The polarographic reduction of vitamin A in N-N-dimethylformamide
by Robert Gene Park

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE in Chemistry
Montana State University

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This thesis is gratefully dedicated to my parents, Mr. and Mrs. E.W. Park.

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ABSTRACT

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The proposed electrode reaction for the two forms of vitamin A is thought to be similar to the mechanism proposed by Given for the reduction of olefins in N,N-dimethylformamide.

INTRODUCTION

The polarograph has for many years been used successfully for inorganic analysis. The convenience, simplicity and wide applicability of the method are well known.

The operation of the polarograph involves the measurement of current as a function of applied potential at a small polarized electrode. The details of operation and theory are briefly outlined below.

The polarographic method of analysis is based upon the measurement of current, the magnitude of which is determined by the rate of diffusion of the oxidizible or reducible ions to the electrode. The driving force for diffusion is the concentration gradient, i.e. a difference in concentration from one point in the solution to another. If the ions are readily oxidized or reduced, a concentration gradient is established in the vicinity of the electrode. The rate of diffusion, as stated by Fick, is proportional to the gradient of concentration of the diffusing substance and is expressed in the following equation:

$$\frac{ds}{dt} = \frac{AD^\circ}{d} (C - C_o)$$

where A = area of the electrode

D° = proportionality factor between the rate of diffusion and the concentration gradient. Called the diffusion coefficient, and expressed in $cm^2\ sec^{-1}$

d = thickness of the hypothetical diffusion layer about the microelectrode, in centimeters

C = bulk concentration of the substance diffusing to the electrode; expressed in millimoles per liter

C_o = concentration of the electroactive ions at the surface of the electrode

If the diffusing ions are readily oxidized or reduced at the surface of the electrode, C_o approaches zero, and the rate of diffusion is then proportional to the bulk concentration of the electroactive ions C, or

$$\frac{ds}{dt} = \frac{AD^\circ}{d} C$$

In addition to diffusion there are two other means by which ions may be transferred to the electrode. These are 1) convection, caused by stirring or uneven temperature, and 2) migration of the ions in an electric field. Since polarographic oxidation or reduction depends on diffusion of the electroactive ions to the electrode, the other means of transfer must be eliminated or greatly reduced. Convection effects can be made negligible by maintaining the solution at constant temperature and taking care that the electrolytic cell is not agitated. Migration effects can be made negligible by adding an excess of inert electrolyte which will carry the bulk of

the current, thus greatly reducing the transference number of the reducible or oxidizible species. The solution to be analyzed polarographically is transferred to a cell for electrolysis where one of the electrodes is a dropping mercury electrode, which is generally used as the cathode. The dropping mercury electrode consists of a small bore glass capillary connected to a mercury reservoir. The other electrode used in this experiment consists of a mercury pool, connected to the instrument by means of a platinum wire immersed in the pool. The applied potential is slowly increased from zero, and from the instrument a record of the current as a function of applied voltage is obtained. At the start of the electrolysis the residual current is small and increases slowly with the increase in applied voltage. When the applied potential approaches the characteristic reduction potential (half wave potential) of the reducible ion, there is a sudden increase in current. As the rate of diffusion of the reducible ions reaches a maximum, the diffusion current reaches a constant maximum value. The plot of the voltage versus current is called a polarogram. The height of the wave is proportional to the concentration of the reducible ions, and the half wave potential is characteristic of the substance being reduced.

The rate of diffusion of the reducible ions determines the magnitude of the current during electrolysis, and the diffusion current is described by the equation derived by Ilkovic

(1) which is:

$$i_d = 706 \quad nm^{2/3} t^{1/6} D_o^{1/2} C^\circ$$

where i_d = maximum current observed due to the reduction or oxidation of the species in solution, expressed in microamperes

n = number of electrons involved in the electrode reaction

m = mass rate of flow of mercury from the dropping mercury electrode in milligrams per second

t = drop time of the mercury from the dropping mercury electrode in drops per second

D_o = diffusion coefficient of oxidizible or reducible substances in cm^2 per second

C° = millimoles per liter of reducible or oxidizible substances

The Ilkovic equation is applicable when the following conditions are fulfilled:

(1) An excess of an inert electrolyte is present in the solution so that the only means of transfer of the reducible or oxidizible ion is by diffusion.

(2) The potential of the dropping mercury electrode is adjusted so that the species is oxidized or reduced as soon as it diffuses to the electrode.

It is seen, therefore, that the diffusion current, under specific experimental conditions, can be used to evaluate C°.

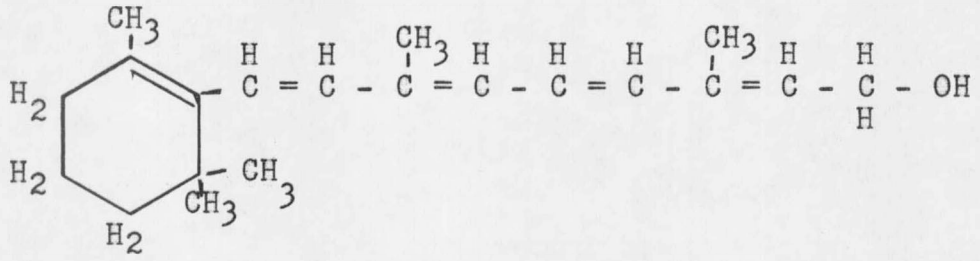
The operation of the polarograph has not been confined to the analysis of inorganic ions, but has also found wide use in the analysis of organic compounds.

The first organic compound to be reduced polarographically was nitrobenzene in 1925 (2). Since that time a great number of organic compounds have been determined with the use of the polarograph, and a great variety of solvents and supporting electrolytes used. Among the organic compounds which can be reduced or oxidized are those with the following groups: conjugated carbon-carbon double bonds; aldehydes; ketones; quinones; nitro, nitroso, amino, oxide and azo groups; quaternary ammonium groups; halogens; disulfides; peroxides; and epoxides (3). Some of the solvents used to carry out these reductions are alcohols, glycols, dioxane, cellosolve, glacial acetic acid and formamide (4).

Among the various organic compounds which can be determined polarographically are the vitamins (5). Heyrovsky and Hasselbach (6) were able to determine provitamin A indirectly by a polarographic technique, by the addition of excess iodine to the provitamin A solution, and then recording the polarographic-anodic wave of the excess iodine. They were unsuccessful in determining vitamin A directly, presumably because of its insolubility in water. So far as is known there has been no direct polarographic

method for vitamin A previously reported.

As mentioned previously, carbon-carbon double bonds can be reduced polarographically if they are conjugate to other double bonds. Therefore, it appeared that vitamin A could be reduced polarographically because of its conjugate unsaturation, as shown by its structure:



If vitamin A could be reduced polarographically, then it might be possible to develop an analytical method more suitable than the Carr-Price method (7) for the determination of vitamin A.

In the Carr-Price procedure, a great deal of time is spent in the saponification of the sample, and also in the exhaustive liquid-liquid extraction of the vitamin A from the saponification solution. It was hoped that by the use of a polarographic method the saponification and extraction steps could be eliminated, thus decreasing the time required for the analysis of vitamin A.

The primary objective of this investigation, then, was to examine the possibilities of determining vitamin A polarographically.

EXPERIMENTAL

A. Apparatus

1. Fisher Scientific Co. Electropode Polarograph, Model No. 9-317
2. Blue M "Magni Whirl" constant temperature bath, Model No. MW-115255A, equipped with a circulating pump, in order to circulate water to the jacket of the polarographic cell

B. Reagents

1. N,N-Dimethylformamide - Matheson, Coleman and Bell, No. 5974
2. Tetraethylammonium Bromide - Eastman Organic Chemicals, No. 1516
3. Electrolyte: 83.5 ml N,N-dimethylformamide saturated with tetraethylammonium bromide diluted to 100 ml with N,N-dimethylformamide
4. Vitamin A alcohol, highest purity - Distillation Products Industries
5. Vitamin A acetate, highest purity - Distillation Products Industries
6. Dioxane - Allied Chemical Co., General Chemical Division, No. 1697
7. Absolute ethyl alcohol
8. Nitrogen gas, liquid pumped - National Cylinder Gas Co.
9. Mercury metal, reagent grade - Fisher Scientific Co.

