CHAPTER II

POTENTIOMETRY AND POLAROGRAPHY

Numerous methods have been used for studying complex formation. Among these, the spectrophotometric method is widely used (18). Since the weak absorption of the essential amino acids in the ultraviolet region, the spectrophotometric method is the unsuitable method. In this work the electrochemical methods, potentiometry and polarography, were resonably chosen for elucidating and determining these complex formation.

2.1 Equilibrium Constants

The equilibria of ligands and complexes in solutions can be defined by equations based on the law of mass action. For the sake of simplicity, the charges are omitted here and in the following. The acid-dissociation of HL from the reaction $HL \rightleftharpoons H + L$ is defined by the acid-dissociation constant (Ka). $Ka = [H]\,[L]/[HL]$ (2.1) The formation of a complex species ML_j from a central ion M, and ions of ligand L, is assumed to be governed by a series of equilibrium constants (19). If many ions of ligand are bound to a central ion, the complex formation will occur stepwise, and the equilibrium will be determined by as many constants as their complexes.

Consequently

Reaction step	Constant
$M + L \rightleftharpoons ML$	K_1
$ML + L \rightleftharpoons ML_2$	K_2
⋮	⋮
$ML_{j-1} + L \rightleftharpoons ML_j$	K_j

where K_1, K_2, --- K_j are the stepwise stability constants of the reaction (20). The product of the stepwise stability constant has been designated by the expression

$$\beta_j = K_1 K_2 K_3 \text{ --- } K_j \qquad (2.2)$$

where β_j is the overall stability constant. Therefore, the following equations can be written

$$M + L \rightleftharpoons ML \quad : \quad \beta_1 = [ML]/[M][L] = K_1$$

$$M + 2L \rightleftharpoons ML_2 \quad : \quad \beta_2 = [ML_2]/[M][L]^2 = K_1 K_2$$

$$\vdots \qquad\qquad \vdots$$

$$M + {}_jL \rightleftharpoons ML_j \quad : \quad \beta_j = [ML_j]/[M][L]^j = K_1 K_2 \text{ --- } K_j$$

Usually $K_1 > K_2 > K_3 \text{---} > K_j$, but exceptions are known (20).

2.2 Potentiometry

The potentiometric method is one of the oldest methods of determining complex formation. It is also used very successfully at the present time and several variations of the potentiometric method are known. The pH-metric titration method (18) is the most useful one for elucidating the composition of complexes and calculating their stability constants.

2.2.1 pH-metric titration of amino acids (15)

All amino acids contain -COOH and $-NH_3^+$ groups that can ionize in aqueous solution, and each group has a specific ionization or dissociation constant. The values of these dissociation constants can be determined by pH-metric titration. In order to illustrate how this might be done, imagine that a solution contains 1 mole of amino acid (contained monocarboxylic and monoamino group in 1 molecule) and that enough strong acid is added so that each ionizable group on amino acid is fully protonated. Next, this solution is titrated with aqueous strong base ; the volume of base added and the pH of the resulting solution are recorded and then plotted as shown in Figure 2.1

