

POLAROGRAPHIC INVESTIGATION OF
B-N-Oxalyl-L- α , β -DIAMINOPROPIONIC ACID,
A NEUROTOXIN FROM THE SEEDS OF
LATHYRUS SATIVUS (GUAYA)

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DEDICATION

To my father Yohannes Anshebo,
my mother Desta Elemo,
my brothers & sisters.

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LIST OF SYMBOLS

- α = Transfer coefficient
 $\bar{\alpha}$ = Average transfer coefficient
 O = Overall stability constant
 A = Surface area of electrode
 C = Concentration
 D = Diffusion coefficient
 D_c = Diffusion coefficient of complex
 D_s = Diffusion coefficient of the simple metal ion
 E = Electrode potential referred to some reference electrode
 E^0 = Formal electrode potential
 $E_{\frac{1}{2}}$ = Half wave potential
 $E_{\frac{1}{4}}$ = Potential where $i/i_a \approx \frac{1}{4}$
 $E_{\frac{3}{4}}$ = Potential where $i/i_a \approx \frac{3}{4}$
 ΔE = Pulse amplitude
 E_p = Peak potential
 F = Faraday's constant
 h = Height of mercury column
 i = Electric current
 i_a = Adsorption current
 i_o = Catalytic current
 i_d = Diffusion current
 \bar{i}_d = Mean diffusion current
 i_k = Kinetic current
 i_∞ = Limiting current

h	=	peak current
j	=	Ligand number
k	=	Rate coefficient
\bar{g}	=	Average rate coefficient
K	=	Equilibrium constant
k_b	=	Rate of backward chemical reaction
k_f	=	Rate of forward chemical reaction
l	=	Capillary length
C_L	=	Ligand concentration
m	=	Rate of mercury flow
W_M	=	Simple metal ion concentration
H	=	Complex concentration
n	=	Number of electrons exchanged
O	=	Oxidized species
r_t	=	Rate of electrode reaction
R	=	Gas constant / Reduced species
t	=	Life of mercury drop
T	=	Absolute temperature
$W_{\frac{1}{2}}$	=	Peak half width

ABSTRACT

Polarographic investigations of β -N-Oxalyl-L-alpha, 0-Diamino-propionic acid, ODAP, a neurotoxin from the seeds of Lathyrus sativus(Guaya) have been made.

Aqueous solutions of ODAP in KCl supporting electrolyte gave a single polarographic wave with a half-wave potential of -1.518V. This wave was found to be due to the irreversible discharge of hydrogen. The reduction wave disappeared on standing and may be due to intramolecular rearrangement leading to lactone formation.

At more positive potential a second peak appears for pH's less than 2. The reduction wave shifts towards more positive values as the pH decreases. This reduction wave could be due to a direct reduction of the protonated amino acid.

Polarograms of aqueous solution of Cu(II), Ni(II), Zn(II), Cd(II), Pb(II) and Co(II) in KCl supporting electrolyte were not affected by the addition of ODAP. However, in ammonia buffer (pH = 8.2) and when ODAP was added only Ni(II) and Co(II) showed larger shifts in the differential pulse polarographic peak potentials after one day.

Polarographic behavior of complexes of ODAP with Ni(II) and Co(II) in borax buffer (pH = 9.2) have been studied. The reduction of both complexes were polarographically irreversible.

The stability constant and ligand number of the Ni(II)-ODAP complex in borax (pH = 9.2) were determined. The simple and complex metal ions were reduced irreversibly. The ligand number was determined to be 2 and the stability constant calculated to be $\log K = 7.4$.

1. INTRODUCTION

Lathyrus sativus is one of the 150 species of the genus Lathyrus, found mainly distributed over north temperate zones but centered on the Eastern Mediterranean Region. The legume is also found in Ethiopia with a local name "Guaya", the main area of cultivation being concentrated in the northern half of the country (192).

"Guaya" is used in the preparation of local foods such as roasted seeds "Kolo", boiled seeds "Nifro", flour made into bread "Kitta", and the flour made into a kind of gravy known as "Shiro", used in the preparation of traditional "Shiro Wot"(2),

Lathyrus sativus has been used by people when there is acute shortage of other staples during times of famine. The crop can be grown with very little agricultural attention and has a high degree of adaptability under extreme conditions such as drought, water-logging and invasion of certain pests. It also gives fairly good and stable yields with practically no inputs in conditions where other crops fail (2-4).

Lathyrus sativus seed provides a nourishing diet of high quality protein and carbohydrates. It contains proteins ranging from 21% to 33%. Lathyrus sativus is deficient in methionine, rich in lysine, and the concentration of the amino acids is within acceptable limits. Its vitamin A and ascorbic acid content is low (2,4-6).

The excess consumption of Lathyrus sativus has been associated for more than 2000 years with an irreversible disease of the nervous system known as human lathyrism or neurolathyrism (7-9). The use of Lathyrus sativus one-third to one-half of the diet for two or three months was considered enough to cause the disease, although not all persons eating the diet seemed to be affected; in families only certain members were attacked. Males were said to be more susceptible than females (2,4,10).

Neurolathyrism is characterized by loss of muscular control, spasticity of leg muscles, convulsions, head retraction, stiffening of neck and in extreme cases, death. In some cases, only a mild effect, such as a slight bending of the knees was observed. In severe cases symptoms such as loss of control of bladder and rectum, and complete impotence, were quite pronounced. In extreme cases, the severity of the disease was of such a high order that patients were reduced to crawling (4,7, 11-16).

The occurrence of neurolathyrism has been reported in many parts of the world, including, Afghanistan, Algeria, Bangladesh, China, Ethiopia, France, Germany, Greece, India, Italy, Russia, Spain and the U.S.A. (2,6,10,12,17).

A number of substances have been isolated from Lathyrus sativus seeds including α -N-Oxalyl-L- α , β -Diarninopropionic acid, water soluble aliphatic amino acid glycoside with a nitrile group, phenolic compounds, a toxic alkaloid, unusual amino acids, glucosides of diaminopropionic acid and Di-N-Oxalyl Diarninopropionic acid (11,18).

The principal causative agent of neurolathyrism isolated from seeds of Lathyrus sativus has been identified as β -N-Oxalyl-L- α , β -Diarninopropionic acid by Rao et al. (19) and Murti et al. (20) independently. The compound in solution (2mg/ml) is highly acidic (pH=2.5) and forms oxalic acid and diarninopropionic acid on acid hydrolysis. It has a specific rotation, $[\alpha]_D^{27}$, of -36.9° (c, 0.66 | 4N HCl) and its apparent pK values are 1.959, 2.959 and 9.25, corresponding to the two carboxyl and one amino functions, respectively (19).

Three different nomenclatures have been suggested for the neurotoxins β -N-Oxalyl Amino-L-Alanine (BOAA), β -N-Oxalyl-L- α , β -Diarninopropionic acid (ODAP or Ox-dabro), and L-5-Oxalyl-amino-2-Aminopropionic acid (OAP), (21,22),

Different methods of detoxification were used such as steeping the seed in hot water for several hours and boiling the seed in water and draining out the supernatant. These methods of reductions of the neurotoxin causes loss of soluble proteins and vitamins (4) #

The electrochemical behaviour of ODAP has not been studied so far. Therefore, in this project, an attempt was made to undertake an electrochemical study of the compound using different technique. Accordingly, normal dc and differential pulse polarography were used to investigate the processes taking place leading to the appearance of reduction waves in aqueous solutions of the amino acid at different pH values. Also, the formation of a complex with Ni(II) and an estimate of the ligand number and stability constant has been made.

2. THEORY

Polarography is the branch of voltammetry in which a dropping electrode is used as the indicator electrode. The unique characteristic of the polarographic technique is that it uses a dropping mercury electrode, such that the electrode surface is continuously renewed in a well defined and regulated manner to give reproducible electrode areas.

Any electrochemical process involves two main types of processes, namely transference of matter through a solution towards and away from electrodes, and electrochemical processes involving electron transfer at the electrode (32). The overall rate at which an electrode reaction proceeds generally depends on the kinetics of these processes. In addition adsorption phenomena may have to be considered, and there may be complications resulting from the coupling of the electron transfer process with purely chemical reactions (33).

This section is intended to give the theoretical background necessary to understand the polarographic behaviour of the system under investigation.

2.1. Types of Polarographic Limiting Current

The variation of current with a continuously changing potential can be used to give a polarogram. In the presence of an electroactive substance increase in current is observed over a particular potential range of the current vs potential curve. At first, as the potential is increased from zero, only a very small current flows, this is called the residual current. This is essentially a charging current arising from the charging of the double layer at each drop. Only the residual current flows until the reduction potential of the electroactive species is reached. At this point the electroactive species starts to be reduced. A steep rise in current is now observed and, with a further small increase in applied potential, the rise will continue. However, the electroactive species arrives at the

D in $\text{cm}^2\text{sec}^{-1}$, C in moles per cubic centimeters, and t in seconds.

The use in polarography of equation (1) can be justified since typical values of drop life time are short and the radius of the drop at maturity is small. The equation dealing with the actual experimental conditions have to consider the growth of each mercury drop which causes the existing diffusion layer to stretch over a still large sphere. This effect of expansion of the mercury drop makes the layer thinner and consequently the concentration gradient at the electrode surface larger than in the case of linear diffusion. This results in a larger current. Therefore the observed current is a function of the growth of the mercury drops and of the diffusion coefficient of the reactive material (35). For the limiting diffusion current at any time during the drop life, the Ilkovic equation may be expressed in the form:

$$i_d = 708.1nD^{\frac{1}{2}}Cm^{\frac{2}{3}}t^{1/6} \quad (2)$$

where i_d is in microamperes, C in terms of millimoles per litre, D in the units $\text{cm}^2\text{sec}^{-1}$, m as mgsec^{-1} , and t in seconds.

The most important consequence of the Ilkovic equation for analytical applications is the linear relationship between the diffusion current and the concentration of the electroactive substance.

In practical polarography knowledge of the variables which govern m and t are important because i_d is very nearly directly proportional to $m^{\frac{2}{3}}t^{1/6}$, equation (2), which in turn is proportional to the square root of the height of the corrected mercury column (36). This dependence of the limiting current on the square root of the corrected mercury column height is one of the most convenient ways to test whether a process is controlled by the rate of diffusion to the dropping mercury electrode.

2.1.2. Kinetic Current

Kinetic current is limited by the rate of a chemical reaction accompanying the electrode process and taking place at or near the surface of the electrode. The general mechanism responsible may be written as



The species O is reduced, at a potential where Y is not. However, in some systems it is possible to reduce directly at more negative potential than is needed to reduce O. Equation (3) and (4) involve the transfer of substances Y and O from the bulk of the solution toward the electrode, chemical transformation of substances Y and O in the vicinity of electrode, and the reduction of substance O. These steps control the current (33). The kinetic limiting current depends on the ratio of the equilibrium concentration of Y and O in the bulk of the solution and the rate constants for their interconversion (37).

For pure kinetic current that is obtained when the equilibrium concentration of O is negligibly small and when the transformation of Y into O is very slow the average kinetic current during the life of the drop is given by (37):

$$i_k = 493nD^{\frac{1}{2}}C_y m^{\frac{2}{3}}t^{\frac{2}{3}} (k_f/k_b^{\frac{1}{2}}) \quad (5)$$

Where i_k is in microamperes and C_y is in millimoles per litre.

From equation (5) it can be seen that the average kinetic current is proportional to $m^{\frac{2}{3}}t^{\frac{2}{3}}$, which indicates that kinetic current does not vary with the height of the corrected mercury

column. This provides a simple test to differentiate kinetically controlled process from that of diffusion controlled one.

Detailed treatment of polarographic kinetic currents are given in references 33, 37 and 38.

2.1.3 Catalytic Current

Catalytic current is current that arises when the electroactive species being consumed in the electrochemical reaction is partially regenerated by some chemical process involving the product of the electrochemical reaction.

The mechanism in which catalytic current arises can be written as



The substance Z is assumed not to be reducible. The addition of the substance acting as a catalyst causes a shift, in the reduction of the electroactive species to more positive potential, or in oxidation, to more negative values. An increase in current is obtained because of regeneration of the active species due to reaction of the product R with the substance Z.

Catalytic currents are characterized by a non linear dependence on the catalyst concentration (39). Different type of current-height dependencies can be obtained depending on the substance investigated and the conditions used. The catalytic current can be dependent on the square root of the height of the mercury column like a diffusion current or it can be independent of the square root of the height of the mercury column like a pure kinetic current. Some times catalytic current increases with decreasing height of the mercury column. This can be obtained only for catalytic currents and can serve as a criterion to identify catalytic controlled process (40).

2.1.4. Adsorption Current

Adsorption currents are those that are limited by the coverage of the surface of the electrode by the electroactive species, by the electrolysis product, or by an intermediate. The adsorbed layer may either enhance the rate of the electrode process, retard it, or completely block a further electrochemical process. Adsorption processes are manifested by a separate wave at potentials either more positive or more negative than the wave for the reduction or oxidation of the nonadsorbed compounds.

The limiting current of the adsorption wave is proportional to the amount of adsorbed substance on the electrode during the life of the drop (37) and is given by;

$$i_a = 13.66nm^{\frac{2}{3}}t^{-\frac{1}{3}} a^{-1} \quad (8)$$

where a is the area covered by each molecule in square angstrom.

From equation (8) the adsorption current is proportional to $m^{\frac{2}{3}} t^{-\frac{1}{3}}$, which is directly proportional to the height of the corrected mercury column. This is the diagnostic criterion for adsorption controlled limiting current. The height of the adsorption waves is usually pH independent and depends to various extents on temperature (39).

2.2. Reversible and Irreversible Electrode Processes

Those electrode processes in which the equilibrium between the reactants and the products are established at every instant during the life of a drop at any potential are said to be reversible, while those which involve slow, activation energy governed chemical and electrochemical steps are irreversible. Between the reversible and irreversible processes there is an intermediate class of reactions that are fast enough to approach equilibrium during the drop life but not quite so fast that they

appear to reach it within the experimental error of the measurements (37). It should be known that reversibility and irreversibility of an electrode process is artificial since a reaction may be reversible toward one technique but irreversible toward another in which the measurements are made more rapidly, so that the lag in the attainment of equilibrium becomes apparent.