C. Solvent System

Due to the insolubility of vitamin A in water, an organic solvent was required to investigate the polarographic reduction of that compound; it should have the following properties:

1. The solvent must be polar so that the resultant solution will conduct current.
2. The solvent must be inert so that there will be no complicating reactions between the solvent and the compound being studied.
3. The compound to be studied and the supporting electrolyte must be soluble in the solvent.
4. The reduction potential of the solvent, if reducible, must be more negative than the compound being studied so it will not be reduced during the determination.

A number of different solvents were tried using tetraethylammonium bromide as the electrolyte. The solvents tried were absolute ethyl alcohol, 10% aqueous solution of ethyl alcohol, dioxane, 10% aqueous solution of dioxane, N,N-dimethylformamide and a 10% aqueous solution of N,N-dimethylformamide. When solutions of dioxane and ethyl alcohol were used, it was observed that at a potential slightly more negative than -1.0 volts versus mercury pool, there was a very sudden increase in current. The current had such a high value that the galvanometer

reading went off scale at the highest shunt ratio on the instrument. At potentials between 0 and -1.0 volts there was no current increase that would indicate the reduction of vitamin A. Of the solvents listed only N,N-dimethylformamide saturated with tetraethylammonium bromide yielded a satisfactory polarogram of vitamin A.

A maximum (Figure 1) was observed with the use of N,N-dimethylformamide saturated with tetraethylammonium bromide. However, by diluting the saturated solution with pure N,N-dimethylformamide at a ratio of 5.5:1 the maximum was eliminated.

D. Determination of Physical Constants

In order to evaluate the Ilkovic equation where $i_d = 706\ nm^{\frac{2}{3}}\ t^{\frac{1}{6}}\ D_o^{\frac{1}{2}}\ C^\circ$ (1), the following constants must be determined:

D_o = diffusion coefficient of oxidizible or reducible substances in cm^2 per second

I = diffusion current constant which is equal to $I = \frac{i_d}{C^\circ m^{\frac{2}{3}} t^{\frac{1}{6}}}$

n = number of electrons involved in the electrode reaction

m = mass rate of flow of mercury from the dropping mercury electrode in milligrams per second

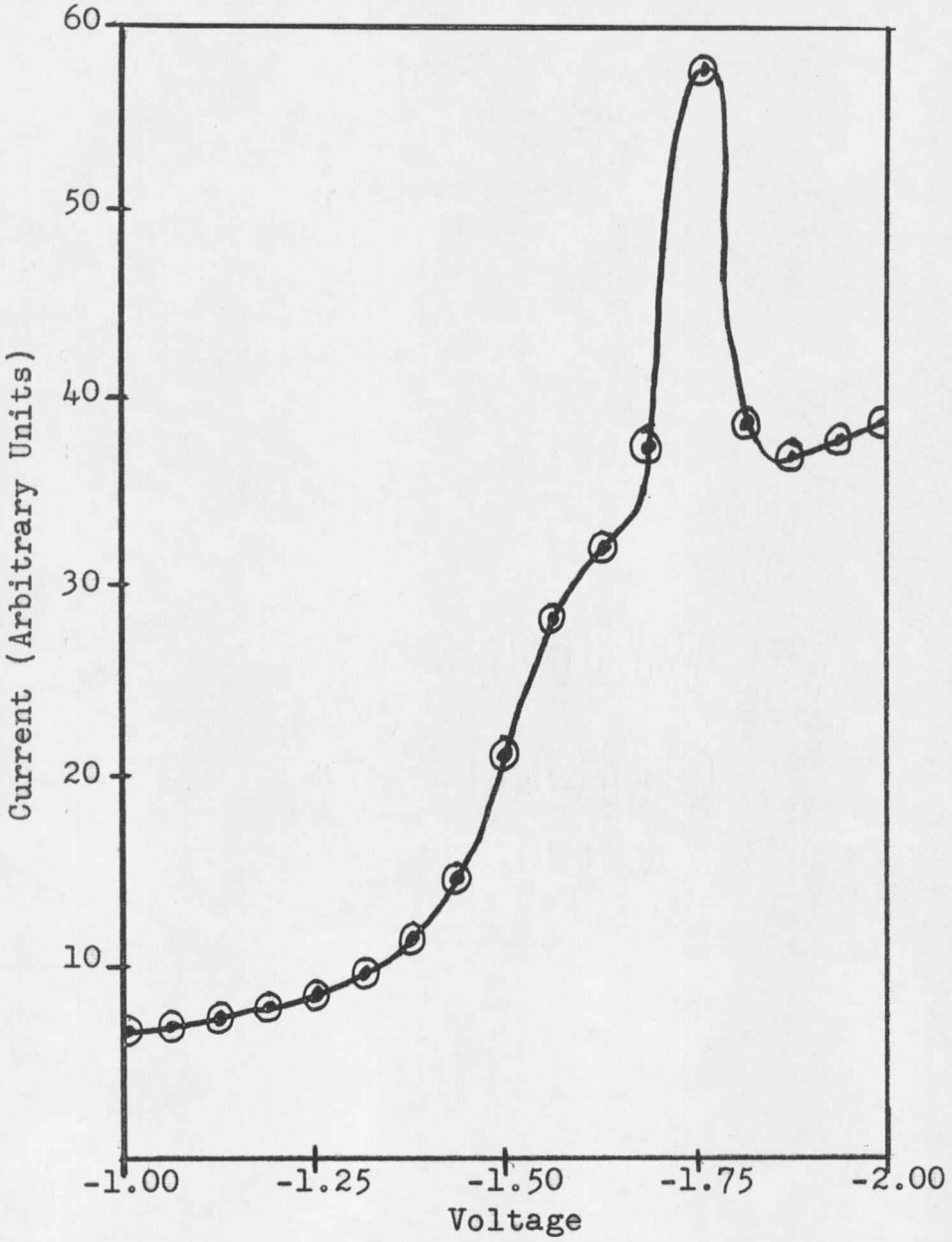


Figure 1. Polarogram Showing Polarographic Maxima of Vitamin A.

t = drop time of the mercury from the dropping mercury electrode in drops per second

C° = millimoles per liter of reducible or oxidizible substances

Solutions of vitamin A acetate and vitamin A alcohol were prepared. Each solution contained 10 mg of the vitamin per 10 ml of the electrolyte. Nitrogen was then bubbled through the solution in order to remove any dissolved oxygen. The temperature of the solution during the polarographic reduction was maintained at 25.0 ± 0.2° C.

The polarograms of the two forms of vitamin A (Figures 2 and 3) were then used for the graphic determination (10) of the diffusion current (i_d) and the half wave potential ($E_{\frac{1}{2}}$) (Table I; Figures 2 and 3). The description of the graphic method is as follows: the upper and lower portions of the curves (Figures 2 and 3) are extended by lines AB and CD. Then lines AC and BD are traced perpendicular to the abscissa axis. The points G and F bisect the lines AC and BD. The line GF is traced and it intersects the polarographic wave at $E_{\frac{1}{2}}$. A line HI is traced perpendicular to the abscissa axis intersecting the curve at $E_{\frac{1}{2}}$. The length of HI is taken as the diffusion current (i_d) and the point I is taken as the residual current (i).

