CHEMICAL SPECIATION OF COMPLEXES OF L-HISTIDINE AND L- GLUTAMIC ACID WITH TOXIC METAL IONS IN DIMETHYLFORMAMIDE-WATER MIXTURE

By

METI MENGISTU

THESIS SUBMITTED TO

DEPARTMENT OF CHEMISTRY

SCHOOL OF APPLIED NATURAL SCIENCE

PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT

FOR DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

OFFICE OF GRADUATE STUDIES

ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

JULY 2019

ADAMA, ETHIOPIA

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DECLARATION

I hereby declare that this thesis entitled "**Chemical speciation of complexes of L-Histidine and L-Glutamic Acid with toxic metal ions in Dimethylformamide-water mixture**" submitted to Adama Science and Technology University for the award of degree of master of science in Chemistry is the result of work carried out under the guidance of Dr. Hadgu Hailekiros and Co- guidance Dr. Rajalakshmanan Eswaramoorthy during the period of September 2018 –July 2019. It is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or currently submitted for any other degree or diploma in Adama Science and Technology University or other Institutions having the same title and content.

Meti Mengistu Date ----/---/2019

Adama, Ethiopia

ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

SCHOOL OF APPLIED NATURAL SCIENCE

DEPARTMENT OF CHEMISTRY

CERTIFICATE

This is to certify that the thesis entitled **"Chemical speciation of complexes of L-Histidine and L-Glutamic Acid with toxic metal ions in DMF-water mixtures"** submitted by **Meti Mengistu** to the Department Chemistry, School of applied natural science, Adama Science and technology University, for the award of the degree of **master of science in Chemistry** is a bona fide record of research work carried out by her under our supervision. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

Advisor	Signature	Date
Co-advisor	Signature	Date

APPROVAL OF BOARD OF EXAMINERS

Department of Chemistry

School of Applied Natural Science

Adama Science and Technology University

Submitted by

Name	Signature	Date

We, the undersigned, members of the board of examiners of the final open defense by **Meti Mengistu** have read and evaluated his thesis entitled **"Chemical speciation of complexes of L-Histidine and L-Glutamic Acid with toxic metal ion in DMF-water mixtures"** and examined the candidate. This is therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the degree of Master of Science in chemistry.

Chair Person	Signature	Date
Internal Examiner	Signature	Date
External Examiner	Signature	Date

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LIST OF ABBREVIATION

CNS	----------	Central Nervous System
COST	----------	Concentration of Solution by titration
DMF	----------	Dimethylformamide
DMSO	----------	Dimethyl sulfoxide
DNA	----------	Deoxyribonucleic acid
E	----------	L-Glutamic acid
EDTA	----------	Ethylenediaminetetra acetate
GI	----------	Gastrointestinal
H	----------	L- Histidine
IUPAC	----------	International Union of Pure and Applied Chemistry
MeHg	----------	Methyl Mercury
MSG	----------	Monosodium glutamic acid
NAP	----------	number of associable protons
NDP	----------	Number dissociable protons
NP	----------	Number of experimental points
ROS	----------	Reactive oxygen species
SD	----------	Standard deviation
TL_O	----------	Total initial Concentration of ligand
TM_O	----------	Total initial Concentration of metal
μ	----------	Ionic strength

ABSTRACT

The protonation equilibria of complexes of Pb (II), Cd (II), and Hg (II) with L-Histidine and L-Glutamic were investigated pH metrically in Dimethylformamide-water mixtures (0-50%v/v) at 310 K and ionic strength of 0.16 moldm^{-3}. The protonation constants have been calculated using the computer program MINIQUAD75. The protonation constants from the best fit models show the formation of LH_3^{2+}, LH_2^{+}, LH and L^{-} in the case of L-histidine and XH_3^{+}, XH_2, XH^{-} and X^{2-} in the case of L-glutamic acid.

The present investigation binary chemical speciation of Pb (II), Cd(II)and Hg(II) complexes of L-Histidine and L-Glutamic acid in 0.0-50 % v/v Dimethylformamide-water mixtures the dominant species detected L-Histidine with metal were ML_2H_4, ML_2H_3, ML_2H_2, ML_2H and ML_2 for Pb(II) and Cd(II) and Hg(II) forms ML_2H_4, ML_2H_3, ML_2 and ML. for L-Glutamic acid MX_2H_4, MX_2H_3, MX_2H_2, MX and MX_2 for Pb(II), Cd(II) and Hg(II) in DMF-water mixtures. Distributions of species, formation equilibria and effect of influential parameters on the stability constants have been presented. Possible structures of the various species present in solution are also indicated based on the analysis of the pH metric data.

Speciation of mixed ligand complexes of Pb(II), Cd(II) and Hg(II) with L-Histidine and L-glutamic acid were studied in Dimethylformamide-Water mixture. Titrations were carried out in the presence of different relative concentrations (M: L: X=1.0:2.5:2.5, 1.0:2.5:5.0, 1.0:5.0:2.5 in the case of Pb (II) and Cd(II) and 1.0:5.0:5.0, 1.0:5.0:10.0, 1.0:10.0:5.0 in the case of Hg(II)) were carried out with 0.4 mol dm^{-3} NaOH as titrant in Dimethylformamide-water mixture. Stability constants of ternary complexes were refined with MINIQUAD75. The species detected were $MLXH_3$, $MLXH_2$, MLXH and ML_2X for Pb(II), MLX_2H_2 and ML_2X for Cd(II) and $MLXH_3$ and $MLXH_2$ for Hg(II). The species distribution diagrams drawn using HYSS HYPERQUAD with pH at different compositions of Dimethylformamide and plausible equilibria for the formation of species were presented

Keywords: Speciation, L-Histidine, L-Glutamic, Stability constants, DMF, MINIQUAD75 and HYSS HYPERQUAD.

CHAPTER 1

INTRODUCTION

1.1 BACK GROUND OF THE STUDY

The term speciation has been used in a number of different ways, including the transformation of species, the distribution of species, or the analytical activity to determine the concentrations of species [1]. To reduce the confusion regarding the usage of the term speciation IUPAC commissioned an interdisciplinary committee to consider the issue, and they came out with the recommendation in 2000 that chemical compounds that differ in isotopic composition, oxidation or electronic state, or in the nature of their complexed or covalently bound substituents, can be regarded as distinct chemical species [2]. This recommendation leads to the following definitions:

Chemical species is specific form of an element defined as to isotopic composition, electronic or oxidation state, or complex or molecular structure. Speciation analysis is analytical activities of identifying and measuring the quantities of one or more individual chemical species in a sample [3]. In addition, speciation of an element is the distribution of an element amongst defined chemical species in a system under specified conditions. Various aspects of structure contribute to identifiable species for a particular element, and may differ in importance depending on the reasons for which a speciation analysis is undertaken. Speciation studies of toxic metal ion complexes are useful in order to understand the role played by the active site cavities in biological molecules and the bonding behavior of their residues with metal ions [4]. The possible ligand groups in proteins are the amino acid side chains, the terminal amino, carboxyl and thiol groups and, in some cases, the amide group in the peptide backbone [5]

Heavy metals are commonly defined as those having a specific density of more than 5 g/cm^3 such as Lead, Mercury and Cadmium [6]. They are generally not required for metabolic activity and are toxic to living organisms even at low concentrations [7]. The mechanism of the toxicity of metals is very complicated. Generally, toxicity of metals results from blocking the essential biological functional groups (–OH, –SH and –N) or modifying the active conformation of bio-molecules (enzyme, DNA, etc.) through binding and displacing the essential metal ions from their natural binding sites of the bio-molecules with a foreign metal ion [8]. Human civilization and the increase in industrial activity has gradually redistribute many toxic metals from the

earth's crust to the environment and increased the possibility of exposure. Lead, cadmium and mercury are among the various toxic heavy metals especially prevalent in nature due to their high industrial use [9].

Lead (Pb) is a highly toxic metal whose widespread use has caused extensive environmental contamination and health problems in many parts of the world. The main sources of lead pollution are the battery industry, leaded petrol and gasoline exhaust. Lead affects every organ of the body, specially the bones and teeth, the kidneys, and the nervous, cardiovascular, immune and reproductive systems [10]. Lead also interferes with the normal metabolism of calcium in cells and causes it to build up within them [11].

Cadmium (Cd) is one of the most toxic metal ions of our environment and is found in air, food and water. Cd ions are absorbed by most tissues of the body and become concentrated mainly in liver and kidney and it has a long biological half-life of 11 to 20 years in humans [12]. The sources of Cd pollution in urban areas are metallurgical plants, Cd plating and battery fabricators. It can enter the environment through natural causes, such as volcanic activity and forest fires [13]. Human exposure to cadmium mainly occurs through cigarette smoking but exposure can also occur through contaminated food, water or air [14].

Mercury (Hg) is very toxic and exceedingly bio accumulative. Major sources of mercury pollution include anthropogenic activities such as agriculture, municipal wastewater discharges, mining, incineration, and discharges of industrial wastewater. Mercury exists mainly in three forms: metallic elements, inorganic salts and organic compounds, each of which possesses different toxicity and bioavailability [15]. These forms of mercury are present widely in water resources such as lakes, rivers and oceans where they are taken up by the microorganisms and get transformed into methyl mercury within the microorganism, eventually undergoing bio magnification causing significant disturbance to aquatic lives. Consumption of this contaminated aquatic animal is the major route of human exposure to methyl mercury [16].

Amino acids are building blocks of protein. More than 300 amino acids are listed in the Practical Handbook of Biochemistry and Molecular Biology, but only 20 amino acids take part in protein synthesis [17]. Amino acids have special importance compared to other chemical compounds in the sense that they are regarded as the foundation stones of living organisms. Fostered by the crucial role of amino acids in our life, studying their structural, chemical and

physical properties becomes very necessary to explain their behavior and potential applications. Among these properties are protonation and stability constants of complexes they form with various metal ions [8].

L-Histidine (H) has been used in the treatment of rheumatoid arthritis, allergies, ulcers and anemia. It is essential for the growth and repair of tissues and important for the maintenance of the myelin sheaths, which protect nerve cells. It is needed for the production of both red and white blood cells and it protects the body from radiation damage. It lowers blood pressure. It aids in the removal of heavy metals from the body and aids in sexual arousal [18].

L-Glutamic acid (E) is a non-essential amino acid, and is interconvertible to glutamine, which is known to be a very important in preventing ammonia intoxication. Adults may ingest 20 to 35 mg per day of this amino acid without any apparent ill effects. It is an excitatory neurotransmitter for the central nervous system, the brain and spinal cord. It is important in the metabolism of sugars and fats. It aids in the transportation of potassium into the spinal fluid. It acts as fuel for the brain and helps correct personality disorders. It is used in the treatment of epilepsy, mental retardation, muscular dystrophy and ulcers [19]

N, N-Dimethylformamide (DMF) is a polar (hydrophilic) aprotic solvent with high boiling point. It is miscible in all proportions with water and most organic solvents. It is also a powerful solvent for a variety of organic, inorganic, in peptide coupling for pharmaceuticals, in the development and production of pesticides and resin products [20]. Most of the speciation studies are performed in aqueous medium under the conditions comparable to those existing in the biological systems. So far, no systematic report has appeared on studies speciation of L-Histidine (H) and L-Glutamic acid (E) with toxic heavy metal in DMF-water mixtures of various concentrations. Hence, in the present study, organic solvent (DMF) is chosen to mimic the permittivity of the biological fluids.

1.2 STATEMENT OF THE PROPOSED STUDY

Most of the metabolic reactions are catalyzed by metalloenzymes or metal activated enzymes. The activity of these enzymes is believed to be due to the metal-enzyme-substrate complexes. Since some toxic heavy metals have, the ability to replace essential metal found at active site of enzymes the speciation study of that heavy metal is needed. Ionic mechanism of action for lead and Cadmium mainly arises due to its ability to substitute other bivalent cations like Ca^{2+},

Mg^{2+}, Fe^{2+} and affecting various fundamental biological processes of the body [21]. Treatment of poisoning by ions such as Pb^{2+} and Cd^{2+} is much more difficult since these are both divalent ions and selectivity is harder to accomplish.

Living organisms exposed environmentally to high metal concentrations follow various mechanisms to counter potential toxicity [22]. Because of this problem, many questions pending to address regarding the exact mechanism of Pb, Cd and Hg-induced damage, we know that Pb, Cd and Hg compete with metal sites in many protective or antioxidants proteins inducing an improper activity and even the total loss of the antioxidant cellular defense. Further, deeply research is needed to figure out the complete mechanism of hepatocellular damage induced by toxic metals in order to set up proper therapeutics approaches for chronic and acute metal-protein detoxification. However, the exact model of the metal-protein system may be difficult to construct in simple bio-molecules like amino acids and peptides due to the complex nature of proteins. However, such models may give a tremendous amount of information about the structure of proteins and function of bio-molecules in biological systems.

Most of the speciation studies are performed under the conditions comparable to those existing in the biological systems. These are taken as models for the systems existing in bio-fluid and natural water [23]. Therefore, DMF-water mixture was selected as media to maintain the dielectric properties of the medium in comparable levels to those of the physiological fluids since the polarity at the active site cavities should generally be applicable when it is possible to compare ligand binding to the metal ion as in protein residues and solvent environment [24].

Large data base of protonation constants values are available, but data for ionic strength dependence is available is only few case moreover there is considerable difference in the values of protonation constants reported under similar conditions. The present study deals with the determination of protonation constants of E and H in DMF-water mixture was discussed in different concentration by maintaining ionic strength 0.16 mol/dm^{-3} at 310 k.

Hence, L-Histidine (H) and L-Glutamic acid (E) are chosen as model compounds to investigate the binding effect of bio-molecules with the toxic metal ions of Pb (II), Cd (II) and Hg (II) in organic media- water mixture under comparable condition to those of biological systems.

1.3 OBJECTIVE OF THE STUDY

1.3.1 General Objective

The overall objective of this study is to:

Investigate the binding effect of biomolecules (Histidine (H) and glutamic acid (E)) with the toxic metal ions Pb (II), Cd (II) and Hg (II) in organic media -mixtures under comparable to those of biological systems.

1.3.2 Specific Objectives

The specific Objectives of this proposed study are to:

i. Determine the protonation constants of H and E in aqueous and organic media-water mixture
ii. Investigate the effect of dielectric constant of solvent on protonation equilibria of H and E in organic media-water mixture.
iii. Predict the distribution diagrams of H and E in aqueous and organic media-water mixture.
iv. Determine the stability constant of binary and ternary complexes of H and E with Pb(II), Cd(II) and Hg(II) in aqueous media and organic media-water mixture
v. Investigate the effect of dielectric constant of solvent on the binary and ternary complexes of H and E with Pb (II), Cd (II) and Hg (II) ions in organic media-water mixture.
vi. To predict the types of species of binary and ternary complexes of H and E with Pb (II), Cd (II) and Hg (II) in aqueous and organic media-water mixture.
vii. Propose the structure of binary and ternary complexes of H and E with Pb (II), Cd (II) and Hg (II) ions.

1.4 SIGNIFICANCE OF PROPOSED STUDY

Knowledge of chemical speciation of metal ions is needed in human biology, nutrition, toxicology, in biomedical applications, metal ions in the environment, extraction metallurgy, food chemistry and metal ions in many industrial process and clinical practice. Because bioavailability, metabolism and excretion, toxicity, biological activity and detoxifying processes are dependent on the particular species considered [25]. In addition, transport of the metal in the body and its final deposition/storing in target organ (e.g. brain, liver and bone) also determined by the speciation of the element because of the possible ligand-exchange reactions with several potential metal ion-binding bio-molecules in the bio-fluids or tissues [8].

Understanding of the protonation-deprotonation equilibria of H and E is necessary before an attempt is made to investigate the metal-ligand equilibria associated with these ligands. Since natural and bio-fluids have a wide range of ionic strength and composition, a different equilibrium constant for a complex would have to be used for each individual system. The constant is used to calculate the speciation of the complex and its dissociation products in the fluid, which determine the mobility and toxicological effects of the complex

Stability constant is well known tool for solution chemist, biochemist, and chemist to help for the determination of the properties of metal-ligand reaction in water and biological system. In addition, determination of protonation constant are important physicochemical parameters can provide critical information about H and E metal complexes as drug properties such as solubility, lipophilicity, acidity, transport behavior, and permeability. Stability constants of the complex formation of biologically active ligands with metal ions in addition to concentration of each species at any pH are very important for the whole understanding of the physiochemical manner of such compounds require determination of their protonation constants [23]. Evaluating physiochemical properties of anew chemical entities at early stage of drug development is mandatory to reduce attrition rates due to poor biopharmaceutical properties. Among these properties, ionization constant (pKa) is needed to predict ADME (Absorption, distribution, metabolism and excretion) behaviour to understand pH related formation

mechanisms and solubility characteristics. Good drug and powerful ligand have an important feature in a common. That means they possess electron donor groups. So, it follow reliable speciation knowledge can be employed to optimize drug efficiency to minimize side reaction and to predict undesirable clinical side effect.

