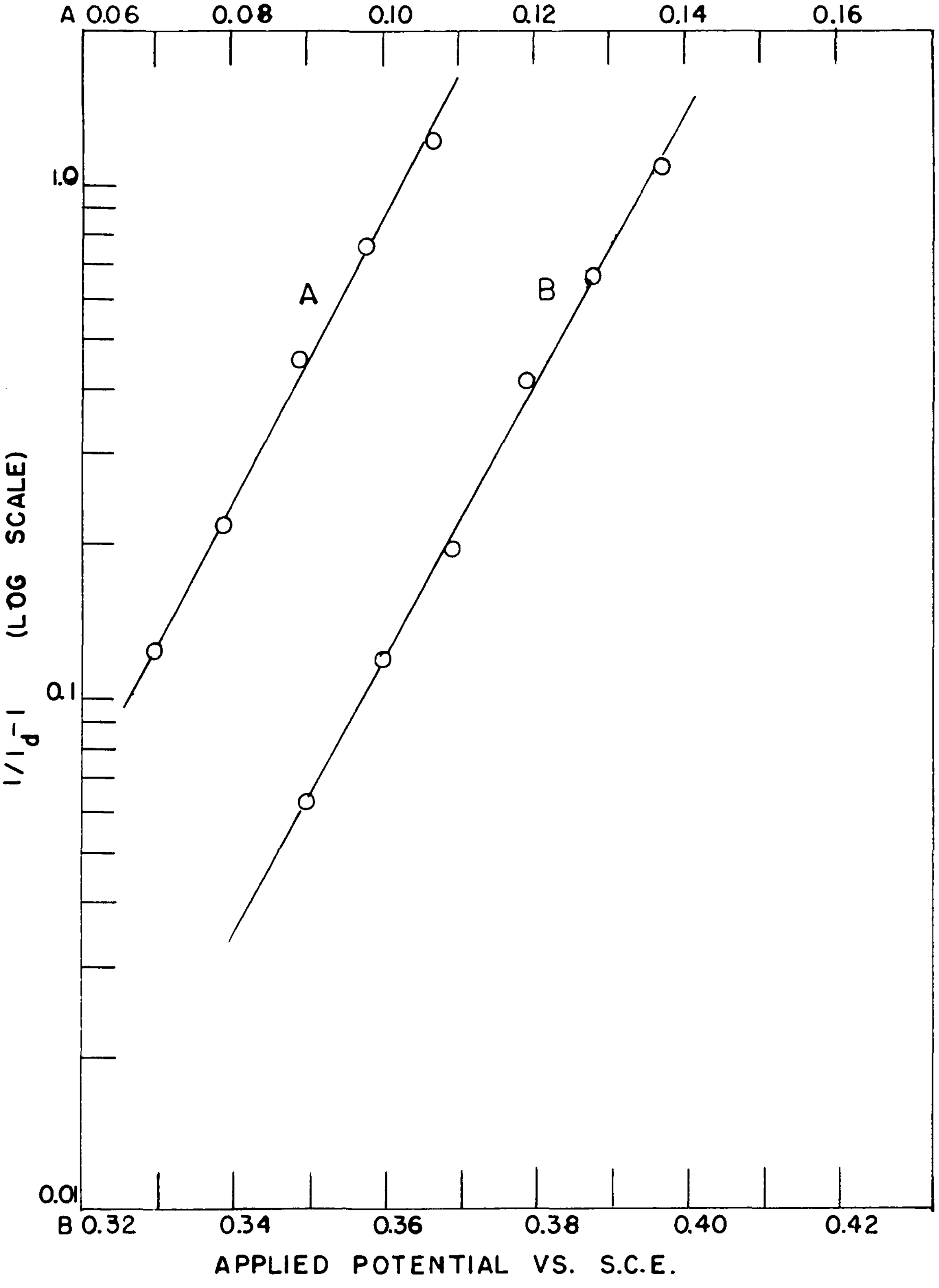

A 0.06
0.08
0.10
0.12
0.14
0.16
1.0
0.1
0.01
A
B
$I/I_d - I$ (LOG SCALE)
B 0.32
0.34
0.36
0.38
0.40
0.42
APPLIED POTENTIAL VS. S.C.E.

Figure 25. Plot of half-wave potential vs. pH of 2,5-dihydroxy-p-benzoquinone.