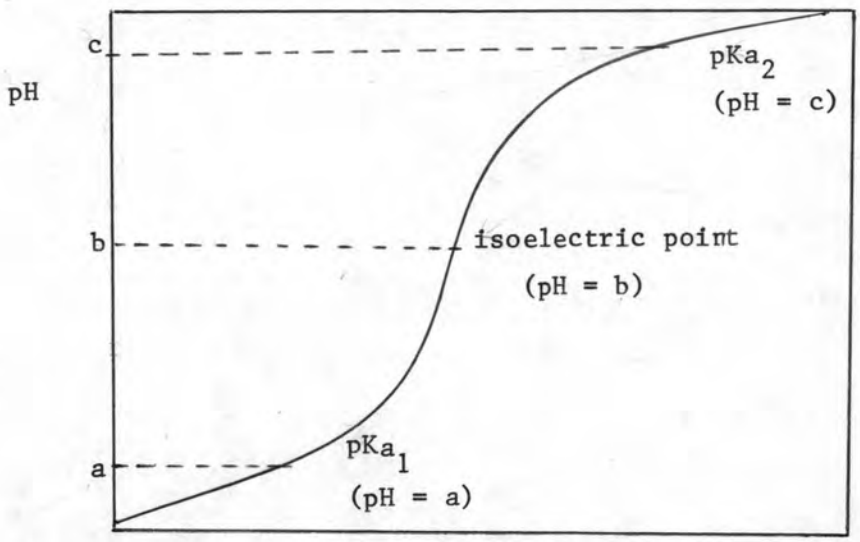
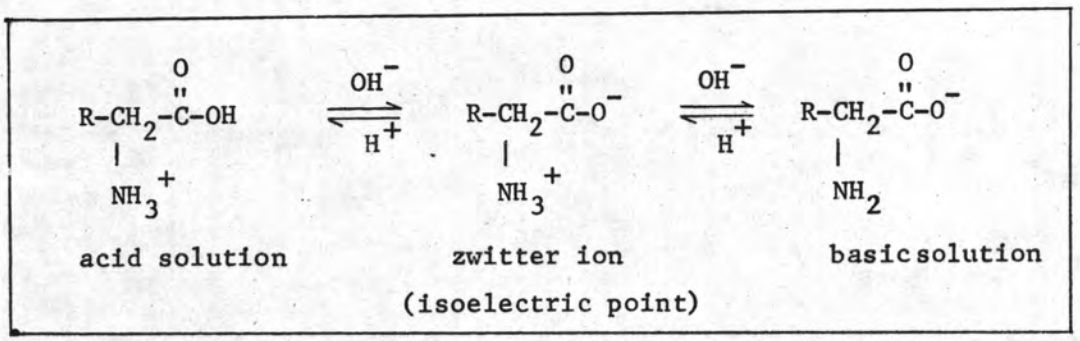


Figure 2.1 Typical pH-metric titration curue of amino acid with strong base

The most acidic group, and the one to react first with added strong base, is-COOH. At pH = a, enough strong base has been added to half-neutralize the -COOH group. The pH at which this half-neutralization occurs is equal to the pKa_1 of the amino acid or of the -COOH group. When 1 full mole of strong base has been added, amino acid is in the zwitterion form and has no net charge. The pH at which a molecule has no net charge is called its isoelectric point, and is denoted by pI (pH = b). Addition of more strong base converts the $-NH_3^+$ group to $-NH_2$. Half-neutralization of $-NH_3^+$ occurs at pH = c, and accordingly the pKa_2 of the amino acid or of the $-NH_3^+$ group. This acid-base behavior of the amino acid is shown below.



In this presentation, the acid-dissociation constants (Ka) can be determined graphically. Because each of the acidic groups of the ligands gives rise to a separate buffer region (see Figure 2.1) thus facilitating the computations. If Ka is defined as equation (2.1), T_L represents the total concentration of the ligand in solution and "a" represents the number of moles of base added per mole ligand in the particular buffer region under consideration, then it follows that (21)

$$T_L = [HL] + [L] \qquad (2.3)$$

$$aT_L + [H] - [OH] = [L] \qquad (2.4)$$

Solving equations (2.3) and (2.4) for $[L]$ and $[HL]$, and substituting into equation (2.1), one obtains (21)

$$Ka = \frac{[H](aT_L + [H] - [OH])}{\{T_L - (aT_L + [H] - [OH])\}} \qquad (2.5)$$

Fitting the equation (2.5) by least square method gives the linear section through the origin and Ka as the slope.

The monoamino, monocarboxylic acids have isoelectric points in the pH range 5.5-6.5. The basic amino acids have isoelectric points at higher pH values (e.g., 9.74 for lysine), and the acidic

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amino acids have isoelectric points at lower pH values (e.g., 3.22 for glutamic acid).