The value of $E_{\frac{1}{2}}$ is obtained with reference to the mercury

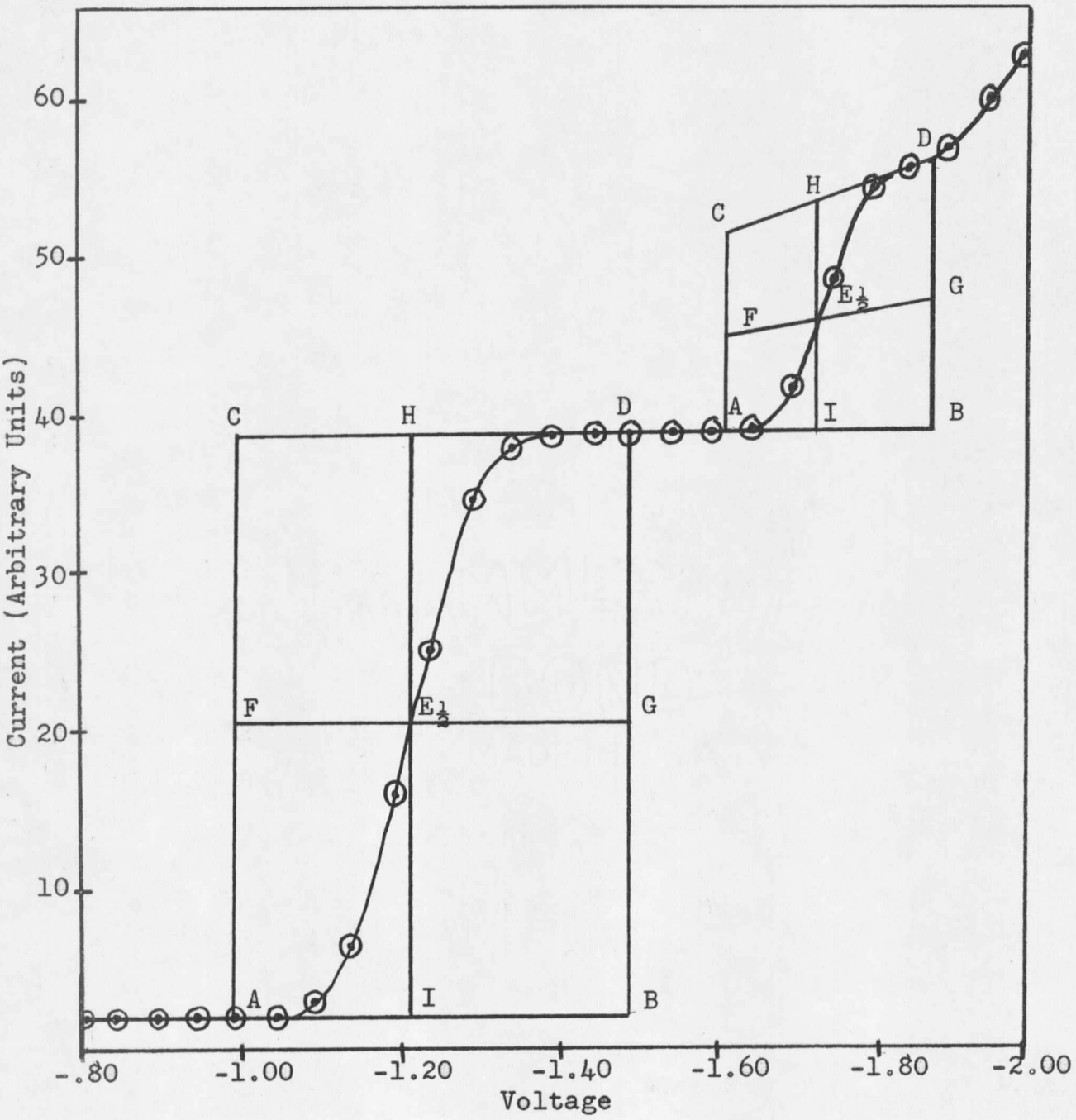


Figure 2. Polarogram of Vitamin A Acetate.

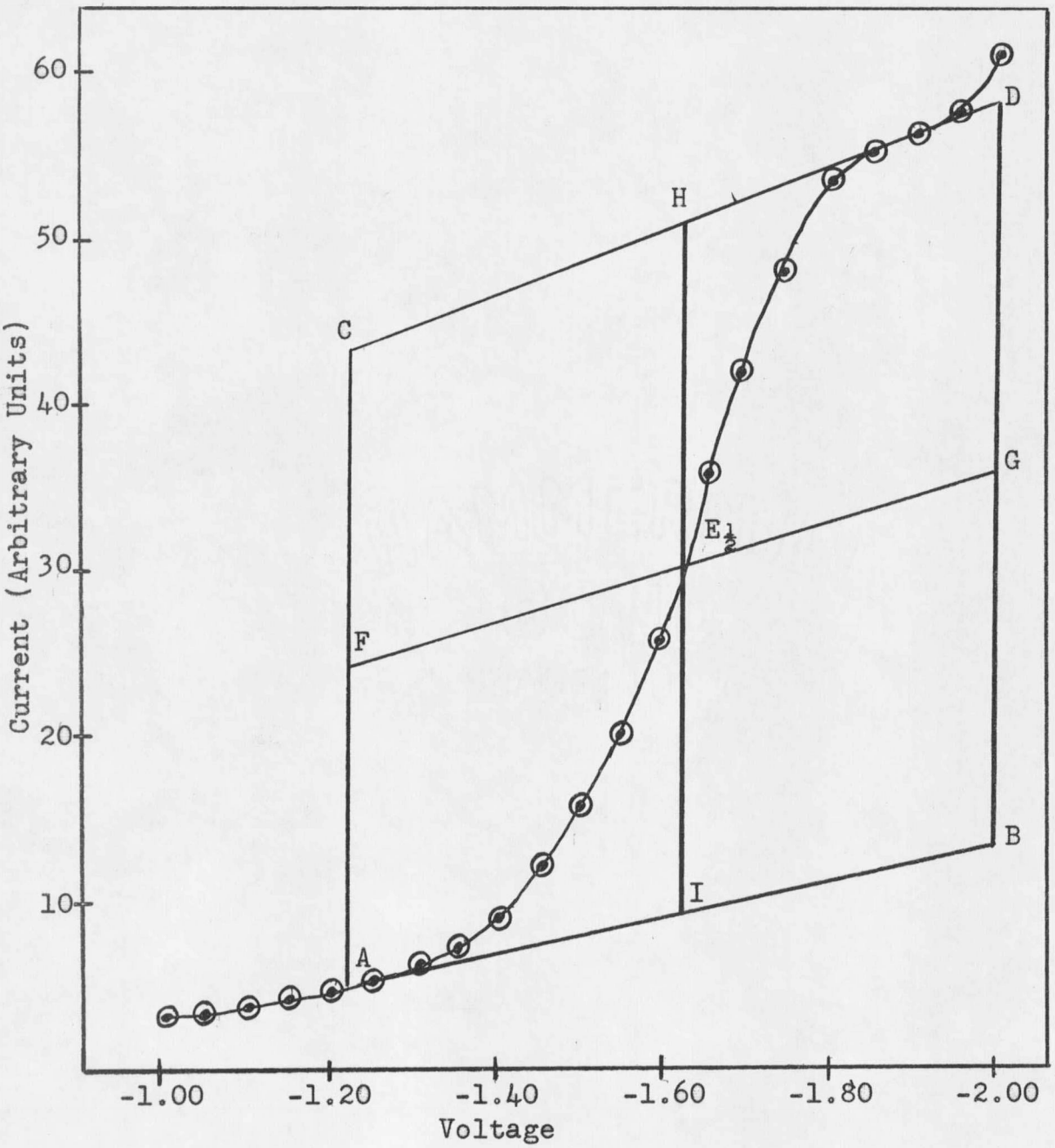


Figure 3. Polarogram of Vitamin A Alcohol.

pool and may not be used as a reliable reference value due to possible polarization at the mercury pool. Therefore, this value for $E_{\frac{1}{2}}$ is quantitatively applicable only to experimental conditions as herein reported.