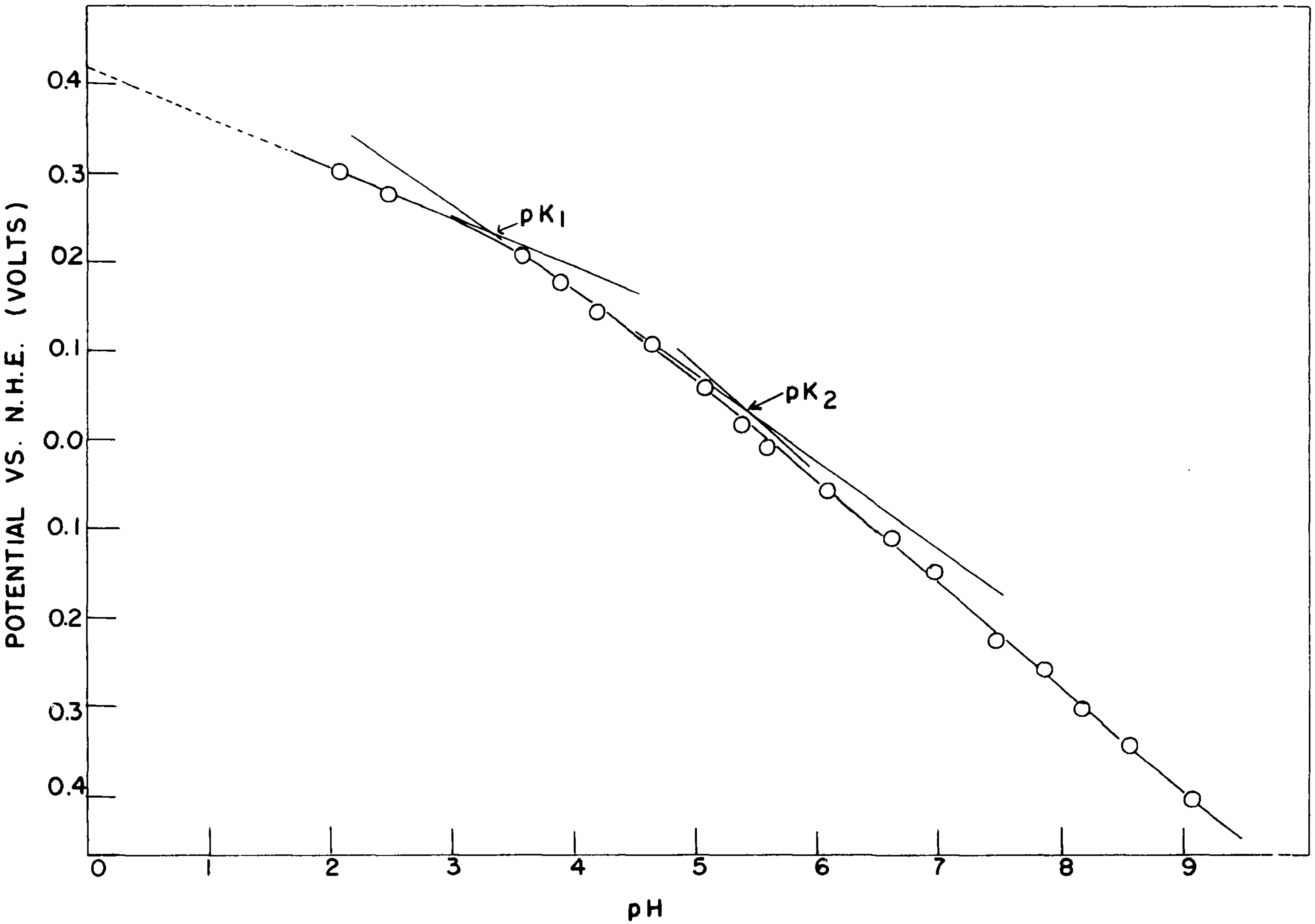

POTENTIAL VS. N.H.E. (VOLTS)
0.4
0.3
0.2
0.1
0.0
0.1
0.2
0.3
0.4
0
1
2
3
4
5
6
7
8
9
pH
pK1
pK2

Table 10. Half-wave potential of 2,5-dihydroxy-p-benzoquinone as a function pH

pH	Buffer System	Half-wave Potential vs. s.c.e.	Half-wave Potential vs. N.H.E.
2.10	McIlvaine	0.060	0.301
2.50	McIlvaine	0.046	0.277
3.60	Walpole (acetate)	-0.036	0.210
3.90	Walpole (acetate)	-0.068	0.178
4.32	Walpole (acetate)	-0.104	0.142
4.69	Walpole (acetate)	-0.137	0.109
5.09	Walpole (acetate)	-0.191	0.055
5.40	Walpole (acetate)	-0.229	0.017
5.65	Sorenson (phosphate)	-0.257	-0.011
6.10	Sorenson (phosphate)	-0.302	-0.056
6.65	Sorenson (phosphate)	-0.355	-0.109
6.98	Sorenson (phosphate)	-0.394	-0.148
7.49	Michaelis (barbituate)	-0.473	-0.227
7.88	Michaelis (barbituate)	-0.502	-0.256
8.21	Michaelis (barbituate)	-0.548	-0.302
8.58	Michaelis (barbituate)	-0.591	-0.345
9.10	Michaelis (barbituate)	-0.660	-0.414

Table 11. Comparison of the acid dissociation constants of 2,5-dihydroxy-p-benzoquinone; the constants expressed as the negative logarithm

	Neutralization	Polarographic	Potentiometric[a]
pK_1	3.50	3.35	2.73
pK_2	5.50	5.25	5.18

[a] Schwarzenbach (32)

The neutralization values of the dissociation constant and those obtained polarographically are only in fair agreement. The pK_2 value of Schwarzenbach and the polarographic method are in fair agreement. The discrepancies may be explained in the way the pH measurements were taken. Schwarzenbach had at his disposal one of the earliest glass electrodes and used one buffer to calibrate it. The modern glass electrode used in this work was calibrated with two standard buffers: Beckman pH 4.01 and 7.00 buffers. These buffers are prepared according to NBS specifications.

The effect of chelation on the oxidation-reduction properties of 2,5-dihydroxy-p-benzoquinone is revealed by the polarographic investigation of the quinone in the presence of varying amounts of aluminum. In the presence of aluminum at pH 3.75, the polarographic wave of 2,5-dihydroxy-p-benzoquinone undergoes striking changes with increasing amounts of aluminum until a well-defined wave is produced for which the half-wave potential is 0.140 volts more negative. The results of the polarographic investigation which bear this out are outlined below.

1. The polarogram of free 2,5-dihydroxy-p-benzoquinone at pH 3.75 gives a well defined wave. The wave is reversible as attested by a plot of the logarithm of I/I_d-I vs. applied potential; the relationship is linear and the slope -0.035 volts, close to the theoretical value of a two-electron reduction. The half-wave potential is -0.065 volts vs. s.c.e.