The studies carried out on these systems under the present experimental conditions are useful to understand (i) the role played by the active site cavities in biological molecules, (ii) the type of complex formed by the metal ion and (iii) the bonding behavior of the protein residues with the metal ion. The species refined and their relative concentrations under the present experimental conditions represent the possible forms of metal ions in the biological fluids and `speciation represents toxicity of these metals in the presence of L-histidine and L-glutamic acid.

CHAPTER 2

LITERATURE REVIEW

2.1 CHEMICAL SPECIATION

The effect of toxic metals depend on strongly the particular form in which the element is present in the system [26]. For example, while both methylmercury and inorganic mercury are toxic, they show different patterns of toxicity. Often these different chemical forms of a particular element or its compounds are referred to as “species”. The notion that the distribution among its effect on the behavior of a particular element has accepted in such diverse fields as toxicology, clinical chemistry, geochemistry and environmental chemistry [4]. Recently, speciation analysis plays a unique role in the studies of biogeochemical cycles of chemical compounds, determination of toxicity and ecotoxicity of selected elements, quality control of food products, control of medicines and pharmaceutical products, technological process control, research on the impact of technological installation on the environment, examination of occupational exposure and clinical analysis [27]. The fields of health and nutrition benefit tremendously from the information that speciation analysis provides [28].

Chemical speciation determination generally relies on utilizing analytical methods in conjunction with chemical speciation models [1]. Laboratory analysis provides a direct measure of different forms of a target chemical, whereas chemical modeling applies known thermodynamic relationships among chemical forms to predict the overall equilibrium distribution. Often a combination is used when chemical analysis can only determine certain forms and modeling is used to predict the other forms relative to the measured form. The wide spread use of equilibrium constant data of metal complexes in solution phase in many fields of research shows the need to calculate precise and accurate values valid over wide range of experimental conditions [4]. A perusal of the literature [29] revealed that potentiometry using glass electrode gained wide popularity in the determination of equilibrium constants in preference to spectrophotometry, because the number of unknown quantities (extinction coefficient and stability constant) is double in the latter. Kaden and Zuberbuhler advocated [30] hyphenated spectro pH meter. pH metric technique in conjunction with ion-selective electrodes is a versatile technique in obtaining reliable information of protonated, hydroxylated and polynuclear species of even multi metal and multi ligand systems. Computer oriented algorithms indispensable for the calculation of equilibrium constants of all the species in such

complex systems. Chemical speciation modeling for labile systems is based on the assumption that all component and derived species are in equilibrium and that reliable stability constants are available at the applicable ionic strength (μ) and temperature [31].

Detailed knowledge of chemical speciation is essential to a full understanding of bioavailability and toxicity of heavy metal ions, and to their adsorption, sedimentation, and transport phenomena in soils, rivers, and aquifers [20]. Generally, from mercury, lead, and cadmium it is clear that metal speciation influences mammalian toxicity [7]. The optimization of many industrial chemical processes, as in hydrometallurgy and pulp and paper processing, relies on a detailed understanding of speciation in often-complicated multicomponent, multiphase systems.

2.2 BIOLOGICAL FUNCTIONS OF AMINO ACIDS

Amino acids are organic molecules containing amino group ($-NH_2$) and carboxylic acid group (-COOH) both attached to the same carbon atom called α carbon. They are the structural units that make up proteins. They join to form short polymer chains called peptides or longer chains called either polypeptides or proteins [32]. The oxidation of amino acids makes a significant contribution towards generation of metabolic energy. This energy varies greatly with the type of organism and with the metabolic situation in which an organism finds itself. The 20 standard amino acids, with a variety of carbon skeletons going into the composition of proteins, have 20 different pathways for degradation. All these pathways taken together, in human beings, account for 10-15% of the body's energy production [33]. Among those amino acid histidine and glutamic acid were discussed as below.

2.2.1 L- Histidine

L-Histidine (H) is one of the strongest metal coordinating ligands among the amino acids and plays an important role in the binding of metal ions. H is an important component of active sites of biomolecules and there is a very likely hood for toxic metals to interact with H [34]. L-histidine is essential at the active sites of many enzymes and biomolecules like superoxide dismutase, ferritin, ceruloplasmin, hemoglobin, metallothionein, and cysteine dioxygenase. It is one of the strongest metal coordinating ligand and plays an important role in the binding of metal ions with proteins. In all these proteins, the major binding group for metal ions is the

imidazole group of histidine. It has three potential metal-binding sites, namely, carboxylate oxygen (Ocaboxyl), imidazole imidonitrogen (Nim) and amino nitrogen (Nam) [35]

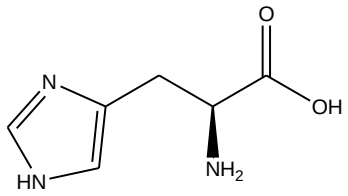


Figure 1. Structure of L- Histidine

Due to the high reactivity of its imidazole group, histidine residue is often found at the active site of enzymes and involve directly in catalysis. It controls the transmission of metals in biological bases [36] and act as a neurotransmitter or neuromodulator in mammalian central nervous system. H is used in the treatment of anemia, allergies and other inflammatory reactions [37]. L-Histidine can be converted to histamine (Figure 2) which is a major neurotransmitter in the brain and throughout the nervous system [38].

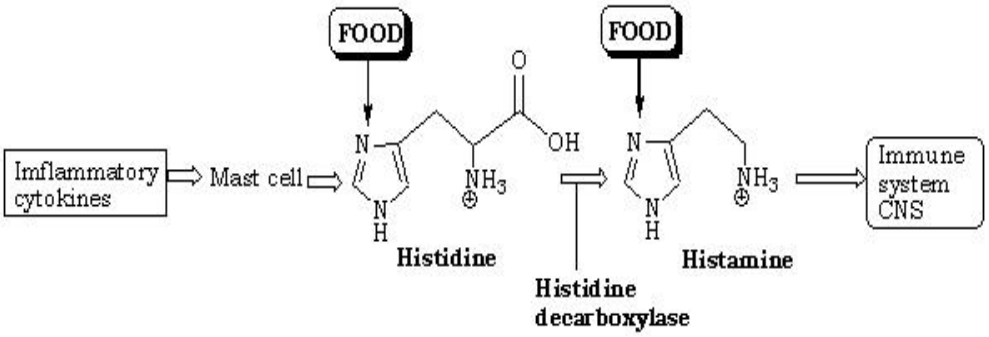


Figure 2. Metabolic pathways that mediate biogenic amine responses

Histamine, the decarboxylation product of histidine, enters the immune and central nervous systems directly from food intake or when released by mast cells in response to allergic reactions, causing edema, itching, hypotension, and bronchoconstriction [32].

2.2.2 L-Glutamic acid

L-Glutamic acid (E) is a non-essential dicarboxylic amino acid, which have molecular formula $C_5H_9NO_4$ and at physiological pH, exists as anion. In the human body, it is converted to glutamine. It is a dicarboxylic acid (Figure 3.) and is grouped under branched chain amino acid with acidic chain [39]. L-Glutamate is a major excitatory neurotransmitter in the mammalian central nervous system that contributes to fast synaptic neurotransmission, and to complex

physiological processes like memory, learning, plasticity and neuronal cell death [40]. E is the only amino acid metabolized by the brain.

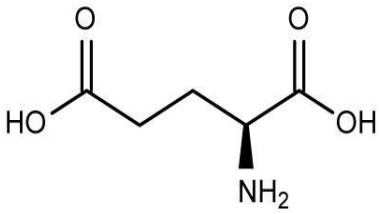


Figure 3. Structure of L-glutamic acid

The bound form of glutamate is linked to other amino acids in proteins present in muscles, hair and skin. Glutamate in the human body is considered essential for protein repair, regeneration, and growth. In an average adult, the body content of glutamate is around 1.5-2.0 kg mainly in the bound form. The monosodium glutamic acid (MSG) is used as a flavor enhancer [41] in savories, soups, salad dressings, processed meats, frozen entrees, ice creams, frozen yogurt, crackers, bread, in low fat foods to make up for flavor lost when fat is reduced or eliminated and it is widely used as a condiment.

2.3 N, N-DIMETHYLFORMAMIDE

N, N-Dimethylformamide is an organic compound with the formula $(CH_3)_2NC(O)H$ Commonly abbreviated as DMF, which is miscible with water and the majority of organic liquids [42]. Dimethylformamide has the largest dielectric constant (36.7) of the common dipolar aprotic solvents and it has been termed the super solvent. Another important phenomenon about the structure of the DMF molecule is its trigonal pyramidal geometry. There is a directional lone pair of electrons at the apex of the pyramid, which helps in complexation. The primary use of DMF is as a solvent with low evaporation rate. It is used as a solvent in peptide coupling for pharmaceuticals, in the development and production of pesticides, and in the manufacture of synthetic leathers, fibers, films, and surface coatings. It is also used as a common reagent and used in the research laboratory [43].

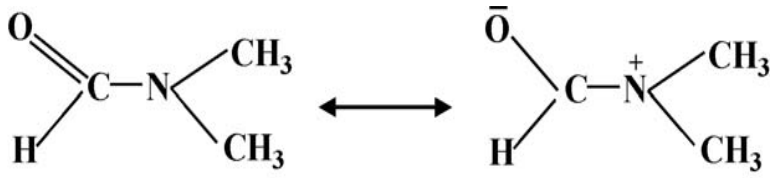


Figure 4. Structure of N, N-Dimethylformamide

The negative pole in DMF is on an oxygen atom that juts out from the rest of the molecule and this oxygen atom is the best hydrogen bond acceptor. Through unshared pairs of electrons on these negatively charged, well-exposed atoms are solvated very strongly. The positive pole on the other hand, is buried within the molecule. In DMF the presence of two electrons repelling $–CH_3$ groups make the lone pair at nitrogen still more perceptible towards donation. Thus, it may be argued that the DMF is actually the donator of nitrogen electron pairs [44].

2.4 TOXIC HEAVY METALS

Heavy metals may enter the body through food, water, air, or absorption through the skin when they are exposed to humans in agriculture and in manufacturing, pharmaceutical, industrial, or residential settings, however the mechanism toxicity of these metals is very complicated. One of the mechanisms by which the heavy metal ion cause toxicity is from blocking the essential biological functional groups (–OH, –SH and –N) or modifying the active conformation of bio-molecules (enzyme, DNA, etc.) through binding [45]. The metal ion can be bound to the coordinating site of the bio-molecules, which may be binding sites for the essential metal ions. Thus entry of the new metal ion to the essential biological functional groups of the bio-molecules are blocked, change conformation of the molecule and they are deactivated and they could not perform their biochemical functions. Metals such as Hg, Cd, and Pb etc. that are generally not required for metabolic activity and are toxic to living organisms at quite low concentrations [46].

The adverse effects of the absorbed and accumulated heavy metals include damaged or reduced mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver, reproduction and other vital organs. Long-term exposure may result in slowly progressing physical, muscular, and neurological degenerative processes that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy, etc. Living organisms exposed environmentally to high metal concentrations follow various mechanisms to counter potential toxicity. In human and other animals, chelation therapy is the preferred medical treatment for

reducing the toxic effects of metals. The toxic heavy metal ions (Pb(II), Cd(II) and Hg(II)) are potent enzyme inhibitors because they are easily bound to enzyme donor groups. Lipophilicity of these metal species usually dictates the extent of the toxicity and the body's ability to excrete them [47]. Metal ions in bio fluids are present in different forms (1) non-exchangeable form in metalloproteinase, (2) labile equilibrium with a variety of bio ligands along with other similar metal ions in solution, and (3) aquatic form.

2.4.1 Lead Toxicity

Lead is a toxic element with no beneficial effects to the human body. Lead affects every organ of the body, especially the bones and teeth, the kidneys, the nervous, cardiovascular, immune and reproductive systems [21]. Lead and other heavy metals create reactive radicals, which damage cell structures including DNA and membranes. Lead also interferes with DNA transcription, enzymes that help in the synthesis of vitamin D, and enzymes that maintain the integrity of the cell membrane [48]. Lead may also be harmful to the developing immune system, causing production of excessive inflammatory proteins; this mechanism may mean that lead exposure is risk factor for asthma in children [49]. Ionic mechanism of action for lead mainly arises due to its ability to substitute other bivalent cations like Ca^{2+}, Mg^{2+}, Fe^{2+} and monovalent cations like Na^{+} (though bivalent cations are more readily substituted), affecting various fundamental biological processes of the body [21]. Treatment of poisoning by ions such as Pb^{2+} and Cd^{2+} is much more difficult since these are both divalent ions and selectivity is harder to accomplish. Lead competes with Iron and Calcium for absorption from the human gastrointestinal tract. Moreover, it can be inhaled and organic (e.g. tetraethyl) Lead can be absorbed via skin.

Lead, even in Pico molar concentration, can replace calcium, thereby affecting key neurotransmitters like protein kinase C, which regulates long term neural excitation and memory storage. It also affects the sodium ion concentration, which is responsible for numerous vital biological activities like generation of action potentials in the excitatory tissues for the purpose of cell-to-cell communication, uptake of neurotransmitters (choline and dopamine) and regulation of uptake and retention of calcium by synaptosomes [21].

2.4.2 Cadmium Toxicity

Cadmium is one of the most toxic metal ions of our environment and is found in air, food and water. Cadmium causes iron deficiency by binding to cysteine, glutamate, aspartate, and

histidine ligands. Cd ions are absorbed by most tissues of the body and become concentrated mainly in liver and kidney and it has a long biological half-life of 11 to 20 years in humans [50]. Cadmium can cause both acute and chronic intoxications [51]. Cadmium is highly toxic to the kidney and it accumulates in the proximal tubular cells in higher concentrations. Cadmium can cause bone mineralization either through bone damage or by renal dysfunction. Studies on humans and animals have revealed that osteoporosis (skeletal damage) is a critical effect of cadmium exposure along with disturbances in calcium metabolism, formation of renal stones and hypercalciuria. Inhaling higher levels of cadmium can cause severe damage to the lungs. If cadmium is ingested in higher amounts, it can lead to stomach irritation and result in vomiting and diarrhea. On very long exposure time at lower concentrations, it can become deposited in the kidney and finally lead to kidney disease, brain, lung, heart, fragile bones and lung damage [52]. Cadmium-induced oxidative damage has been demonstrated by the increased lipid peroxidation and inhibition of enzymes required to prevent such oxidative damage. It has been suggested that the mechanisms of acute Cd toxicity involve the depletion of glutathione and protein-bound sulfhydryl groups, resulting in enhanced production of reactive oxygen species (ROS) such as superoxide ion, hydrogen peroxide, and hydroxyl radicals. ROS has been implicated in chronic Cd nephrotoxicity, immune toxicity, tissue damage and carcinogenesis [53].

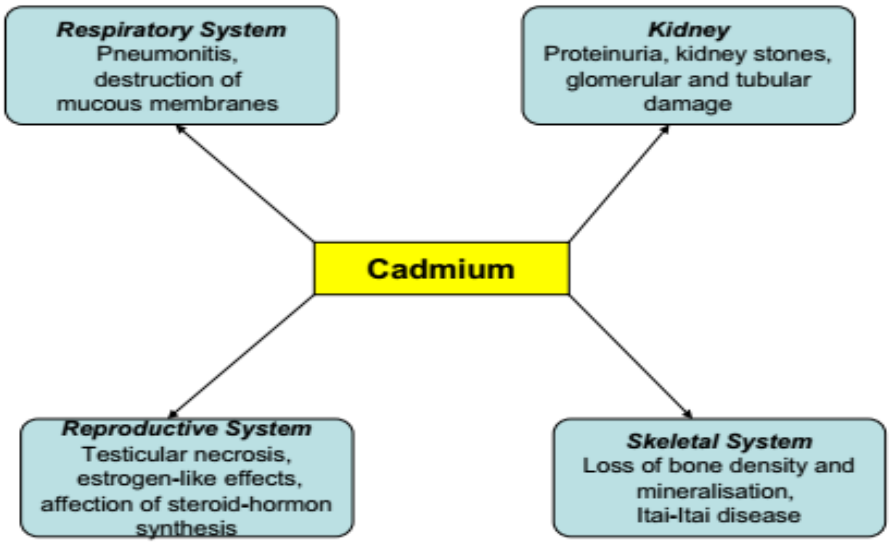


Figure 5. Effect of Cadmium on several organ systems

In food, only inorganic cadmium salts are present. Organic cadmium compounds are very unstable. In contrast to lead and mercury ions, plants readily absorb cadmium ions. They are equally distributed over the plant. Cadmium is taken up through the roots of plants to edible

leaves, fruits and seeds. During the growth of grains such as wheat and rice, cadmium taken from the soil is concentrated in the core of the kernel. Cadmium also accumulates in animal milk and fatty tissues [54]. Therefore, people are exposed to cadmium when consuming plant- and animal-based foods.

2.4.3 Mercury Toxicity

Mercury exposure is related to the release of mercury forms (elemental Hg^0, inorganic Hg (II) and organic Hg) into the environment by both natural and fabricated activities [55]. Mercury is a highly toxic element because of its accumulative and persistent character in the environment and living organisms. It affects the immune system, alters genetics and enzyme systems, and damages the nervous system, and the senses of touch, taste and vision [56].

