INTRODUCTION

Understanding the mechanisms of enzymes has been the focus of numerous research areas in the past few years. In order to study these enzymes, complexes have been synthesized and studied to model the active sites of enzymes. Such complexes have provided valuable information that has increased our knowledge of enzyme mechanism. In biological systems, the chemical makeup of many enzymes involves the use of 1st and 2nd row transition metal ions. Such metal ions include Zn, Fe, Co, Cu, Mn and Mo. It has been shown that metal ions play an important role in enzyme activation. Understanding the exact chemistry behind how metal ions activate enzymes is still an area of ongoing research.

Zinc has proven to be an extremely crucial 1st row transition metal. Zinc has a role as a nutrient in biological systems. With the significance that zinc has in biological systems, it becomes important to study the behavior of zinc in a biological environment. One of the most interesting areas of recent research is the crucial role zinc plays in activating metalloenzymes.[1] Metalloenzymes are enzymes that utilize a metal ion located at the active site to catalyze biological processes. Examples of metalloenzymes that use zinc in order to function are carbonic anhydrase, an enzyme that interconverts CO_2 and bicarbonate, and alcohol dehydrogenase, an NAD^+/NADH dependent enzyme that catalyzes reversible oxidation of alcohols to aldehydes or ketones. Both of these enzymes require a zinc metal ion complexed at the active site in order to function.

Despite known involvement of zinc in enzyme activity, many of the mechanisms governing coordination chemistry of zinc with metalloenzymes still remain unclear. To

give a better picture of the fundamental chemical interaction of zinc with these

metalloenzymes, we have focused our research on understanding the Lewis acid

characteristics of zinc in aqueous solutions through potentiometry, differential pulse

voltammetry and X-ray diffraction. The work presented in this thesis has two primary

objectives: to show the manipulation of the chemistry of Zn (II) in relation to the HSAB

theory (discussed below) and to present preliminary work with a new (N_2S (thiolate))

zinc-hydroxide complex for potentially modeling active sites on metalloenzymes.

Hard and Soft Acid-Base (HSAB) Theory[2]

Hard and soft acid and base (HSAB) theory has had a very rich and complicated

past which has spanned over five decades. HSAB theory has been used to rationalize

trends in chemistry, such as enthalpy calculations and ionization energies. It has also

been useful in predicting the feasibility of chemical reactions. The pioneer work on

uncovering this useful chemical principle came from the research of Ahrland, Chatt, and

Davies.[3] Earlier work done by Sidgwick generated data on the affinity of a few common

ligands with various acceptor molecules. From this data, Ahrland, Chatt, and Davies

deduced that there was no single rule governing the affinities for ligands with acceptor

molecules. Rather, the ability to complex depends on the molecule to which the ligand

bonds. Ahrland discovered that for Ga(III), the order of complexing atoms from Group V

and VI are N > P > As > Sb and O > S < Se > Te, respectively. However, with Pt(II), the

order is as follows: N << P > As > Sb and O <<< S >>> Se < Te. Additional work with

Ag(II) gave N << P > As and O << S < Se < Te. This data is by no means consistent, but

it leads to two observations. The first is that there is a great difference in affinity of the

first and second element in Groups V, VI, and VII (N and P, O and S, F and Cl). The

second and perhaps the more important observation made by Ahrland et. al. was that

there are two classes of acceptor atoms, (a) and (b). Those that form the most stable

complexes with the first atom of each Group were deemed (a), while those that form the

most stable complexes with the second or succeeding atom were deemed (b). Table 1

shows the breakdown of the Periodic Table according to class (a), (b) or border region[2].

The border region exists due to the varying oxidation states of those atoms, which in turn

results in varying chemical behavior.

It is important to note that most metals and hydrogen are in class (a) while the

limited number of class (b) atoms form stable complexes with olefin[2]. Therefore, π-

bonding with the d-orbitals on the metal must play a role in class (b) characterization.

This is now known to be true due to the involvement of Pd and Pt in catalysts. This

research was the first of its kind to rationalize the affinity of ligands for acceptor

molecules and ions. One major drawback of this classification of acceptor atoms was the

lack of extensive experimental data to support these theories. Soon after, numerous

research papers[2, 4-22] were published dealing with practical experimental applications of

this classification system. Many of these articles dealt with determining reactivity of

chemical reactions and the factors that play a major role. One such article[2] by John O.