2. The polarogram of the solution having a ratio of 3:1 2,5-dihydroxy-p-benzoquinone to aluminum showed three ill-defined waves. The approximate half-wave potentials were -0.06, -0.14, -0.2 volts vs. s.c.e., respectively. The first wave is due to the reduction of free 2,5-dihydroxy-p-benzoquinone and the other two to the reduction of mixed complexed species of aluminum and reagent. The polarogram of the solution having a ratio of 2,5-dihydroxy-p-benzoquinone to aluminum of 3:2 shows two waves. The waves are not well-defined but were of equal height. The half-wave potentials were approximately -0.15 and -0.2 volts vs. s.c.e., respectively. The wave for the reduction of free 2,5-dihydroxy-p-benzoquinone was eliminated by the formation of mixed complexed species.

3. The polarogram of the solution having a ratio of 1:1 of 2,5-dihydroxy-p-benzoquinone to aluminum showed only one ill-defined wave. The half-wave potential was about -0.2 volts vs. s.c.e. The poorly defined wave is probably caused by incomplete chelation of the metal or possibly by the presence of some mixed chelates.

4. The polarogram of the solution having a ratio of 3:5 2,5-dihydroxy-p-benzoquinone to aluminum shows a wave which is well-defined, Figure 26. The half-wave potential is -0.205 volts vs. s.c.e. The chelation is complete at this ratio as indicated by one wave. Using the data of Figure 26,

a plot of the logarithm of I/I_d-I against the applied potential shows a slope of -0.033 volts, thus indicating a two-electron reduction for the aluminum-2,5-dihydroxy-p-benzoquinone compound, Figure 27.

5. The polarograms of the solutions having ratios of 3:10, 3:20, and 3:40 2,5-dihydroxy-p-benzoquinone to aluminum show only one well-defined wave. The half-wave potentials of all these solutions remained at -0.205 volts.

The results show that there is, indeed, a shift in the half-wave potential of 2,5-dihydroxy-p-benzoquinone when aluminum is present. This shift is a result of the formation of a compound between aluminum and 2,5-dihydroxy-p-benzoquinone. The results also point out that an increase in the concentration of aluminum does not cause the half-wave potential to shift any further than -0.205 volts vs. s.c.e.

In Table 12 are shown the results of the experiment for the determination of the combining ratio of aluminum and 2,5-dihydroxy-p-benzoquinone. The polarograms indicate two waves after the ratio of 0.5:1, the mole ratio of quinone to aluminum. Wave I is due to the reduction of the uncomplexed 2,5-dihydroxy-p-benzoquinone, and wave II is due to the reduction of the aluminum-2,5-dihydroxy-p-benzoquinone compound. At a mole ratio of 0.5:1 only one wave is present. This is due to the reduction of the aluminum-2,5-dihydroxy-p-benzoquinone complex and shows that the combining ratio of the aluminum to the quinone is 2:1. The proposed structure is

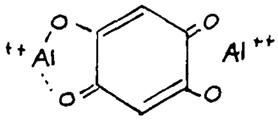


Figure 26. Polarogram of 2,5-dihydroxy-p-benzoquinone. The ratio of the quinone to aluminum is 3:5.