Mercury has no known essential functions to the body but elemental mercury and its metabolites have biochemical damage to tissues and genes through diverse mechanisms, such as interrupting intracellular calcium homeostasis, disrupting membrane potential, denaturing and altering protein synthesis, inhibiting enzymes and interrupting excitatory amino acid pathways in the central nervous system [57]. Mitochondrial damage, lipid peroxidation, microtubule destruction, and the neurotoxic accumulation of serotonin, aspartate, and glutamate are all mechanisms of methyl mercury neurotoxicity [15]. The divalent mercury in the brain leads to strange symptoms, including erythrism. Mercury is also found to be the process linking behavioral symptoms and Alzheimer's disease. An acute mercury exposure may give rise to lung damage [58].

Mercury has ability to block the active sites of hemoglobin; the oxygen-carrying pigment of red blood cells reduces the ability of the circulatory system to deliver oxygen to the tissues and further compromises the body’s energy demands [15]. The poison also destroys red blood cell membranes and damages the lining of blood vessels. Its accumulation in the heart damages the heart muscle and the valves, which regulate blood flow. Consequently, it is no surprise that mercury poisoning is well associated with irregularities in blood hemoglobin, chest pains and tachycardia (abnormally rapid heartbeat) [59]. This is part of the reason that there is so little known about chronic, low-level mercury exposure. The common associated with sulfhydryl Reactive metal toxicity symptoms are discussed in table 1. [60]

Table 1: common associated with sulfhydryl reactive metal toxicity symptoms

Metals	Symptoms
Mercury	chronic fatigue; depression; poor memory and cognitive function; emotional instability; peripheral numbness or tingling; decreased senses of touch, hearing or vision; hypersensitivity and allergies; compromised immune function; cardiovascular disease
Lead	fatigue; loss of appetite; headaches; poor memory; inability to concentrate; aberrant behavior; decreased coordination; irritability; pain in abdomen, bones and muscles; gout; anemia
Cadmium	hypertension; fatigue; muscle and joint pain/ osteomalacia; anemia; lumbar pain; atherosclerosis; kidney damage with associated urinary loss of essential minerals, amino acids and protein

2.5 PROTONATION CONSTANT

. A review of literature has revealed that a little is reported for protonation constants of glutamic acid and histidine in organic-water solvents. Raju Sala et al [61] reports the determination of protonation constants of E and H in DMSO-water compositions maintaining an ionic strength of 0.16 mol/L with sodium chloride at 303.00 $\pm$0.05 K. With these ideas, in view the literature was surveyed for the protonation constants of H and E (Table 2).

Table 2: Protonation Constants of H and E Reported in Literature

Sl. No	**log β**			**Ionic strength (mol dm^{-3})**	**Computer program used**	**Instrumental method**	**Ref**
	011	012	013				
L-Histidine							
1	8.77	14.64	16.40	0.15	MINIQUAD	Potentiometry	[62]
3	9.08	15.08	--	0.1	MINIQUAD	pH metry	[63]
4	9.05	15.15	--	0.1	MINIQUAD	Potentiometry	[64]
8	9.19	15.11	17.08	0.1	MINIQUAD	pH metry	[65]
L-Glutamic acid							
2.	9.75	13.99	16.27	0.1	MINIQUAD	pH metry	[66]
3.	9.56	13.71	15.96	0.5	-	pH metry	[67]
4.	9.52	13.69	16.00	1.0	-	pH metry	[68]
8.	9.67	13.19	16.23	2.25	SQUAD	pH metry/	[69]

2.6 BINARY STABILITY CONSTANTS

Several literature reports have been devoted to the study of stability constants of metal-L-Histidine and L-glutamic acid complexes. His complexes of Co(II), Ni(II) and Cu(II) were studied by Durrani [70] pH metrically. Martin and Edsall [71] studied potentiometrically the association of divalent Cu, Zn, Co and Cd with histidine derivatives. Some of these data are recorded in Table 3. Due to the multiple biological roles of glutamic acid, speciation studies of E with Co(II), Cu(II) and Zn(II) in DMF–water mixtures and Pb(II), Cd(II), Hg(II), Co(II), Ni(II), Cu(II) and Zn(II) in urea–water mixtures were reported [72]. Ionic strength dependence of the formation constants of E with uranium (VI) and beryllium (II) and pH-metric and spectrophotometric studies of oxovanadium (IV) with E, aspartic acid and imidazoles were also reported [73]. Maheswara et al. [74] Also studied the chemical speciation of E complexes of Pb(II), Cd(II), Hg(II), Co(II), Ni(II), Cu(II) and Zn(II) in 1,2-propanediol–water mixtures were reported. K. Bharath et al. [75] studied the chemical speciation of L-Histidine Complexes of Pb(II),Cd(II), and Hg(II) in DMSO-Water Mixtures at 303K and 0.16moldm^{-3} ionic strength. Some of these data are recorded in Table 4.

Table 3 Stability constants of metal-histidine complexes reported in literature

Metal ion	log β_{mlh}					μ(mol dm^{-3})	Calculation method	Instrumental technique	Ref
	110	111	120	121	122				
Pb(II)	6.70	---	---	---	---	0.16	MINIQUAD	pH metry	[76]
Cd(II)	4.10	---	7.05	---	22.36	0.16	MINIQUAD	pH metry	[76]
Hg(II)	12.13	14.81	---	---	---	0.16	MINIQUAD	pH metry	[76]
Co(II)	4.68	---	---	---	---	0.1	---	Potentiometry	[77]
Ni(II)	5.87	---	---	---	---	0.1	---	Potentiometry	[77]

Table 4: Stability constants of metal-glutamic acid complexes reported in literature.

Metal ion	log β_{mlh}					μ(moldm^{-3})	Calculation method	Instrumental technique	Ref
	110	111	120	121	122				
Cu(II)	10.12	14.16	18.10	23.85	---	0.1	EQUISPEC	Potentiometry	[78]
Cd(II)	6.48	---	11.10	---	---	3.0	---	---	[75]
Ni(II)	7.56	---	14.11	---	---	0.1	MINIQUAD75	Potentiometry	[75]
Pd(II)	18.83	---	---	23.98	---	0.1	SUPERQUAD	Potentiometry	[74]
Hg(II)	7.70	15.30	13.00	---	22.40	---	GEOCHEM-PC	---	[72]
Zn(II)	6.51	---	12.01	---	---	0.2	SCOGS	pH metry	[72]

2.7 STABILITY CONSTANTS OF TERNARY COMPLEXES

The formation constant of ternary complexes was calculated in a similar way using modified Irving equation. Simplified equations were arrived for the calculation of formation constants of mixed ligand complexes, when the two ligands simultaneously interacted with the metal ion [79]. These are valid only if the protonation constants of primary and secondary ligands are comparable. However, non-linear least squares algorithms were found to be suitable for studying the unprotonated or protonated mixed ligand complexes, even though the corresponding binary systems contain hydroxylated and/or protonated species.

Formation constants of mixed ligand complexes of Cu (II), Ni(II), Co(II) and Zn(II) with Aspartic or glutamic acid as primary ligand and imidazole as secondary ligand were determined potentiometrically [80]. Voltammetry technique was used to study the binary and ternary complexes of cadmium with L-glutamic acid and L-aspartic acid as primary ligands and L-ascorbic acid as secondary ligand [81]. Imam Ahmed [82] determined the stability constants of ternary systems of Zn(II), Ni(II), Co(II), Cd(II), Pb(II), and La(III) pH-metrically with N-(2-acetamido) iminodiacetic acid and dicarboxylic amino acids (aspartic and glutamic) as primary ligands and 3-amino-5-mercapto-1, 2, 4-triazole as a secondary ligand. Stability constant of mixed ligand complexation of Mn, Fe and Ni with H as a primary ligand and E, proline and aspartic acid as secondary ligands, 1:5:5 metals to ligand ratios were studied pH metrically using 0.1 mol dm^{-3} $NaClO_4$ as the ionic strength [83]. A brief account of some of the typical systems reported in literature is given in Table 5.

Table 5: Mixed ligand complexes reported in literature.

System	log β_{mlxh}							
	1110	1111	1112	1120	1121	1210	μ(moldm^{-3})	Ref.
Co(II)-Glu-Met	7.76	-	-	10.33	-	-	0.1	[84]
Ni(II)-Glu-Met	9.62	16.67	-	-	-	12.98	0.1	[84]
Cu(II)-Glu-Met	15.18	19.12	23.99	-	-	-	0.1	[84]
Cd(II)-Gly –His	9.11	-	-	-	-	-	0.2	[85]
Ni(II)-Gly Leu-His	16.91	-	-	-	-	-	0.2	[85]
Zn(II)-Cys-His	15.23	21.60	-	-	26.50	-	0.15	[86]
Cu(II)-Cys-His	18.51	25.80	30.69	-	39.15	-	-	[86]
Cu(II)-Glu-Asp	15.63	-	-	-	-	-	-	[87]
Cu(II)-Glu-Gly	15.10	-	-	-	-	-	-	[87]
Zn(II)-Glu-Pyr	6.97	-	-	10.64	-	-	0.01	[88]
Hg(II)-Glu-Met	16.32	-	25.12	-	-	-	-	[88]

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials and Apparatus

Burette, burette stand, funnel, mass balance, measuring cylinder, Volumetric pipette, pH meter, 250mL, 500ML and 1000mL Erlenmeyer flak, test tube, magnetic stirrer, stirrer bar, 100ml & 250ml Beakers

3.2 Chemicals and Reagents

Triply distilled deionized water, Eriochrome black-T, Nitric acid, EDTA, Methanol, Acetone, Xylenol orange, hexamethylenetetramine powder, Sodium Hydroxide pellet, sodium nitrate, Oxalic acid, potassium hydrogen phthalate, Zn(II) sulphate, borax, diethyl ether, DMF, L-Histidine, L-Glutamic acid, Lead Nitrate, Cadmium Nitrate and Mercury Nitrate

3.3 EXPERIMENTAL

3.3.1 Preparation of Solutions

All the solutions were prepared in distilled water. 'A' grade glassware was used throughout the experimentation.

3.3.1.1 L-Histidine and L-Glutamic Acid Solutions

0.05 mol dm^{-3} aqueous solutions of L-Histidine GR grade (SRL, India) and L-Glutamic acid GR grade (SRL, India) were prepared by dissolving sample in distilled water. To increase the solubility of ligands, 0.05 mol dm^{-3} nitric acid concentration was maintained in the solutions. The pessimistic errors in the preparation of the ligand solutions by weight method do not exceed 0.1%. The computer program COSWT was determine the probable errors that may creep into the concentrations of the stock solutions of the ligands [89].

3.3.1.1.2 EDTA Solution

Disodium salt of EDTA was purified by precipitation from aqueous solution using methanol. The precipitate was washed with acetone, diethyl ether and finally air-dried. An approximately

0.1 mol dm^{-3} solution was prepared and standardized complexometrically, with a 0.1 mol dm^{-3} standard Zn (II) Sulphate solution using Eriochrome Black-T as indicator [90].

3.3.1.1.3 Metal Ion Solutions

0.1 mol dm^{-3} aqueous solutions of lead (II), cadmium (II) and mercury (II) nitrates were prepared by dissolving their respective salts in triply distilled deionized water. The stock solutions make slightly acidic to repress hydrolysis of the metal ions. The concentrations of the metal ions were determined complexometrically by titrating against a standard solution of EDTA using the Xylenol orange as indicator and hexamethylenetetraamine powder as buffer to maintain the pH at 5-6 [91]. The free hydrogen ion concentration in the stock solution was determined by Gran plot method.

3.3.1.1.4 Sodium Hydroxide

A stock solution of 2.0 mol dm^{-3} sodium hydroxide was prepared by dissolving GR grade (Merck, India) sodium hydroxide pellets in triple distilled water. The solution was further diluted to the required concentration of 0.4 mol dm^{-3}. The strength of alkali was determine using the Gran plot method. So as to assess the errors that might have crept into the determination of the concentrations, the data were subjected to analysis of variance of one way classification (ANOVA) using the computer program COSWT [92].

3.3.1.1.5 Nitric Acid and Sodium Nitrate

An approximately 2-mol dm^{-3} nitric acid and sodium nitrate solution was prepared and diluted further to 0.2 mol dm^{-3}. The concentration of stock Nitric acid (GR grade, India) solution was calculated from its specifications (specific gravity, purity and molecular weight) and diluted to the required concentration by dissolving in triple distilled water. Its strength was determined by titrating with a standard sodium hydroxide and borax solutions. In most equilibrium studies of metal complexes formation, the equilibrium constant was evaluated from measurements on solutions containing various concentration of M^{2+} and L^{-}. Such concentration changes was produced changes in the ionic strength (μ) of the solutions. To keep the ionic strength constant, a large excess of an unreactive ionic salt is added to the solutions. The salt is added only to maintain the ionic strength of the medium and should not interact directly with M^{2+} or L^{-}. In this experiment, aqueous solution of 0.2-mol dm^{-3} sodium nitrate was prepared to maintain the ionic strength in the titrand.

3.3.2 N, N-Dimethylformamide

N, N-Dimethylformamide (Qualigens, India) of 99% purity were used as received.

3.3.3 Electrometric Titration Assembly

An ELICO (Model Ad8000, India) pH meter of readability (-2.00 to 16.00 pH) in conjunction with a glass combination pH electrode was used to monitor changes in hydrogen ion concentration during either acido-basic or complex equilibria. The accuracy of the instrument was ±0.01pH unit and 310 K temperature maintain for all the titrations. The solutions left to stand for about 5 minutes before each titration. A magnetic stirrer will used during the titrations.

3.3.3.1 Calibration of Glass Combination pH. Electrode

For very precise work, the pH meter calibrated before each measurement by standard buffer solutions that span the range of pH values to be measured. In these studies, potassium hydrogen phthalate (0.05 mol dm^{-3}) and borax (0.01 mol dm^{-3}) solutions was used in order to preliminarily check the response of the glass electrode in acidic (pH=4.01) and basic (pH=9.18) regions [93]. The electrode system was calibrated in terms of hydrogen ion concentrations instead of activities. It is assumed that the activity coefficient is constant, an assumption usually justified by working in a medium of a constant ionic strength (0.16 $moldm^{-3}$ sodium nitrate). In electrometric studies, the glass electrode response was established over a wide range of pH. The acid-base titrations were analyzed by using the Gran plot method [45]. In the gran plot method the functions ϕ and ϕ' are calculated by using the formulae

$\phi = (V_0+V_i)\ 10^{-pH}$ applied for the pH data before equivalence point,

$\phi' = (V_0+V_i)\ 10^{pH}$ applied for the pH data after equivalence point.

A typical Gran plot is given in Figure. 2.1.

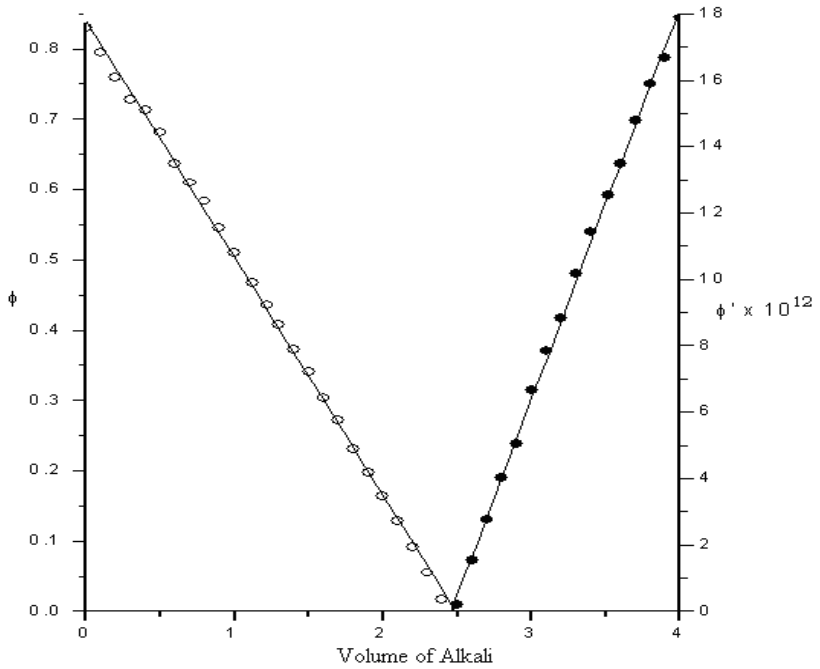


Figure 6. Gran plot for the titration of nitric acid with alkali

3.3.3.2 Equilibration of pH meter in Aqua-Organic media

In order to obtain steady and reproducible results in aqua-organic media the electrode has to be pre-equilibrated with the respective media. In the present study, the glass combination pH electrode was immersed in a well-stirred mixture of required composition of DMF-water mixture for two days. The electrode was tested intermittently by titrating a strong acid with sodium hydroxide in the medium of identical composition. The electrode equilibration was continued if the residuals between the pH meters dial readings and the calculated pH values were not constant. Before each titration in organic media, the electrode was pre-conditioned for at least one hour in a solution of identical composition as that of the titrand. This practice resulted in a reasonably consistent and appropriate hydrogen ion activity in the outer layer of the glass membrane.