Edwards and Ralph G. Pearson dealt with the factors that determined the reactivity of

nucleophilic reagents. In this work, it was deduced that there are three major components

that govern reactivity: basicity, polarizability and unshared pairs of electrons on the

atom. In some instances, reactivity may be determined solely by one factor instead of a

Table 1: Classification of acceptor atoms in their normal valent states[3]

      = Class (a)   = Class (b)   = Border region

He	H																
Ne	Li	Be											B	C	N	O	F
Ar	Na	Mg											Al	Si	P	S	Cl
Kr	K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br
Xe	Rb	Sr	Yt	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I
Rn	Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At
	Fr	Ra	Ac	Th	Pa	U											

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collaboration of all three. However, for the most part, reactivity is affected by many factors. Therefore, the class system developed by Arhland, et. al.[3] needed to be extended to incorporate additional factors.

In 1963, Pearson developed a groundbreaking classification of Lewis acids based on ionic and covalent σ-bonding, π-bonding, electron correlation phenomena and solvation effects. In this work,[23] Pearson divided acids and bases into two categories, those that are polarizable, or "soft" and those that are nonpolarizable, or "hard". This classification was based upon the work by Ahrland[3] in that all metal ions were divided into classes depending on whether they form the most stable complex with the first ligand atom of each group, class (a), or whether they formed the most stable complex with the second or a subsequent atom of each group, class (b). Table 2 shows the trend found by Pearson, which is consistent with Ahrland[3].

Following work done by Chatt and Edwards, it was concluded that other ligand atoms may be analyzed and classified as either (a) or (b). Tables 3 and 4 contain various Lewis acids and bases, respectively, separated into the categories of class (a) or hard, class (b) or soft, and borderline. It should be noted that the criteria for classification are based upon those used by Ahrland, Chatt, and Davies along with data available at the time.[3] From analyzing the Lewis acids in Table 1, class (a) acids are of relatively small size as well as having a high oxidation state, while class (b) acids all have large size and very low oxidation states. Since the high oxidation states of class (a) acids cause them to have low polarizability, they are best termed "hard" acids and class (b) acids with high polarizability termed "soft" acids. Following this trend, it can be stated that "*hard acids prefer to bind to hard bases and soft acids prefer binding to soft bases*".[24] This

Table 2: Complex stability patterns of Groups V, VI, VII with metal ions[25]

Group	Class (a)	Class (b)
V	N >> P > As > Sb > Bi	N << P > As > Sb > Bi
VI	O >> S > Se > Te	O << S ~ Se ~ Te
VII	F >> Cl > Br > I	F < Cl < Br << I

Table 3: Classification of Lewis Acids[2]

Class (a) or hard	Class (b) or soft
H^+, Li^+, Na^+, K^+	Cu^+, Ag^+, Au^+, Tl^+, Hg^+, Cs^+
Be^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Sn^{2+}	Pd^{2+}, Cd^{2+}, Pt^{2+}, Hg^{2+}, CH_3Hg^+
Al^{3+}, Sc^{3+}, Ga^{3+}, In^{3+}, La^{3+}	Tl^{3+}, $Tl(CH_3)_3$, BH_3
Cr^{3+}, Co^{3+}, Fe^{3+}, As^{3+}, Ir^{3+}	RS^+, RSe^+, RTe^+
Si^{4+}, Tl^{4+}, Zr^{4+}, Th^{4+}, Pu^{4+}, VO^{2+}	I^+, Br^+, HO^+, RO^+
UO_2^{2+}, $(CH_3)_2Sn^{2+}$	I_2, Br_2, ICN, etc.
$BeMe_2$, BF_3, BCl_3, $B(OR)_3$	Trinitrobenzene, etc.
$Al(CH_3)_3$, $Ga(CH_3)_3$, $In(CH_3)_3$	Chloranil, quinines, etc.
RPO_2^+, $ROPO_2^+$	Tetracyanoethylene, etc.
RSO_2^+, $ROSO_2^+$, SO_3	O, Cl, Br, I, R_3C
I^{7+}, I^{5+}, Cl^{7+}	M^0 (metal atoms)
R_3C^+, RCO^+, CO_2, NC^+	Bulk metals
HX (hydrogen bonding molecules)	