For a simple electrochemical process written as



the variation of current with potential for reversible process is given by

$$E = E_1 + \frac{RT}{nF} \ln \frac{i_d - i}{i} \quad (10)$$

Equation (10) is the basis for the experimental criteria of polarographic reversibility and can be used to determine the number of electrons involved in a reversible electrochemical reaction. Those systems for which the half-wave potentials for the reduction of the oxidized form is identical with that for the oxidation of the reduced form and equal to the standard potential of the couple are described as polarographically reversible (40).

The half-wave potential characterizes the reacting material and is independent of the bulk concentration. Hence, it is characteristic for a given substance under a specific set of conditions and serves for qualitative analysis.

The most widely applicable and commonly used criteria is a plot of $\log \frac{i_d - i}{i}$ vs potential, called "log plot". For a polarographic reversibility the slope of the log plot should be $\frac{59.2}{n}$ mv at 25°C (37).

The effect of drop time on half-wave potential can also be used as a subsidiary criterion for reversibility or irreversibility of the electrochemical system. If the half-wave potential is nearly independent of the drop time the process is reversible (37), while it is an irreversible cathodic process if the half-wave potential becomes more positive as the drop time increases (37, 43, 44).

The dependence of $E_{\frac{1}{2}}$ on drop time is given by (37):

$$\frac{dE_{\frac{1}{2}}}{d(\log t)} = \frac{0.02957}{en} \quad (15)$$

for a totally irreversible wave at 25°C.

2.3. Differential Pulse Polarography

In differential pulse polarography a linearly increasing dc voltage ramp is applied to the system. Near the end of the drop life, a small amplitude pulse of approximately 50mv is superimposed onto the ramp. The current is sampled before and after the application of the pulse and the difference in the current is recorded, giving a peak shaped polarogram, the peak occurring near the half-wave potential, if the pulse amplitude is sufficiently small (41, 45-48).

Of the modern polarographic methods differential pulse polarography is the most useful and has a greater sensitivity. This is due to its ability to discriminate between faradaic and charging current (45).

If the reversible polarographic current-potential relationship written in equation (24) is differentiated with respect to potential and the Cottrell expression, equation (1), substitute for the limiting diffusion controlled current the expression,

$$\Delta i = \frac{n^2 F^2}{RT} AC(-\Delta E) \left(\frac{D}{\pi t} \right)^{\frac{1}{2}} \frac{P}{(1+p)^2} \quad (16)$$

can be obtained representing the change in current which results when a potential pulse ΔE is applied to the electrode. P in equation (30) is given by

$$P = \exp \left(E - E_{\frac{1}{2}} \right) \frac{nF}{RT} \quad (17)$$

Equation (16) is valid for a small amplitude, because the derivative is being approximated by a differential. For small pulse amplitudes the maximum peak current is given by

$$i_p = \frac{n^2 F^2}{4RT} AC (-\Delta E) \left(\frac{D}{\pi t} \right)^{\frac{1}{2}} \quad (18)$$

The peak half-width which is defined as the width of the peak at the point where the peak current is one-half its maximum height is given by

$$W_{\frac{1}{2}} = \frac{3.52RT}{nF} \quad (19)$$

and is equal to 90.4mv for $n=1$ at 25°C . Equation (19) is derived from equations (17) and (18) by solving for p at the conditions that $i = \frac{i_p}{2}$. From p two values of E are obtained and the difference between these values gives the peak-half width. Equation (19) is valid only for small pulse amplitude and is a criterion of differential pulse polarographic reversibility (41, 47).

Parry and Osteryoung (47) have shown that within experimental errors, the peak potential is related to the half-wave potential by the expression:

$$E_p = E_{\frac{1}{2}} - \frac{\Delta E}{2} \quad (20)$$

ΔE is negative for reduction.

Thus for a reduction process, the peak potential is shifted in anodic direction as the pulse amplitude increases. For infinitely small pulses the peak potential will occur at the polarographic half-wave potential

2.4 Polarographic Study of Amino Acids

Amino acids normally do not produce a reduction wave at the dropping mercury electrode unless a reducible group is present, for example iodine in thyroxine (49). However, there are amino acids which do produce polarographic waves.

Cysteine and cystine give polarographic wave at a dropping mercury electrode due to oxidation of the thiol group for cysteine and reduction of the disulfide group for cystine (50-52). The study of the electrochemical characteristics of both cysteine and cystine was carried out using different polarographic techniques such as dc, ac and differential pulse polarography. Waves up to three were obtained depending on the concentration of the electroactive species and pH.

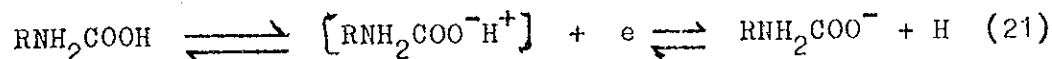
Other amino acids yield cathodic waves attributed to the reduction of hydrogen in aqueous and aprotic media (53-55).

According to (53) polarographic reduction of amino acids such as asparagine, hydroxyproline, isoleucine, methionine, phenylalanine, proline, threonine and tryptophan give single waves involving one electron reduction of the proton of the carboxyl group in dimethyl sulfoxide. The amino acids, dihydroxyphenylalanine, glutamic acid, aspartic acid, glutamine, and cysteine hydrochloride give multiple waves.

Aqueous solution of amino acids have also been studied (55). Based on the pH dependence of polarographic currents for the amino acids, a polarographic wave was observed due to the free H^+ at pH's from 2 to 4. A sub wave was also obtained over the shoulder of the main wave. This sub wave is due to the reduction of the protonated amino acid $R(NH_3^+)CO_2H$ to give $R(NH_3^+)CO_2^-$ and H (55).

Polarographic behaviour of the amino acids in dioxane and water mixture was also studied (54). The reduction mechanism of

the amino acids in 80% dioxane solution is based on the direct reduction of H^+ from the nondissociated condition:



It is not possible to use the cathodic wave obtained due to the reduction of hydrogen for polarographic determination of this amino acids. Therefore, the determination of the amino acids is carried indirectly. This is not the case for cysteine and cystine which can be determined directly by polarography.

Indirect polarographic determination of the amino acids can be done by complexation procedures.

2.4.1. Complexation of Amino Acids with Metal Ions

Polarography have played an important part in investigation of the composition and stability of complexes, rates and mechanism of complex reaction, and relation between structure and redox behaviour of such compounds.

Complexation have been employed in polarographic studies (a) if the molecule is polarographically inactive unless complexed with metal ions (b) if complexation results in a well defined wave, than before complexing (c) if it remove interfering waves (56).

Stable complex formation of amino acids with metal ions has long been known. The amino acids have both nitrogen and oxygen sites available for coordination and they form stable complexes. The copper salt of leucine was first prepared in 1854 by Gossmann and understanding of this and similar compounds was subsequently developed by Hofmeitster in 1877 and by Werner in 1891 (57).

Upto the year 1949, very few qualitative investigations had been made. The only bivalent amino acids studied were glycine

complexed with copper, nickel, cobalt, zinc, cadmium, mercury and magnesium; and alanine complexed with copper. In the year 1950 quantitative investigations of glycine, alanine, valine and leucine with copper, zinc, cobalt and manganese were done by Albert (58).

The main reason for such neglect in quantitative investigation of complexation of amino acid with metal ion was the lack of understanding of the nature of the equilibria concerning such complex formation. This problem was solved in the year 1941. It was concluded that the equilibrium between a complex forming agent and an ion is usually thermodynamically reversible and these equilibria are correctly represented by mass-action equation (58).

The complexes of rare earth's with amino acids have been studied extensively (59). Metals of group II complexed to glycine, its derivatives and other amino acids with only two ionizing groups were also studied (60).

The tendency of amino acids to chelate with the alkali metals is relatively slight and for the most part the reaction with such ions as Na^+ , K^+ , Ca^{2+} and Ba^{2+} results in production of simple salts (57).

Copper (II) complexes of amino acids have been studied in detail and although some derivatives are considered to coordinate solely through nitrogen or oxygen the majority contain Cu - O and Cu - N bonds (61).

Silver reacts to form complexes with all amino acids, and mercuric salts complex with most α -amino acids (57).

The polarographic behaviour of Ni(II) and Co(II) have been studied by several workers, in different media and also with different complexing agents (62).

The majority of non-transition metals have greater affinity for oxygen than for nitrogen while the reverse is true for transition metal complexes. The factors responsible for this behaviour are the electronegativity of the ligand atom, repulsion between metal d-electrons and ligand atom lone pair electrons, and the crystal field splitting brought by the ligands (63).

Extensive potentiometric studies of the complexes formed between metal ions and different amino acids were made by Williams et al. (64-69) in series of papers entitled "Thermodynamic Considerations in Co-ordination". These potentiometric studies were used to determine stability constants and thermodynamic variables of complex formed.

Formation constants of complexes of metal ions such as Mn(II), Fe(II), Co(II), Ni(II), Cu(II), and Zn(II) with glutamate and serinate (64), Pb(II) and Cd(II) (65, 66) with asparaginate, aspartate, cysteinate, glutamate, histidinate, phenylalanate, serinate, and tryptophanate, and Cd(II), Pb(II), and Zn(II) with ethylenediaminetetra-acetic acid, 1,2 - di(2-amino-ethoxy) ethanetetra-acetic acid, glutathione, cysteine, and D-Penicillamine (67) have been determined.

Estimation of Gibbs free-energy changes, enthalpies, and entropies of formation of complexes of glycinate, glycyglycinate, glycyglycyglycinate, cysteinate and glutathionate with Pb(II) (68), Mn(II), Co(II), Fe(II), Ni(II), and Zn(II) with asparaginate (69), and Ni(II) and Cu(II) with glutamate (69) were made. The values of these thermodynamic variables were used to suggest structures for the various complexes present in solution (68).

2.4.2. Determination of Stability Constants by Polarography

When a metal ion in solution undergoes complexation with ligands changes occur in the half wave potential and in the limiting current. Usually the half wave potential becomes more negative and the limiting current becomes smaller.

The polarographic study of stabilities of metal complexes is in general based on the shifts of half wave potentials and on the changes of limiting currents due to complexation. The first expressions for the half wave potentials of complex ion reduction were derived on thermodynamic bases by Lingane (70). The expressions were obtained when the reduction of the metal ion complex takes place reversibly at the dropping mercury electrode and is applicable to systems involving essentially one very stable complex. The mathematical analysis of the shift of half wave potential with ligand concentration for reversible system, which makes possible the identification of the successive complex ions formed and the evaluation of their stability constants, was derived by Deford and Hume (71).

The treatment of Lingane is restricted to the case where the complexing agent is present in excess. Methods of calculating stability constants have also been developed when a metal is polarographically reduced in the absence of excess complexing agent (72-74).

The foregoing methods are applicable only when the electrode processes of both simple and complexed species occur reversibly. Therefore, utilization of irreversible waves for the determination of stability constant requires a different approach than that of the reversible one, since the relationships are substantially kinetic. A method has been proposed by which stability constants of complexes that are irreversibly reduced at the dropping mercury electrode, while the uncomplexed metal undergoes reversible reduction can be determined (75). The method was also extended to evaluate stability constants of mixed complexes (76). Another method of determining stability constants has been worked out by Biernat et al. (77), when both simple and complexed species are reduced irreversibly. This method was employed for determination of stability constant of iron (II) oxalato complex (78).

The determination of stability constant according to Biernat et al. (77) will be the basis for the present work, in view of the finding that the simple and complexed species are reduced irreversibly. Therefore, the derivation of the equations for this particular case will be reproduced below.

To derive the relationship between the rate of an electrode reduction process and the complexation equilibrium the following assumption may be made.

- (a) Only mononuclear complexes are formed in the solution.
- (b) Complexation reactions are sufficiently fast.
- (c) In an irreversible complex reduction each form may be independently subjected to electrode reaction.
- (d) The totally irreversible polarographic wave equation,

$$\frac{\bar{i}_{irr}}{\bar{i}_d - \bar{i}_{irr}} = 0.886t^{\frac{1}{2}}D^{-\frac{1}{2}} K^0 \exp \left[\frac{-\alpha n F}{RT} (E - E^0) \right] \quad (22)$$

is taken into account.

- (e) In solution there are no specific adsorptive species in the investigated potential region.

The rate of the electrode reduction is then the sum of the reduction rates for each particular form,

$$r_t = r_0 + r_1 + r_2 + \dots + r_N \quad (23)$$

Equation (23) can be expressed for first order heterogenous reaction as a product of rate coefficient k and concentration c of a particular complexes on the electrode surface,

$$r_t = k r_t = k_0 c_0 + k_1 c_1 + k_2 c_2 + \dots + k_N c_N, \quad (24)$$

in which,

$$c_t = c_0 + c_1 + c_2 + \dots + c_N \quad (25)$$

and

$$\bar{k} = \frac{k_0 c_0 + k_1 c_1 + k_2 c_2 + \dots + k_N c_N}{c_t} \quad (26)$$

The value of \bar{k} for a given solution is constant. This is because the excess ligand concentration causes a constancy of concentration ratios of particular complexes, disregarding the change of the electroactive concentration on the electrode surface.

The complexation equilibria is represented by



where j varies from zero to N and the ionic charges are omitted for simplification.