The polarograms were also used to determine the number of electrons involved in the reduction by applying the following equation, which is applicable to a reversible reaction:

$$E_{de} = E_{\frac{1}{2}} - \frac{.0591}{n} \log \frac{i}{(i_d - i)} \qquad (11)$$

where E_{de} = applied potential

$E_{\frac{1}{2}}$ = half wave potential

n = number of electrons involved in the reaction

i = residual current

i_d = diffusion current

By making a plot of E_{de} vs. $\log \frac{i}{(i_d - i)}$ and using the method of least squares for positioning the line (Figures 4, 5, and 6), a straight line is produced with a slope equal to .0591/n (12). From this relationship the values of n for the different forms of vitamin A were determined (Table I). The logarithmic analysis of the reduction waves for vitamin A acetate gives slopes of .0533 for the first wave and .0469 for the second wave. Because these values are close to the theoretical value of .0591 for one electron change, the value

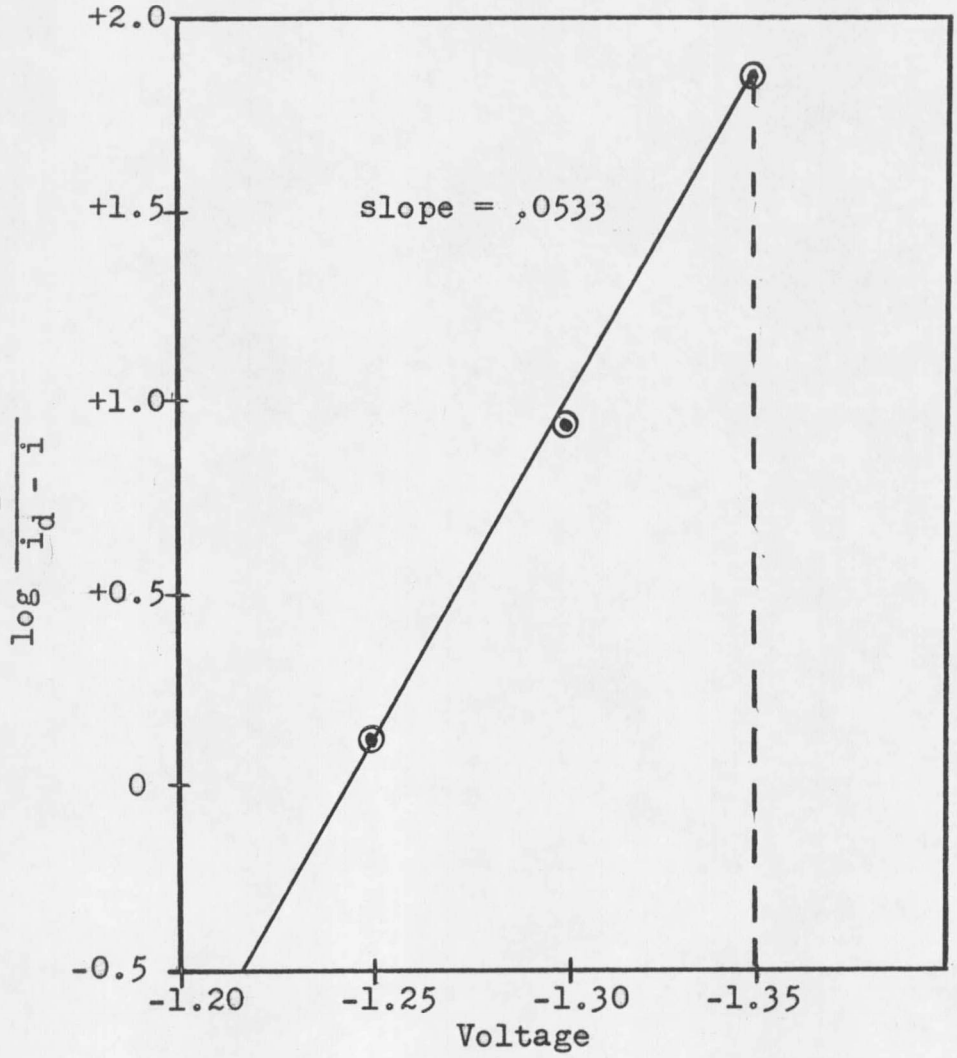


Figure 4. Logarithmic Analysis of Wave No. 1 of Vitamin A Acetate.

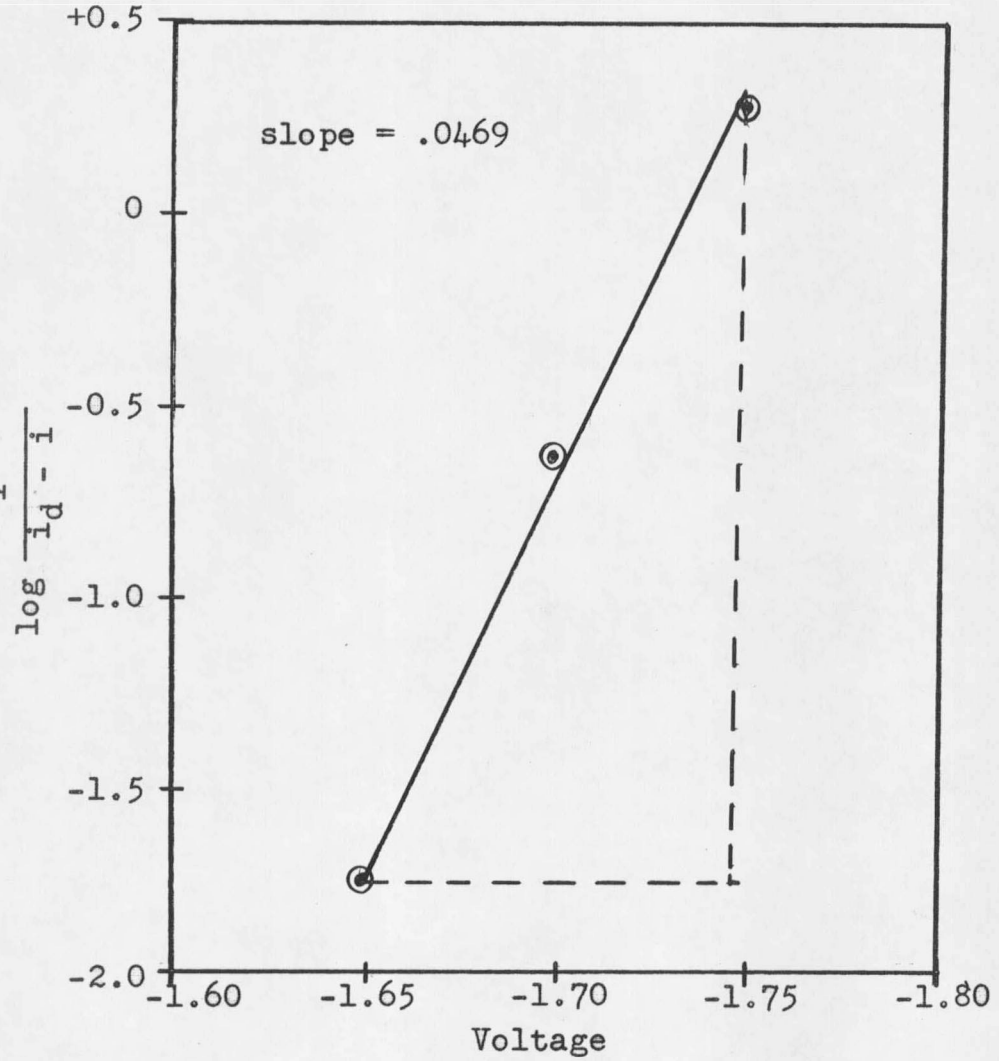


Figure 5. Logarithmic Analysis of Wave No. 2 of Vitamin A Acetate.