Borderline

Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}
$B(CH_3)_3$, SO_2, NO^+

Table 4: Classification of Lewis Bases[2]

Hard	Soft
H_2O, OH^-, F^-	R_2S, RSH, RS^-
$CH_3CO_2^-$, PO_4^{3-}, SO_4^{2-}	I^-, SCN^-, $S_2O_3^{2-}$
Cl^-, CO_2^{2-}, ClO_4^-, NO_3^-	R_3P, R_3As, $(RO)_3P$
ROH, RO^-, R_2O	CN^-, RNC, CO
NH_3, RNH_2, N_2H_4	C_2H_4, C_6H_6, H^-, R^-
Borderline	
$C_6H_5NH_2$, C_5H_5,N, N_3^-, Br^-, NO_2^-, SO_3^{2-}	

conclusion has been substantiated with the help of complex formation research. In Table 5, formation constants of Ag^+, Pb^{2+}, and In^{3+} with the halide ions are given.[26]

From the data given in Table 5, it becomes clear that there is a strong correlation between electronegativity, polarizability and hardness/softness. As electronegativity decreases, the polarizability of the ligand increases, which in turn increases softness. In addition, as electronegativity increases, the ability to become polarized decreases which increases the hardness of the ligand. This rational is shown in Table 5. The complex formation constants of Ag^+ (soft) with the halide ions increase with decreasing electronegativity. A similar trend is also seen with In^{3+} (hard). As the electronegativity of the halides increases, the formation constants of the halides with In^{3+} also increase. It can also be noted that Pb^+ (intermediate) forms relatively stable complexes with all of the halides, independent of electronegativity. The simple principle that hard acids prefer to bind to hard bases and soft acids prefer to bind to soft bases is the fundamental statement of the HSAB theory. It is important to note some common misconceptions about this statement. It implies that hard acids simply prefer hard bases and soft acids prefer soft bases. It does not state that hard acid-soft base/soft acid-hard base complexes never arise. Hydroxide, which is a hard base, forms very stable complexes in water with positively charged acids, either hard or soft. An excellent example[24] to demonstrate the principle is with CH_3Hg^+, a soft acid, and H^+, a hard acid. In solution, both form stable complexes with OH^-, a hard base. Given the following competition reactions:

$$H^+ + CH_3HgOH \rightleftharpoons H_2O + CH_3Hg^+ \qquad\qquad K_{eq} = 10^{6.3}$$

$$H^+ + CH_3HgS^- \rightleftharpoons HS^- + CH_3Hg^+ \qquad\qquad K_{eq} = 10^{-8.4}$$

Table 5: Formation constants of hard, soft and intermediate metal ions with halide ions. Classification is based on Ahrland, Chatt, and Davies[3] and Pearson[2].

Log K_1	F^-	Cl^-	Br^-	I^-	Classification
Ag^+	0.4	3.31	4.68	6.58	(b) or soft
Pb^{2+}	1.26	0.90	1.06	1.30	Intermediate
In^{3+}	4.6	2.32	2.04	1.64	(a) or hard

It becomes apparent that the proton prefers the hard base and the CH_3Hg^+ prefers the soft base. Following the preliminary work done by Pearson[2, 23-29], focus was placed on quantifying hardness and softness in an attempt to measure these quantities. Hardness and softness scales were created which took into account various factors such as ionization energies, heats of hydration, formation constants and equilibrium data.[30-34] It becomes apparent that multiple factors must be taken into account in order to quantify the hardness and softness of acids and bases. Even with the evolution of quantitative scales for hardness and softness, the HSAB Theory still remains a qualitative analysis of the interactions between hard and soft acids and bases.