The over-all equilibrium constant is

$$\beta_j = \frac{[ML_j]}{[M][L]^j} \quad (28)$$

and hence the complex concentration is

$$[ML_j] = \beta_j [M] [L]^j = c_j \quad (29)$$

substituting equation (29) into equation (26); one obtains

$$\bar{k} = \frac{k_0 + k_1 \beta_1 [L] + k_2 \beta_2 [L]^2 + \dots + k_N \beta_N [L]^N}{1 + \beta_1 [L] + \beta_2 [L]^2 + \dots + \beta_N [L]^N} \quad (30)$$

To relate the average rate factor to potential, for irreversible process equation (31) can be used.

$$k_j = k_j^0 \exp \left[\frac{-\alpha_j n F}{RT} (E - E^0) \right] \quad (31)$$

Substituting equation (31) into equation (30) gives

$$\bar{k} = \frac{\sum_0^N k_j^0 \beta_j [L]^j \exp \left[\frac{-\alpha_j n F}{RT} (E - E^0) \right]}{\sum_0^N \beta_j [L]^j} \quad (32)$$

Introducing equation (32) into equation (22), an over-all irreversible wave equation for complex reduction is obtained, given as

$$\frac{\bar{i}_{irr}}{\bar{i}_d - \bar{i}_{irr}} = 0.886 \frac{t_1^{\frac{1}{2}} \sum_0^N k_j^0 \beta_j [L]^j \exp \left[\frac{-\alpha_j n F}{RT} (E - E^0) \right]}{\bar{D}_c^{\frac{1}{2}} \sum_0^N \beta_j [L]^j} \quad (33)$$

where the average complex diffusion coefficient \bar{D}_c is given by

$$\bar{D}_c = \frac{\sum_0^N D_j \beta_j [L]^j}{\sum_0^N \beta_j [L]^j} \quad (34)$$

Equation (33) is not suitable for calculation of stability constants from experimental data. Therefore the partial derivatives of equation (33) can be used.

Taking the partial derivative with respect to E of the log of equation (33) at constant ligand concentration gives,

$$\frac{\partial}{\partial E} \ln \frac{\bar{i}_{irr}}{\bar{i}_d - \bar{i}_{irr}} = \frac{-nF}{RT} \quad (35)$$

where $\bar{\alpha}$ is the average transfer coefficient given by

$$\bar{\alpha} = \frac{\sum_0^N \alpha_j \beta_j k_j^0 [L]^j \exp \left[\frac{-\alpha_j nF}{RT} (E - E^0) \right]}{\sum_0^N \beta_j k_j^0 [L]^j \exp \left[\frac{-\alpha_j nF}{RT} (E - E^0) \right]} \quad (36)$$

Equation (35) enables the evaluation of the numerical value of $\bar{\alpha}$ and the number of electrons participating in the elementary event of electrode reduction. The value of $\bar{\alpha}$ can be obtained if the number of electrons is known. The latter is frequently equal to the valency charge. Values of the average transfer coefficient suggest the electrode mechanism. If the value of α_j for two different waves are the same then it suggests that the reduction mechanism is the same and enables one to expect that the same molecule undergoes reduction which simplifies the calculation. The variability of value obtained from equation (35) for different waves would indicate that the two electrode reactions with different α_j take place.

Equation (35) gives information as to which complex takes part in the direct reduction. If the direct reduction of only one species is involved the irreversible reduction proceeds through the free central ion or through one of the complexes.

Since the system in which this study was done involves irreversible reduction proceeding through the free central ion, only this system will be dealt here.

The main proof that the reduction proceeds through the free ion is the constant value of equation (35), not only in a given wave but also in all waves, disregarding the ligand concentration.

If the reduction proceeds through the free ion,

equation (33) can be written as

$$\frac{\bar{i}_{irr}}{\bar{i}_d - \bar{i}_{irr}} = 0.886 t_1^{\frac{1}{2}} D_c^{-\frac{1}{2}} k_0^0 \frac{\exp\left[-\frac{\alpha n F}{RT} (E - E^0)\right]}{\sum_0^N \beta_j [L]^j} \quad (37)$$

The half-wave potential of complex reduction is

$$(E_{\frac{1}{2}})_c = E^0 - \frac{RT}{\alpha n F} \ln (0.886 t_1^{\frac{1}{2}} D_c^{-\frac{1}{2}} k_0^0) - \frac{RT}{\alpha n F} \ln \sum_0^N \beta_j [L]^j \quad (38)$$

Subtracting equation (38) from that giving the half-wave potential for an uncoordinated cation,

$$(E_{\frac{1}{2}})_s = E^0 + \frac{RT}{\alpha n F} \ln (0.886 t_1^{\frac{1}{2}} D_c^{-\frac{1}{2}} k_0^0), \quad (39)$$

One obtains the expression which relates the difference in half wave potentials to the complexation equilibrium constants.

$$\Delta E_{\frac{1}{2}} = \frac{RT}{\alpha n F} \ln \sum_0^N \beta_j [L]^j - \frac{RT}{\alpha n F} \ln (D_s^{\frac{1}{2}} / D_c^{\frac{1}{2}}) \quad (40)$$

The second term in the right hand side of equation (40) may be written as

$$\frac{RT}{\alpha n F} \ln \left[(\bar{i}_d)_s / (\bar{i}_d)_c \right] \quad (41)$$

Using equation (41), equation (40) can be written as

$$\Delta E_{\frac{1}{2}} = \frac{RT}{\alpha n F} \ln \sum_0^N \beta_j [L]^j - \frac{RT}{\alpha n F} \ln \left[(\bar{i}_d)_s / (\bar{i}_d)_c \right] \quad (42)$$

Since the reduction in this case proceeds through the free ion, equation (42) resembles that employed for reversible system. The difference is in the presence of the transfer coefficient. Hence the calculations are very similar (70).

3. EXPERIMENTAL

All polarographic experiments were performed using a Metrohm polarecord E 506 coupled with a Metrohm polarographic stand E 505 recorder.

A three electrode cell was used, which consisted of a dropping mercury electrode, a platinum auxiliary electrode and a silver /silver chloride/ saturated KCl reference electrode. The mercury for the indicator electrode was purified thoroughly using procedure given in reference 79. Doubly distilled water was used as a solvent.

The working electrode had a controlled drop time of one second, except during the examination of drop time dependence on peak potential and peak current in differential pulse polarographic technique. It was operated at a scan rate of 1mv or 4mv per second depending on the conditions required. A pulse amplitude of 50mv was used in experiments done by differential pulse polarography. A mercury reservoir height of 54.5cm was maintained in all the experiments except in studies of mercury reservoir height dependence on the limiting current using the normal dc polarographic technique.

pH measurements were taken using a Beckman Chem-Mate pH meter.

All solutions were deaerated prior to each study with pure grade nitrogen for about 10 minutes and were protected with an atmosphere of water-saturated nitrogen while recording the polarograms.

All measurements were performed at room temperature, i.e., $(20 \pm 2)^{\circ}\text{C}$.

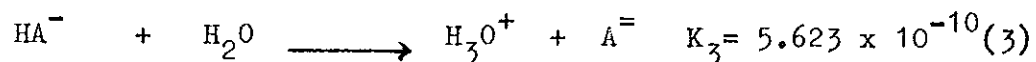
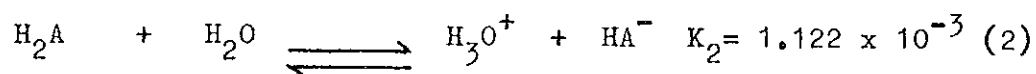
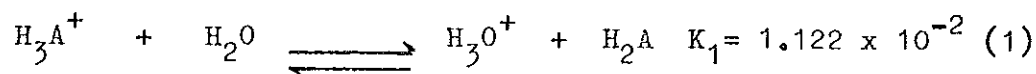
The amino acid, β -N-Oxalyl-L- α , β -Diaminopropionic acid, that was used in the study was a synthetic sample obtained by courtesy of Dr. Berhanu Abegaz of the chemistry department, AAU.

The chemicals used were, KCl , KNO_3 , NaNO_3 , NH_4Cl , $\text{Na}_2\text{B}_4\text{O}_7$, $\text{Na}_3\text{H}_5\text{C}_6\text{O}_7$, KH_2PO_4 , Na_2HPO_4 , HCl , CH_3COOH , NaOH , KOH , NiSO_4 , ZnSO_4 , and CuSO_4 , and were of Analar grade (BDH) with the exception of $\text{KHC}_8\text{H}_4\text{O}_4$, NaCl , NH_3 solution, $\text{Cd}(\text{NO}_3)_2$, PbCl_2 , $\text{Co}(\text{NO}_3)_2$ and $(\text{CH}_2\text{COOH})_2$ which were of normal laboratory reagent grade.

4. RESULTS AND DISCUSSIONS

4.1. Composition of Aqueous Solution of ODAP as a Function of pH

According to Rao et al.(19) the amino acid, β -N-Oxalyl-L- α , β -Diaminopropionic acid, has apparent pK values of 1.95, 2.95, and 9.25 corresponding to the two carboxyl and one amino functions, respectively. The ODAP-water equilibria can be represented by:



Making use of these apparent pK values the composition of aqueous solution of ODAP as a function of pH of the solution can be plotted, (see(80) for a general discussion of the method employed).

Let α_3 be defined as the fraction of the anion containing species existing at a particular pH.

From equation (1),

$$K_1 = \frac{[\text{H}_3\text{O}^+][\text{H}_2\text{A}]}{[\text{H}_3\text{A}^+]} \quad (4)$$

which gives, after rearranging,

$$[\text{H}_3\text{A}^+] = \frac{[\text{H}_3\text{O}^+][\text{H}_2\text{A}]}{K_1} \quad (5)$$

By definition let,

$$\alpha'_0 = \frac{[H_3A^+]}{C_T}, \quad \alpha'_1 = \frac{[H_2A]}{C_T}, \quad \alpha'_2 = \frac{[HA^-]}{C_T}, \quad \alpha'_3 = \frac{[A^-]}{C_T} \quad (13)$$

The sum of the α'_i value for a system must be unity; that is,

$$\alpha'_0 + \alpha'_1 + \alpha'_2 + \alpha'_3 = 1 \quad (14)$$

Substituting equation (12) into equation (13) gives,

$$\alpha'_0 = \frac{[H_3O^+]^3}{Y} \quad (15)$$

$$\alpha'_1 = \frac{K_1 [H_3O^+]^2}{Y} \quad (16)$$

$$\alpha'_2 = \frac{K_1 K_2 [H_3O^+]}{Y} \quad (17)$$

$$\alpha'_3 = \frac{K_1 K_2 K_3}{Y} \quad (18)$$

where

$$Y = K_1 [H_3O^+]^2 + [H_3O^+]^3 + K_1 K_2 K_3 + K_1 K_2 [H_3O^+] \quad (19)$$

A calculation of α'_i values at any desired pH is possible and can be used to plot composition of aqueous solution of ODAP as a function of pH. This is shown in Fig. 1. Fig. 1 can serve to indicate which species exists at a particular pH.

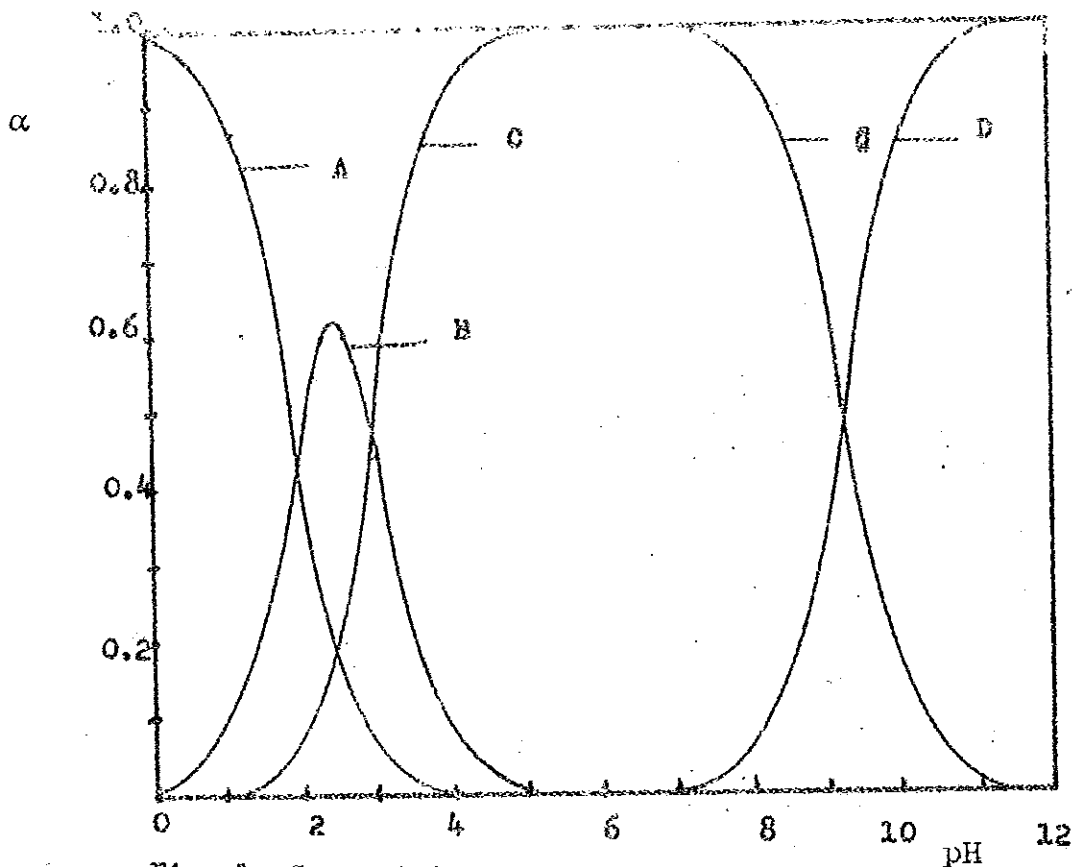
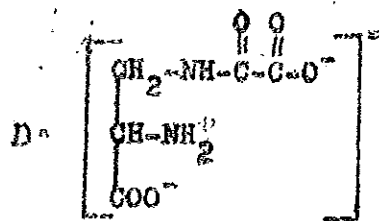
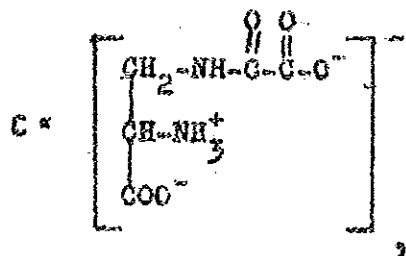
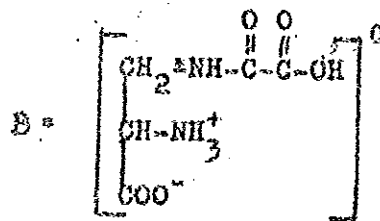
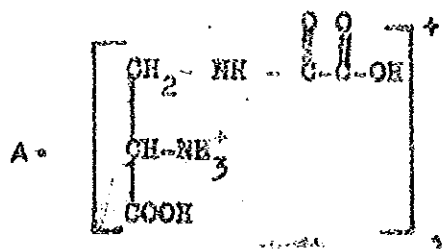


Fig. 1. Composition of aqueous solution of ODAP as a function of pH.