2.2.2 pH- metric titration of metal-amino acid complexes system

The pioneering work of Bjerrum (22) on metal ion-ammine complexes showed how the pH-metric titration method could be used in the study of complex formation in solution for mononuclear metal complex. Under conditions when the ligand has acid-base properties, if the complex formation between metal and ligand occurred, hydrogen ions are liberated from the ligand. Analytical determination of the hydrogen-ion concentration in solution is commonly performed by titrating of the resulting solution with a standardized solution of a strong base (23). The solution titrated is, for example, a solution of a metal ion mixed with a ligand and a solution of a strong base is added to it from a buret, thus changing the pH. The glass electrode is commonly used for measuring pH (18). The acid-dissociation constant of the ligand is determined previously under the same temperature and the same ionic strength condition on the solution of ligand that the metal ion is omitted. The analytical concentration of metal ion, ligand and strong base are known from the experimental conditions and the pH of the solution is determined experimentally. By plotting the volume of strong base added vs. resulting pH of the solution from the pH-metric titration data, the "titration curve" is obtained and showed that the complex formation takes place or not. When a mixture of metal-amino acid which do not mutually form a complex is titrated with strong base, the curve obtained reproduces, in turn, the component

curves, as shown by curve B in Figure 2.2 (1).

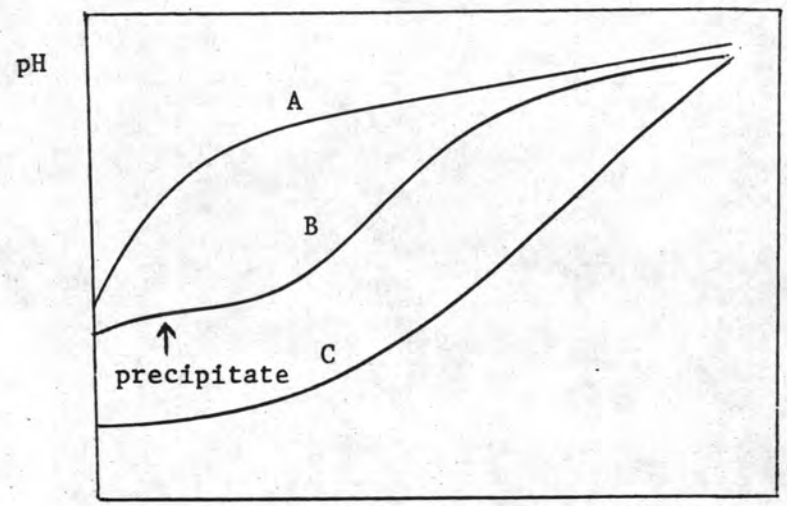

Figure 2.2 The typical titration curve of A = ligand only, B = metal + ligand when no complex takes place (additive effect) and C = metal + ligand when the complex takes place (non-additive effect) (1)

When, however, the two substances can form the complex by liberation of hydrogen ions (as in the case of amino acids), an entirely different picture is seen (see curve C in Figure 2.2). The new curve no longer traverse, in turn, the component curves, but strikes a path that is independent of the ligand curve. Most important of all, the usual precipitates of metal hydroxides (or basic salts) no longer take place upon the first addition of strong base (1). According to Albert (1) when the complex takes place in solution, the degree of formation or average ligand number ($\bar{n}$), introduced by Bjerrum (22), defined as the average number of ligand molecules or ions bound by one metal ion can be calculated from pH-metric titration data by the equation

$$\bar{n} = \frac{T_L - \alpha [L]}{T_M} \qquad (2.6)$$

where T_L is the total concentration of the ligand before the metal ion was added, $[L]$ is the concentration of free ligand (unprotonated form), T_M is the total concentration of metal (free and combined) and α has the value given by the equation

$$\alpha = \frac{[H]}{Ka} + 1 \qquad (2.7)$$

$[L]$ can be determined by the simplified equation

$$\log [L] = (pH-pKa) + \log\{T_L-[BOH]\} \qquad (2.8)$$

where $[BOH]$ is the concentration of added strong base in the complex solution. The "formation curve", introduced by Bjerrum (22), was provided by plotting the $\bar{n}$ vs. pL as shown in Figure 2.3.