Due to the abundant nature of the acid-base reaction, much focus has been placed on the classification of the elements with reference to acids and bases. This focus has stemmed from trying to understand basic acid-base reactions in aqueous solution. Almost all chemical reactions are based on the simple, generalized acid-base reaction

$$A + :B \leftrightarrow A:B$$
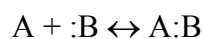
Where A is a Lewis acid, or an electron pair acceptor, :B is a Lewis base, or an electron pair donor and A:B is the complex formed by donation of the electron pair from :B to A. Underneath this basic chemical reaction lies the fundamental principles that govern almost every branch of chemistry. This simple interaction can be expanded to principles of inorganic[4-16] and organic[17-21] chemistry, as well as electrochemistry.[22] Pearson's HSAB Theory has been applied to hundreds of research papers, due to its versatility in predicting behavior in chemical reactions and for becoming a fundamental principle in understanding the nature of chemistry.

Previous work has shown that the properties of a hard or soft acid or base may be altered due to the chemical environment in which the metal ion is placed.[26] This is to say that a borderline species may exhibit softer or harder properties under certain circumstances. The transition of one classification of Lewis acid or base to another classification would give tremendous insight into the understanding of chemical reactions, as well as a healthy extension to Pearson's HSAB Theory. It is the purpose of this research to extend Pearson's HSAB Theory to predicting and studying the Lewis acid properties of zinc in a modified aqueous environment. From Table 3, Zn^{2+} is classified as an intermediate species, according to the classification of Ahrland, Chatt, Davies[3] and Pearson.[23] Therefore, it would be predicted that Zn^{2+} forms relatively stable complexes with both hard and soft bases. However, if the chemical environment in which zinc is placed is altered, a transition of zinc from an intermediate to a soft acid is possible.

Zinc metalloenzymes

Previous work has shown that chemical environment plays an important role in governing whether a metal ion is considered hard, soft or intermediate in the HSAB Theory. pH is a factor in whether or not an enzyme that uses a metal ion for activation will function properly. For example, Zn^{2+} is an intermediate in the HSAB theory and when Zn^{2+} exists as an aquo ion, it has a log K_1 (OH^-) of 4.6.[26] When Zn^{2+} is complexed to a metalloenzyme such as carbonic anhydrase or alcohol dehydrogenase, the log K_1 is greater than 6.0. This indicates an increase in acidity of the Zn^{2+} ion, which becomes important at biological pH. Through complexation, the chemical environment of Zn^{2+} changes. This altered behavior of Zn^{2+} can be attributed to delocalization of electron

density, coordination geometry and coordination number forced onto Zn^{2+} by binding in the metalloenzyme. This increase in log K indicates that Zn^{2+} has increased in strength as a Lewis acid, altering the chemistry of the zinc metal ion. The increase in acidity is important when metalloenzymes rely on metal ions bound to the active site in order to function at physiological pH. It is believed that through this change in the Lewis acid characteristic of Zn^{2+}, ligands may be coordinated to a Zn-L complex much more easily than if Zn^{2+} existed as an aquo ion in solution.

As mentioned before, complexes have been previously studied in order to gain insight into enzyme behavior. These complexes have proven useful in modeling active sites of metalloenzymes. By studying these models, we are able to make a correlation between simple compounds and complex macromolecules. Many compounds have been studied, but we have focused our research on two ligands: cyclen and PATH.
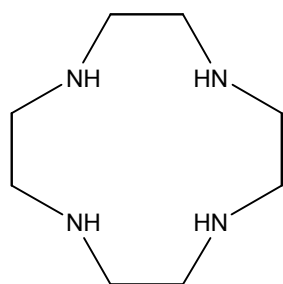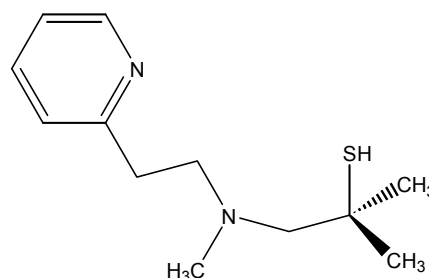
Cyclen   PATH

Macrocycles have long been the backbone upon which models of enzymes have been built. There are several advantages with using macrocycles for models. One advantage is that they provide a coordination sphere in which metals can be sequestered. This cavity allows for thermodynamically and kinetically stable complexes due to the limited flexibility of macrocycles. The size of the coordination sphere can be easily

varied with different macrocycles. This becomes important in size-match selectivity.