4.2. Investigation of Aqueous Solution of ODAP Using Polarographic Techniques

To study a compound polarographically, it should be electroactive i.e., it should either be oxidized or reduced at certain potential region. In view of this, the first work that was done was to investigate aqueous solution of ODAP in different supporting electrolytes such as KNO_3 , KCl , NaCl , and NaNO_3 . Aqueous solutions of the amino acid, ODAP, showed a single polarographic wave in these electrolytes.

From the differential pulse polarographic investigation (Fig.2), the aqueous solution of 0.1mM ODAP in 0.1MKCl had a peak potential of -1.508v and a peak half-width of 132mv. A peak half-width greater than the expected 90.4mv (for $n=1$, $T=298\text{k}$) indicates irreversibility. The half-wave potential obtained from the normal dc polarogram (Fig.3) was -1.518v for 0.1mM ODAP in 0.1MKCl.

The effect of concentration upon the differential pulse polarographic peak current was studied in 0.1M NaCl, at concentrations of ODAP between 0.1mM and 1mM. The peak current increases linearly with concentration, as shown in Fig. 4.

In studying the reaction of organic compounds it is desirable to work with buffer solution (49). For the investigation of ODAP, solutions of borax, $\text{Na}_2\text{B}_4\text{O}_7$ (pH = 6.8, 7.9, 9.1), citrate, $\text{HCl-Na}_3\text{H}_5\text{C}_6\text{O}_7$, (pH = 4.2), phosphate, $\text{KH}_2\text{PO}_4\text{-Na}_2\text{HPO}_4$, (pH = 5.0), acetate, $\text{CH}_3\text{COOH-NaOH}$ (pH = 4.8), phthalate, $\text{NaOH-KHC}_8\text{H}_8\text{O}_4$, (pH = 5.0), ammonia, $\text{NH}_3\text{-NH}_4\text{Cl}$, (pH = 7.9), and succinic acid-borax (pH = 3.4, 4.5) were prepared. In borax and ammonia buffer the polarographic wave for ODAP could not be seen. In all the other buffer systems, the reduction wave in the presence of ODAP coincided with that of the supporting electrolyte and these systems could not be used.

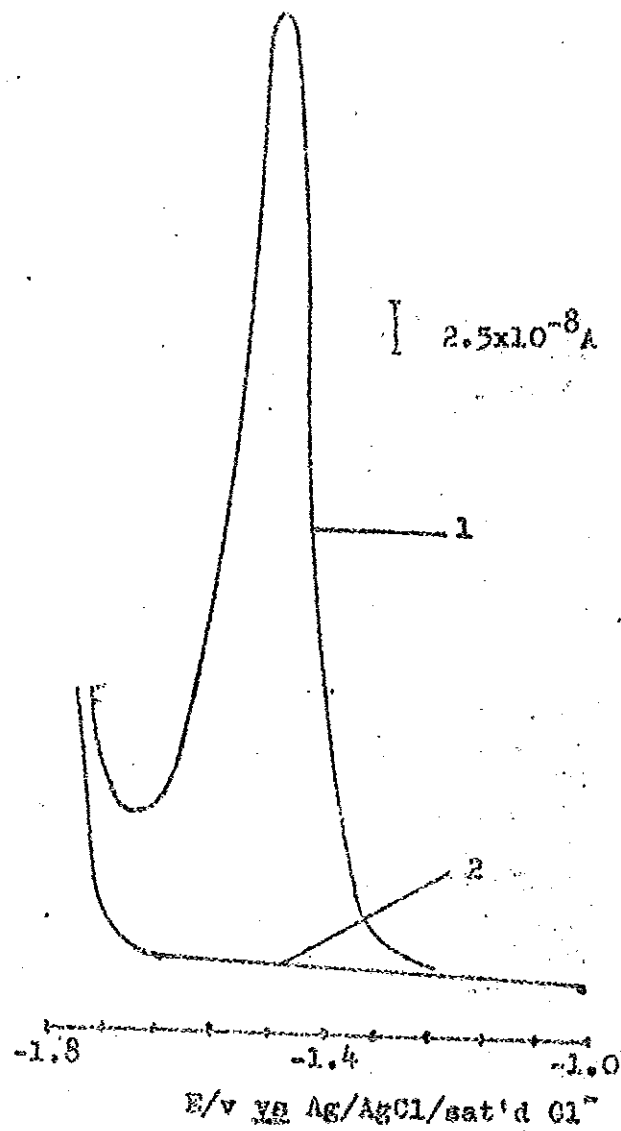
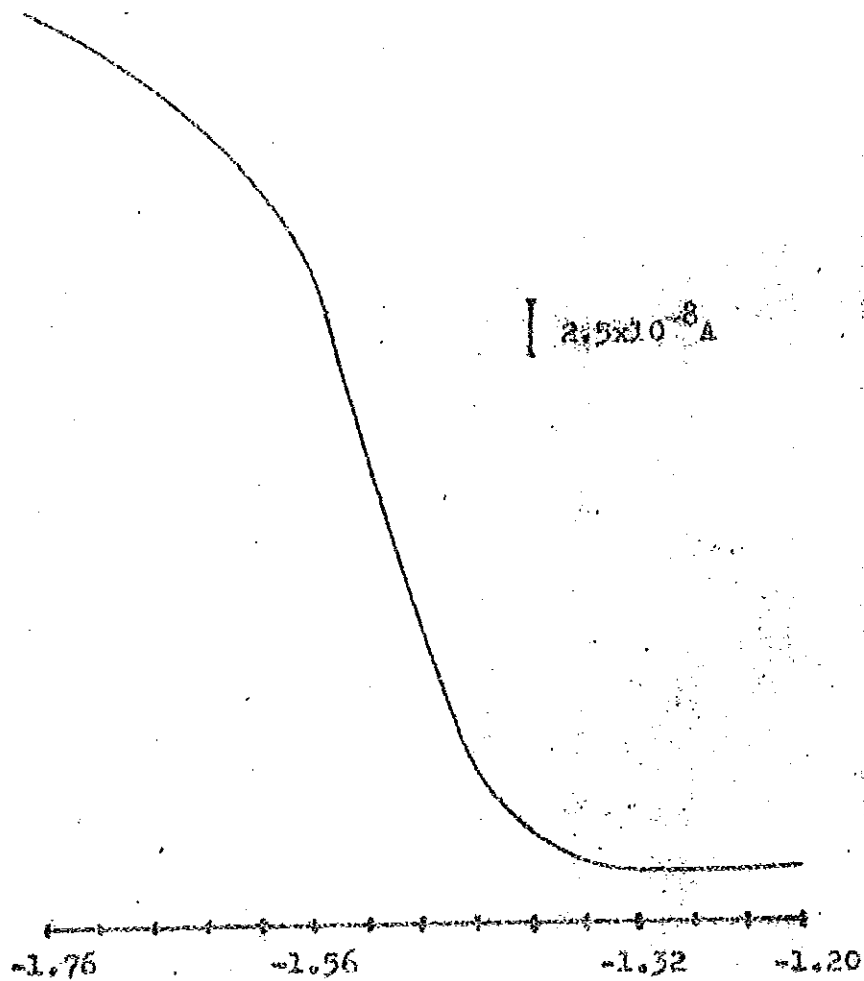


Fig. 2. Differential pulse polarogram of
1. 0.1mM ODAP in 0.1M KCl
2. 0.1M KCl



E/V vs Ag/AgCl/sat'd Cl⁻

Fig. 3. dc polarogram of 0.1mM ODAP in 0.1M KCl.

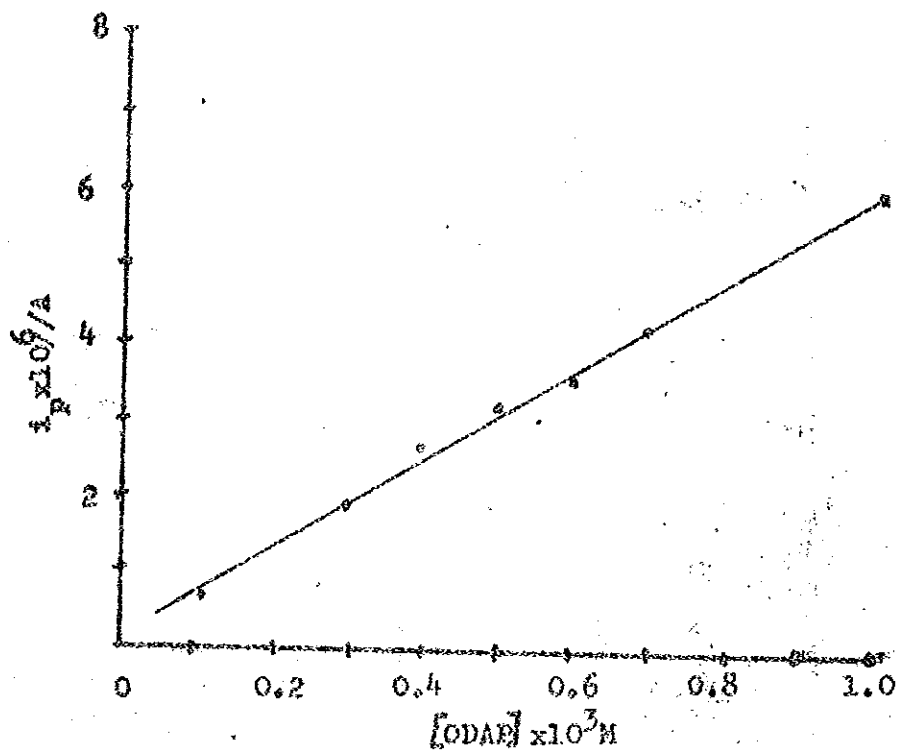


Fig. 4, Plot of peak current vs ODAE concentration
in 0.1M NaCl,

Diffusion currents are a linear function of the square root of the height of the mercury column, while kinetic currents are independent. Catalytic current can be independent of the height of the mercury column like a kinetic current, or can be proportional to its square root like a diffusion current (see chapter 2, sections, 2.1.1, 2.1.2, and 2.1.3). In order to characterize the nature of the polarographic wave normal dc polarograms were recorded for 0.1mM ODAP in 0.1MKCl by varying the height of the mercury column. The variation of the limiting current as a function of the square root of the corrected mercury column height was linear (Fig. 5). This linear relationship observed for the amino acid indicates the reduction process to be either diffusion or catalytic controlled.

For polarographic reversibility the slope of the log plot should be $59/n$ mv at 25°C (37). To investigate the reversibility of the wave observed in the presence of the amino acid, normal dc polarogram of 0.1mM ODAP in 0.1MKCl was used to plot potential vs $\log \frac{i_d - i}{i}$. This is shown in Fig. 6. The slope was found to be 94mv decade^{-1} . Since the slope is different from that expected for reversible reduction process the ODAP system seems to be irreversible.

The half-wave potential of a reversible wave is nearly independent of the drop time, while that of an irreversible cathodic wave becomes more positive as the drop time increases (37). To see the peak potential variation with drop time differential pulse polarogram was recorded with drop time between 0.4 and 1.4sec (Fig. 7). The peak potential shifts towards more positive values with an increase in drop time. All the above observations, support the fact that the reduction process is irreversible.

To see the effect of pH of the reduction wave of the amino acid, polarogram was recorded in NaOH. No reduction wave was seen. Further attempt was made to find the pH range where the reduction peak of the amino acid is affected. The pH of 0.1MKCl

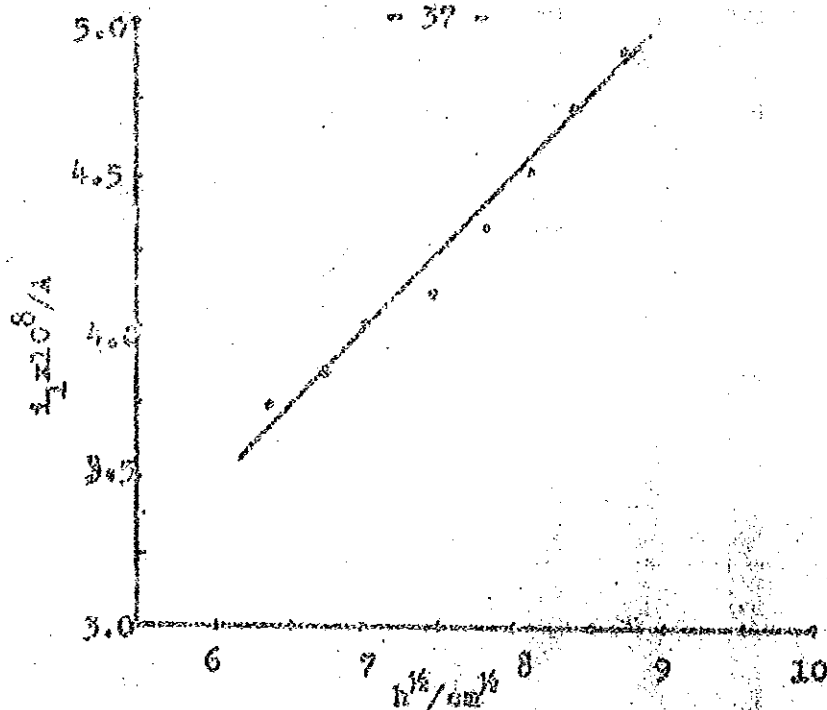


Fig. 5. Plot of $\log i_p$ vs $h^{1/2}$ for 0.1M ODAP in 0.1M KCl.