3.3.3.3 Determination of Correction Factor

The effect of variation in asymmetry, liquid junction potential, activity coefficient, sodium ion error on the response of pH meter is to be consider for accurate determinations. Correction factor proposed by McBryde and others [93] to account for the above parameters used in the present electrometric titrations. The simulated acid-base titrations data (pHC_i) calculate by SCPHD program will used to compute correction factor (log F) for each of the solvent

compositions [94]. The log F value is used to convert pH meter dial reading into logarithm of reciprocal of hydrogen ion concentration (pHEi) according to the equation.

$$\text{Log F} = \text{pHCi} - \text{pHEi}$$

Log F was found to remain constant only in a narrow pH range (≈1.7-2.8) as the pH meter dial readings become unreliable after about 80% neutralization of the strong acid [95]. Each point in the titration data past the equivalence point was corrected for sodium ion error (~<0.05 units) using the data available for aqueous system.

3.4 TITRATION PROCEDURE

Initially strong acid was titrated against alkali at regular intervals to check the complete equilibration of the glass electrode. Titrations were carried out in media containing varying amount of organic solvent (0.0-50.0% v/v) maintaining an ionic strength (μ) of 0.16 mol dm^{-3} with $NaNO_3$ at 310 K. In these titrations, the titrand consist of mineral acid (HNO_3) and ligand (L- Histidine and L- Glutamic acid), in the absence and presence of metal ion, in a total volume of 50 cm^3. It will be keep mixed by stirring with magnetic stirrer. Titrations were performed by adding each time 0.1cm^3 portions of sodium hydroxide (0.4 mol dm^{-3}) to the titrand. The pH meter reading was record only after a constant value was displayed [96]. Typical duplicate titrations showed that equilibration is fast and titration data do not differ by more than 0.02 units

3.4.1 Proton - Ligand Equilibria

The requisite amounts of sodium nitrate (to give overall concentration of 0.16 mol dm^{-3}) water and organic solvent (DMF) were added such that the overall percentages of DMF were in the range of (0.0-50% v/v). The amount of mineral acid maintained was 1 mmol, while the concentration of ligand was of the order of 0.25-0.50 mmol in different experiments. The initial concentrations of the ingredients used in the experiments are summarized in Table 6.

Table 6 : Total initial concentration of ingredient (in mmol) in proton ligands. [NaOH]=0.4 moldm^{-3}, V_0=50cm^3; Temperature 310 K; Mineral acid =1mmol ionic strength = 0.16 mol dm^{-3}

(% v/v) of DMF	No of titration	TL0	
		L-Histidine	L-Glutamic acid
0.0	3	0.252	0.250
		0.376	0.376
		0.501	0.503
10.0	3	0.251	0.250
		0.374	0.374
		0.502	0.502
20.0	3	0.254	0.250
		0.377	0.376
		0.503	0.501
30.0	3	0.250	0.251
		0.378	0.375
		0.501	0.502
40.0	3	0.253	0.252
		0.374	0.375
		0.500	0.501
50.0	3	0.250	0.252
		0.374	0.375
		0.501	0.501

TL_O = Number of mmols of ligand.

3.4.2. Metal-Ligand Complexes

In each of the titrations, the titrand consisted of a mineral acid (nitric acid) of approximately 1 mmol in a total volume of 50 cm^3 and the ionic strength was adjusted at 0.16 mol dm^{-3} with sodium nitrate. Solutions containing metal ions and ligands (metal to ligand ratios being in the range of 1:2.50, 1:3.75 and 1:5.0 for Pb (II), Cd (II) and Hg (II)) were titrated with 0.4 mol dm^{-3} sodium hydroxide. In these entire titrations, metal ion was the last component added to the titrand.

The actual concentrations of the constituents in different percentages of the DMF are given in Table 7. In order to minimize local build-up of alkali concentration which results in hydrolysis of the species, the burette tip was kept as near the solution as possible, exercising care to avoid diffusion of the titrand into burette. The upper pH limit of rejecting the data even for pre-processing was determined by the appearance of opalescence in the reaction mixture leading to precipitation. A downward drift of pH meter dial readings also indicated the initiation of precipitation.

Table 7: Total initial concentrations of ingredients (in mmol) for metal - ligand titrations in DMF-water mixtures. [NaOH] = 0.4 mol dm^{-3}; V_0 = 50 cm^3; Temperature = 310 K, Mineral acid = 1.0 mmol; μ =0.16 mol dm^{-3}

% v/v DMF	No of titration	TM0			TLo		$TL_0:TM_0$
		Pb(II)	Cd(II)	Hg(II)	H	E	
0.0	3	0.102	0.098	0.101	0.2500	0.2504	2.50
					0.3740	0.3743	3.75
					0.5013	0.5004	5.00
10.0	3	0.102	0.098	0.101	0.2504	0.2515	2.50
					0.3740	0.3762	3.75
					0.5010	0.5011	5.00
20.0	3	0.102	0.098	0.101	0.2503	0.2515	2.50
					0.3751	0.3771	3.75
					0.5022	0.5066	5.00
30.0	3	0.102	0.098	0.101	0.2500	0.2491	2.50
					0.3711	0.3784	3.75
					0.5020	0.4990	5.00
40.0	3	0.102	0.098	0.101	0.2485	0.2495	2.50
					0.3732	0.3760	3.75
					0.4900	0.4991	5.00
50.0	3	0.102	0.098	0.101	0.2496	0.2492	2.50
					0.3741	0.3760	3.75
					0.4972	0.4994	5.00

3.4.3 Mixed-Ligand Complexes

Titrations were carried out in the presence of different relative concentrations of metal (M) to primary (H, L) and secondary (E, X) ligands (M: L: X = 1:5:5, 1:5:10, 1:10:5) with sodium hydroxide. The other conditions are similar to those mentioned earlier. The actual concentrations of each of the ingredients are given in Tables 8. A preliminary investigation of alkalimetric titrations of mixtures of H and E, in different mole ratios, in the presence of mineral acid and inert electrolyte confirmed that these two ligands do not form any condensed species.

Table 8: Total initial concentrations of ingredients (in mmol) for mixed ligands (H and E) titrations in DMF-water mixtures; [NaOH] = 0.4 moldm^{-3}; V_0 = 50 mL; Temperature = 310 K; Mineral acid = 1.0 mmol; μ = 0.16 moldm^{-3}

% v/v DMF	TM_0			TL_0	TX_0	TM_0:TL_0:TX_0
	Pb(II)	Cd(II)	Hg(II)	H	E	
0.0	0.102	0.098	0.101	0.2497	0.2501	1:2.5:2.5
				0.2498	0.5000	1:2.5:5.0
				0.5001	0.2501	1:5.0:2.5
10.0	0.102	0.098	0.101	0.2497	0.2498	1:2.5:2.5
				0.2501	0.5000	1:2.5:5.0
				0.4997	0.2501	1:5.0:2.5
20.0	0.102	0.098	0.101	0.2501	0.2500	1:2.5:2.5
				0.2500	0.4995	1:2.5:5.0
				0.5001	0.2500	1:5.0:2.5
30.0	0.102	0.098	0.101	0.2499	0.2499	1:2.5:2.5
				0.2500	0.5001	1:2.5:5.0
				0.4996	0.2500	1:5.0:2.5
40.0	0.102	0.098	0.101	0.2500	0.2501	1:2.5:2.5
				0.2497	0.4995	1:2.5:5.0
				0.5001	0.2501	1:5.0:2.5
50.0	0.102	0.098	0.101	0.2501	0.2501	1:2.5:2.5
				0.2495	0.4999	1:2.5:5.0
				0.4997	0.2501	1:5.0:2.5

3.5. PROCESSING OF DATA AND MODELING STRATEGY

3.5.1. Speciation by Computer Modeling

Chemical speciation is often studied by using experimental methodology; however, an alternative approach involves the application of theoretical chemical concepts to predict the distribution and transformations of chemical species, usually by calculating the concentrations of species in equilibrium. For equilibria in solution, usually the equilibrium constants are measured for the individual reactions and the species concentrations are calculated by solving the mass-balance equations [97]. Thus, it is essential that the formation constants used in any chemical speciation modeling be critically evaluated. In evaluating formation constants from any source, factors that are considered include experimental method, purity of reagents, background electrolyte, reagent concentrations, ionic strength and computational procedures.

3.5.2 Software for Modeling Complex Equilibria

Glass electrode measures protons associated with the solvent (including those in looser combinations). G . R a m a [1] revealed that potentiometry using glass electrode gained wide popularity in the determination of equilibrium constants in preference to Spectrophotometry, because the numbers of unknown quantities (extinction coefficient and stability constant) are double in the latter. However, with the advances in optimization methods for a large number of variables, model-free data treatment and automation in data acquisition systems, Kaden and Zuberbuhler advocated [98] hyphenated Spectro-pH meter. Hence, the approximate protonation constants of H and E were calculated with the computer program SCPHD [99]. The best-fit chemical model for each system investigated was achieved at using non-linear least-squares computer program, MINIQUAD75 [100], which exploits the advantage of constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm. The variation of stepwise protonation constants (logK) with the dielectric constant of the medium was analyzed on electrostatic grounds for the solute-solute and solute-solvent interactions.

3.5.3 Formulation of Mathematical Model

For simplicity of mathematical treatment, the interaction of a metal ion with a ligand (LH_h) is considered and the same treatment can be extended to other species encountered in complex equilibria of mononuclear complexes summarized [101] in Table 9.

Table 9: Types of species likely to be encountered in complex equilibria of mononuclear complexes

Species	Stoichiometric coefficients			
	m	l	h	x
Metal hydrolysis	1	0	<0	0
Binary complexes (a) Unprotonated, unhydroxylated (ML_n)	1	>0	0	0
(b) Protonated	1	>0	>0	0
(c) Hydroxylated	1	>0	<0	0
Ternary complexes	1	>0	>0	>0

M, l, x, h: Stoichiometric coefficients of metal, primary ligand, secondary ligand and hydrogen, respectively

Eq. 3.1 represents the interaction of metal ion, with most anionic form of the ligand and proton in solution phase results in NB complexes and the equilibrium for the formation of the jth species.

$$m(j)\,M + l(j)L + h(j)H \rightleftharpoons M_{m(j)}L_{l(j)}H_{h(j)} \qquad \text{----------}\mathbf{3}.1$$

Then the formation constant for the j^{th} species can be written as

$$\beta_{m(j)l(j)h(j)} = \frac{M_{m(j)}L_{l(j)}H_{h(j)}}{FM_i^{\,m(j)} * FL_i^{\,l(j)} * FH_i^{\,h(j)}} = \frac{CS_{j,i}}{FM_i^{\,m(j)} * FL_i^{\,l(j)} * FH_i^{\,h(j)}} \qquad \text{----------}3.3$$

Where, FM_i and FL_i are the free concentrations of metal ion and ligand and $CS_{j,i}$ is the concentration of j^{th} complex at i^{th} experimental point: h(j) is negative for hydroxylated species. A titrand of total volume, V_0 containing an indifferent electrolyte, V_m cm^3 of metal ion (C_M mol dm^{-3}) and V_l cm^3 of ligand (C_L mol dm^{-3}) is titrated with alkali of concentration, ALK. If β_i is the pH meter dial reading after equilibration (detected by successive reading not differing by 2 or 3 times the readability of pH meter) for V_i cm^3 of alkali added, the total concentrations at i^{th} experimental point are given by

$$\left.\begin{aligned} TME_i &= V_m * C_M / (V_0 + V_i) = TMO * V_0 / (V_0 + V_i) \\ TLE_i &= V_l * C_L / (V_0 + V_i) = TLO * V_0 / (V_0 + V_i) \\ THE_i &= \frac{V_A * C_A}{V_0 + V_i} - \frac{ALK * V_i}{V_0 + V_i} + NDP * TLE_i + OH_i \end{aligned}\right\} \qquad \text{----------}3.3$$

Where TM_O and TL_O are the analytical concentrations of metal and ligand in V_0 cm^3 of solution. The calculated total metal ion concentration is

$$TMC_i = FM_i + \sum_{j=1}^{NB} m(j) * \beta_m(j)l(j)h(j) * FM_i^{\,m(j)} * FL_i^{\,l(j)} * FH_i^{\,h(j)} = FM_i + \sum_{j=1}^{NB} m(j) * CS_{j,i} \qquad \text{----------}3.4$$

Similarly, for ligand and hydrogen ion, the corresponding equations are

$$\left.\begin{aligned} TLC_i &= FL_i + \sum_{j=1}^{NB} l(j) * CS_{j,i} \\ THC_i &= FH_i + \sum_{j=1}^{NB} h(j) * CS_{j,i} + OH_i \end{aligned}\right\} \qquad \text{----------}3.5$$

If the free concentrations of all the three ingredients (ligand, metal and hydrogen ion) are monitored, it is sufficient to consider NB points. Solving of simultaneous linear equations [1] gives the formation constants. If several data points NP, are available then ${}^{NP}C_{NB}$ sets of

stability constants result and therefore, some statistical criteria are to be applied to arrive at the best parameters. In many cases, the determination of metal ion and free ligand concentration becomes difficult. Then hydrogen ion concentration is the only parameter that can be monitored and hence, non-linear mass balance equations have to be solved.

A large number of computer programs are available ranging in application from calibration of glass electrode to modeling strategies [102] applicable to binary complexes containing protonated, hydroxylated and polynuclear species. All the programs adopt this mathematical model for the above tasks.

3.5.4 Preprocessing Of Volume versus pH. Data

For the overlapping equilibria of the formation of two proton-ligand complexes shown in Equi. 3.6 and 3.7.

$$L + H \rightleftharpoons LH; \quad K_1^H = [LH]/[L][H] \quad \text{----------3.6}$$

$$LH + H \rightleftharpoons LH2; \quad K_2^H = [LH_2]/[LH][H] \quad \text{----------3.7}$$

Secondary formation function ($\bar{n}_H$) - average number of protons bound per mole of ligand and Eq. 3.8 relates the equilibrium constants.

$$\bar{n}_H = \frac{\beta_{011} * FH_i + 2 * \beta_{012} * FH_i^2}{1 + \beta_{011} * FH_i + 2 * \beta_{012} * FH_i^2} \quad \text{----------3.8}$$

Where, $\beta_{011} = K_1^H$ and $\beta_{012} = K_1^H * K_2^H$ and FH_i is the free hydrogen ion concentration.

One of the several algebraic transformations [103] useful for the determination of equilibrium constants is:

$$\frac{\bar{n}_{Hi} - 1}{(2 - \bar{n}_{Hi}) * FH_i} = \frac{\beta_{012}}{\beta_{011}} - \frac{\bar{n}_{Hi}}{(2 - \bar{n}_{Hi}) * FH_i^2} * \frac{1}{\beta_{011}} \quad \text{----------3.9}$$

It may be represented as

$$Y_i = \beta_{012} * X_i + (-\beta_{011}) \quad \text{----------3.10}$$

The unweighted linear least squares treatment of Eq. 3.9 finds wide applicability in the estimation of β_{011} and β_{012}. For non-overlapping equilibria ($|\log K_{i+1} - \log K_i| > 3$), stepwise stability constants at each point can also be calculated as

$$\log K_1 = pH_i + \log[\bar{n}_H / (1 - \bar{n}_H)] \text{ and}$$

$$\log K_2 = pH_i + \log[(\bar{n}_H - 1)/(2 - \bar{n}_H)]$$

Alternatively, the generalized equation can be written as

$$\log K_n = pH_i + \log\left[\left(\bar{n}_H - n + 1\right)/\left(n - \bar{n}_H\right)\right]$$ ---------- --3.11

The computer program, SCPHD [89] employs Eq. 3.12.

$$\bar{n}_{Hi} = (NDP_i + TLE_i + E_i + OH_i - ALK_i - FH_i)/TLE_i$$ ----------3.12

For the calculation of paired data ($\bar{n}_{Hi,}$ pH_i). Here NDP, TLE_i, E_i and OH_i are the number of dissociable protons of the ligand, total ligand concentration, total acid concentration and concentration of hydroxide ion due to auto ionization of water, respectively. From this secondary data, the stepwise stability constants are calculated from Eq. 3.11.

The output of SCPHD in the present investigation was used (i) to prune the data in different experiments so that it contains more information than noise and (ii) to utilize log β_{011}, log β_{012} etc. as approximate (or initial) values for the refinement. Similar to $\bar{n}_H$ for proton-ligand complexes, secondary formation function $\bar{n}_i$ (average number of ligands bound per mole of metal ion) can be calculated by Eq. 3.13.

$$\bar{n}_i = \frac{\sum_{l=1}^{NML} l * (ML_l)_i}{FM_i + \sum_{l=1}^{NML} (ML_l)_i} = \frac{\sum_{j=1}^{NML} j * \beta_j * FL_i^{l(j)}}{1 + \sum_{j=1}^{NML} \beta_j * FL_i^{l(j)}}$$ ----------3.13

Eq. 3.13 can be transformed into Eq. 3.14

$$\bar{n}_i = \frac{TLE_i * \left(\bar{n}_{Hi} - NDP\right) - (E_i + OH_i - ALK_i - FH_i)}{\bar{n}_{Hi} * TME_i}$$ ----------3.14

The value of $\bar{n}_{Hi}$ at FH_i is calculated using the Eq. 3.12, with the stability constants of proton-ligand complexes in the best-fit model and the concentration of the free ligand (hence PL) is calculated from Eq. 3.15.

$$FL_i = \frac{TLE_i - \bar{n}_i * TME_i}{1 + \sum_{j=1}^{NML} \beta_j * FL_i^{l(j)}}$$ ----------3.15

This algorithm was used in SCPHD to calculate the paired data ($\bar{n}_i$ Vs PL_i).