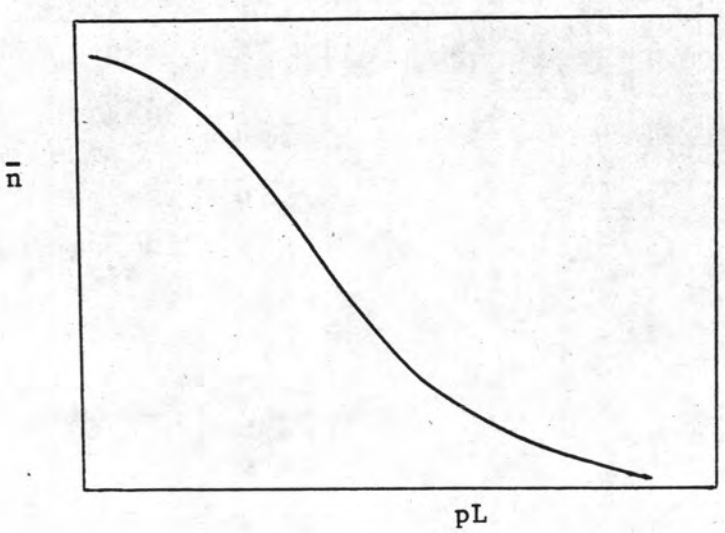

Figure 2.3 Typical formation curve for a metal-ligand system

The formation curve are valuable because it provides evidence as to whether the reaction under consideration is stepwise or not. If the formation curve decreased smoothly, the stepwise complex formation in solution can be pointed out. Finally, the calculation of stepwise stability constants from $\bar{n}$ functions for mononuclear metal complexes was determined by the given equations (1)

$$K_1 = \frac{\bar{n}}{(1-\bar{n})\ [L]} \qquad (2.9)$$

and

$$K_2 = \frac{\bar{n}-1}{(2-\bar{n})\ [L]} \qquad (2.10)$$

Albert's method (1) looked so simply and was widely used, but some disadvantages was encountered, it was derived on the approximation. Lenz and Martell (21) introduced the new computerized method that gave the correct results and was widely applied in the present time. This method was chosen reasonably to apply in this presentation. If K_1 of equimolar metal-ligand complexes is defined as

$$K_1 = \frac{[ML]}{[M]\ \ [L]} \qquad (2.11)$$

with T_M defined as total metal in solution, then

$$T_L = [ML] + [HL] + [L] \qquad (2.12)$$

$$T_M = [ML] + [M] \qquad (2.13)$$

$$aT_L + [H]-[OH] = [L] + [ML] \qquad (2.14)$$

Solving equations (2.1), (2.12) and (2.14) for $[L]$ gives

$$[L] = \frac{(T_L-aT_L-[H] + [OH])Ka}{[H]} \qquad (2.15)$$

Solving equations (2.1) , (2.12) and (2.13) for [M] and [ML] and substituting into equation (2.11) gives

$$K_1 = \frac{T_L - [L]X}{[L]^2 X} \qquad (2.16)$$

where $X = 1 + \frac{[H]}{Ka}$

If K_2 of 2:1 molar ratio of ligand to metal complexes is defined as

$$K_2 = \frac{[ML_2]}{[ML]\ [L]} \qquad (2.17)$$

Then, solving as above,

$$K2 = \frac{T_M - [L]X}{[L]^2 X} + \frac{T_L - [L]\ X}{K_1[L]^3 X} \qquad (2.18)$$

Fitting the equation by least squares gives K_2 as the intercept and $-1/K_1$ as the slope.

2.3 Polarography

2.3.1 Polarographic principle

Polorography is an electrochemical method based on the electrolysis with a dropping mercury electrode. The current flowing during this electrolytic process is recorded as a function of the applied potential. In the presense of a substance that can undergo reduction or oxidation at the surface of the mercury electrode, this

current-voltage curve is called a polarogram. One of the characteristic features of a polarographic wave is the region in which the current levels off after a sharp rise and becomes essentially independent of the applied voltage; this is called a limiting current (i_l). The i_l is the result of a restriction in the rate at which the analyte in the electrode process can be brought to the surface of the microelectrode; with proper control over experimental conditions, this rate is determined almost exclusively by the velocity at which the analyte diffuses. Under these circumstances the i_l is given a special name, the diffusion current (i_d). Ordinary, the i_d is directly proportion to the concentration of analyte (24). One other important term, the half-wave potential ($E_{\frac{1}{2}}$) ; this is the potential corresponding to a current that is equal to one-half the i_d. The $E_{\frac{1}{2}}$ is important for qualitative identification of the analyte (see Figure 2.4).