Size-match selectivity is the premise that metal ions will form the most stable complexes

with ligands that provide a coordination geometry similar to that required by the metal

ion. Another advantage of macrocycles is the incorporation of donor atoms which aid in

complex stability. Nitrogen, sulfur, and oxygen donor atoms are widely used in models

because of their common appearance in the active site of catalytic enzymes. One such

macrocycle that incorporates nitrogen donor atoms in its structure is cyclen (1, 4, 7, 10-

tetraazacyclododecane).

Cyclen (Figure 1) is a 4-coordinate aza-macrocycle with 4 nitrogen donor atoms

attached by 4 ethylene bridges. Cyclen and its derivatives have been used in gene

controlling and recognition[35-40], biological sensors and receptors[41-44] and in

pharmaceutical agents[45-49] for treatment of various ailments. All known research has

focused on measuring rate constants of a cyclen-Zn complex reacting with a competing

ligand. Once cyclen complexes Zn^{2+} in solution, the complex orients itself to a square

pyramidal geometry, which Zn^{2+} prefers conformationally, with a water occupying the

axial site. A square pyramidal geometry is attained where the zinc sits on top of the

complex, out of the plane of the coordination sphere. This geometry offers several

advantages for a stable complex. Since the zinc is complexed out of the plane of the

nitrogen atoms, there is less steric hindrance for a second ligand to bind. This allows for

bulky ligands to bind to the complexed zinc where in the aquo ion form this is not

possible. Along with bulky ligands binding to the complexed zinc, there is an increase in

the formation constant for zinc with bound ligands. With these observations, it is

apparent that the chemistry of Zn^{2+} in solution becomes altered when complexed to
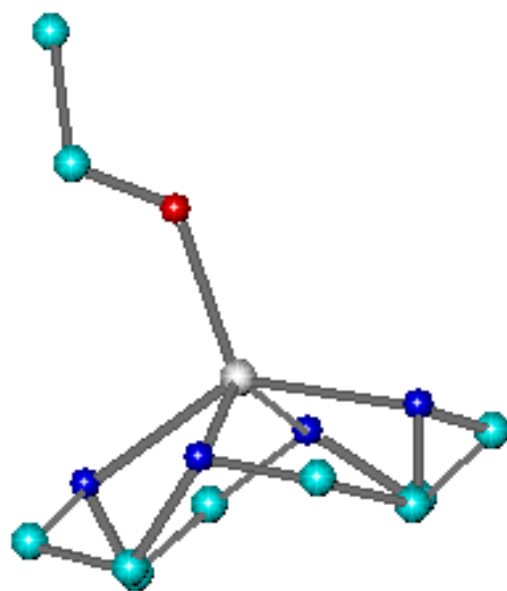
Figure 1: Complex of Zn(cyclen) with ethanol

cyclen. As stated before, it is believed that the reason why the cyclen-Zn system is so effective is due to the transition of Zn^{2+} from an intermediate in the HSAB theory to a softer acid, which effectively is also a stronger Lewis acid. Table 6 contains log K values for the macrocycle 12-aneN$_3$ with ligands F$^-$, Br$^-$ and I^{-}[50]. There is an obvious increase in the formation constants for these ligands with 12-aneN$_3$ over those with the zinc aquo ion. This increase in the formation constants can be attributed to the altered chemical environment placed on the zinc ion by the macrocycle, which is similar to that of cyclen.

The use of simple macrocycles like cyclen gives valuable insight into understanding metalloenzyme mechanisms. The use of these compounds leads to the idea that other compounds may also serve as a tool for modeling the active sites of metalloenzymes. Organic and inorganic synthesis has given us an avenue for generating some of the most insightful ligands for modeling metalloenzymes. Synthesis allows for the manipulation of a chemical entity to model the enzyme of interest. This becomes useful when a ligand needs to be preorganized to sequester a metal ion in solution. Due to the preorganized nature of the ligand, little energy is needed to obtain the lowest energy conformation or on rearrangement of the ligand. In addition to minimizing energy expenditures, arrangement of atoms in the ligand becomes an important factor. In order to successfully model the active site of an enzyme, a ligand must possess similar donor atoms arranged in a manner that mimics the target enzyme. Goldberg from The Johns Hopkins University has recently synthesized and studied[51] a new complex for modeling the zinc form of the enzyme peptide deformylase (PDF). This new type of ligand is a pyridine-amine-thiolate ligand, PATH (Figure 2). PATH (2-methyl-1-[methyl(2-pyridin-2-ylethyl)amino]propane-2-thiolate) provides a N$_2$S(thiolate)