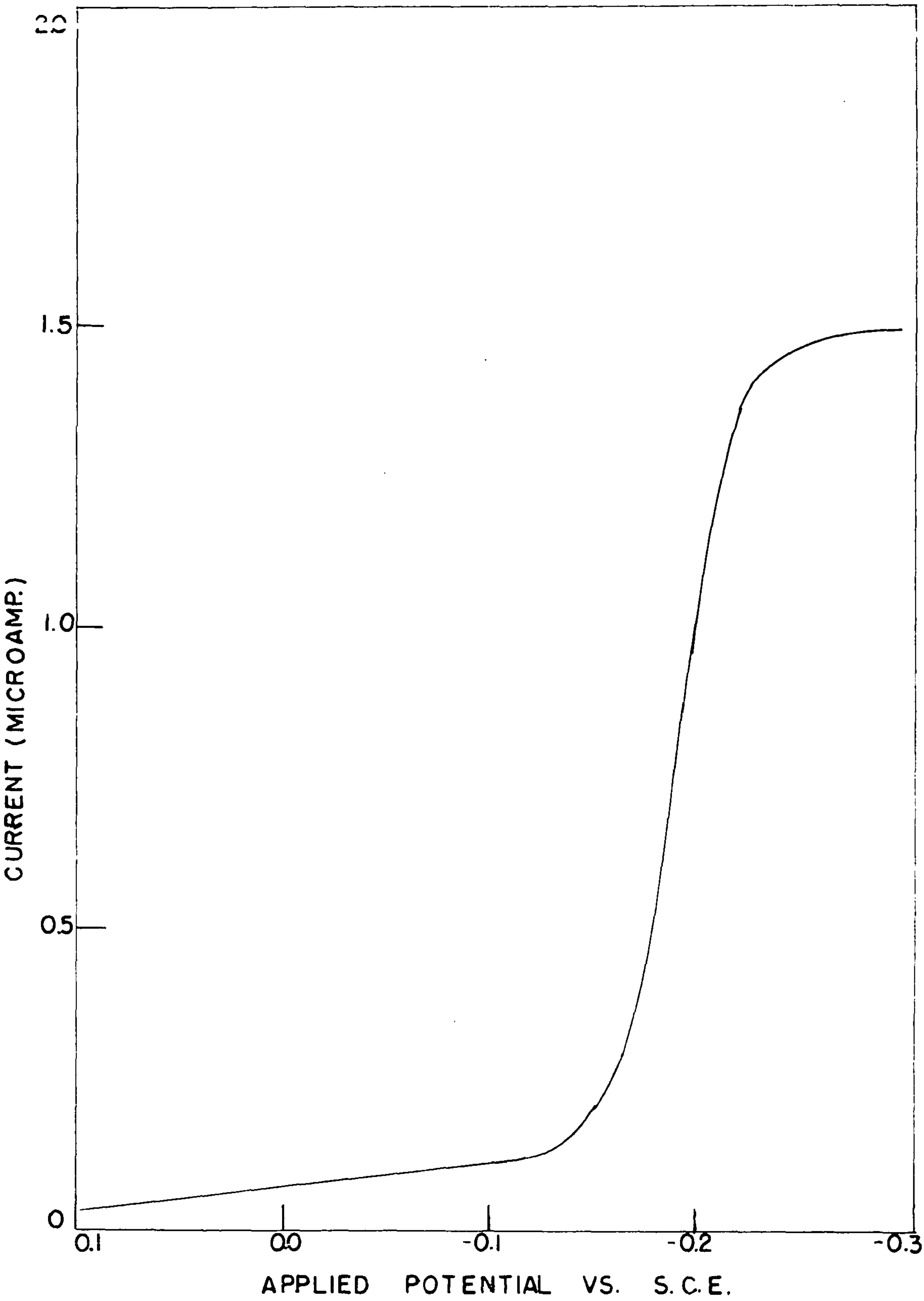
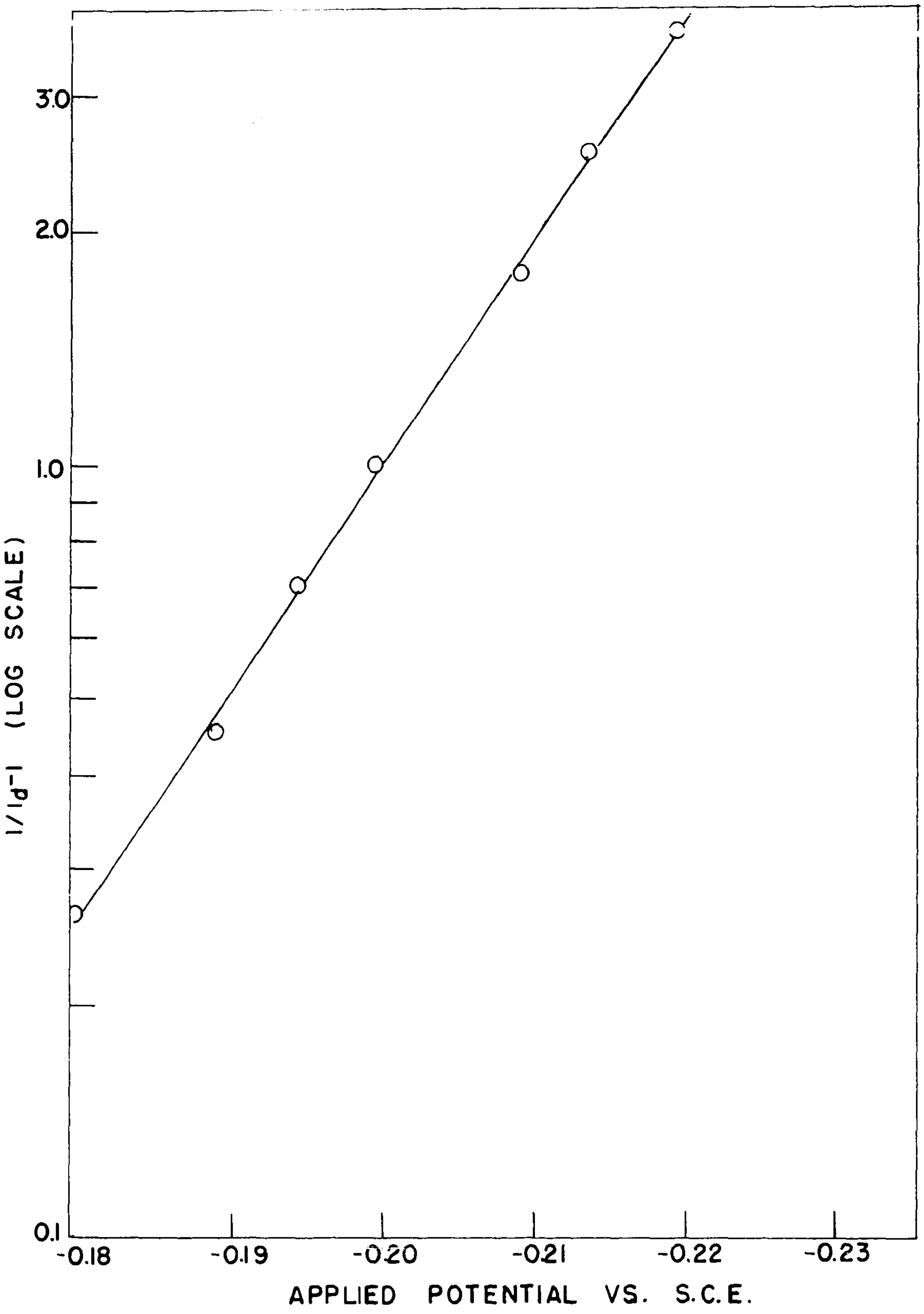
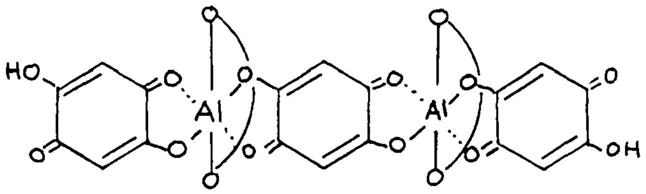

2.0
1.5
1.0
0.5
0
CURRENT (MICROAMP.)
0.1
0.0
-0.1
-0.2
-0.3
APPLIED POTENTIAL VS. S.C.E.