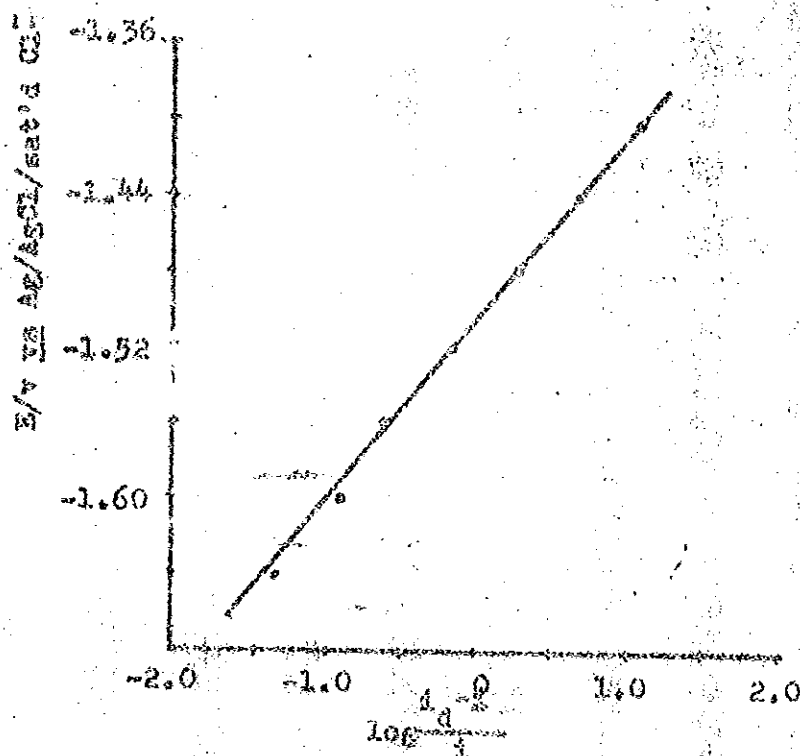


Fig. 6. Plot of potential vs $\log \frac{i}{i_0}^{1-1}$ for 0.1M ODAP in 0.1M

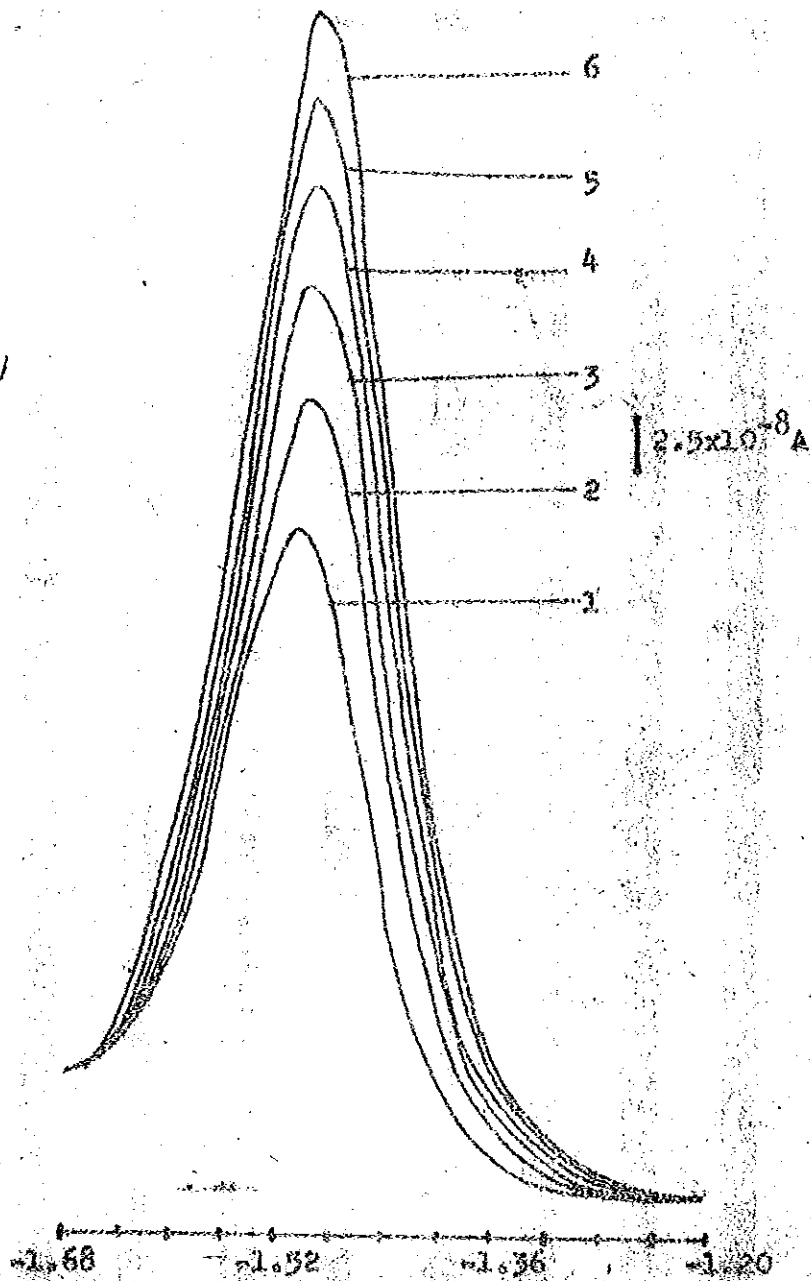


Fig. 7. Polarograms of 0.1mM ODAP in 0.1M KCl at various drop times; 1, 0.4; 2, 0.6; 3, 0.8; 4, 1.0; 5, 1.2; 6, 1.4; sec.

was 5.7; when the amino acid is added, however, a decrease in pH from 5.7 to 4.4 was observed. The pH was then adjusted to 3.3, 3.8, 5.7, 7, and 8 by adding HCl or KOH. The reduction peak of the amino acid could not be observed at pH of 5.7, 7 and 8, while it could be seen at pH of 3.3, and 3.8. It was therefore, concluded that the reduction wave appears only in the lower pH regions. In basic system the reduction peak disappears.

A polarogram was recorded after adding HCl to 0.1mM ODAP in 0.1MKCl (pH=4.4) system to lower the pH to 4.0. The polarogram indicated an increase in peak current while the peak potential remained nearly constant compared to the polarogram of 0.1mM ODAP in 0.1MKCl (pH=4.4). This implies that the addition of HCl has influence only on the peak current. To study this effect further, the polarogram of 0.1mM HCl in 0.1MKCl (pH=3.3) without adding ODAP was recorded. A reduction wave was observed at a peak potential of -1.521v. The pH of the system was then increased to the pH of 0.1MKCl (pH=5.7) by adding KOH. The polarogram was recorded and no peak was observed. The results of these comparative studies are given in Table 1.

Table 1. Comparison between (a) 0.1mM ODAP in 0.1MKCl and (b) 0.1mM HCl in 0.1MKCl

System	-Ep(v)	$W_{\frac{1}{2}}$ (mv)	slope of log plot(mv)	i_p vs $h^{\frac{1}{2}}$
HCl/KCl	1.521	136	96	linear
ODAP/KCl	1.513	132	94	linear

The reduction wave obtained for 0.1mM HCl in 0.1MKCl was due to hydrogen discharge (36). Similarly, the reduction wave of 0.1mM ODAP in 0.1MKCl seems to be due to hydrogen. Polarograms of (a) 0.1mM ODAP in 0.1MKCl, (b) 0.1mM HCl in 0.1MKCl, and (c) 0.1mM ODAP + 0.1mM HCl in 0.1MKCl were recorded (Fig.8) and the data obtained from the polarograms are summarized in Table 2.

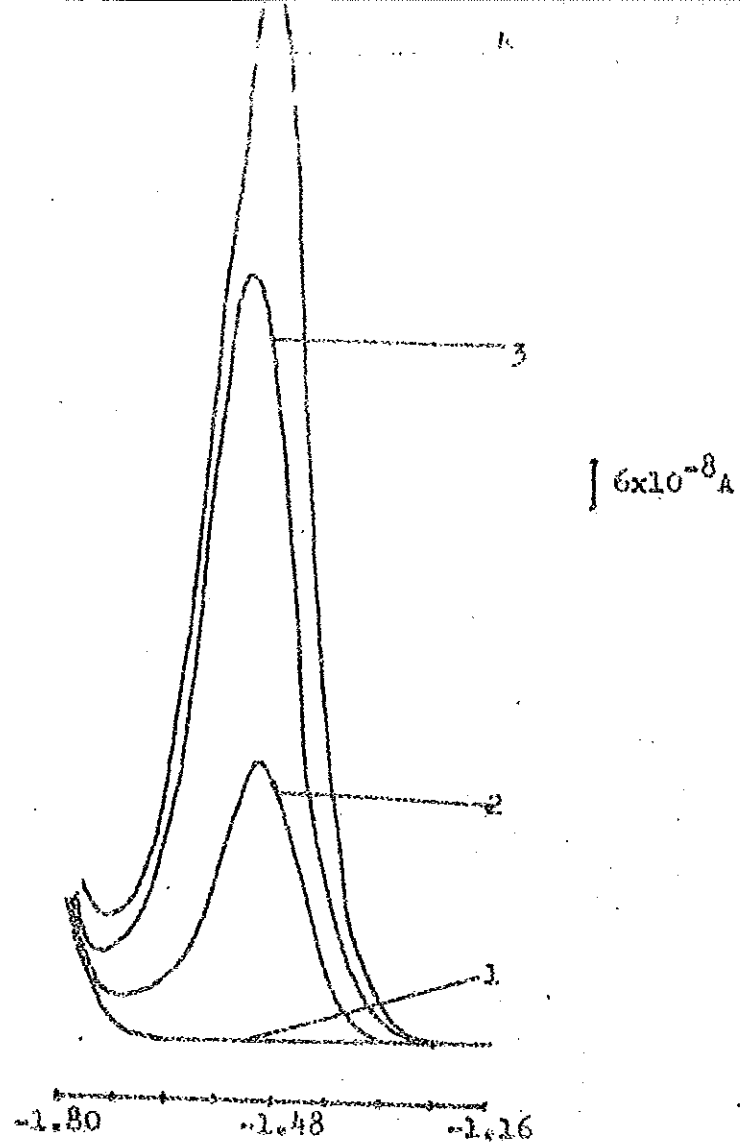


Fig. 8. Polarograms of ; 1, 0.1M KCl; 2, 0.1mM ODAP/0.1M KCl;
 3; 0.1mM HCl/0.1M KCl; 4, 0.1mM ODAP/0.1mM HCl/0.1M KCl.

Table 2 Values of peak potentials and peak currents for

(a) ODAP in KCl, (b) HCl in KCl and (c) ODAP+HCl in KCl

System	$-E_p$ (V)	$i_p \times 10^{-7} A$
0.1MKCl	-	-
0.1mMODAP/0.1MKCl	1.513	3.12
0.1mMHCl/0.1MKCl	1.526	8.70
0.1mM ODAP/0.1mMHCl/0.1MKCl	1.516	12.00

As can be seen from Table 2, the peak potential is nearly the same in all cases, while the peak current of 0.1mMHCl in 0.1MKCl increased by $3.3 \times 10^{-7} A$ when 0.1mM ODAP was added. This difference is approximately the same as the peak current of 0.1mM ODAP alone in 0.1MKCl. This indicates that the reduction peak of ODAP in KCl is due to hydrogen. This is in agreement with the general finding (55) that the polarographic wave of aqueous solution of amino acids is due to the reduction of hydrogen.

The variation of pH and peak current of differential pulse polarogram of ODAP in KCl with time were also studied. The reduction peak of the amino acid disappeared after seven hours (Fig.9) while the pH increased from 4.2 at the beginning to 5.7 when the reduction peak disappeared (see Table 3). The fact that fresh solution of ODAP in a media of pH=5.7 also showed no polarographic wave is to be noted from earlier data.