Table 6: Log of formation constants of ligands with 12-aneN$_3$ and Zn^{2+}(H$_2$O)[50]

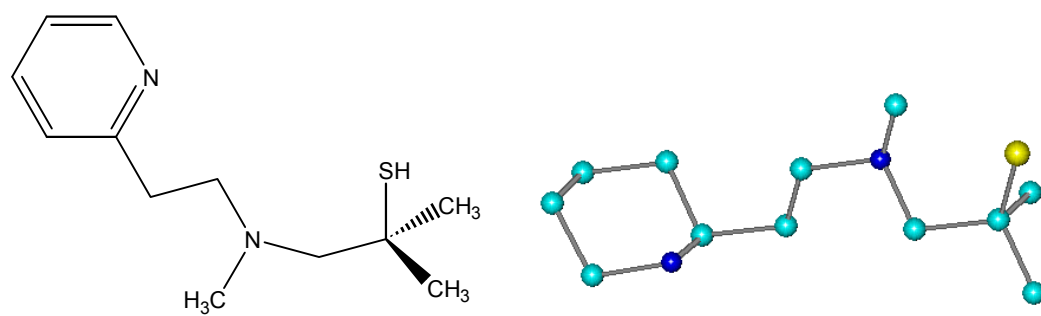| Ligand	12-aneN$_3$	Zn^{2+}(H$_2$O)
I$^-$	1.6	-1.5
Br$^-$	1.5	-0.6
F$^-$	0.8	0.8

Figure 2: PATH (2-methyl-1-[methyl(2-pyridin-2-ylethyl)amino]propane-2-thiolate) ligand

coordination sphere that resembles that of the PDF active site. From the research of

Goldberg, PATH has been shown to be the first and only working model for the zinc

form of the active site of PDF (Figure 3). It is also the first example of a 4-coordinate

zinc complex that contains an alkylthiolate donor in the coordination sphere. This

orientation leads to an excellent model for the active site for PDF. Both cyclen and

PATH generate chemical environments similar to the ones found in biological systems.

Therefore, these systems are well suited to give us insight into accurately modeling the

active site of metalloenzymes in biological systems and to gain a better understanding of

catalytic enzyme mechanisms.

Techniques for Analysis

It was pointed out above that the main techniques used in this research included

potentiometry and polarography. Potentiometry has proven to be an invaluable tool for

studying solution chemistry. Its versatility in monitoring chemical reactions has made it

a mainstay in the chemical laboratory. Potentiometry relies on measuring the potential in

an electrochemical cell. This method of measurment has been useful in observing

endpoints in titrations and monitoring progress of a chemical reaction. This can generate

very useful data which can be used to calculate formation constants. This is the main

focus of the use of potentiometry in this research. The potential and pH of a titration of a

metal-ligand complex with a secondary ligand complexing will be measured and from

this data, formation constants of PATH and cyclen with various metal ions will be

calculated. It is clear that potentiometry is the most widely used technique for analysis.

However, another technique has re-entered the chemical arena as a useful analytical tool.
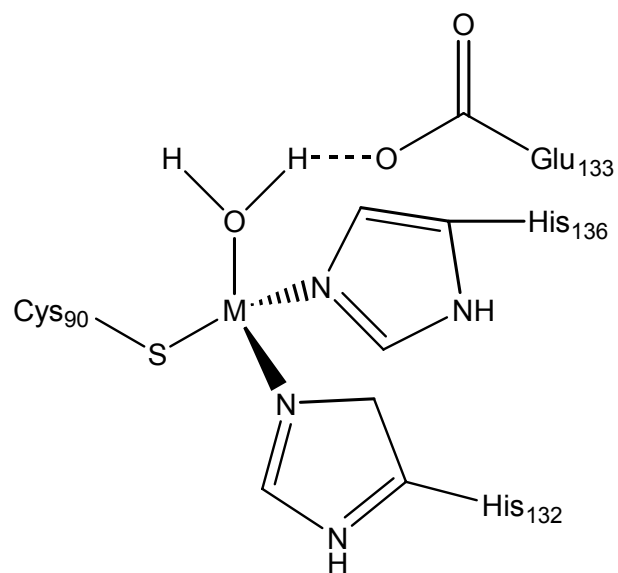
Figure 3: Structure of active site of PDF (M = Fe^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+})