CHAPTER 4

RESULTS AND DISCUSSIONS

SECTION I

4.1 PROTONATION EQUILIBRIA OF L-HISTIDINE AND L-GLUTAMIC ACID

Knowledge of the acido-basic properties of amino acids is extremely important in understanding and analyzing the properties of proteins. Further, separation, identification, and quantification of different amino acids and determining their sequence in proteins are based on their acido-basic behavior. Some amino acids act as tridentate ligands. When the third donor site of the amino acid is a nitrogen atom, marked tridentate behavior is frequently found [32]. L-Histidine (H) and L-Glutamic acid (E) act as tridentate ligands resulting in six membered ring structures. L- Histidine is a basic amino acid, containing weakly basic imidazole group. It has three potential metal-binding sites, namely, carboxylate oxygen ($O_{caboxyl}$), imidazole imido nitrogen (N_{im}) and amino nitrogen (N_{am}) [35].

E has three functional groups and all are protonated, in the order of amino followed by two carboxylate groups. E coordination may also occur from the carboxylate oxygen and thus overall it may show tridentate behavior. Nitrogen donor atoms can associate with hydrogen ions in physiological pH ranges and there is often significant competition between hydrogen and metal ion for the donor sites [39]. This situation results in the simultaneous existence of a number of equilibria, producing an array of successively protonated complexes. Therefore, an understanding of the protonation-deprotonation equilibria of H and E is necessary before an attempt is made to investigate the metal-ligand equilibria associated with these ligands.

4.1.1 Preprocessing of Data

The alkalimetric titration curves for H and E in DMF-water mixtures are shown in Figures 7 - 9. A perusal of the titration curves reveals that the acido-basic equilibria are active in the pH range 2 -10. The computer program SCPHD [99] was used to prune the data obtained in different experiments so that it contains more information than noise and the log βs obtained from the program are used as initial values for final refinement.

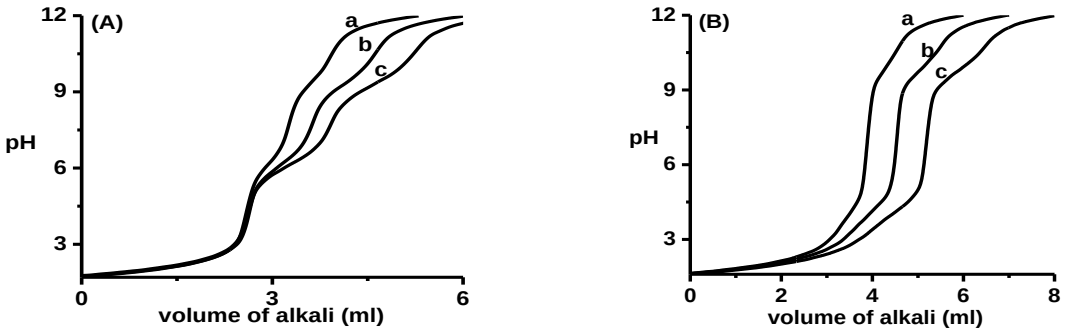


Figure 7: Alkalimetric titration curves for (A) H and (B) E in aqueous medium where: Number of mmols of H and E are a) 0.250, b) 0.375 and c) 0.500.

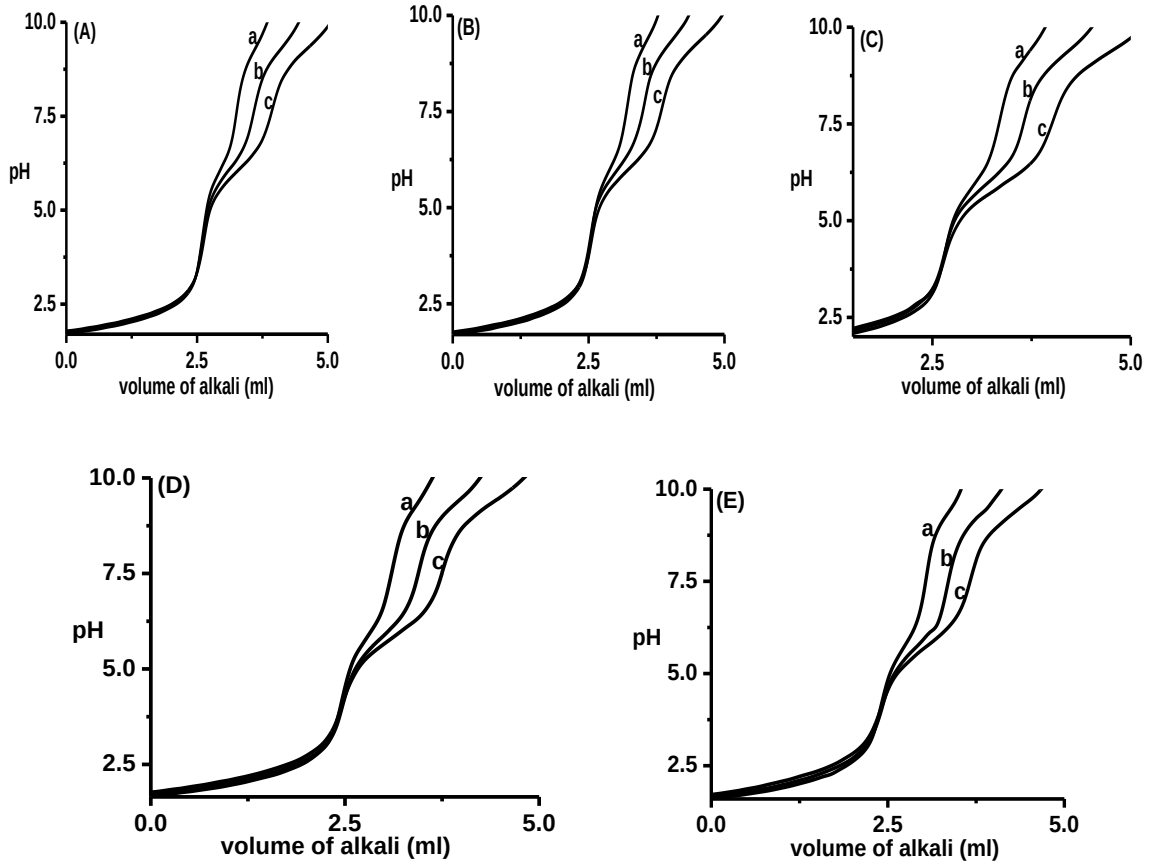


Figure 8: Alkalimetric titration curves for L-Histidine in DMF-Water mixtures; (A) 10%, (B) 20%, (C) 30%, (D) 40%, and (E) 50%. Number of mmols of H: a) 0.250, b) 0.375 and c) 0.500.

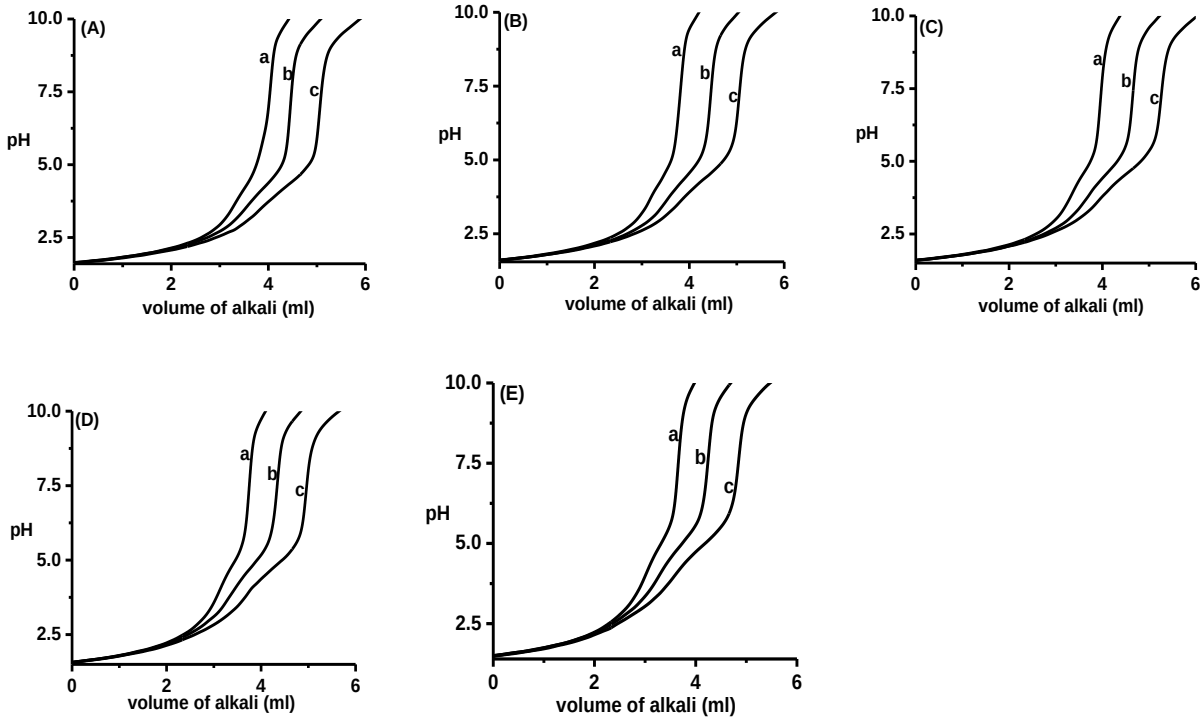


Figure 9: Alkalimetric titration curves for L-glutamic acid in DMF- Water mixture: A) 10 %, (B) 20%, (C) 30 %, (D) 40%, and (E) 50%. Number of mmols of H: a) 0.250, b) 0.375 and c) 0.500.

Secondary formation functions

The alkalimetric titration data are simulated using the model parameters given in Table 11 and they are compared with the experimental data, to verify the sufficiency of the model. The overlap of the typical experimental and simulated titration data Figure 10 indicates that the proposed models represent the experimental data.

Most of the numerical and graphical methods developed for the calculations of equilibrium constants are based on or related to the quantity called the complex formation function. Complex formation involves disappearance of certain ions, which form a complex compound. Simultaneous neutralization and proton liberation are due to auto-ionization of ligand. Irving and Rossotti [104] systematized the formulae representing these two parallel reactions by introducing a function, formation function, $\bar{n}_H$. It is the average number of moles of protons bound per mole of uncomplexed ligand. It is a useful parameter for the detection of polymeric species (L_2H_x). If there are no polymeric species a plot of $\bar{n}_H$ versus pH should overlap. Any deviation indicates the presence of polymeric species. Figure 11 shows that there is no

polymerization of H and E. The pH values at half integrals of $\bar{n}_H$ values correspond to the pKa values of the amino acids. The pH metric method is used for the determination of protonation constants or stability constants only when ligands are either weak organic acids or organic bases. If they are acids (eg. carboxylic acids and phenols), they have dissociable protons (NDP), equal to the number of functional groups. If the ligand contains basic functional groups like amino group, they abstract protons corresponding to the number of such functional groups from the medium. Such number is called number of associable protons (NAP). Another secondary function figure 11 and 13 relevant to protonation equilibria is the number of moles of alkali consumed per mole of ligand, **a**. We can detect whether the given ligand solution contains acid or not by using **a**. The negative values of **a** correspond to the excess of mineral acid present in the titrand and the number of dissociable protons whereas the positive values correspond to the associable protons. Figure 11C shows that one equivalent of alkali is consumed before attaining pH 4.2 and Figure 10D shows that two equivalent of alkali are consumed before attaining pH 7.0, indicates that deprotonation of the one carboxylic in H and two carboxylic groups in E. Another equivalent of alkali consumed after pH 7.0 corresponds to the deprotonation of the amino group. Figure 12 shows that one equivalent of alkali is consumed after attaining pH 7 this indicate that the deprotonation of amino groups.

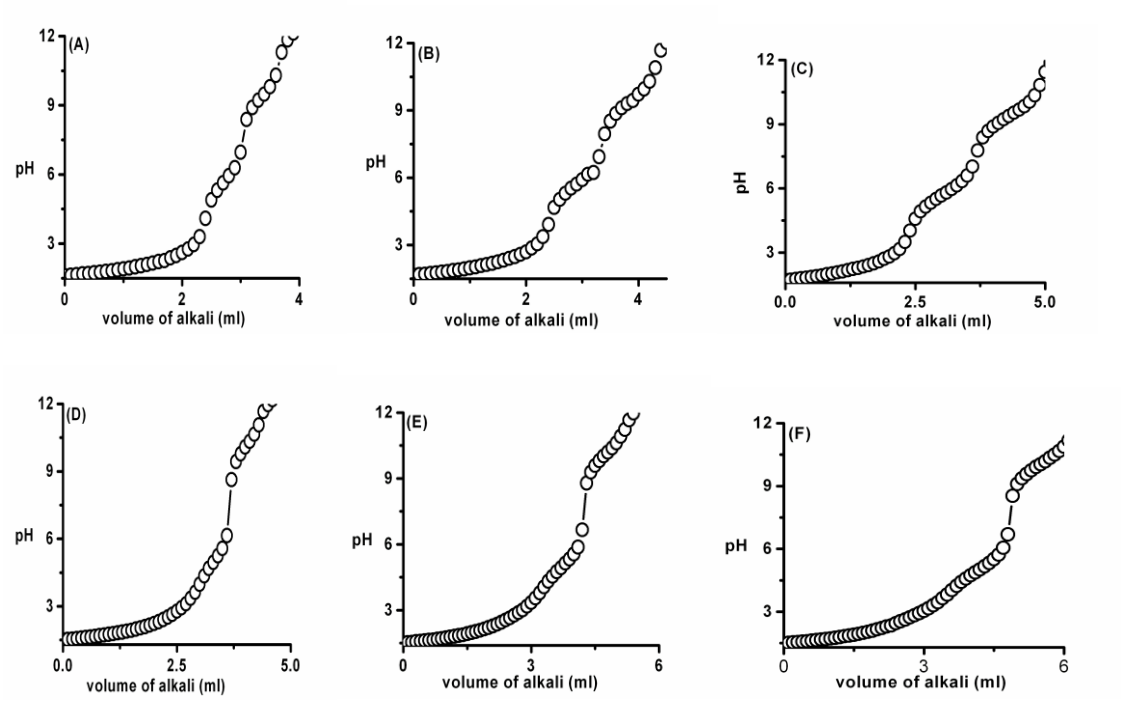


Figure 10: Simulated (solid line) and experimental (O) alkalimetric titration curves in 50% v/v DMF -water-mixtures: (A), (B) and (C) for 0.25, 0.375 and 0.50 mmols of H and (D), (E) and (F) for 0.25, 0.375 and 0.50 mmols of E respectively.

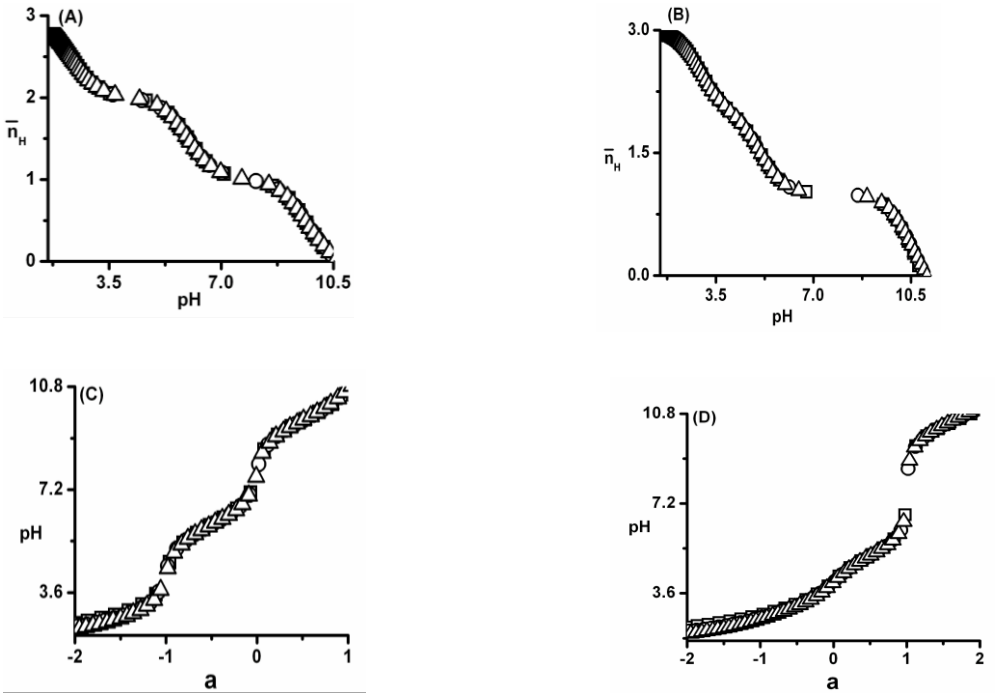


Figure 11: Formation curves of (A) H (B) E and number of moles of alkali (**a**) versus pH curves of (C) H (D) E in 40% v/v DMF-water mixture; . Ligand concentrations in mmols are 0.25(□-□), 0.375(O-O) and 0.5(Δ-Δ).