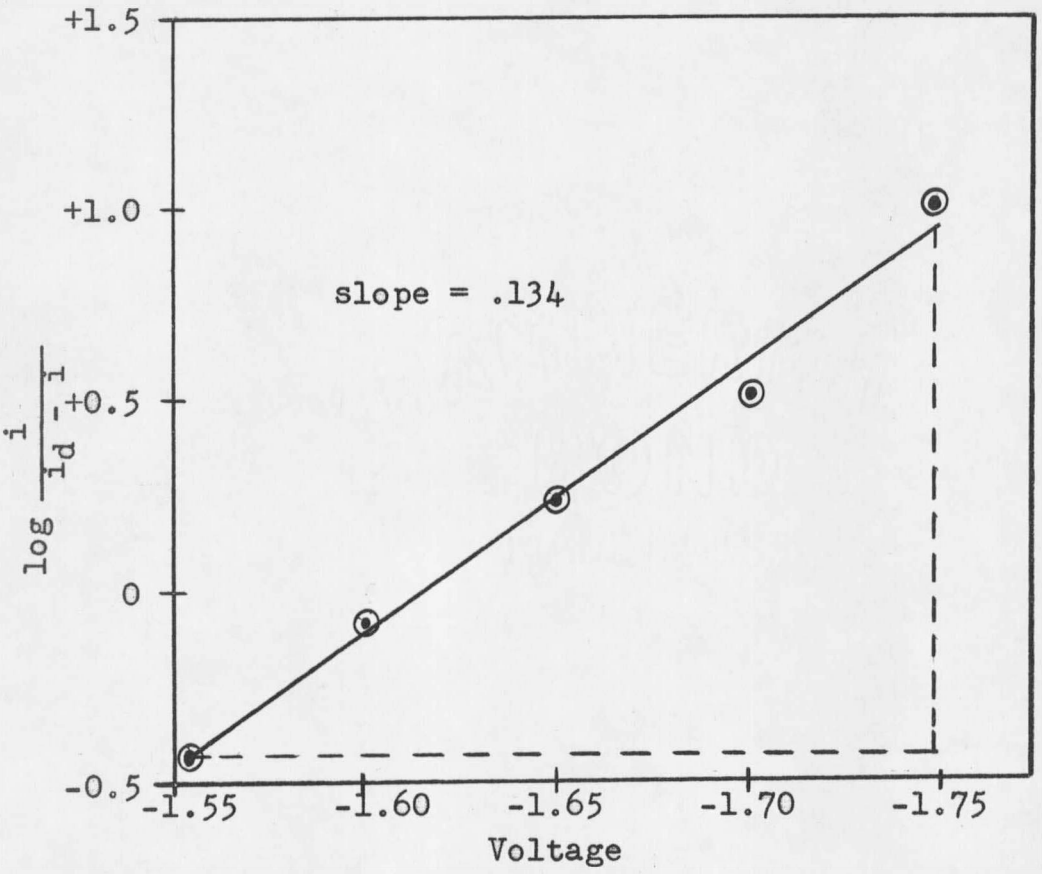


Figure 6. Logarithmic Analysis of the Vitamin A Alcohol Polarogram.

of one was assigned to n for each wave. The slope calculated for vitamin A alcohol, .134, deviates from the theoretical value of .0591, which might indicate that the reduction was irreversible and the above equation not applicable. In order to check this possibility, the polarographic reduction was run in reverse, i.e. the potential at the beginning of the electrolysis was more negative than the half wave potential. Then the potential was increased in a positive direction. The resultant polarographic curve was identical to that obtained in the first determination. This result indicates that the reduction was reversible. The unexpectedly high slope of the curve is unexplained. However, because of the similar molecular structure to that of vitamin A acetate, and the fact that the reaction is reversible, the value of one, which is more reasonable than a value of $\frac{1}{2}$, was also assigned to n for the reduction of vitamin A alcohol.

TABLE I

PHYSICAL CONSTANTS

	n	i_d	I	D_o	slope	$E_{\frac{1}{2}}$ vs. Hg pool
Vit.A Acet.,Wave 1	1	23.6	10.5	2.24×10^{-4}	.0533	-1.245
Vit.A. Acet.,Wave 2	1	9.6	4.28	3.94×10^{-5}	.0469	-1.735
Vit.A Alcohol	1	26.7	10.5	2.24×10^{-4}	.134	-1.615

E. Relationship Between the Diffusion Current and Concentration

A series of samples of varying concentration were run in order to determine the proportionality between diffusion current and concentration of the two forms of vitamin A. For each form of the vitamin it was shown that the wave height or diffusion current is proportional to the concentration (Table II; Figures 7 and 8).

The polarograms of vitamin A acetate show two curves, both proportional to the concentration. As will be explained later, the second curve is attributed to acetate ions.

TABLE II

PROPORTIONALITY BETWEEN DIFFUSION CURRENT AND CONCENTRATION

	concentration mg/10 ml	wave height wave No. 1	wave height wave No. 2
Vitamin A Acetate	2.5 mg	97	35
" " "	5.0 mg	185	70
" " "	10.0 mg	370	147
" " "	20.0 mg	730	290
Vitamin A Alcohol	2.5 mg	105	
" " "	5.0 mg	210	
" " "	10.0 mg	437	
" " "	20.0 mg	830	

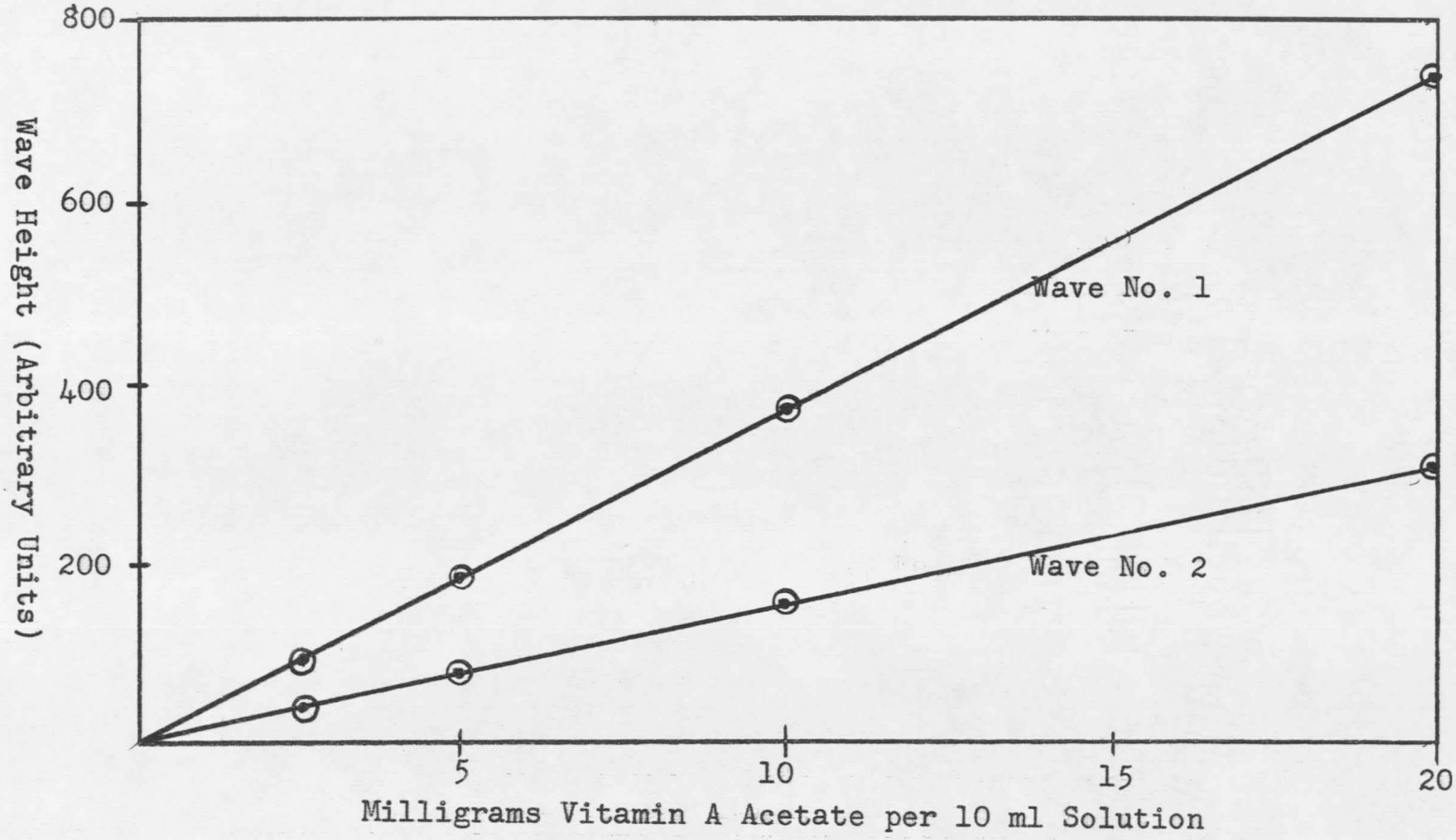


Figure 7. Proportionality Between Diffusion Current and the Concentration of Vitamin A Acetate.