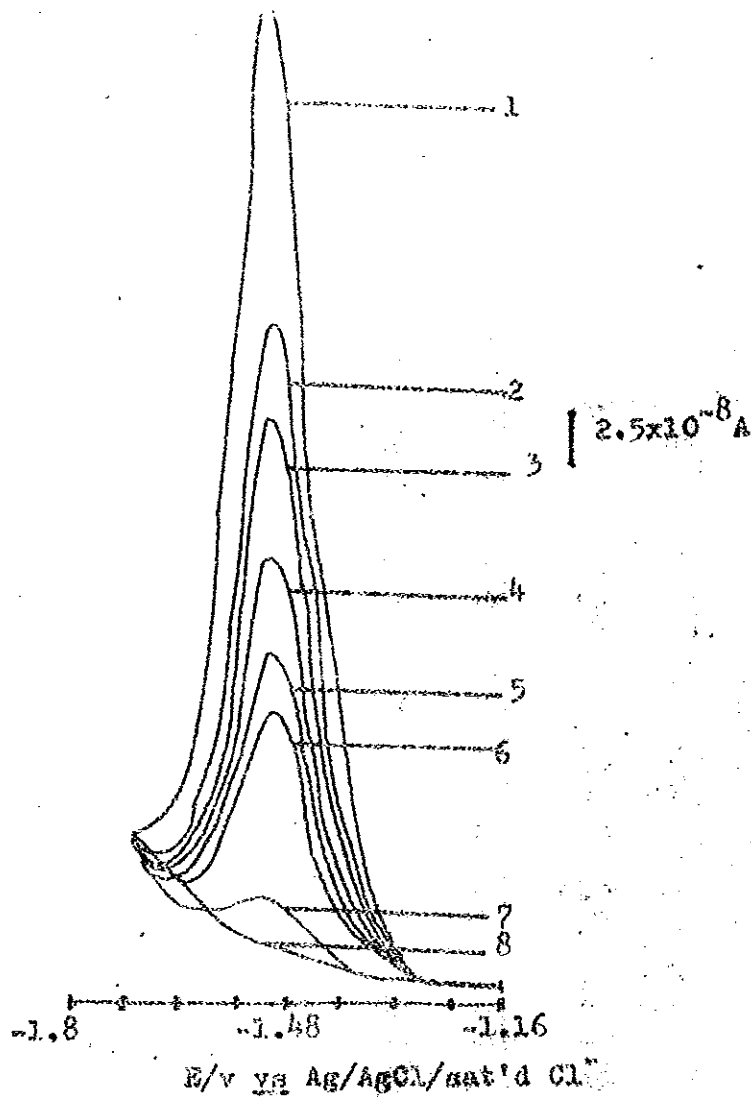
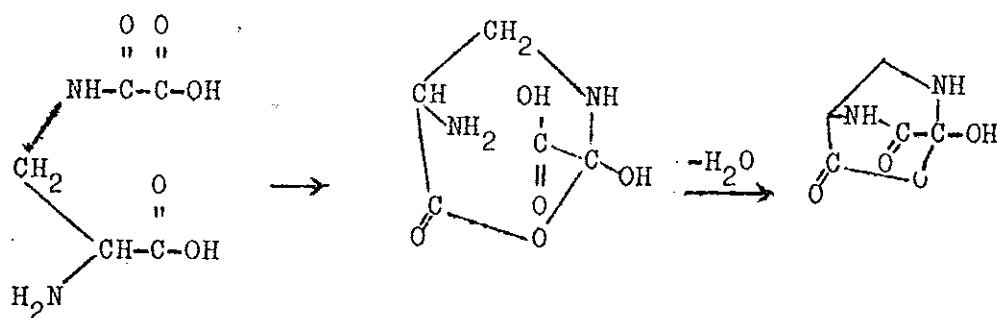


Fig. 9. Polarograms of 0.1mM ODAP in 0.1M KCl, at various times: 1, 0; 2, 1; 3, 2; 4, 3; 5, 4; 6, 5; 7, 6; 8, 7; hours.

Table 3. Variation of pH and Peak Current with time for 0.1mM ODAP in 0.1MKCl

Time(hours)	pH	$i_p \times 10^8 A$
0	4.2	44.5
1	4.3	29.5
2	4.4	25.0
3	4.5	18.3
4	4.7	13.4
5	4.9	11.0
6	5.4	2.3
7	5.7	0.0

As shown in Table 3 the reduction peak disappeared on standing. This is not an unusual phenomena; for example, the behaviour of isatin, hydroxy acids and lactones (49) at the dropping mercury electrode are dependent upon the period of time the solution is allowed to stand before the experiment was carried out. The disappearance of the reduction peak of aqueous solution of ODAP on standing might be due to the formation of the following lactone, although no confirmatory tests have yet been made.



The peak potential (at $E_p \sim -1.520v$) of ODAP-KCl system showed no significant shift in pH range of 2.6 to 3.9 as shown in Fig. 10.

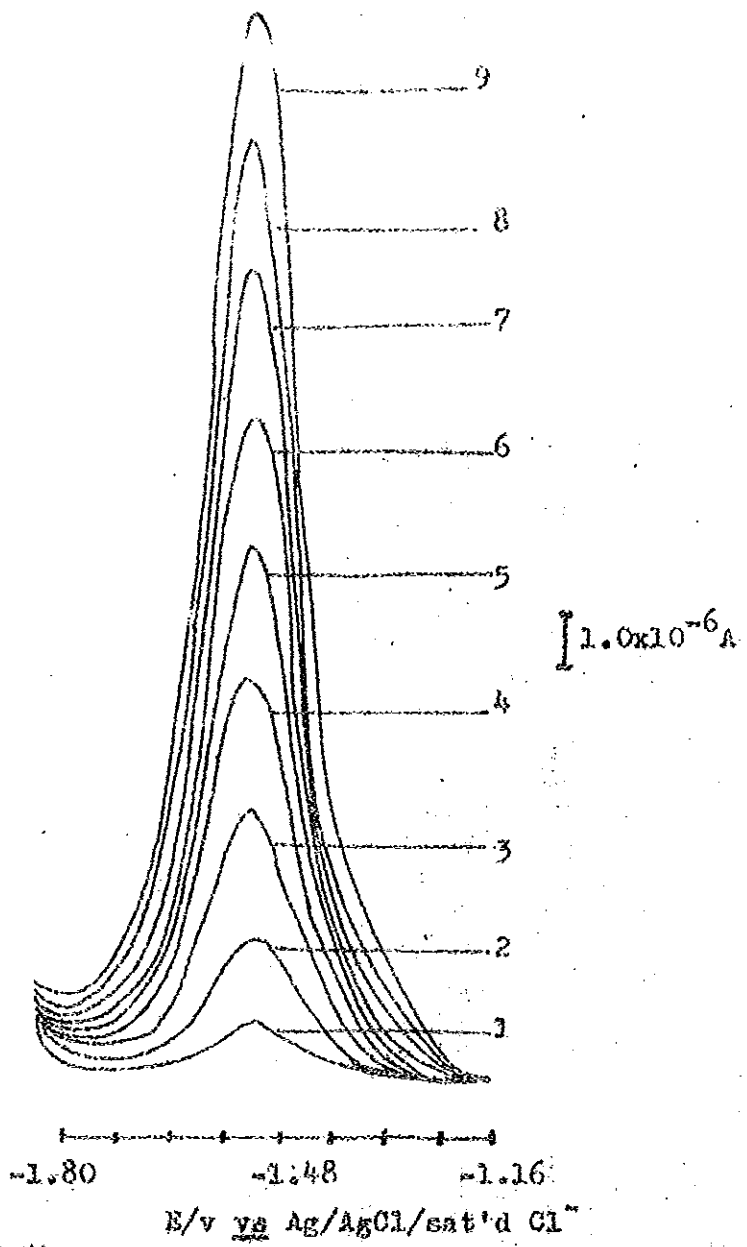


Fig. 10. Polarograms of ODAP in KCl at various pH: 1, 3.9; 2, 3.5; 3, 3.2; 4, 3.0; 5, 2.9; 6, 2.85; 7, 2.75; 8, 2.7; 9, 2.6.

The polarographic behaviour of ODAP in 0.1M HCl was also investigated. Addition of 0.1mM ODAP to the base electrolyte (0.1M HCl) gave rise to a peak at -0.960v (Fig. 11) which was, however, dependent on the pH of the solution. Further investigations at pH less than 2.0 were made in ODAP/KCl system by addition of HCl to gradually lower the pH upto 0.3 and the results are given in Table 4.

Table 4. Peak potential dependence on pH for the second reduction peak at lower pH region, for 3mM ODAP in KCl (pH = 2.4) after addition of HCl.

pH	$-E_p(v)$
1.6	1.072
1.3	1.024
1.0	0.992
0.7	0.956
0.3	0.950

It thus appears that at very lower pH's a characteristic peak is observed for the amino acid, where as at pH greater than 2.4 the H^+ discharge wave (at E_{p0} , -1.520v) described in detail above is observed. Characterization of the peak at pH less than 2.0 was, however, not possible since normal dc polarogram could not be taken due to the ill-formed nature of the wave. As reported for other amino acids (55) this reduction wave could be due to the reduction of the protonated amino acid. Further studies at very low pH's seem desirable to gain further insight on the characteristic polarographic wave observed in the presence of ODAP.

4.3. Polarographic Investigation of Metal-ODAP Complexes

The half-wave potentials of metal ions become more negative and the limiting current becomes smaller as a result of complex formation.

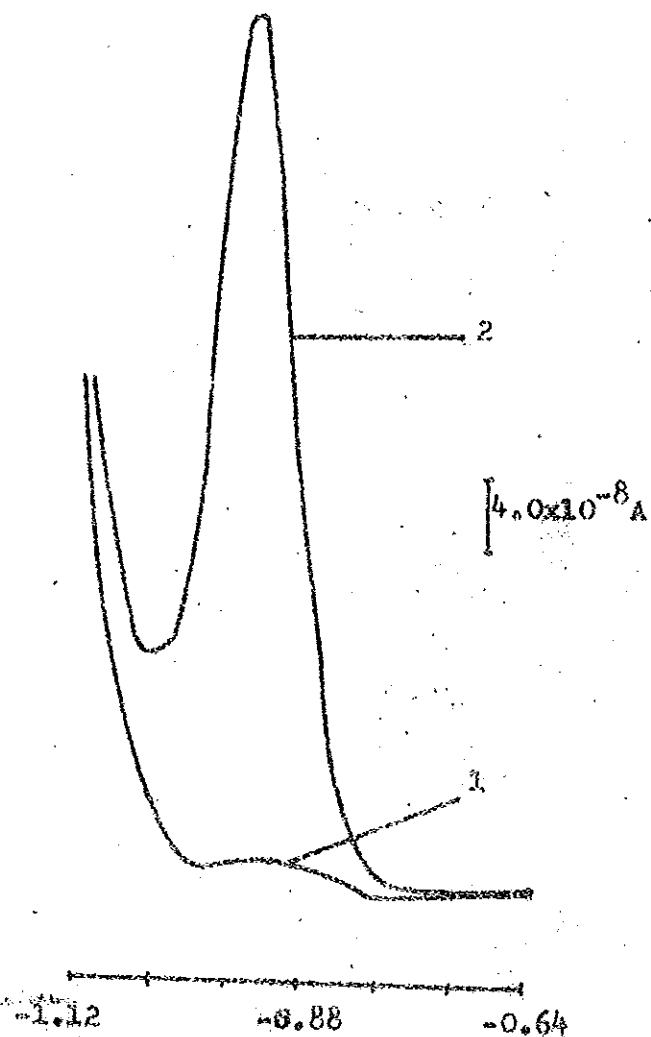


Fig. 11. Polarograms of : (1) 0.1M HCl
 (2) 0.1mM ODAP in 0.1M HCl.

The first task that was therefore done to investigate the complex formation of ODAP with different metal ions was to see the effect of adding the amino acid on the polarogram of the pure metal ions.

Metal ions such as Cu(II), Ni(II), Zn(II), Cd(II), Pb(II) and Co(II) in KCl were used and polarograms were compared before and after addition of ODAP. No substantial changes were noted in the polarograms of the above metal ions and it was therefore assumed that complex formation did not take place in this system. Ammonia buffer (pH = 8.2) was then used as the supporting electrolyte. The differential pulse polarograms of Zn(II), Cu(II), Pb(II), and Cd(II) showed no changes upon addition of ODAP. However, with Ni(II) a decrease in peak current and a broadening of the reduction wave was seen, while the peak potential remained unchanged. After one day there was a decrease in the peak current and a shift in the peak potential for Ni(II). The peak potential of Ni(II)-ODAP system after one day was 144mv more negative than that of the simple metal ion.

Similarly, the Co(II)-ODAP system was studied in ammonia buffer (pH = 8.2). The metal ion Co(II) in ammonia buffer had two reduction peaks at peak potentials of -1.12v and -1.536v respectively. A freshly prepared mixture of Co(II) and ODAP in ammonia buffer (pH = 8.2) did not show a variation in the polarograms. However, after one day there was a shift in peak potential for the first reduction wave by 96mv and a decrease in peak current as compared with that of the simple metal ion, while the peak at -1.536v showed no significant shift.

A study of the polarographic behaviour of the Ni(II)-ODAP and Co(II)-ODAP systems were then carried out in borax buffer (pH = 9.2).

The data obtained from the differential pulse polarograms (Fig. 12, and 13) of 0.02mM Ni(II), and 0.02mM Co(II) and after adding 0.2mM ODAP to each simple metal ions in borax buffer are collected in Table 5.

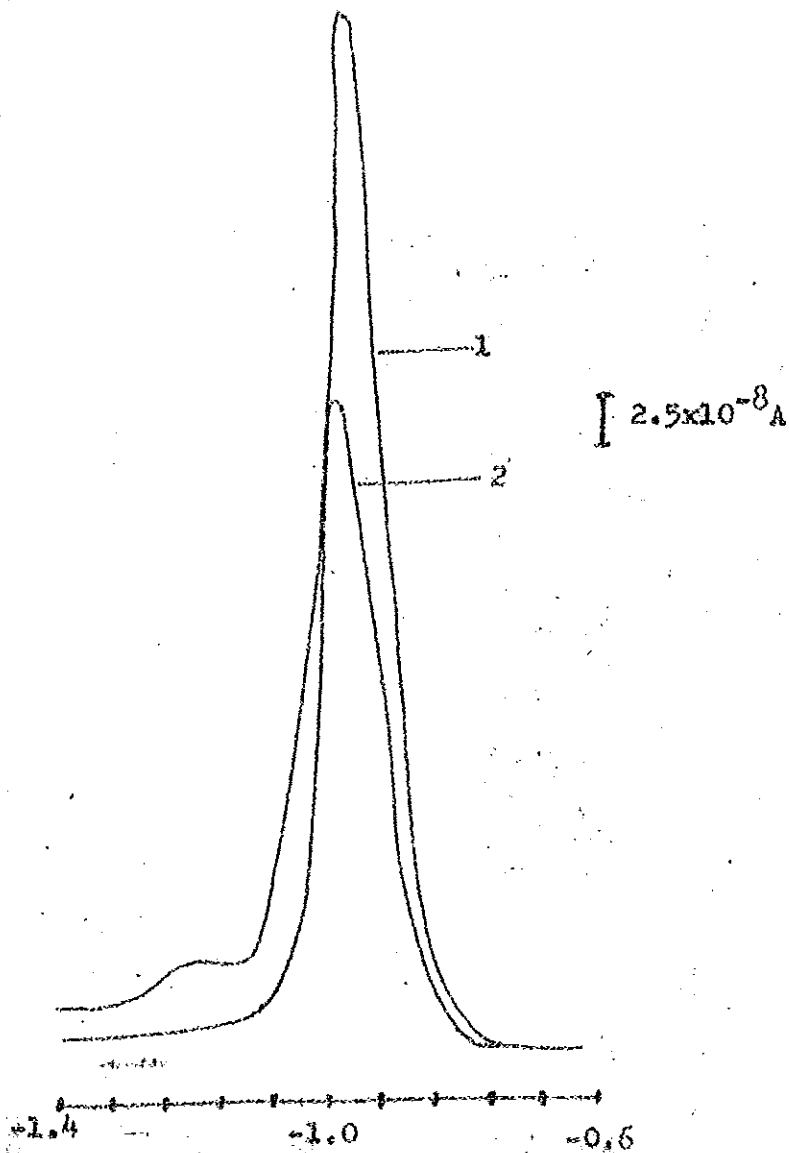
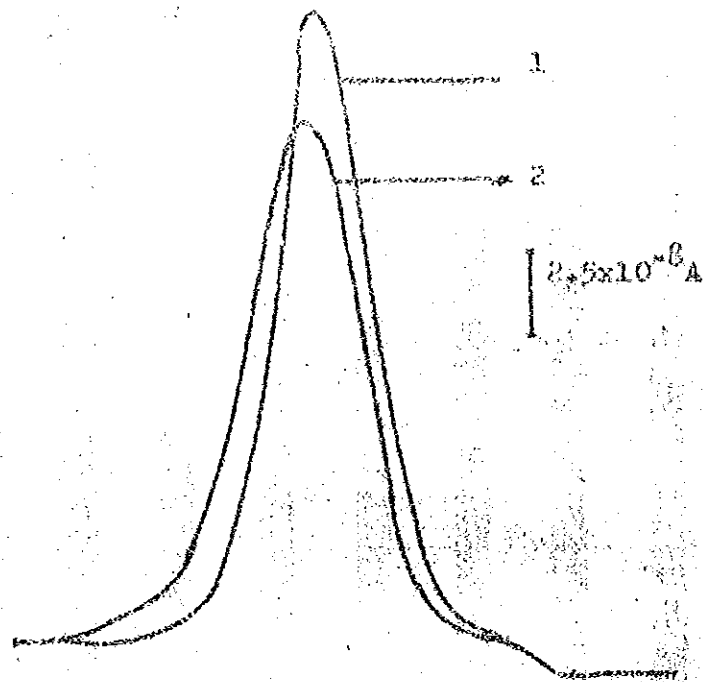