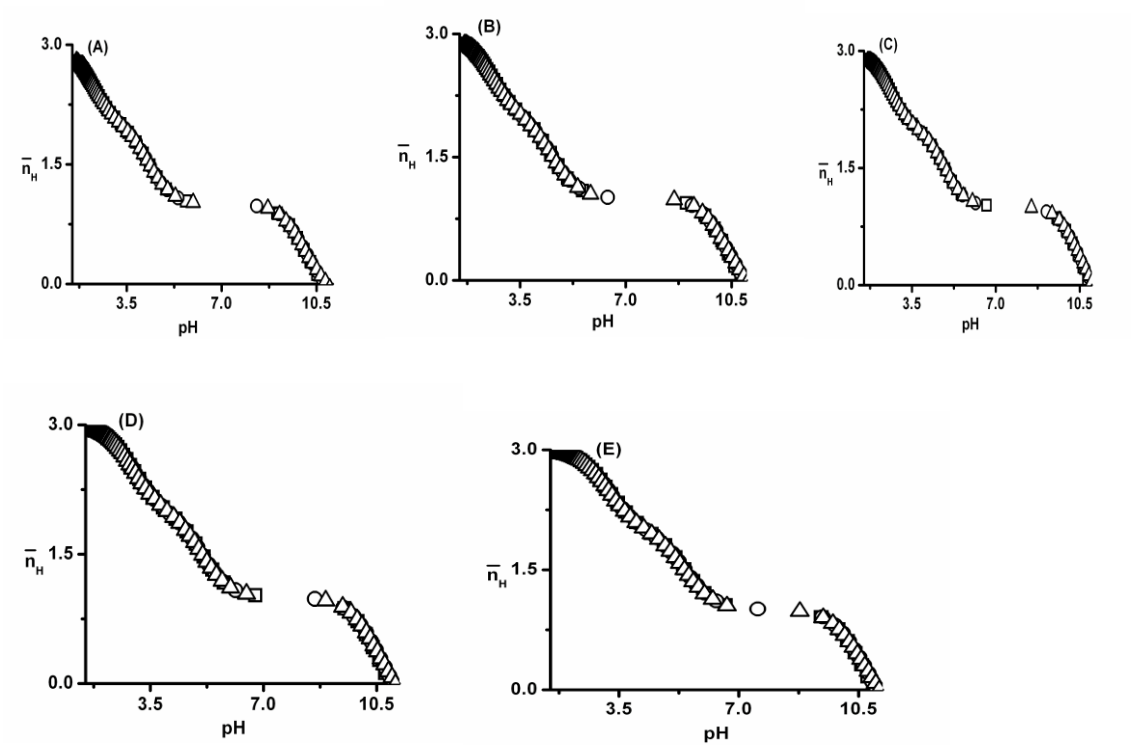


Figure 12: formation curves of E in DMF-water mixtures (% v/v); Where: (A) 10.0, (B) 20.0, (C) 30.0, (D) 40.0 and (E) 50.0. Ligand concentrations in mmols are 0.25(□-□), 0.375(O-O) and 0.5(Δ-Δ).

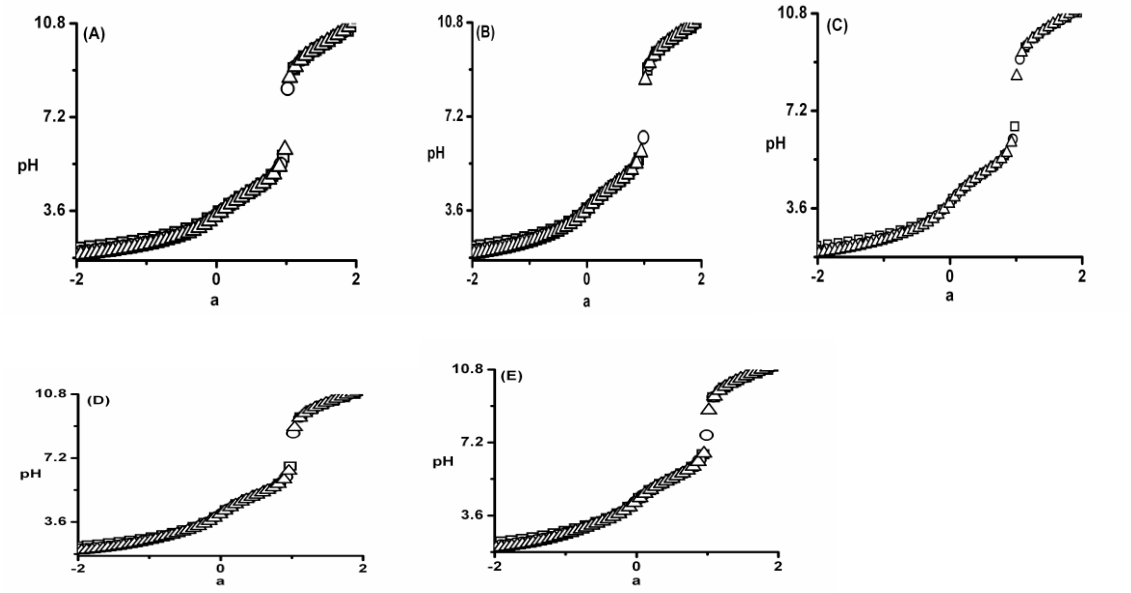


Figure 13: Variation of pH with a of E in DMF-water mixtures (% v/v); Where: (A) 10.0, (B) 20.0, (C) 30.0, (D) 40.0 and (E) 50.0. Ligand concentrations in mmols are 0.25(□-□), 0.375(O-O) and 0.5(Δ-Δ)

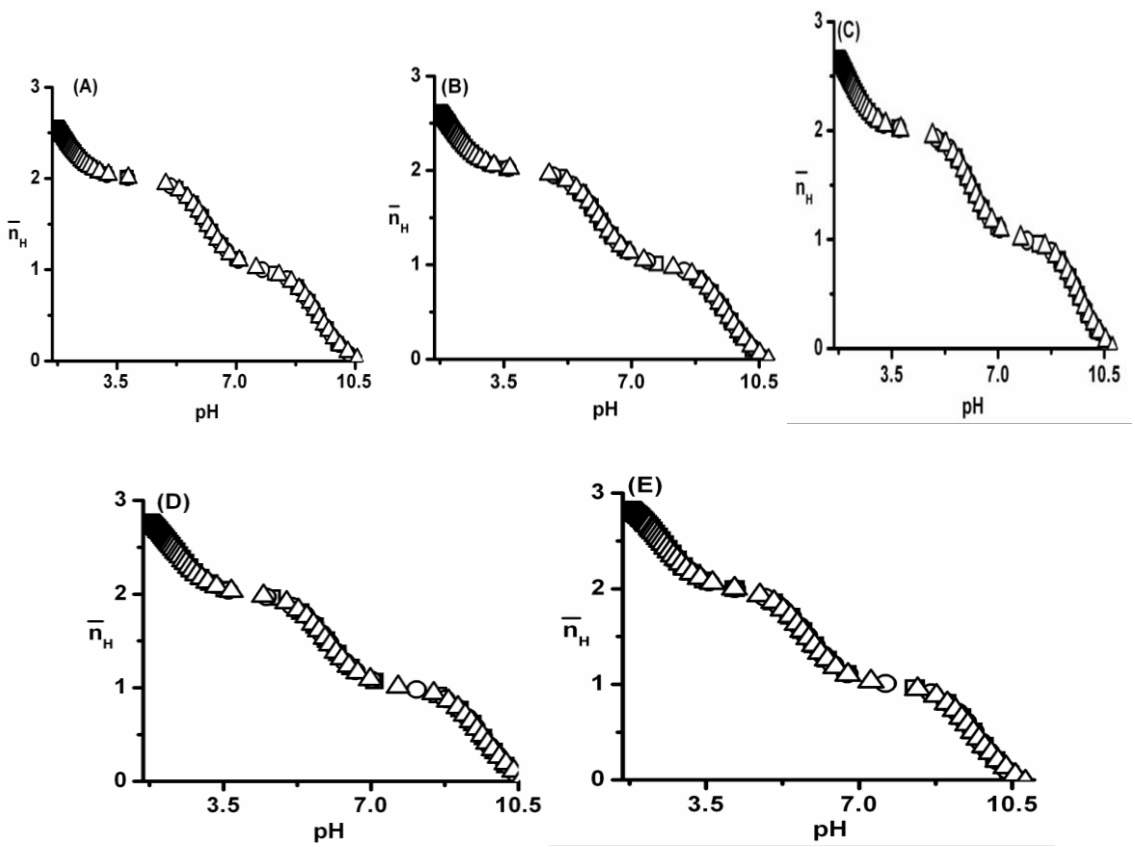


Figure 14: Formation Curves of H in DMF-water mixtures (% v/v);Where: (A) 10.0, (B) 20.0, (C) 30.0, (D) 40.0 and (E) 50.0 Ligand concentrations in mmols are 0.25(□-□), 0.375(O-O) and 0.5(Δ-Δ).

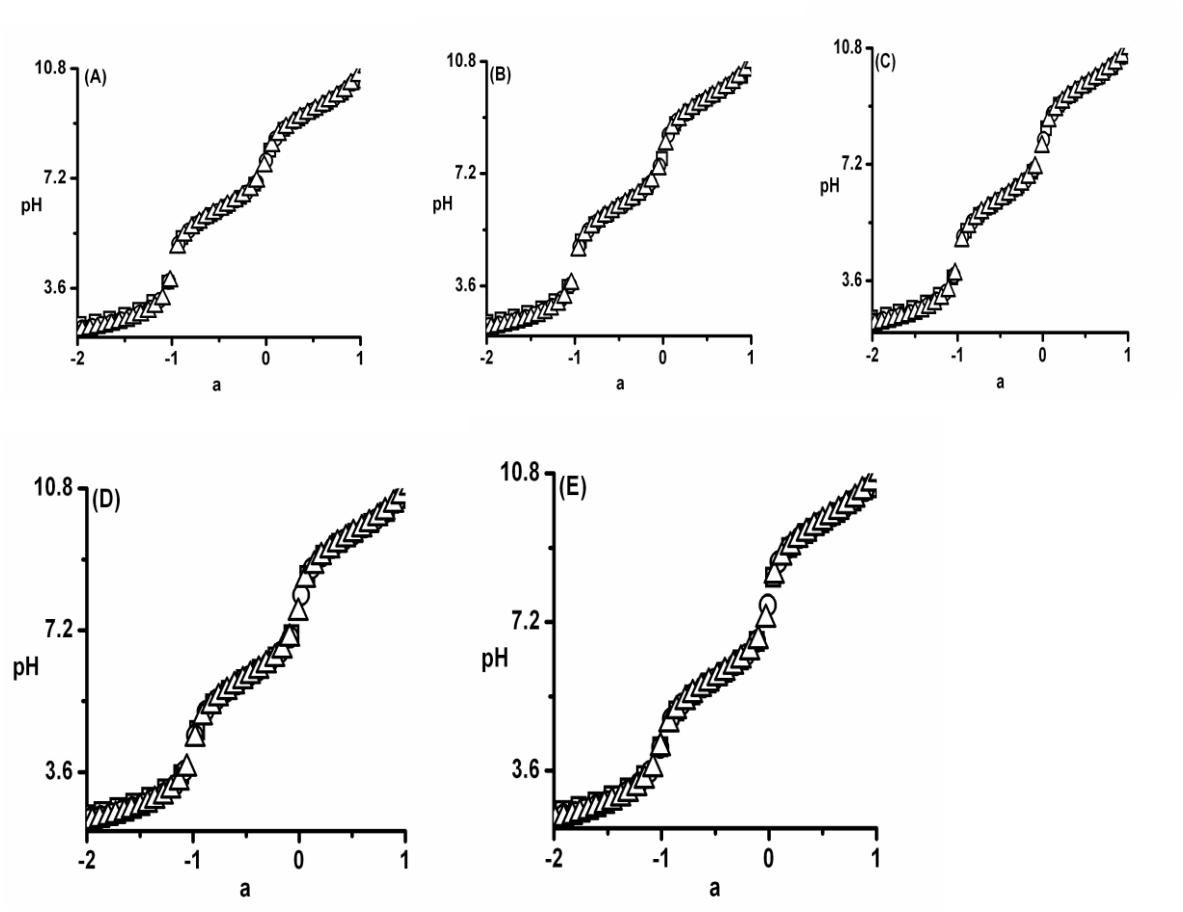


Figure 15: Variation of pH with **a** of H in DMF-water mixtures (% v/v); Where: (A) 10.0, (B) 20.0, (C) 30.0, (D) 40.0 and (E) 50.0. Ligand concentrations in mmols are 0.25(□-□), 0.375(O-O) and 0.5(Δ-Δ).

4.1.2 Mathematical Models for Proton Ligand Equilibria of L-Histidine and L-Glutamic Acid

The computer program MINIQUAD75 [89, 100] was used to refine protonation constants of ligand with DMF-water mixtures. The results of the best-fit model along with some of the important statistical parameters of H and E are given in Tables 11.

Table 10: Best-fit chemical models of protonation equilibria of L-Histidine and L- Glutamic acid in DMF-water mixtures

% of DMF	log β_{mlh}(SD)			NP	U_{corr} x10^8	Skewness	Kurtosis	$\chi 2$	R-factor
	LH	LH_2	LH_3						
H (pH range 1.72-10.28)									
0.0	9.24(0)	15.43(4)	17.16(1)	8.5	1.553	-0.32	3.26	12.87	0.0080
10.0	9.23(1)	15.30(1)	17.10(5)	8.5	1.342	-0.35	2.66	12.01	0.0092
20.0	9.27(3)	15.27(1)	17.23(4)	9.2	1.232	0.06	3.43	12.04	0.0034
30.0	9.35(3)	15.39(2)	17.40(4)	8.6	1.92	-0.83	3.31	12.33	0.0097
40.0	9.46(9)	15.40(8)	17.71(0)	9.9	2.44	-0.22	3.87	12.52	0.0042
50.0	9.48(4)	15.36(1)	17.72(1)	10.0	1.34	-0.37	2.99	11.99	0.0187
E (pH ranges 1.6-10.60)									
0.0	9.75(1)	14.00(1)	16.22(1)	80	3.27	-0.09	3.83	12.33	0.0084
10.0	9.83(0)	14.15(8)	16.40(0)	90	1.97	-0.02	3.03	11.97	0.0032
20.0	9.91(0)	14.50(9)	17.19(1)	77	3.24	-0.06	3.10	12.05	0.0036
30.0	10.06(1)	14.91(0)	17.73(3)	75	3.44	0.08	3.81	12.06	0.0045
40.0	10.23(6)	15.33(2)	18.26(5)	66	3.67	0.14	2.93	11.96	0.0072
50.0	10.21(9)	15.50(0)	18.70(6)	72	3.05	0.32	3.06	12.07	0.0067

U_{corr} = U/ (NP-m), m = number of species; NP =Number of experimental points; SD= Standard deviation

4.1.2.1 Statistical parameters of the best-fit models

A very low standard deviation in logβ values indicates the precision of these parameters. The small values of U (sum of squares of deviations in the concentrations of ligand and hydrogen ion at all experimental points) corrected for degrees of freedom indicates that the experimental data can be represented by the models [89].

In data analysis with least squares methods, the residuals (the differences between the experimental data and the data simulated based on the model parameters) are assumed to follow Gaussian or normal distribution. When the data are fit into the models, the residuals should be ideally equal to zero. If the statistical measures of the residuals and the errors assumed in the model are not significantly different from each other the model is said to be adequate [89]. Further, a model is considered adequate only if the residuals do not show any trend. Respecting the hypothesis of the least squares analysis, the residuals are tested for normal distribution. Such tests are $\chi 2$, skewness, kurtosis and R-factor.

A choice of an equilibrium model was based on adopting the lowest values of χ2, R-factor and Ucorr, taking into consideration the errors in experimental quantities and rules of propagation of errors. These statistical parameters of the present data show that the best-fit models portray the acido-basic equilibria of H and E in DMF-water mixtures as discussed below.

χ^2 test

χ^2 is a special case of gamma distribution whose probability density function is an asymmetrical function. This distribution measures the probability of residuals forming a part of standard normal distribution with zero mean and unit standard deviation. If the χ2 calculated is less than the table value, the model is accepted [89].

Crystallographic R-test

Hamilton's R factor ratio test [105] is applied in complex equilibria to decide whether inclusion of more species in the model is necessary or not. In pH metric method the readability of pH meter is taken as the R_{limit}, which represents the upper boundary of R beyond which the model bears no significance. When these are different, numbers of species the models whose values are greater than R-table are rejected. The low crystallographic R-values given in Tables 10 indicate the sufficiency of the model.