Figure 27. Plot of I/I_d-I (log scale) vs. applied potential for the aluminum-2,5-dihydroxy-p-benzoquinone. Data from Figure 26.


3.0
2.0
1.0
0.1
i/id-i (LOG SCALE)
-0.18
-0.19
-0.20
-0.21
-0.22
-0.23
APPLIED POTENTIAL VS. S.C.E.

The remaining positions about the aluminum atom are probably filled by water. With increasing quantities of 2,5-dihydroxy-p-benzoquinone, wave II remains, but the diffusion current is increasing. This means that more of the quinone is being coordinated to the aluminum. Wave I, which is the wave due to the uncomplexed 2,5-dihydroxy-p-benzoquinone, makes its appearance at a ratio of 1:1. The diffusion current of wave II increases until it reaches a constant value at a ratio of 2.5:1. Thus, a species consisting of 5 molecules of quinone to 2 atoms of aluminum must be present. The proposed structure of such a species is



where  represents a molecule of 2,5-dihydroxy-p-benzoquinone. The remaining coordinating positions of aluminum are now filled with 2,5-dihydroxy-p-benzoquinone fitting in perpendicular to the plane of the chain. The experiment shows that aluminum forms a polynuclear coordinated compound with 2,5-dihydroxy-p-benzoquinone. Wave I cannot be rationalized quite as simply. With increasing amounts of 2,5-dihydroxy-p-benzoquinone, the diffusion current for the reduction of the quinone does not show a definite trend. The variance of the half-wave potential, however, gives a clue to what may be happening. Previous experiments in this work have shown that the half-wave potential of 2,5-dihydroxy-p-benzoquinone is in the neighborhood of -0.06 to -0.07 volts vs. s.c.e. Although wave I has a half-wave potential of -0.12 volts, it eventually attains a value of -0.06 volts. The explanation may be that wave I is actually the wave for the reduction of an aluminum-2,5-dihydroxy-p-benzoquinone of undetermined ratio, being superimposed on the reduction wave of uncomplexed 2,5-dihydroxy-p-benzoquinone. Upon increasing concentration of 2,5-dihydroxy-p-benzoquinone, these mixed species revert to the 5:2 compound, and wave I becomes a pure wave for the reduction of the excess 2,5-dihydroxy-p-benzoquinone.

Table 12. Data for the determination of the combining ratio of aluminum and 2,5-dihydroxy-p-benzoquinone; pH 3.75

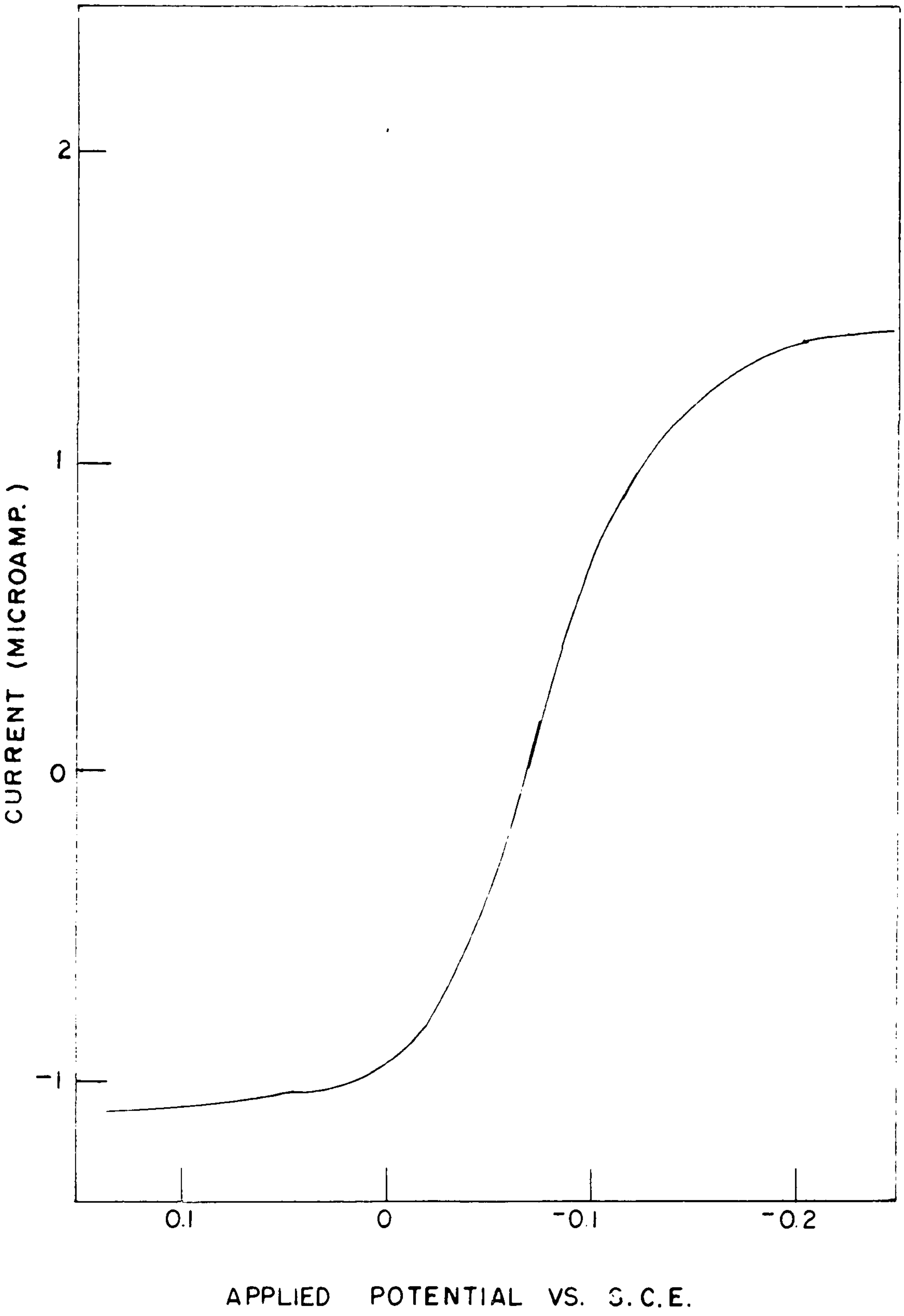
Mole ratio of 2,5-dihydroxy-p-benzoquinone to aluminum	Wave I[a]		Wave II	
	$-E_{\frac{1}{2}}$	I_d	$-E_{\frac{1}{2}}$	I_d
0:1	-	-	-	-
0.5:1	-	-	0.18	0.62
1.0:1	0.11	0.46	0.18	1.54
1.5:1	0.12	1.10	0.19	1.83
2.0:1	0.08	0.72	0.19	2.90
2.5:1	0.06	1.14	0.18	3.28
3.0:1	0.06	2.55	0.19	3.26