Fig. 12. Ni(II) wave in 0.01M borax (pH = 9.2), 0.02mM Ni(II),
 [ODAP] = 1, 0 mM; 2, 0.2mM.



-1.48

-1.16

-0.76

E/v vs Ag/AgCl/sat'd Cl⁻

Fig. 13. Co(II) wave in 0.01M borax (pH = 9.2), 0.02mM Co(II),
 [ODAP] = 1, 0 mM; 2, 0.2mM.

Table 5. Peak potential, peak current and peak half-width for Ni(II), Co(II) and their complex with ODAP

System	$-(E_{P+0.004})v$	$i_P \times 10^8 A$	$W_{\frac{1}{2}}(mv)$
Ni(II)/borax	0.940	50.50	104
Ni(II)-ODAP/borax	0.968	31.50	128
Co(II)/borax	1.136	20.75	120
Co(II)-ODAP/borax	1.160	18.25	144

The peak potential of the metal ions became more negative when the amino acid was added. The shifts were $(28 \pm 4)mv$ and $(24 \pm 4)mv$ for the Ni(II)-ODAP and Co(II)-ODAP systems respectively. There was also a decrease in peak current as shown in Table 5 for both systems. This shift of peak potential and the corresponding decrease of the peak height of the simple metal ion reveals the formation of a complex.

In order to characterize the nature of the polarographic wave of the complexed metal ions normal dc polarograms were recorded at various heights of the corrected mercury column. In both the Ni(II)-ODAP and Co(II)-ODAP systems the limiting current was linearly dependent on the square root of the height of the mercury column (Fig. 14 and 16). These linear relationships indicate that the reduction process is not kinetically controlled but it may either catalytic or diffusion controlled.

To investigate the polarographic reversibility of the reduction, potential vs $\log \frac{i_{-1}}{i}$ were plotted as shown in (Fig. 15 and 17). The slope of the \log plots were determined and these are tabulated in Table 6. The slope shows that the reduction of the complexed metal ions is irreversible. To confirm this fact drop time variation on peak potential was checked. As shown in Fig. 18 and 19, as drop time increases the peak potential of the complexed metal shifts towards more positive direction. This

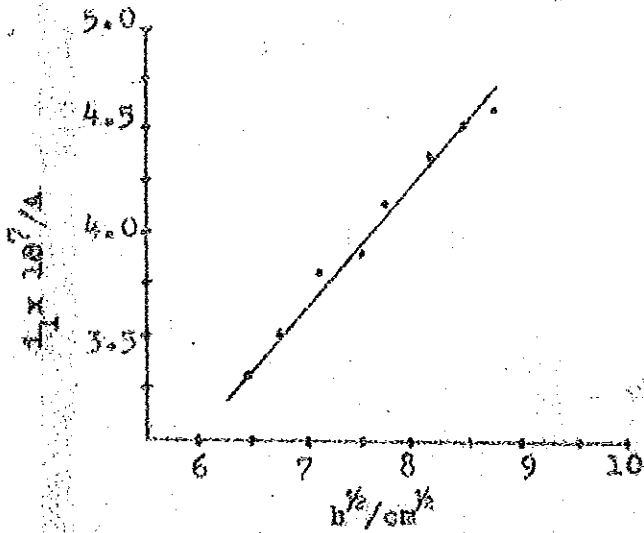


Fig. 14. Plot of I_p vs $h^{1/2}$ for 0.2mM ODAP, 0.02mM Ni(II) in 0.01M borax (pH = 9.2).

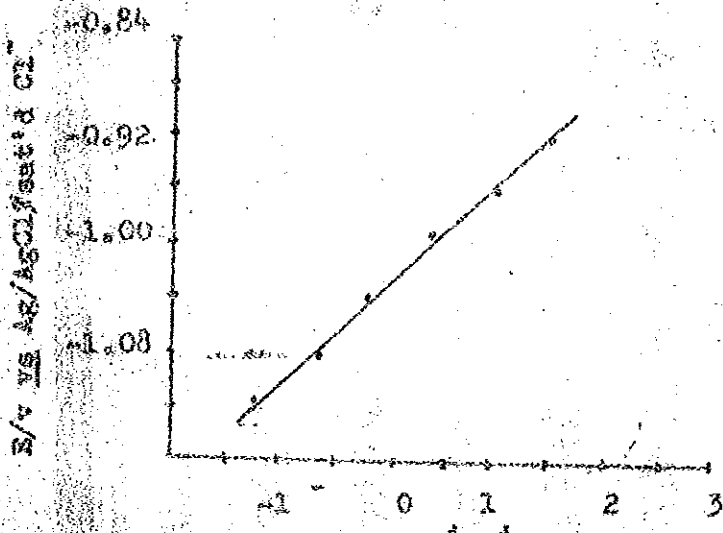


Fig. 15. Plot of E vs $\log \frac{i}{i_0}^{-1}$ for 0.2mM ODAP, 0.02mM Ni(II) in 0.01M borax (pH = 9.2).

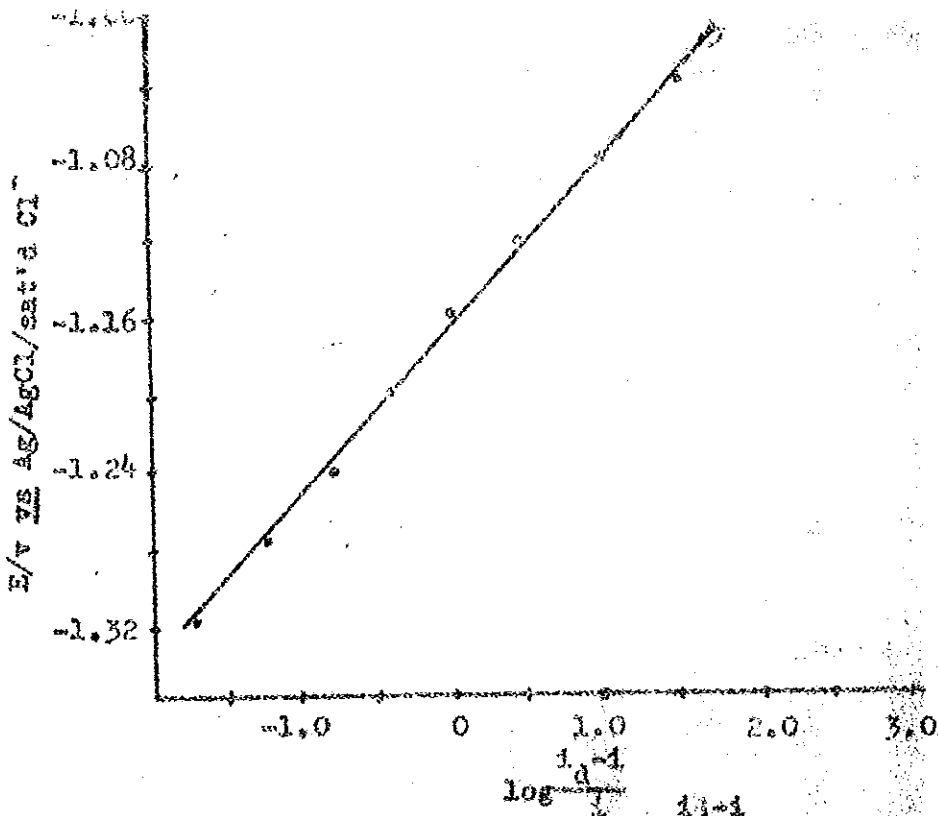


Fig. 16. Plot of potential vs $\log \frac{i_1 - 1}{i_1}$ for 0.2mM ODAP, 0.02mM Co(II) in 0.01M borax (pH = 9.2).

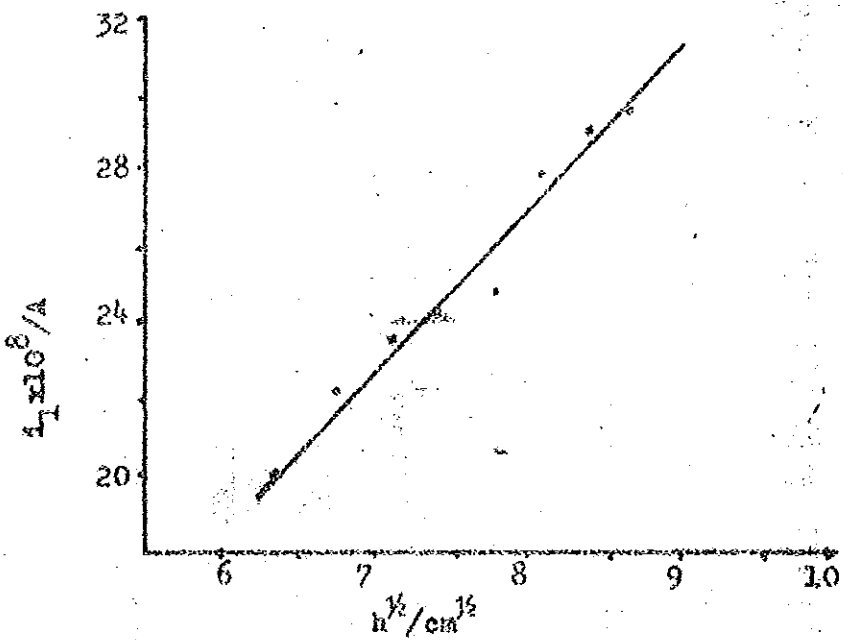
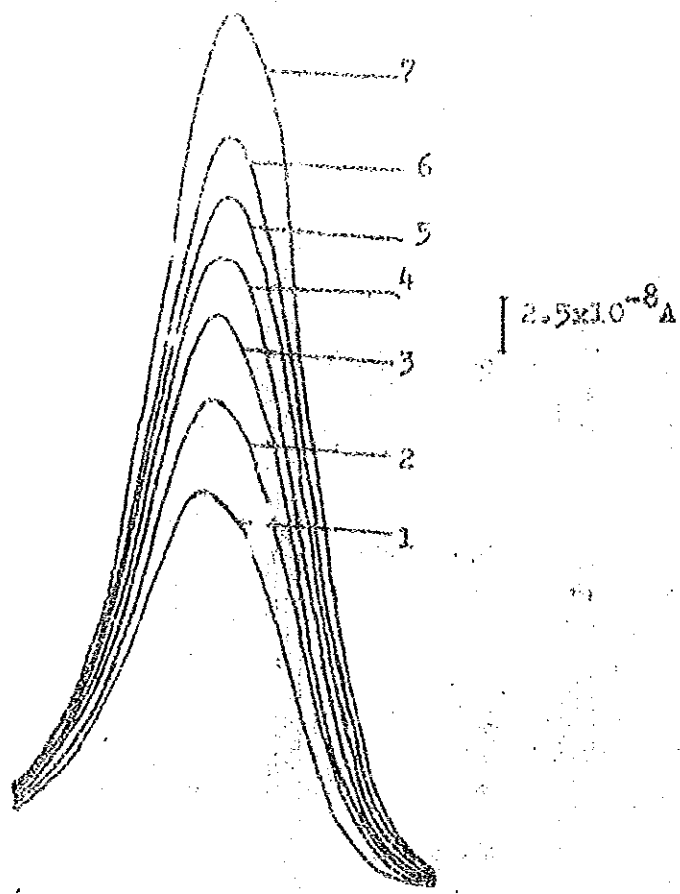


Fig. 17. Plot of i_1 vs $h^{1/2}$ for 0.2mM ODAP, 0.02mM Co(II) in 0.01M borax (pH = 9.2).



$-1.12 \qquad -0.96 \qquad -0.80$
 E/v vs. Ag/AgCl/sat'd Cl⁻

Fig. 18. Polarograms of 0.2mM DDAP, 0.02mM Ni(II), 0.01M borax (pH = 9.2), at various drop times: 1, 0.4; 2, 0.6; 3, 0.8; 4, 1.0; 5, 1.2; 6, 1.4; 7, 2.0; sec.