Skewness

It is a dimensionless quantity indicating the shape of the error distribution profile. A value of zero for skewness indicates that the underlying distribution is symmetrical. If the skewness is greater than zero, the peak of the error distribution curve is to the left of the mean and the peak is to the right of the mean if skewness is less than zero. The values of skewness recorded in Tables 10. are between -0.83 and 0.06 for H and -0.0 9 and 0.32 for E respectively. These data evince that the residuals form a part of normal distribution; hence, least-squares method can be applied to the present data.

Kurtosis

It is a measure of the peakedness of the error distribution near a model value. For an ideal normal distribution kurtosis value should be three (mesokurtic). If the calculated kurtosis is less than three, the peak of the error distribution curve is flat (platykurtic) and if the kurtosis is greater than three, the distribution shall have sharp peak (leptokurtic) [89]. The kurtosis values in the present study for both H and E indicate that the residuals form mainly leptokurtic pattern.

4.1.3 Effect of systematic errors

Any variations in the parameters like concentrations of ingredients affect the magnitudes of equilibrium constants. Such parameters are called influential or dangerous parameters. In order to rely upon the best-fit chemical model for critical evaluation and application, a brief investigation was made by introducing pessimistic errors in the concentrations of alkali, mineral acid, ligand and volume. This type of investigation is useful because the data acquisition was done under varied experimental conditions with different accuracies. Results of a typical system are given in Tables 11 emphasize that protonation constants associated with carboxyl proton are more affected. This may be due to their lower magnitude than those for amino protons. Similarly, errors in concentration of alkali and acid, affect more than volume. With the introduction of errors, the SD's are found to increase inferring the appropriateness of experimental conditions and correctness of analytical concentrations.

Table 11: Effect of errors in influential parameters on protonation constants in 50% v/v DMF-water mixture.

Ingredient	% Error	$\log\beta_{mlh}$(SD)					
		Histidine			**Glutamic acid**		
		LH	LH_2	LH_3	XH	XH_2	XH_3
	0	9.48(4)	15.36(1)	17.72(1)	10.21(9)	15.50(0)	18.70(6)
Alkali	-5	Rejected	16.15(48)	18.36(49)	10.64(35)	15.98(69)	Rejected
	-2	9.55(44)	14.60(77)	16.80(34)	10.33(41)	15.39(91)	18.55(66)
	+2	9.29(98)	14.22(34)	Rejected	Rejected	14.63(44)	17.60(90)
	+5	9.97(99)	Rejected	16.90(39)	Rejected	14.99(23)	17.99(26)
Acid	-5	9.32(88)	14.69(33)	Rejected	9.75(74)	14.29(76)	16.67(34)
	-2	9.27(80)	15.09(85)	16.88(69)	Rejected	14.74(44)	17.41(14)
	+2	9.66(40)	15.68(69)	16.81(99)	9.23(41)	15.29(99)	17.20(94)
	+5	9.72(59)	Rejected	18.57(55)	9.44(56)	15.73(77)	Rejected
Ligand	-5	9.33(28)	15.36(25)	17.59(32)	10.94(15)	15.77(10)	18.62(12)
	-2	9.37(27)	15.32(23)	17.50(36)	10.03(15)	14.90(19)	17.70(11)
	+2	9.41(26)	15.30(22)	17.38(31)	10.15(17)	15.07(12)	18.81(15)
	+5	9.44(27)	15.33(23)	17.30(35)	10.23(39)	15.19(16)	17.88(19)
Volume	-5	9.39(14)	15.38(19)	17.73(10)	10.18(20)	15.47(19)	18.83(23)
	-2	9.39(15)	15.38(11)	17.68(13)	10.19(27)	15.48(12)	18.78(15)
	+2	9.39(29)	15.38(14)	17.70(17)	10.19(26)	15.49(12)	18.72(14)
	+5	9.39(39)	15.38(18)	17.64(21)	10.20(10)	14.99(17)	18.67(21)

4.1.4 Effect of Dielectric Constant of Solvent on Protonation Equilibria

Ligand protonation constants are strongly affected by the dielectric constant of the medium because of the fact that at least one of the constituents is charged and other either is charged or has a dipole moment. Seleem *et al.* [106] and others reported the effect of the reaction medium on protonation equilibria of different ligands. The change in dielectric constant varies the relative contributions of electrostatic and non-electrostatic interactions.

The presence of co-solvent in the medium influences the protonation-deprotonation equilibria in solution due to change in the dielectric constant of the medium. The change in dielectric constant varies the relative contributions of electrostatic and non-electrostatic interactions. The values of stepwise protonation constants are given in Table 12. H contains carboxylic, imidazole imidonitrogen and amino protons. The different forms of H are LH_3^{2+}, LH_2^{+}, LH and L^{-} in the pH ranges 1.8-4.0, 1.8-6.0, 6.0-7.5 and 6.5-11.0, respectively. In the case of E contains two carboxylic and amino protons. The different forms of E are XH_3^{+}, XH_2, XH^{-} and X^{2-} in the pH ranges 2.0-4.2, 3.5-5.5, 3.0-10.5 and 7.4-11.0, respectively.

Table 12: Stepwise protonation constants of H and E in aqua-organic mixtures.

% v/v solvent	**L- Histidine**			**L- Glutamic acid**		
	log K_1	log K_2	log K_3	log K_1	log K_2	log K_3
0.0	0.046	0.222	0.965	0.063	0.157	0.989
10.0	0.048	0.219	0.965	0.064	0.158	0.992
20.0	0.052	0.216	0.967	0.073	0.165	0.996
30.0	0.053	0.216	0.970	0.075	0.170	1.002
40.0	0.060	0.211	0.975	0.075	0.175	1.009
50.0	0.062	0.209	0.0976	0.081	0.181	1.009

The variation of $\log K^M$ or change in free energy with co-solvent content depends upon two factors and can be represented as the sum of the changes in ΔG due to electrostatic and non-electrostatic factors.

$$\Delta G^t = -RT (\ln K^M - \ln K^W) \quad \text{----------4.1}$$

$$= G_{el} + \Delta G_{nel}$$

Born's and Classical treatment [107] holds good in accounting for the electrostatic contribution to the free energy change. According to this treatment, the energy of electrostatic interaction is related to dielectric constant, D of the medium and is given by

$$\Delta G_{el} = \frac{Ne^2}{Z} * \frac{1}{D} * \frac{1}{r} \quad \text{----------4.2}$$

Hence, Eqs. 4.1 and 4.2 suggest that logarithm of protonation constant should vary linearly as a function of reciprocal of the dielectric constant of the medium. The change in dielectric constant varies the relative contribution of electrostatic and non-electrostatic interactions, which, in turn, vary the magnitudes of protonation constants. If the electrostatic forces alone operate, $\log K^M$ varies linearly as a function of reciprocal of the dielectric constant of the medium. In the present study, logKs of H and E increased linearly (Figure. 16) as a function of the reciprocal of the dielectric constant (1/D) of DMF-water mixtures. Since it has no polar groups, specific solvent-water interaction, charge dispersion and specific interaction of co-solvent with solute (indicated by the changes in the solubility of different species in the aqua-organic mixtures) account for the deviation of classical linear relationship of logK with 1/D

The cation stabilizing nature of co-solvents specific solvent-water interactions [108], charge dispersion and specific interactions with solute indicated by the changes in the solubility of different species in aqua-organic mixtures account for the deviation of classical linear relationship of logK with 1/D.

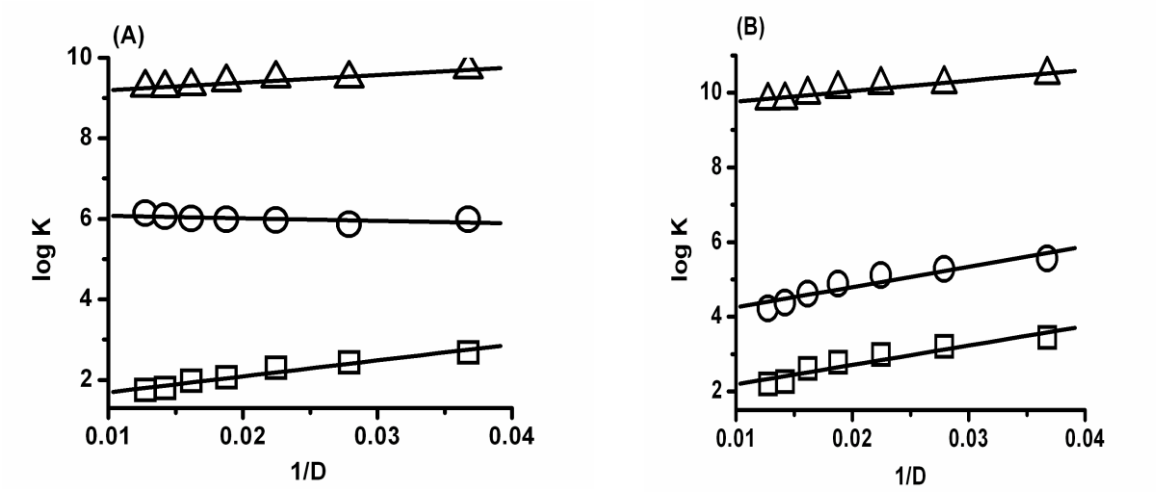


Figure 16: Variation of stepwise protonation constant (logK) with reciprocal of dielectric constant (1/D) of solvent; where: (A) H and (B) E in DMF-water mixtures; (□) log K_1, (o) logK_2 and (Δ) logK_3.

The protonation-deprotonation equilibria of H and E are shown in Figure 17. In these equilibria, H and E can exist in anionic, zwitterionic and cationic forms.

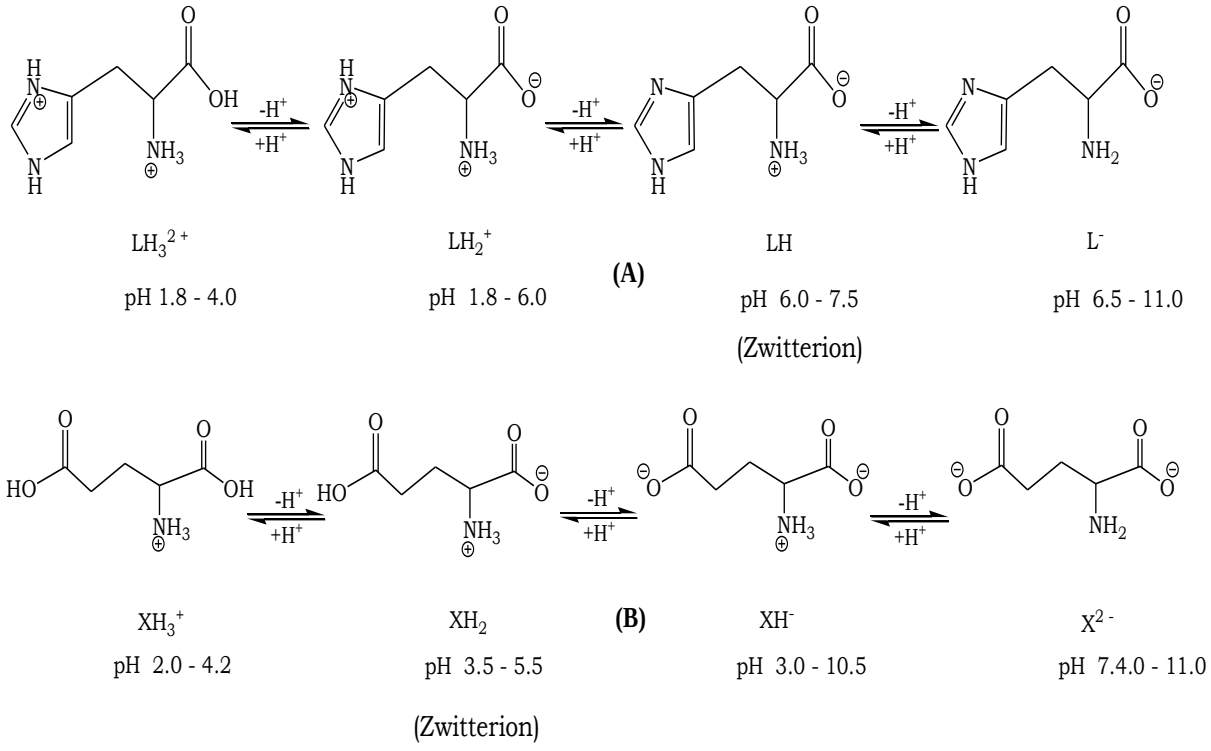


Figure 17: Protonation-deprotonation equilibria of (A) H and (B) E.

These observations can be explained as follows. When H (Equi. 1 -3) and E (Equi. 4 - 6) cations are successively deprotonated, the charge of the species is decreased, and low electric medium favors the protonation reaction, due to dominant electrostatic interactions. Thus, decrease in dielectric constant of the medium should increase the protonation constants.

L-Histidine

$LH_3^{2+} \rightleftharpoons LH_2^{+} + H^{+}$ -- ----------(1)

$LH_2^{+} \rightleftharpoons LH + H^{+}$ - ----------(2)

$LH \rightleftharpoons L^{-} + H^{+}$ ----------(3)

L-Glutamic acid

$XH_3^{+} \rightleftharpoons XH_2 + H^{+}$ ----------(4)

$XH_2 \rightleftharpoons XH^{-} + H^{+}$ ----------(5)

$XH^{-} \rightleftharpoons X^{2-} + H^{+}$ ----------(6)

4.1.5 Distribution diagrams

Distribution diagrams - plots of percentage or fraction of species as a function of free concentrations of ingredients (pH, pL or pM) are not only of theoretical interest but find practical utility in a variety of chemical problems. They are useful in 1) arriving at optimum conditions for the synthesis of solid complexes of a definite composition, 2) preparing ligand or metal buffers, 3) determining relative concentrations of different forms of ligands under a given set of experimental conditions, 4) deciding whether a species exist as a major or minor one under a set of conditions, and 5) detecting stoichiometric asymmetry and over-compensating effects of a chemical reaction.

H has three functional groups are amino, imidazole and carboxyl, in the case of E has three functional groups (amino and two carboxyl) all of which participate in protonation equilibria. The distribution plots (Figure. 18-20) produced using the protonation constants from the best fit models (Table 8) show the formation of LH_3^{2+}, LH_2^{+}, LH and L^{-} in the case of H and XH_3^{+}, XH_2, XH^{-} and X^{2-} in the case of E. The most protonated species (LH_3^{2+} in the case of H and XH_3^{+} in the case E) exist in the pH range 2.0-4.0 and 2.0-5.0 respectively. LH_3^{2+} is deprotonated with increasing pH to form LH_2^{+}, LH and L^{-} in the pH ranges 2.0-7.0, 5.0-10.0 and above 8.0 respectively, in the case of H and XH_3^{+} is deprotonated with increasing pH to form XH_2, XH^{-} and X^{2-} in the pH ranges 2.0-6.0, 3.0-11.0 and above 8.0 in the case of E.

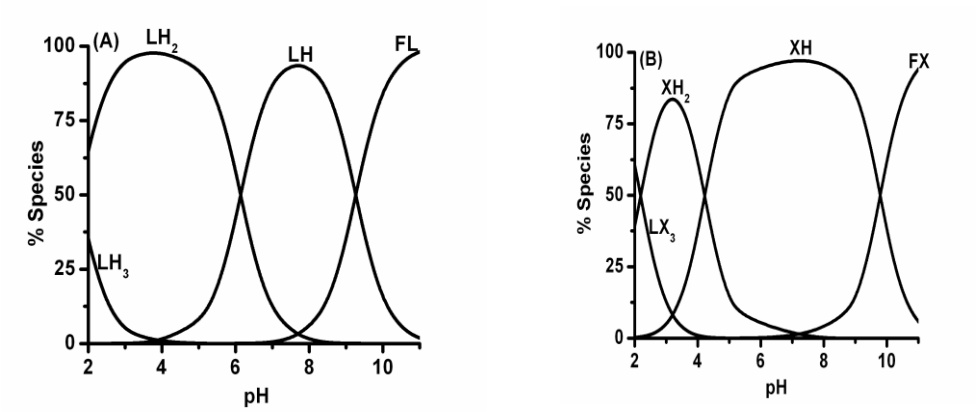


Figure 18: Distribution diagrams of (A) H and (B) E in aqueous medium. Where: Amount of H and E is 0.375 mmol.