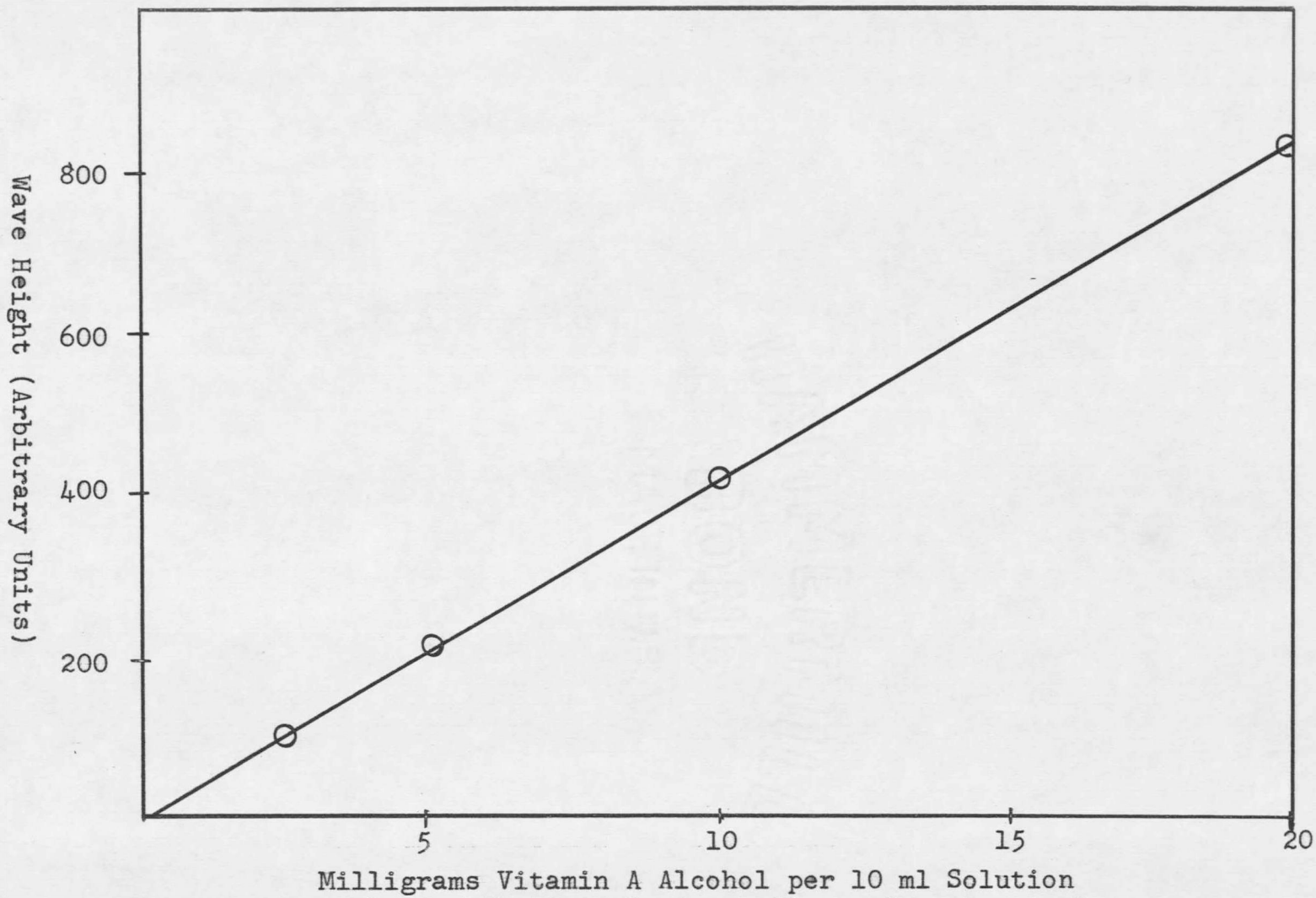


Figure 8. Proportionality Between Diffusion Current and the Concentration of Vitamin A Alcohol.

DISCUSSION

A. Electrode Reaction

The electroreduction of vitamin A is probably similar to the reaction described by Given (13) of olefins in N,N-dimethylformamide. It was suggested by Given that, at a voltage approaching the half wave potential, the ion R^- is formed by reversible addition of one electron. This first addition of an electron determines the potential of the reduction and also the slope of the polarographic wave. The ion R^- then reacts with a proton to form a radical RH· which then adds an electron to become RH^-. The addition of an electron to RH· will occur without a further change in potential because the reduction potential of the free radical will be less negative than that of the molecule from which it was derived. Upon an addition of an electron to RH· the resulting negative ion will then add another proton $RH^- + H^+ \longrightarrow RH_2$ to complete the reaction.

This mechanism is similar in certain respects to that proposed by Laitinen and Wawzonek (14) for olefins in neutral or alkaline solution. The reaction is as follows:

Step 1. $R + e^- \longrightarrow R^-$ (reversible and potential determining)

Step 2. $R^- + e^- \longrightarrow R^=$ (irreversible)

Step 3. $R^= + 2H_2O \longrightarrow RH_2 + 2\ OH^-$ (irreversible and rapid)

The first step is similar to the mechanism as proposed by Given in that the first reduction is potential determining. The addition of an electron to R^- to form $R^=$ is unlikely because the ion $R^=$ would be in an abnormally high energy state. Instead, the R^- would probably react with a proton to form the free radical RH· and the reaction would then proceed in the manner suggested by Given.

In the large scale reduction of unsaturated compounds in anhydrous dimethylformamide, Wawzonek et al (15) found CO and H_2 among the reduction products. They explained the presence of these two gases by the reaction of the organic ion with the dimethylformamide, which is as shown:

$$\geq C:^- + HC(=O) - N(CH_3)_2 \longrightarrow \geq CH + \left[C(=O) - N(CH_3)_2\right]^-$$

$$\left[C(=O) = N(CH_3)_2\right]^- \longrightarrow CO + \left[N(CH_3)_2\right]^-$$

$$\left[N(CH_3)_2\right]^- - e \xrightarrow{\text{anode}} \cdot N(CH_3)_2$$

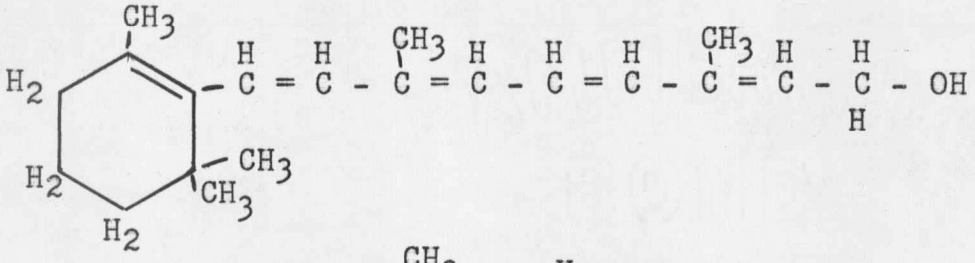
$$2 \cdot N(CH_3)_2 \longrightarrow H_2 + 2CH_3N{=}CH_2$$

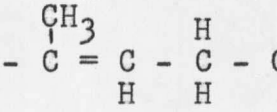
Given (13), in his work with conjugated systems in dimethylformamide, assumed that water might be a contaminant, and thus a source of protons. By the addition of small amounts of water he showed that there was no effect upon the polarogram. Therefore he, too, concluded that the protons were furnished by the dimethylformamide.