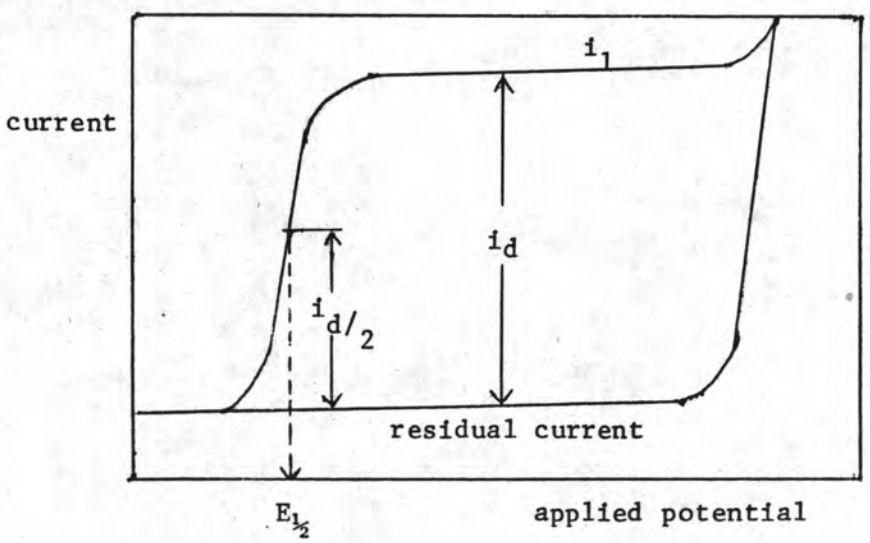

Figure 2.4 Typical polarogram for Cd (II) ion. The upper curve is for a solution of Cd(II) ion in KCl. The lower curve is for a solution of KCl only.

The rate of transport of the analyte to the surface of the electrode is determined by its diffusion and the current follows the Ilkovic derived equation (25).

$$i_d = 0.627\, nFCD^{\frac{1}{2}} m^{\frac{2}{3}} t^{\frac{1}{6}} \qquad (2.19)$$

where C = the concentration of the analyte in moles cm^{-3}

D = the diffusion coefficient in cm^2 sec^{-1}

F = the Faraday constant (96,484 coulombs)

m = the outflow velocity of mercury in g sec^{-1}

n = the number of electron transferred

t = the drop time in sec

i_d = the diffusion current in ampere

The most important consequence of the Ilkovid derived equation for analytical applications is the linear relationship between the i_d and the square root of the height of the mercuty column ($h^{\frac{1}{2}}$) and the temperature (25).

In addition to the i_d there are also other types of polarographic current as following.

(i) Kinetic current (i_k) is the polarographic limiting current, caused by an electroactive species other than the substance present in bulk solution. It is limited by the rate of a chemical reaction when the reducible particle is formed in a chemical reaction in the neighborhood of the electrode and consequently undergoes reduction or oxidation at the dropping mercury electrode. In such instances, the rate of a chemical process is slow enough to be determining step of the electrode process. Kinetic currents are

usually proportional to concentrations of the analyte; in most cases they increase markedly with rise in temperature and they are independent of the mercury pressure (21).

(ii) Catalytic current (i_c) is a limiting current, caused by the presence of a catalyst. The reduction of the analyte is shifted to more positive potential or the oxidation is shifted to more negative value. Catalytic waves are characterized by a non-linear dependence on the catalyst concentration. In buffered solutions, catalyfic waves are often a function of pH and frequently increase with increasing buffer concentration. It also depends on the mercury pressure.