[a] $E_{\frac{1}{2}}$ is the half-wave potential in volts vs. s.c.e., and I_d is the diffusion current in microamperes

The preparation of 2,5-dihydroxyhydroquinone went smoothly by the reduction of 2,5-dihydroxy-p-benzoquinone with zinc in sulfuric acid solution. The product was extracted from the body of the solution with ether. A white crystalline product was obtained which was stable in air. The melting point of the 2,5-dihydroxyhydroquinone showed that it sublimed at 165° and finally melted at 240°. The polarogram of 2,5-dihydroxyhydroquinone at pH 3.75 showed only an anodic wave that confirms the presence of only hydroquinone species.

The quinhydrone of 2,5-dihydroxy-p-benzoquinone and 2,5-dihydroxyhydroquinone was prepared by reacting equimolar amounts of the two compounds in ether. Evaporation of the ether solvent gave a compound which was black and crystalline. A melting point showed that white crystals were sublimed from the black material at 160° and that bulk of the material had not melted below 300°. The black material appeared to be highly soluble in water giving a light red solution. A polarogram of the solution of the black material at pH 3.75 showed both a cathodic and anodic portion on the diffusion wave, Figure 28. The half-wave potential is -0.070 volts vs. s.c.e. This confirmed the presence of quinhydrone. The cathodic and the anodic portion of the diffusion wave are in the ratio of 1.2:1. This ratio indicates that the diffusion coefficient of the 2,5-dihydroxy-p-benzoquinone is slightly greater than that of 2,5-dihydroxyhydroquinone. Müller (11) reports that the ratio of the cathodic and anodic portion of quinhydrone (quinone-hydroquinone) are in the ratio of 1.1:1. Therefore, the product isolated in this work has about the same characteristics as its parent compound; although it appears that the solubility is much greater. After four hours another polarogram of the solution of the quinhydrone showed that the ratio of the cathodic and anodic portion remained constant, and that the portions had not decreased. It thus appears that the quinhydrone of 2,5-dihydroxy-p-benzoquinone and 2,5-dihydroxyhydroquinone is stable with respect to decomposition in aqueous solution.

Figure 28. Polarogram of the quinhydrone of 2,5-dihydroxy-p-benzoquinone and 2,5-dihydroxyhydroquinone; pH 3.75.


CURRENT (MICROAMP.)
2
1
0
−1
0.1
0
−0.1
−0.2
APPLIED POTENTIAL VS. S.C.E.

VII. SUMMARY OF RESULTS

1,4-Dihydroxyphenylmethyleneiminodiacetic acid has been prepared by the condensation of formaldehyde, iminodiacetic acid and hydroquinone.

1,4-Dihydroxyphenylmethyleneiminodiacetic acid has been found to contain two replaceable hydrogen atoms. The compound is a zwitter ion, and the acid dissociation constants are, for the carboxyl proton $10^{-2.95}$ and for the ammonium proton $10^{-8.13}$.

1,4-Dihydroxyphenylmethyleneiminodiacetic acid is oxidized smoothly and reversibly in 1.0 M sulfuric acid by potassium dichromate, the formal reduction potential being 0.737 volts vs. N.H.E. The index potential and the logarithm of the formation constant for the semiquinone have been found to be 0.014 volts and -0.03, respectively.

The polarography of 1,4-dihydroxyphenylmethyleneiminodiacetic acid at pH 6.0, acetate buffer, has been investigated. The oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid does not appear to be reversible, inasmuch as the slope of log I/I_d-I vs. the applied potential was found to be -0.040 volts.

The polarography of 1,4-dihydroxyphenylmethyleneiminodiacetic acid at pH 8.3, borate buffer, has also been investigated. The oxidation wave is not reversible.