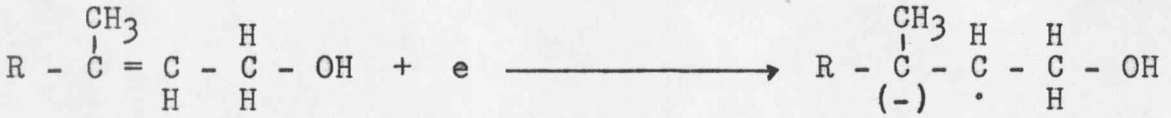
In Given's work he did not attempt to determine the amount of water present as a contaminant in the dimethylformamide. The water additions may have been less than that already present as a contaminant. This evidence is therefore not conclusive, that water is not a source of protons.

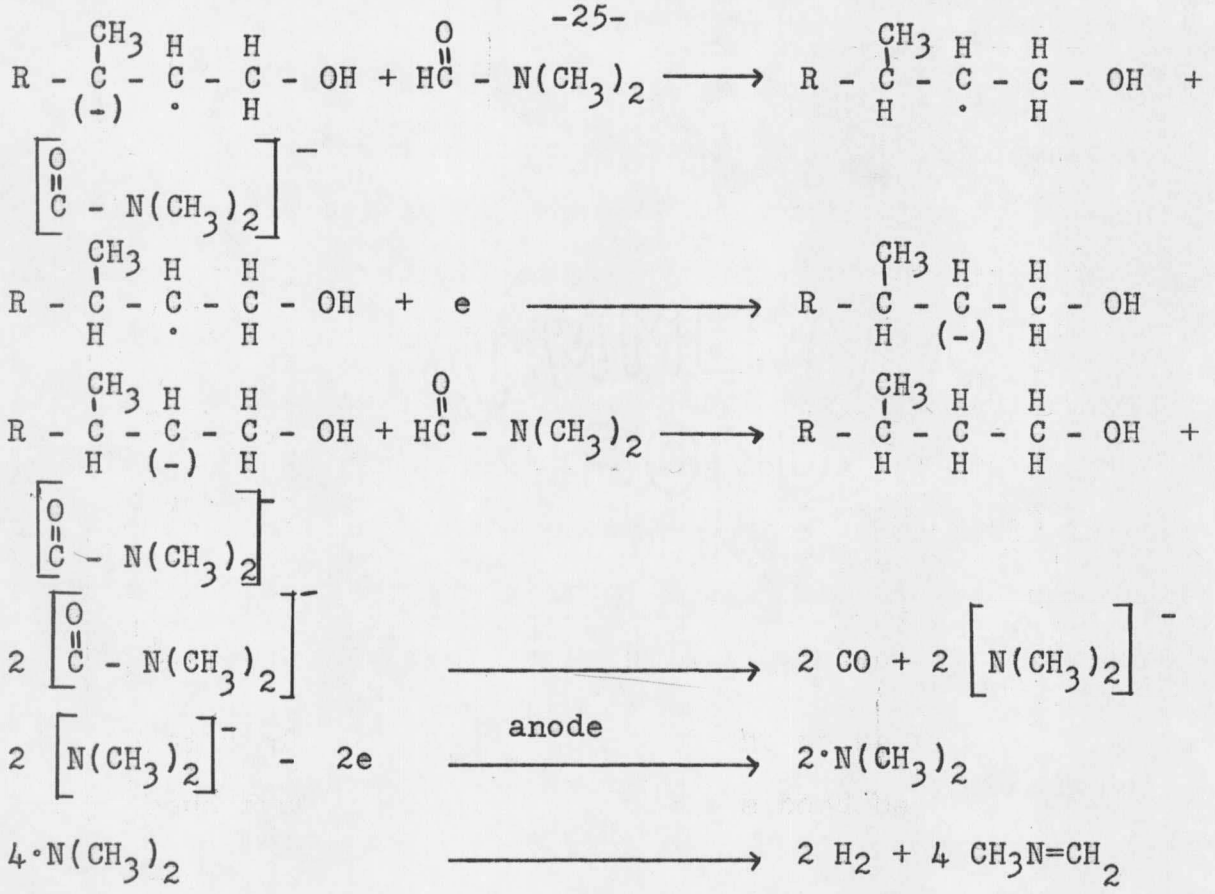
The work of Wawzonek indicates that the dimethylformamide is a source, but perhaps not the only source, of protons in the polarographic reduction of unsaturated compounds. In the proposed reaction dimethylformamide will be used as the source of protons, but it should be kept in mind that water may also be a source of the protons.

The proposed reaction of vitamin A in dimethylformamide is outlined below. The reduction mechanism is that suggested by Given and the source of protons is dimethylformamide, as suggested by Wawzonek. The formula for vitamin A,

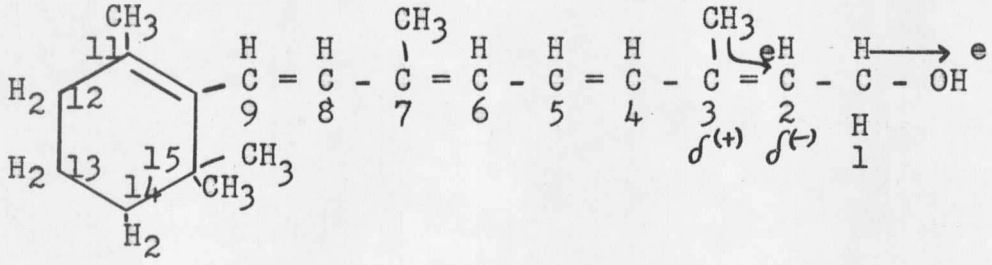


is abbreviated to  for simplicity.





The site of the reduction is thought to be at the first double bond from the OH group, i.e. the bond between carbons 2 and 3.



The maximum electron density would be found toward the middle portion of the molecule, due to the overlapping of the P orbitals (16), thus concentrating the charge away from the

ends of the molecule. Therefore, it might be assumed that the site of the reduction would be either between carbons 2 and 3, or 10 and 11. The inductive effects from the adjacent groups would then determine which bond would be reduced. At the double bond between carbons 10 and 11 there is a positive inductive effect from the methyl groups on carbons 11 and 15, forcing electrons toward the double bond. This effect would tend to cancel any charge that might develop on either carbon. Thus, neither carbon would be more positive or negative than the other. At the double bond between carbons 2 and 3 there is a positive inductive effect from the methyl group on carbon 3, and a negative inductive effect from the alcohol group drawing electrons toward it. The results would be a relatively positive charge on carbon 3. Due to this partial positive charge on carbon 3, the incoming electron from electrolysis would probably add to carbon 3 in preference to carbons 2, 10, or 11. After this first addition, a proton would be added to carbon 3 forming a free radical, followed by another electron forming a negative ion, which in turn would add a proton, thus completing the reaction.

In the reduction of vitamin A acetate, two curves were observed, one at $E_{\frac{1}{2}} = -1.245$, the other at $E_{\frac{1}{2}} = -1.735$. Based upon the following considerations, the curve at -1.735 volts was thought to be that of the acetate ion.

A solution of ammonium acetate was prepared using the

same solvent-electrolyte system as that used for the vitamin A. This solution exhibited a wave at the same half wave potential as the second wave of vitamin A acetate, and the curve had the same wave height per millimole of acetate as that of the vitamin A acetate. Therefore, it was concluded that the second curve of vitamin A acetate is a polarogram of the acetate ion.

B. Maximum and Its Explanation

It was noted with the use of N,N-dimethylformamide saturated with tetraethylammonium bromide that a maximum was observed. The occurrence of a maximum is a commonly observed characteristic of the polarographic wave. By the addition of certain substances, for example gelatin, organic dyes, and some inorganic anions, the maxima may be eliminated and a well defined curve obtained. The explanation for the occurrence, and also for the elimination of maxima, has not been thoroughly determined.

There have been a number of theories for the origin of maxima (9), and the one which seems to be applicable to this experimentation was proposed by Heyrovsky. He stated that a polarogram will not exhibit a maximum if the electroreduction potential ($E_{\frac{1}{2}}$) corresponds to the potential of the electrocapillary zero, i.e. when the mercury is uncharged and the interfacial tension is at a maximum value (Figure 9).