(iii) Adsorption current (i_a) is a polarographic limiting current, caused by the adsorption of the oxidized or reduced form of the analyte at the surface of the electrode. The current which is limited by the rate of the formation of the surface of the electrode is covered by the adsorbed layer. Adsorption process is manifested by separated wave at potentials either more positive or more negative than the wave for the reduction or oxidation of the non-adsorbed compound (26). Many organic compounds exhibit anomalous prewave at potentials less negative than the main waves, which are observed at high concentrations. Adsorption currents are most frequently observed with organic substances of high molecular weights and with substances that form compounds with mercury. Thelimiting current reaches its maximum value, and the height of such wave is proportional directly to the height of the mercury and depends to various extents on temperature (26).

Other currents occurred during the polarographic process are the charging and migration currents (27). Current owing to the charging of the electrode to the respective potential is called the charging or condenser current. It is observed even in a pure supporting electrolyte. In the absence of any supporting electrolyte, if charged electroactive particles are present in the solution, the migrations of these charged particles in the electric field between the two electrodes take placed, providing migration currents. In polarographic study, migration currents are undesired and are eliminated by an addition of a supporting electrolyte. Normally, the molar concentration of the supporting electrolyte is more than twenty times higher than that of the analyte.

Another important characteristic of a polarogram is the reversibility of the electrode reaction. A reversible system is one in which both the rate of the reduction and that of the oxidation are fast. In irreversible process, some of the steps of the total process are slow.

In polarography, the relationship between potential and current is generally described as (28)

$$E_{de} = E_{\frac{1}{2}} - 2.303 \frac{RT}{nF} \log \frac{i-(i_d)_a}{(i_d)_c - i} \qquad (2.20)$$

where E_{de} = the potential at the dropping electrode (V)

i = the current at any point on the wave (μA)

$(i_d)_a$ = the anodic diffusion current (μA)

$(i_d)_c$ = the cathodic diffusion current (μA)

When only the oxidized form is present in the solution, $(i_d)_a = 0$, equation (2.20) is simplified to

$$E_{de} = E_{\frac{1}{2}} - 2.303 \frac{RT}{nF} \log \frac{i}{(i_d)_c - i} \qquad (2.21)$$

To proof that the system is reversible and that it follows the above equation, the i_d is measured first. Then the current i is measured at each of several potentials E on the rising portion of polarogram. The value of log $i/(i_d-i)$ is computed for each point and plotted against the corresponding E. For a reversible system, a linear graph is given with the slope of $-2.303 \frac{RT}{nF}$. Another criterion of reversibility has already been employed, that is the value of $E_{\frac{3}{4}}-E_{\frac{1}{4}}$ which should be equal to -56/n mV at 25°C and -57/n mV at 30°C in the reduction (29,30).

2.3.2 Polarographic metal complexes

The study of complex metal ions by the polarographic method rests on the fact that the reduction potentials of metal ions at the DME are shifted (usually to a more negative value) by complex formation, and by measuring this shift as a function of the concentration of the ligand both the formulas and the stability constants of the complex can be determined. It should be emphasized that this information can only be obtained when the reduction or oxidation of the metal ion complex is reversible at the DME (31). Therefore it is essential to establish the reversibility of the electrode reaction in any given case, before attempting to draw quantitative conclusions from the experimental data.

Generally, when metal ions in solution undergo complexation with ligands other than water molecules, their polarographic reduction waves are altered in two, quite distinct, ways. Firstly, the $E_{\frac{1}{2}}$ is shifted, almost invariably in the more negative direction of applied potential. Secondly, the i_d changes and usually becomes smaller. Studies of the stabilities of metal complexes polarographically involve the determination of shifts in $E_{\frac{1}{2}}$ of metal ions in the presence of increasing amounts of ligands. When the reduction of a metal ion occurs reversibly, both in the absence and presence of ligands, the shift of $E_{\frac{1}{2}}$ is always towards the negative, more cathodic, direction, the shift increasing with increasing ligand concentration. From a simple viewpoint, this may be attributed to the more bulky complexed ion requiring more energy in order to undergo the reduction process at the DME, the rate of the electron exchange process, however, remaining relatively fast with respect to that of diffusion so that the latter is still rate-determining (32).