It has been shown that 1,4-dihydroxyphenylmethyleneiminodiacetic acid forms compounds with calcium, magnesium, nickel, copper, aluminum and bismuth. The polarography of 1,4-dihydroxyphenylmethyleneiminodiacetic acid in in the presence of calcium, magnesium, copper, nickel, aluminum and bismuth has been investigated at pH 6.0. Aluminum and bismuth are the only metals which affect the oxidation wave of 1,4-dihydroxyphenylmethyleneiminodiacetic acid.

Two types of bonding have been proposed for the formation of compounds with 1,4-dihydroxyphenylmethyleneiminodiacetic acid; a compound in which the ligand is a tridentate using the iminodiacetic acid groups, and the other in which the ligand is a tetradentate, using the phenolic oxygen ortho to the methyleneiminodiacetic acid group as the fourth coordinating group.

A polarographic investigation of the combining ratio of nickel, copper and aluminum with 1,4-dihydroxyphenylmethyleneiminodiacetic acid at pH 6.0, has revealed that these metals form 1:1 compounds.

The formation constant of 1,4-dihydroxyphenylmethyleneiminodiacetic acid with calcium, magnesium and copper have been determined. The values, expressed as the logarithm of the formation constant, are 3.71, 3.87, and 8.83 for calcium, magnesium, and copper, respectively. The logarithm of the formation constant, in which 1,4-dihydroxyphenylmethyleneiminodiacetic acid acts as a tetradentate with copper by using the hydroxyl group ortho to the methyleneiminodiacetic acid group, has been found to be 11.8 . The value of the acid dissociation constant of the ortho hydroxy group, determined polarographically, is 9.75 (expressed as the negative logarithm).

Oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid with potassium dichromate, hydrogen peroxide, potassium bromate, and sodium chlorate failed to give the corresponding 1,4-dioxophenylmethyleneiminodiacetic acid.

The oxidation products were gums indicating extensive polymerization.

The oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid with air was followed polarographically. No corresponding quinone was formed. Supplementary experiments on the oxidation of quinhydrone by air in acidic and basic solutions has shown that air decomposes benzoquinone. Presumably the same phenomena occurs in the oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid.

Oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid with potassium persulfate was followed polarographically. The oxidation proceeded to produce the corresponding quinone as it was observed as a cathodic wave on the polarogram.

A large scale oxidation of 1,4-dihydroxyphenylmethyleneiminodiacetic acid with potassium persulfate gave a highly impure compound. The polarogram of this highly impure compound indicated that the compound contained both 1,4-dihydroxyphenylmethyleneiminodiacetic acid and the corresponding quinone. The color of the compound, purple, also gave a clue as to the composition of the isolated product. The infrared spectrum showed that this product contained some bands characteristic of a quinhydrone. The impurity of the compound precluded any definite statement as to the exact composition.

The purple material gave upon heating two compounds. These were identified by infrared analysis as quinhydrone and hydroquinone, which apparently resulted from decomposition of the 1,4-dioxophenylmethyleneiminodiacetic acid. Further evidence of the instability of the purple material was obtained by a polarographic study. With increasing time a solution of the purple material was shown to decompose, the cathodic wave decreasing on standing. With all of these observations there is no doubt that 1,4-dioxophenylmethyleneiminodiacetic acid cannot be obtained in the pure state although it could be shown by the polarographic method to exist briefly.

The preparation of 5-hydroxy-1,4-naphthoquinone (juglone) has been accomplished with some modifications of the Teuber and Götz method using potassium nitrosodisulfonate to oxidize 1,5-dihydroxynaphthalene.

The polarographic behavior of juglone has been investigated. Juglone undergoes a reversible two-electron reduction. A prewave, which is not diffusion controlled, is also present in the polarogram.

It has been found that juglone reacts with nickel, magnesium and manganese at pH 8.48 to form purple, soluble compounds. Further, it was shown that the half-wave potential of the juglone is shifted 0.05 volts in the negative direction in the presence of the nickel. The corresponding shift in the presence of magnesium and in the presence of manganese is 0.01 volts.

The shift in the half-wave potential of the juglone in the presence of these metals is not directly related to increasing metal ion concentration.

The combining ratio of the juglone and nickel and of the juglone and manganese compounds was found to be 2:1 and 3:1, respectively.

A spectrophotometric study of the juglone-nickel complex also revealed that a 2:1 compound was formed. The stability constant, estimated by the method of continuous variations, was found to be 7.15×10^7.

The molar absorptivity of the nickel-juglone compound was found to be

3060 at 530 mμ, a value too low to be of any analytical significance.

The quinhydrone of juglone and its reduced species, α -hydrojuglone could not be prepared. The product isolated from the reaction of juglone and α -hydrojuglone at reflux temperatures in ether solvent, was identified as juglone.

New values have been found for the acid dissociation constants for 2,5-dihydroxy-p-benzoquinone, $pK_1 = 3.50$ and $pK_2 = 5.50$ using the data from a titration with sodium hydroxide. The same constants were obtained from a study of the polarography of 2,5-dihydroxy-p-benzoquinone at varying values of pH; the values found are $pK_1 = 3.35$ and $pK_2 = 5.25$. The values are not in good agreement with earlier values obtained from oxidation-reduction studies.

The polarography of 2,5-dihydroxy-p-benzoquinone has been investigated. 2,5-Dihydroxy-p-benzoquinone undergoes a reversible, two-electron reduction at the dropping mercury cathode, the half-wave potentials varying with pH.

The half-wave potential of 2,5-dihydroxy-p-benzoquinone is shifted by the presence of aluminum, the shift being 0.140 volts more negative. No relationship was found that related the shift of the half-wave potential to the concentration of the aluminum.