Some ions, such as Cl^-, Br^-, I^-, CN^- and S^-, are classed as electrocapillary active, i.e. they will cause a shift in

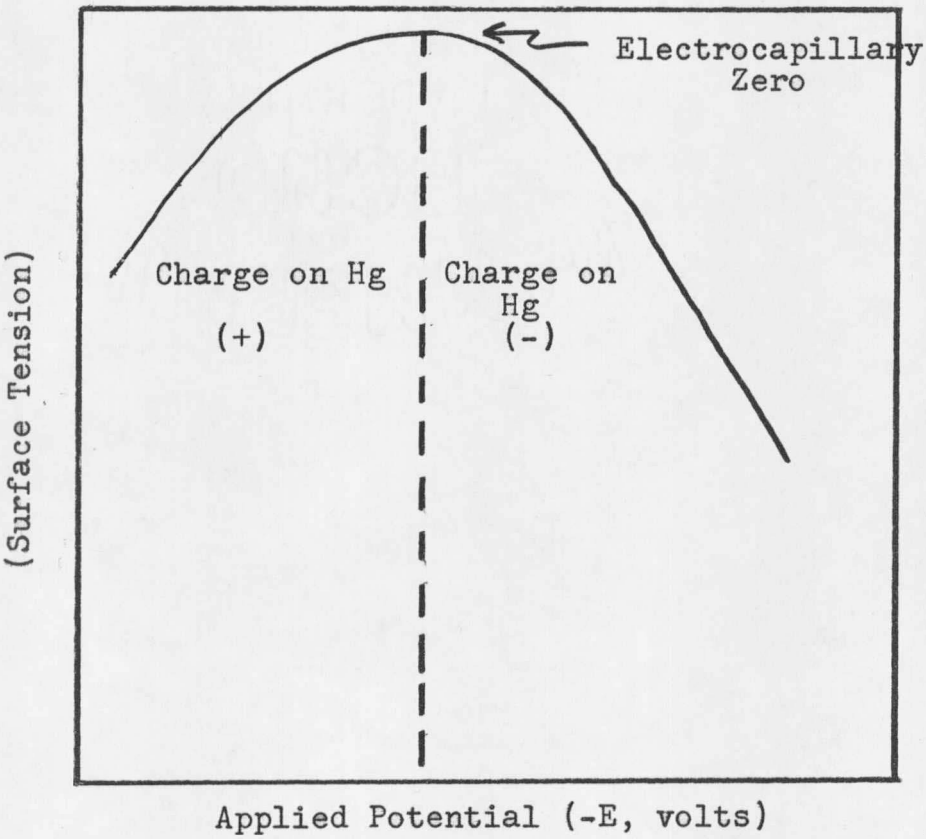


Figure 9. Electrocapillary Curve of Mercury.

the potential of the electrocapillary zero. This shift is due to the adsorption of the ions on the mercury surface. If the ions are negatively charged, they will repel electrons from the surface into the interior of the mercury. The mercury surface would then have a positive charge. The effect of this charge would counteract the interfacial tension of the solution by coulombic effects and the electrocapillary zero would no longer be at the original potential. In order to remove the positive charge on the surface of the mercury, the applied potential must be made more negative, in this way neutralizing the charge. This would allow the surface tension, or interfacial tension, to reach a maximum value, and a new electrocapillary zero would be established.

In the case of the maximum exhibited by vitamin A, it seems likely that by dilution of the saturated solution the reverse of the above would happen. If the half wave potential from vitamin A in the saturated solution were located at E_1 (Figure 10) and the electrocapillary zero at E_2, then, according to Heyrovsky, a maximum would be observed. If the solution were diluted with solvent, then the effect of the electrocapillary active ions (Br^-) would be somewhat diminished, and a negative charge would result on the surface of the mercury. To counteract this change, the potential of the electrocapillary zero would be shifted to a more positive value, E_3. The value of E_3 would correspond to E_1, or the half wave potential of

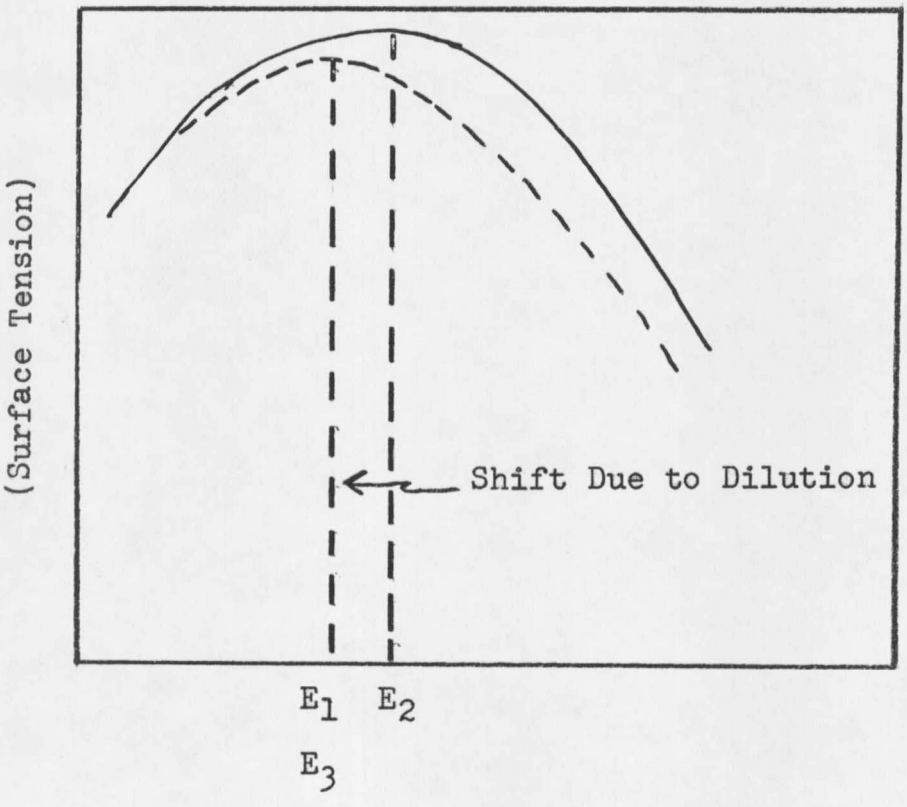


Figure 10. Shift in Electrocapillary Zero Due to Dilution of Solution.

vitamin A, thus eliminating the maximum exhibited by vitamin A.

In any event, by the use of this diluted solution, well defined polarograms of vitamin A alcohol and vitamin A acetate were obtained (Figures 2 and 3).

SUMMARY AND CONCLUSIONS

In the work described here, it has been shown that vitamin A can be reduced polarographically in dimethylformamide using tetraethylammonium bromide as the electrolyte, and that a well defined curve is obtained. The polarographic reduction of vitamin A alcohol yields one wave, whereas that of vitamin A acetate gives two. The curve at the more negative potential is attributed to the acetate ion.

The electrode reaction of the two forms of vitamin A is considered to be similar to that proposed by H.P. Given (13), in which the vitamin A is reduced step-wise. The first step would involve the addition of one electron at the double bond closest to the functional group, i.e. the OH group on the alcohol and the $\overset{O}{\overset{\|}{C}} - CH_3$ group on the acetate, followed by the addition of a proton. This initial reaction would be reversible and potential determining. The second step would involve the addition of an electron at the same site and would be irreversible, followed by the addition of another proton. The resulting organic ions extract protons from the dimethylformamide solvent by a reaction proposed by S. Wawzonek (15).

The results of this experimentation indicate that vitamin A can be determined polarographically, and the results could be applied to an analytical method where the concentration of vitamin A in the electrolytic solution is in excess of about 0.5 mg/10 ml. Since the Carr-Price method requires a concentration range of vitamin A in the final solution of 1 μg to

10μg per 10 ml, a polarographic method would be more suitable for analysis of materials having a high concentration of vitamin A, for example nearly pure vitamin A. Due to the low sensitivity of the polarographic method, it would not be applicable to samples containing low concentrations of vitamin A.

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