Polarography has been considered by many as a "lost art" in the study of complexation of metal ions in solution. It has been used in many avenues of research such as pharmaceutical development,[52, 53] drug-release study,[54,55] toxic metal ion removal[56,57] and coordination chemistry.[57-60] Its use has diminished due to the ease of interpreting potentiometric data, which can be analyzed easily with computer programs. However, polarography can offer advantages over potentiometry such as the ability to detect micromolar amounts of metal ions in aqueous solution. This low level metal ion concentration nearly eliminates hydroxide precipitation, which can prevent determination of formation constants with easily hydrolyzable ions. This technique produces a current wave as a function of applied potential as the species being analyzed becomes reduced at the surface of a mercury drop electrode.[26] A system where the equilibrium rate between the ligand, metal ion and complex is slow on the polarographic time-scale is called non-labile. This slow equilibrium allows for separation of peaks which is of great value in species identification. From this data, polarographic peaks are obtained that correspond to free metal, metal-complex and other complex species. As the pH of the system is increased, shifts in these peaks may be obtained. Some examples of typical differential pulse polarograms[26] for a Bi^{3+}-15-aneN$_4$ system are shown in Figure 4. As can be seen from the polarograms, excellent separation of peaks is available with polarography, which simplifies identification of species. In (a) of Figure 4 at pH = 1.0, there is one peak which corresponds to Bi^{3+}. As the pH increases to 2.55 in (b), a peak of $BiHL^{4+}$ and BiL^{3+} appears. At pH = 3.58 (c), the BiL^{3+} peak has increased and is the predominant peak at pH = 4.05 (d). As the pH is increased, the BiL^{3+} peak shifts with a slope of 59 mV per decade.[26] A labile system is one in which the equilibrium is very rapid between
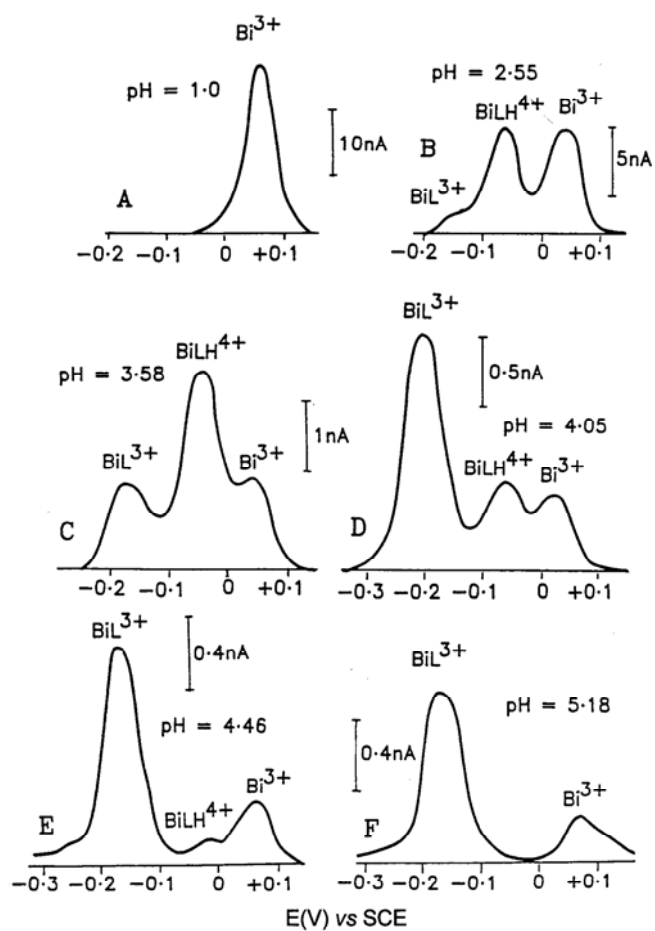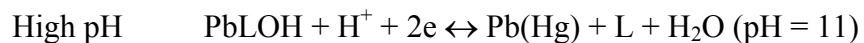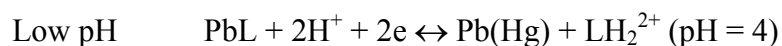
Figure 4: Differential pulse polarograms [(a) to (f)] for the Bi^{3+}-15-aneN$_4$ system as a function of pH[26]. This set of diagrams illustrates non-labile behavior of a metal-ligand system in polarography.