Aluminum and 2,5-dihydroxy-p-benzoquinone have been found to form two distinct compounds as indicated by polarography. A compound consisting of two aluminum atoms to one of 2,5-dihydroxy-p-benzoquinone, and another with a ratio of two aluminum atoms to five molecules of 2,5-dihydroxy-p-benzoquinone, have been identified.

The reduced form of 2,5-dihydroxy-p-benzoquinone, 2,5-dihydroxyhydroquinone, has been prepared by the reduction of the quinone with zinc. 2,5-dihydroxyhydroquinone is a white crystalline compound which sublimes at 165°.

The quinhydrone of 2,5-dihydroxy-p-benzoquinone and 2,5-dihydroxyhydroquinone has been prepared by reacting equimolar amounts of the quinone and its hydroquinone in ether at reflux temperatures. The quinhydrone is black and highly crystalline and does not melt below 300°. The quinhydrone appears to be stable in aqueous solution and is very soluble.

An investigation of the effect of metal ions on the oxidation-reduction properties of certain organic couples has been carried out. It has been found that, in general, where the metal is incorporated directly into the oxidation-reduction function of the molecule, a shift in the half-wave potential is observed. The magnitude of the shift and the dependency on the concentration of the metal have not been correlated.

VIII. BIBLIOGRAPHY

1. Marshall, C. E., _J. Phys. Chem._ _43_, 1155 (1939).

2. Ives, D. J. G. and Janz, Geo. J., Editors, Reference Electrodes, Theory and Practice, New York, N. Y., Academic Press, 1961.

3. Wyllie, M. R. J., _J. Phys. Chem._ _58_, 67 (1954).

4. Gregor, H. P. and Sollner, Karl, _J. Phys. Chem._ _58_, 409 (1954).

5. Gregor, H. P. and Shonhorn, H. J., _J. Phys. Chem._ _81_, 3911 (1959).

6. Lohman, F. H., An Indicator Electrode for Metal Ions, Unpublished Ph.D. Thesis, Ames, Iowa, Library, Iowa State University of Science and Technology, 1955.

7. Diehl, H. C., _Chem. Rev._ _21_, 39 (1937).

8. Haber, F. and Russ, R., _Z. physik Chem._ _24_, 257 (1904).

9. Biilmann, E., _Ann. Chim._ _15_, 109 (1921).

10. Steinbach, O. F. and King, C. V., Experiments in Physical Chemistry, New York, N. Y., American Book Company, 1950.

11. Müller, Otto, H., The Polarographic Method of Analysis, 2nd ed., Easton, Pa., Chemical Education Publishing Company, c1951.

12. Vogel, A. I., A Text-Book of Quantitative Inorganic Analysis, 3rd ed., New York, N. Y., John Wiley and Sons, Inc., 1961.

13. Michaelis, L., _Chem. Rev._ _22_, 437 (1938).

14. Berniejo, F. and Prieto, A., Aplicaciones Anlitical Del AEDT y Simlares, Santiogo de Compostela, Spain, Imprento Del Seminario Conciliar, 1960.

15. Chaberek, S., Jr. and Martell, A. E., _J. Am. Chem. Soc._ _74_, 5052 (1952).

16. Abichandi, C. T. and Jatkar, S. K. K., _J. Indian Inst. Sci._ _A21_, 417 (1938).

17. Schwarzenbach, G., Kampitsch, E., and Steiner, R., _Helv. Chim. Acta_ _28_, 1133 (1945).

18. Schwarzenbach, G., Ackermann, H., and Ruckstahl, P., _Helv. Chim. Acta_ _32_, 1175 (1949).

19. Cason, James, *Organic Reactions 4*, 305 (1948).

20. Bernthesen, A. and Semper, A., *Ber. dtsch. Chem. Ges. 20*, 934 (1887).

21. Teuber, H. J. and Götz, W., *Chem. Ber. 87*, 1936 (1954).

22. Feiser, L. F., *J. Am. Chem. Soc. 50*, 439 (1928).

23. Friedheim, E. A. H., *Biochem. J. 28*, 180 (1934).

24. Ciusa, F., *Ann. Chim. Applicata 16*, 127 (1926).

25. Zuman, P., *Collection Czechoslov. Chem. Comm. 19*, 1140 (1954).

26. Voxburg, W. C. and Cooper, C. R., *J. Am. Chem. Soc. 63*, 437 (1941).

27. Willstätter, R. and Wheeler, A. S., *Ber. dtsch. Chem. Ges. 47*, 2796 (1914).

28. Mylius, F., *Ber. dtsch. Chem. Ges. 18*, 2567 (1884).

29. Frank, R.L., Clark, Geo. R., and Coker, J. N., *J. Am. Chem. Soc. 72*, 1827 (1950).

30. Jain, B. D. and Singhal, S. P., *Talanta 4*, 178 (1960).

31. Moeller, Therold and Kobish, Ed. H., *J. Am. Chem. Soc. 72*, 2777 (1950).

32. Schwarzenbach, G. and Suter, H., *Helv. Chim. Acta 24*, 617 (1941).

33. Jones, R. G. and Shonle, H. A., *J. Am. Chem. Soc. 67*, 1034 (1945).

IX. ACKNOWLEDGMENTS

The author wishes to acknowledge and thank the many people whose help has enabled the author to carry out this work. In particular he wishes to thank his wife, Nancy, for encouragement and patience.

The author wishes to thank Professor Harvey Diehl for the suggestion of the problem and the very helpful guidance during this work.

Thanks also to the author's parents who gave him much encouragement and helped take care of the author's family during the latter part of this work.

Acknowledgment is due also to the Texaco Company for the financial aid during part of this work.

Finally, thanks to Gerald Scheppers who spent time proof reading the manuscript.