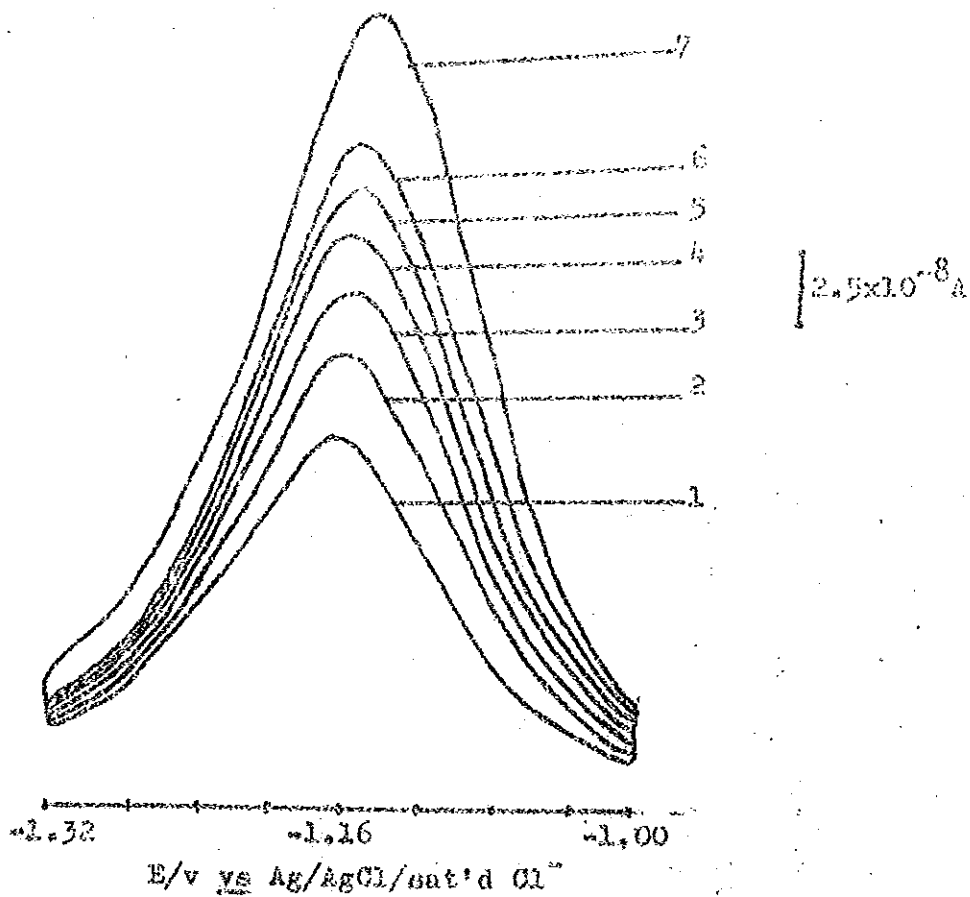


Fig. 19. Polarograms of 0.2mM ODAP, 0.02mM Co(III), 0.01M borax (pH = 9.2), at various drop times: 1, 0.4; 2, 0.6; 3, 0.8; 4, 1.0; 5, 1.2; 6, 1.4; 7, 2.0; sec.

variation in peak potential with drop time and the value of peak half-width (Table 5) supports the fact that both systems, Ni(II)-ODAP and Ca(II)-ODAP are reduced irreversibly.

Table 6. slope, height dependence and half-wave potential of Ni(II)-ODAP and Co(II)-ODAP complexes.

System	Slope of Log plot(mv)	i_1 vs $h^{\frac{1}{2}}$	$-E_{\frac{1}{2}}$ (v)
Ni(II)-ODAP	78	linear	0.992
Co(II)-ODAP	88	linear	1.168

Further studies were made on the Ni(II)-ODAP system, to determine the ligand number and the stability constant of the complex, and these are reported in section 4.4.

4.4 Determination of Stability Constant for Ni(II)-ODAP Complex in Borax Buffer (pH = 9.2)

The polarographic technique for the determination of stability constants of complexes is in general based on the shift of half-wave potentials. The shift increases with increasing ligand concentration.

Since both the simple metal and the complexed metal are reduced irreversibly, the method of Biernat et al. (77) was utilized for determination of stability constant.

At a pH of 9.2 there are two species C and D as shown in Fig. 1. The value of α' at this pH is nearly the same for C as for D, indicating that the two species exists in equal ratio. The formation of the complex depends on the stability of the anions towards the metal ion. Since both C and D have the same value of α' at pH=9.2, their concentration will be the same.

Therefore, the determinations of ligand number and stability constant were made using the concentration of one of the species.

The log plot analysis was performed for the different reduction waves at various ligand concentration. The plots of potential vs $\log \frac{i}{i_d - i}$ depicted in Fig. 20.

The slope of the log plot, the half-wave potential and the transfer coefficient of the simple metal as well as for the different complexed metal ions are summarized in Table 7. The half-wave potential shifts towards more negative value with increasing concentration of the ligand.

Table 7. Half-wave potential, slope and transfer coefficient of 0.02mM Ni(II) and Ni(II)-ODAP complexes in borax buffer (pH=9.2)

[D] mM	$E_{1/2}$ (v)	Slope	α
-	0.968	0.0780	0.374
0.05	0.984	0.0778	0.375
0.10	0.992	0.0781	0.373
0.20	1.000	0.0778	0.375
0.30	1.008	0.0775	0.376

According to (77) if the value of the transfer coefficients are the same for different reduction waves obtained at various concentration of ligands, then this suggests that the electrode reduction mechanism is the same. Hence, the reduction proceeds through the free central ion.

As shown in Table 7 the transfer coefficient of the simple metal and the complexed metal ion is nearly the same. This tells us that the reduction of Ni(II)-ODAP complex takes place through the free Ni(II) ion with the same reduction mechanism for the different ligand concentrations.

Equation (40) enables the determination of the ligand number, j , and the stability constant. This equation can be simplified, since in most cases, the ratio D_s/D_c is usually close to one and may be neglected (70). Equation (40) can then be written as

$$\Delta E_{\frac{1}{2}} = \frac{RT}{2.303nF} \ln \beta_j [L]^j \quad (20)$$

As shown in Fig. 21 the half-wave potential vs $\log [D]$ plot is a straight line. This plot enables the calculation of ligand number of the Ni(II)-ODAP complex using the following simplified form of equation (20).

$$j = \frac{2.303nF \cdot c}{RT} \frac{\Delta E_{\frac{1}{2}}}{\Delta \log [D]} \quad (21)$$

Using equation (21) the ligand number of Ni(II)-ODAP complex was found to be 1.95.

Using the ligand number obtained and equation (20) the average stability constant for Ni(II)-ODAP complex was calculated to be $\log \beta = 7.4$.

It is interesting to compare the determined value of $\log \beta$ with stability constants of other Ni(II)-amino acid complexes. Values of $\log \beta$ for nickel complex with some amino acids were determined by Albert (81). These values are given in Table 8.

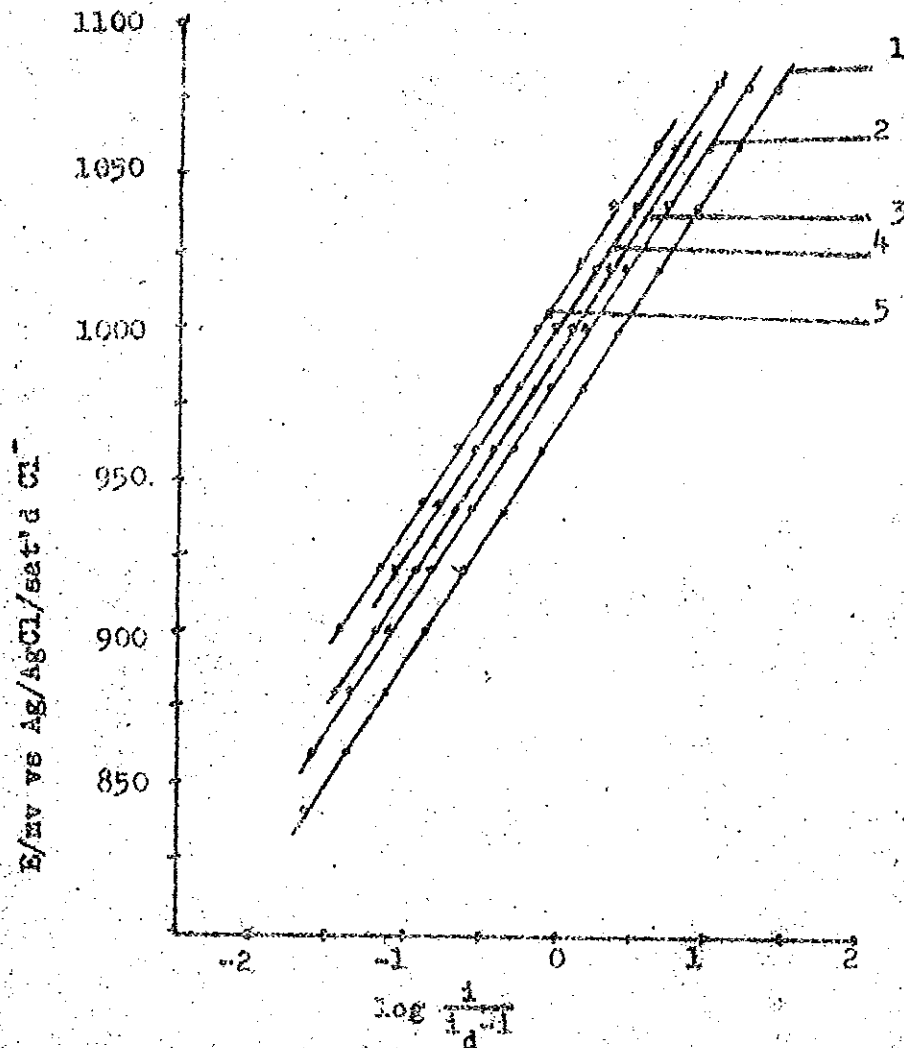


Fig. 20. Plot of $E_{1/2}$ vs $\log \frac{1}{D - D_\infty}$ for Ni(II)-ODAP complexes, $[ODAP] = 1, 0;$
2, 0.1; 3, 0.2; 4, 0.4; 5, 0.6M.

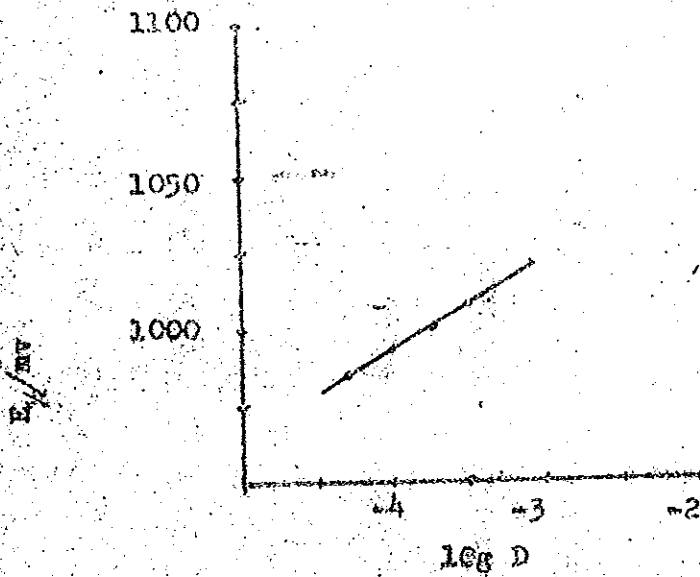


Fig. 21. Dependence of the half-wave potential on $\log[D]$.

Table 8. Values of $\log\beta$ for Ni(II) complexes of some amino acids (81)

Amino acid	$\log\beta$
L - Tyrosine	10.1
DL - Cysteine	19.3
DL - β - Aminoalanine	15.2
DL - γ - Aminobutyryne	16.4
DL - Ornithine	8.3
L -- Lysine	8.8
L - Arginine	9.2
L - Histidine	15.9
Histamine	11.7
L - Aspartic acid	12.7
L - Glutamic acid	10.3

Comparison of the values of $\log\beta$ of the amino acids (Table 8) with that of ODAP reveals that the stability constant of the Ni(II)-ODAP complex is relatively small.

According to (63) the first stability constant ($\log\beta_1$) of Ni(II)- aspartic acid complex is 7.12 ± 0.02 . This is comparable with the value obtained for Ni(II)-ODAP complex. In view of the similarity in structure between ODAP and aspartic acid, this correspondence in $\log\beta$ values is not unduly surprising.

There are a number of sites for complexation of metal ions with ODAP. The nitrogen and oxygen atoms are possible sites. According to (63) the nitrogen atom has greater affinity than oxygen for complexing with transition metal ions. Therefore, nitrogen atoms in the amino acid investigated form linkage with nickel more preferentially than oxygen. At pH = 9.2 the amino acid exists in one of the two forms, C or D (Fig. 1), so that complexation reaction takes place with these anionic forms.

Since the ligand number determined for Ni(II)-ODAP complex at a pH of 9.2 was nearly 2, the complex formed with anion C may have a form NiL_2 and with that of D may have a form $[NiL_2]^-$. Where L is the ligand which is complexing with the metal ion.

To know the coordination number and the exact structure of the complex, further investigation such as electronic spectra are required.

The ligand concentration range used was narrow because if the concentration of the ligand increases beyond 0.2mM a second peak starts appearing with a peak potential of $(-1.172 \pm 0.004)v$. Resolution of the first peak was difficult for ODAP concentration greater than 0.6mM, because of the interference of the second wave.

The stability constant of Ni(II)-ODAP complex in borax buffer was determined using the method of Biernat et al. (77), since both the simple and complexed metal ions were found to be reduced irreversibly. The value obtained was, $\log K = 7.4$. The ligand number of the Ni(II)-ODAP was determined to be 2.

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