the ligand, metal ion and complex. An example[26] of a labile system is shown in Figure 5, where the ligand N,N'-dipicolylethylenediamine, DPA2, with Pb^{2+} exhibits various peak potentials with increased pH. By graphing the peak potential as a function of pH, the composition of the species involved can be identified. The slopes of the E vs. pH relationships obey the Nernst equation.

$$E = E^o - \frac{RT}{nF} \ln \left[M^{n+} \right]$$

where E is the reduction potential, E^o is the standard electrode potential, R is the gas constant (8.316 J/mol·K), T is the temperature in K, n is the number of moles of electrons involved in the reduction process, F is the Faraday constant (96,485 coulombs/mole of electrons and M is the metal ion involved in the reduction process.

The changes in slope are due to the number of protons involved in the reduction at the mercury electrode. The potential, E, responds to pH because the M^{n+} concentration responds to pH in equilibria such as

Low pH $\quad$ $PbL + 2H^+ + 2e \leftrightarrow Pb(Hg) + LH_2^{2+}$ (pH = 4)

High pH $\quad$ $PbLOH + H^+ + 2e \leftrightarrow Pb(Hg) + L + H_2O$ (pH = 11)

where L is the DPA-2 molecule. As the slope changes at $pK_2 = 5.51$, this brings about a change in slope of 29.58 which corresponds to only one proton in the reduction process. The slope changes again at $pK_1 = 8.40$ where there is a mix of metal-ligand and metal-ligand hydroxide complex present. Above pH = 8.40, the slope is 29.58, again indicating one proton involved in the reduction process. Once the pH rises above 12, the slope reaches a value of 59.16 mV per pH unit, which indicates the involvement of two protons in the reduction process.
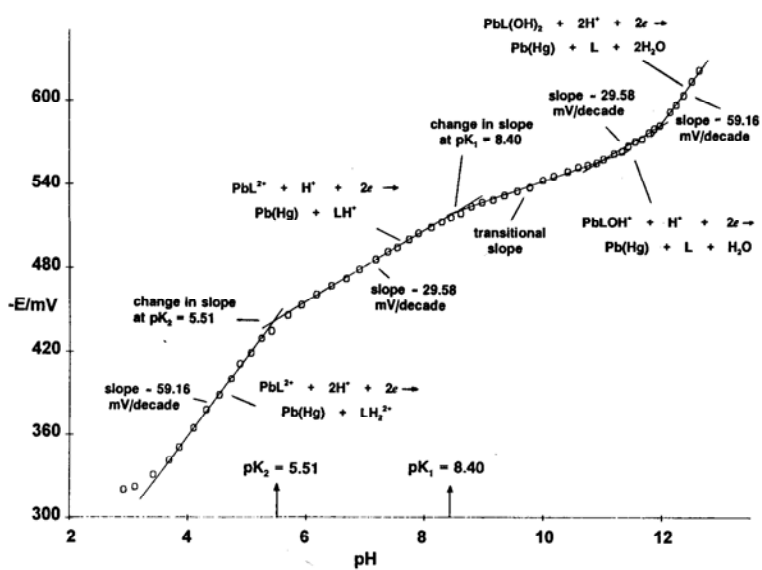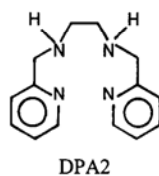
Figure 5: Variation of the polarographic peak potential (E) as a function of pH for DPA2-Pb complex[26].

The ability of polarography to study complexes at low pH and low total metal ion concentration makes this technique invaluable in the study of complexes in aqueous solution. Its use, combined with glass electrode potentiometry, provides the main analytical tools necessary to study models of metalloenzymes in aqueous solution.