There are many methods for determination of stability constants and formulas of metal complexes by polarographic technique, depended on the types of metal-ligand system. Three main types of metal-ligand system had been known. (i) Those in which a single complex species is formed to the virtual exclusion of all others over the entire ligand concentration working range. (ii) Those in which several complexes are formed in a stepwise manner, but whose stabilities differ to such an extent from one another that each tends to predominate between fairly sharply defined limits of ligand concentration. (iii) Systems in which a set of mobile equilibria exist between the various complexes and the aquo ion. In such systems, several types of complexes are

present at every ligand concentration, the proportion of higher species increasing with increasing proportions of ligand until, finally, the predominant species is that with the highest possible coordination number.

The method of Lingane (31) is widely used for the determination of stability constants and coordination numbers of metal complexes. The simplified method (32) from the Lingane's method is widely applied in the present time and was chosen for this investigation. Consider the reduction of a complexed ion to the metallic state (as an amalgum) at the DME. The electrode reaction may be expressed as $MLj + ne^- + Hg \Longleftrightarrow M(Hg) + jL$ where MLj is the complex of metal ion M with ligand L. (An arrow of the form $\Longleftrightarrow$ will be used to denote a reversible electrode reaction). For convenience, this overall process may be represented as being made up of two reactions, the first involving prior dissociation of the complex ion into aquo ions and ligand, the second being the electrochemical reaction of the aquo ion itself, thus :

$$MLj \rightleftharpoons M + jL$$

$$Hg \Big\updownarrow ne^-$$

$$M(Hg)$$

If these processes take place reversibly and much more rapidly than the rate of natural diffusion of ions to the electrode surface, then the derived equation for the shift in $E_{\frac{1}{2}}$ ($\Delta E_{\frac{1}{2}}$), produced by the presence of an excess of free ligand, L, is given by (32):

$$\Delta E_{\frac{1}{2}} = E_{\frac{1}{2},s} - E_{\frac{1}{2},c} = \left(2.303 \frac{RT}{nF}\right) \log \beta_j + j\left(2.303 \frac{RT}{nF}\right) \log [L] \quad (2.22)$$

where $\Delta E_{1/2}$ = the shift in $E_{1/2}$

$E_{1/2},s$ = the $E_{1/2}$ of simple ions

$E_{1/2},c$ = the $E_{1/2}$ of metal-ligand complexes

The concentration of free ligand, $[L]$, was calculated by equation (2.1). $[H]$, the hydrogen-ion concentration, is obtained directly from pH of the solution. $[HL]$, the concentration of the remaining ligand at equilibrium, is represented by T_L because of the negligible values of Ka. The plot of equation (2.22) of $\Delta E_{1/2}$ versus log $[L]$ should be linear of a slope $j(2.303 \frac{RT}{nF})$ from which the coordination number, j, of the complex, MLj, may be found. Once the complex stability constant, log β_j, is found from the intercept, $(2.303 \frac{RT}{nF})$ log β_j. In the system that complexes are formed in a stepwise manner, the above plot, instead of being a single linear portion, consists of a series of segments each corresponding to different value of the slope for the various value of j. Stability constants and j-values may be found from each segment using the equation (2.22). Caution is advised in case where the segment linearity is in doubt as it is all too easy to assume that a graph has linear portions when, in fact this is unjustifiable due to the presence of the various complexes overlapping one another. It has been shown (32) that it is usually necessary for the stability constants of the complex species to differ by at least a power of 10 in order for use of the equation (2.22) to be justifiable. A continuous curve is encountered in many systems, indicating the presence of a series of complex equilibria. Even so, the limiting slope of such a curve may be sufficiently linear to make it possible

to determine the maximum coordination number and thus the formula and the stability constant of the highest complex formed.

In the derivations of equation (2.22) it has been assumed that the concentration of the free ligand, $[L]$, is so large that $[L] \approx T_L$, i.e., the same both at the electrode surface and in the bulk of the solution (32). Therefore, the importance of the ligand being in excess is very essential in the experimental conditions. If this is not the case then determined $E_{\frac{1}{2}}$ will be unreliable. It has been shown (32) that it is a fairly simple matter to calculate the necessary excess of ligand that is required for particular cases. It also can be notified from the polarogram that if the ligand is not in sufficient excess, the reversible wave becomes distorted and may even split into two or more parts.