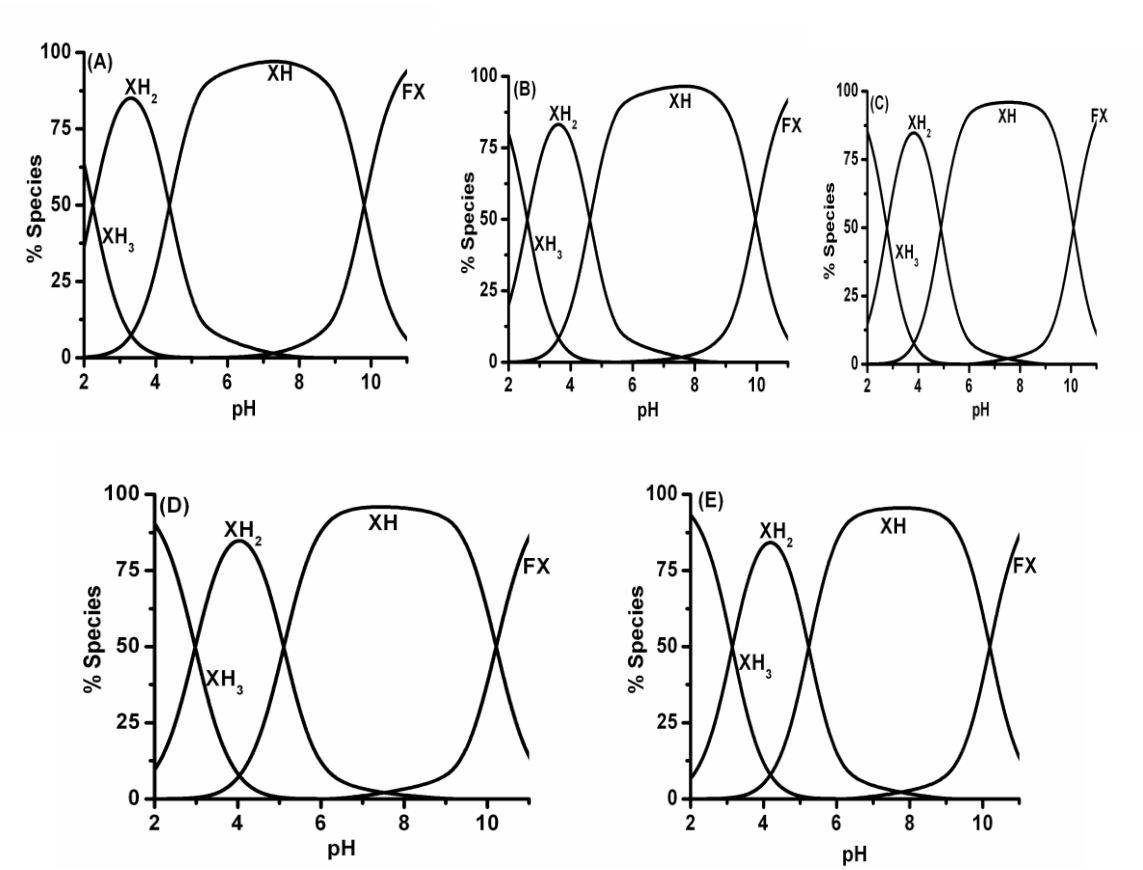


Figure 19: distribution diagrams of L-Glutamic acid in DMF-water mixtures (% v/v); (A) 10.0, (B) 20.0, (C) 30.0, (D) 40.0 and (E) 50.0. Amount of E is 0.375 mmol.

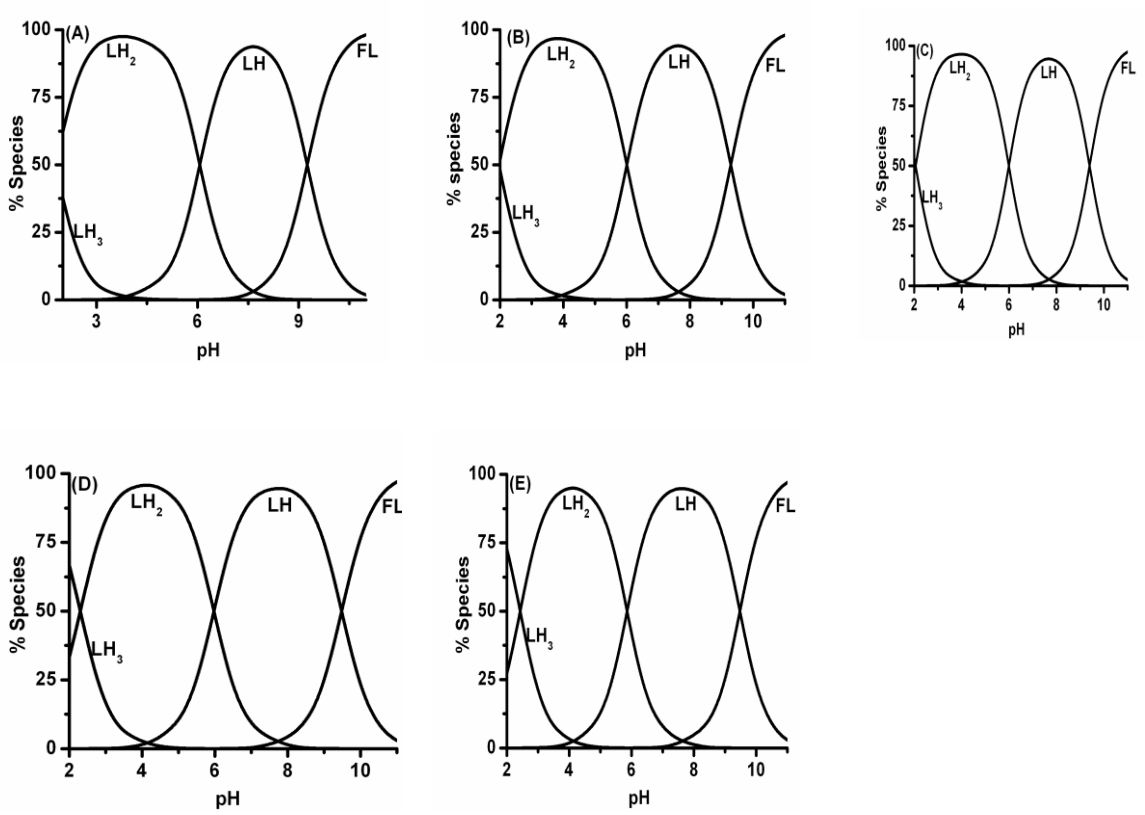


Figure 20: Distribution diagrams of L-Histidine in DMF-water mixtures (% v/v); (A)10, (B) 20.0, (C) 30.0, (D) 40.0 and (E) 50.0. Amount of H is 0.375 mmol.

SECTION-II

4.2 DETERMINATION OF BINARY STABILITY CONSTANTS

The stability of complexes can be quantified as equilibrium constant, which is also called as stability constant or formation constant (Eq. 4.3).

$$mM + lL + hH \rightleftharpoons M_mL_lH_h \; ; \; \beta_{mlh} = \frac{M_mL_lH_h}{[M]^m[L]^l[H]^h} \qquad \text{----------4.3}$$

The earlier literature reports and the distribution patterns of different forms of H and E in DOX- and DMSO-water media [75] presented in the previous chapter, indicate a high probability for the formation of various protonated metal complexes of different stoichiometries. DMF used as a solvent in peptide coupling for pharmaceuticals, in the development and production of pesticides. So far, no systematic report has appeared on studies speciation of L-Histidine and L-Glutamic acid with toxic heavy metal in DMF-water mixtures of various concentrations. Hence, stability constants and chemical speciation studies of H and E with some toxic metals like Pb, Cd and Hg in DMF-water mixtures are reported in this paper. This type of study throws light on 1) the effect of electrostatic and non-electrostatic interactions (denaturation) on complex formation in vitro, 2) the role played by the active site cavities in biological molecules, 3) detection of specific solute-solvent interactions, 4) understanding the selectivity and sensitivity of various protonated complexes and relative abundance of free components, 5) the type of complex formed by the metal ion, and 6) the bonding behavior of the protein residues with the metal ion.

4.2.1 Formation of species

As it can be seen from, the data reported in the literature, there were several instances where different species were reported for the same chemical system by different researchers. The probes utilized and the computational procedures adopted by them were sometimes the same and sometimes different. In order to rationalize the contradictory models reported by different authors, greater details are required regarding the 1) ingredient concentration, 2) method of pruning primary data for the refinement, 3) pH range and the number of points in each sub range and 4) proportion of the points corresponding to each species in the model.

In many chemical systems it was observed that the predicted species based on the pH range and the form of the ligand were in fact not detected. This unexpected behavior could be attributed to i) the hard and soft acid base theory of the metal ion and ligand, ii) coordination number of the metal ion, and iii) stabilities of the complexes formed. If the ligand contains one or more donor atoms than those participating in complexation, there is possibility for the formation of protonated species, MLlHh, in addition to unprotonated complexes, MLl. With progressively increasing pH, the protonated species lose protons to form MLl and it may undergo hydrolysis resulting in MLl(OH)k. Finally, at high pH the metal ion precipitated as hydroxide. Typical alkalimetric titration curve are given in Figure 21 from which the stability constants of binary metal-ligand complexes were determined.

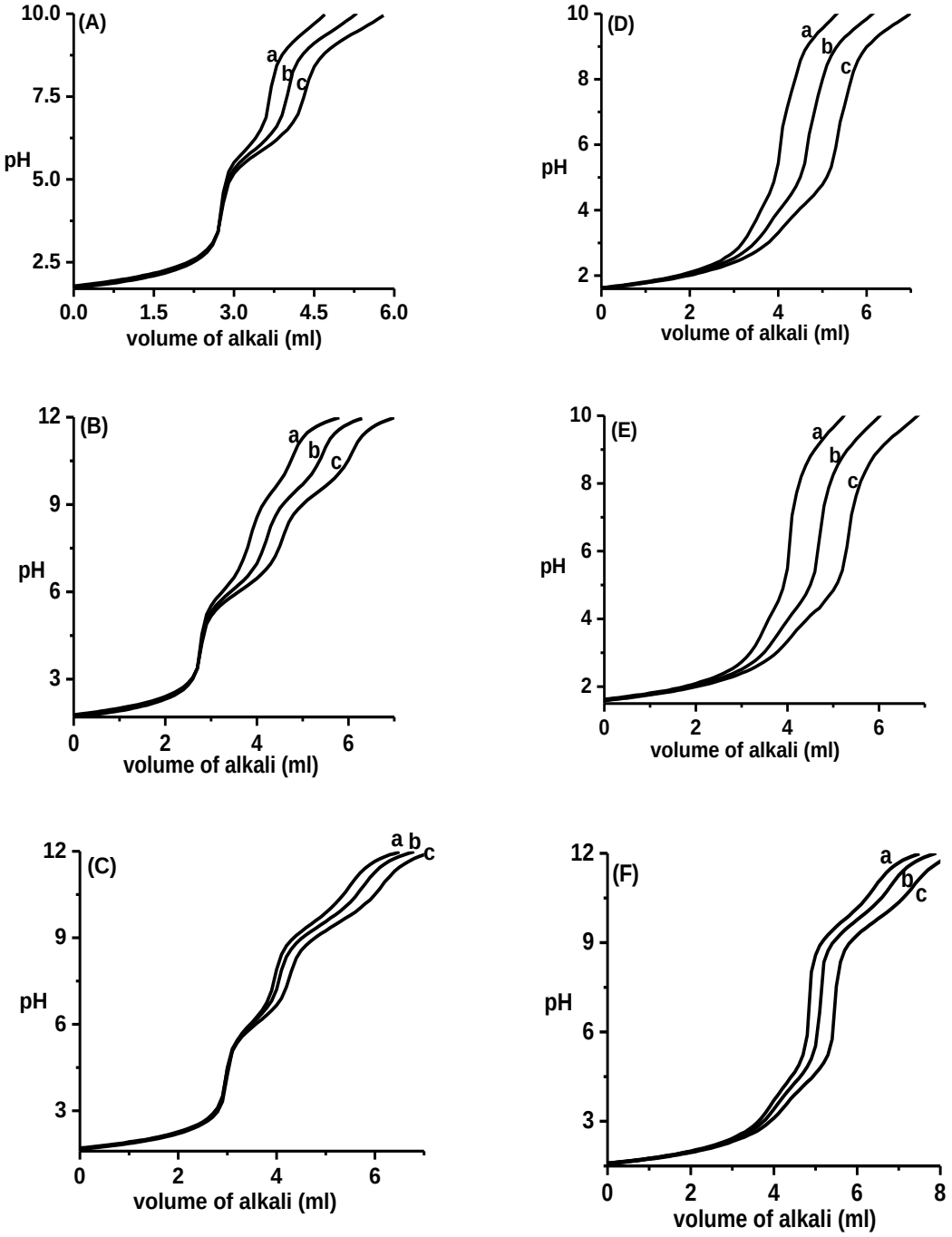


Figure 21: Alkalimetric titration curves for H complexes and E in 20% (v/v) DMF-water mixture; (A) Pb(II), (B) Cd(II), (C) Hg(II) complex for H and (D) Pb(II), (E) Cd(II), (F) Hg(II) for E; amount of the ligand (a) 0.250 (b) 0.375 (c) 0.500 mmol.

These curves indicate that the precipitation of the H complexes of Pb(II) in DMF-water mixtures takes place in the pH range 9.0-9.5 and Pb(II)-E system in 6.9-7.2. It suggests that Pb(II)-E complexes are hydrolyzed at lower pH when compared to Pb(II)-H system. Cd (II)-H complexes in DMF- water mixtures was observed in the pH above 11.5. Cd(II)-E system in

DMF-water mixtures in the pH range 10.0-11.5. In the case of Hg (II), a different situation was observed in DMF- water mixtures for both the ligands (H and E); the precipitation was observed at very lower pH range i.e. 2.3-2.8. It indicates that the hydrolysis of mercuric ion takes place at very low pH in the presence of H and E.

Since the ratio of TL_0 to TM_0 is greater than unity (TL_0: TM_0 are 2.5, 3.75 and 5.0) in all the experiments (Table 6 of Chapter 3), the formation of polynuclear complexes was not considered. Polymeric species is possible if TL0: TM0 $\leq$ 2 [4]. The formation function (the number of moles of ligand bound per mole of metal ion) is a good parameter for the detection of protonated species. If there are no protonated species the plots of versus pH at all the ligand concentrations, with fixed metal ion concentration should overlap. Any deviation indicates the presence of the protonated species. Some typical plots given in Figure 22 show more spread in some pH regions, indicating the presence of protonated metal complexes, but in A, B, and F the curves are slightly overlapped with each other, indicating the absence of the protonated complexes. Little deviation from overlapping is due to the formation of hydroxyl species or due to varying concentration of mineral acid. In all these curves the maximum observed value is less than 2 which indicates consumption of two mole of ligand per mole of metal ion during the complexation. Figure 23 give the relation between the number of moles of alkali consumed per mole of metal ion (a) and the pH. The number of protons released in the equilibrium can be guessed depending on the moles of alkali consumed.

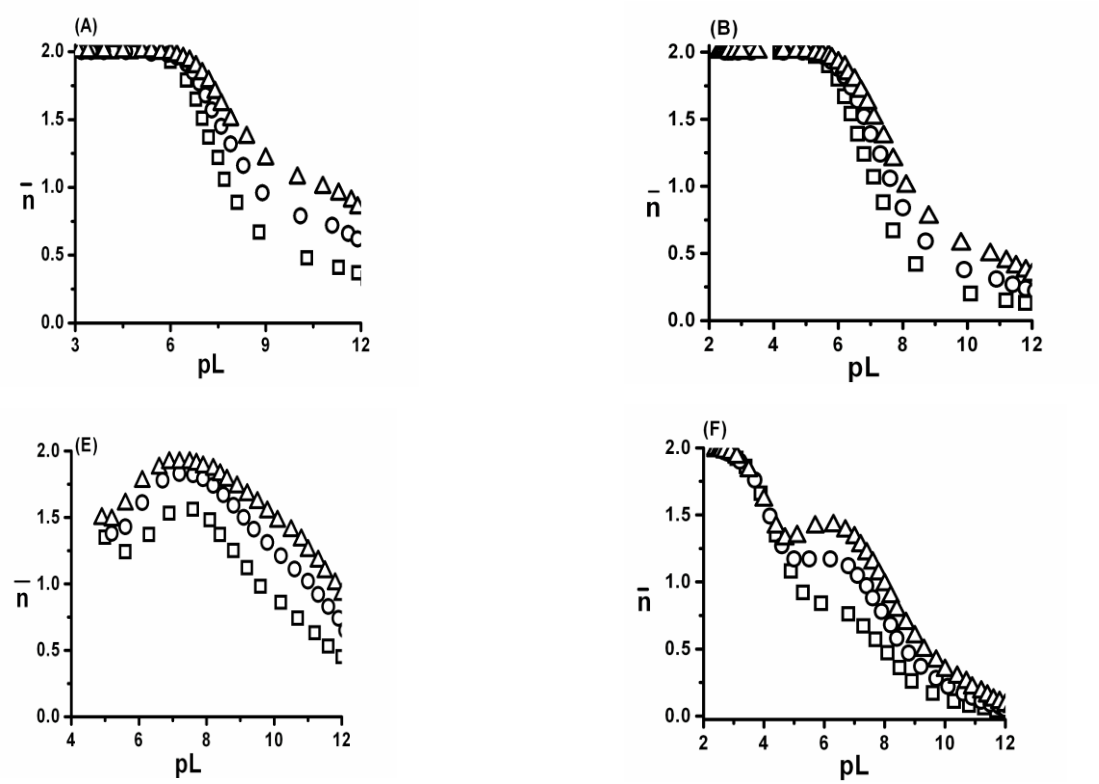


Figure 22: Formation curves H and E complexes in 50% v/v and 10% v/v DMF-water mixture; (A) Pb(II), (B) Cd(II) for H complexes in 50% v/v DMF-water mixture and (E) Pb(II), (F) Cd(II) E complexes in 10% v/v DMF-water mixture; ligand concentrations in mmols are 0.250(□-□), 0.375(○-○) and 0.5(▵ - ▵).

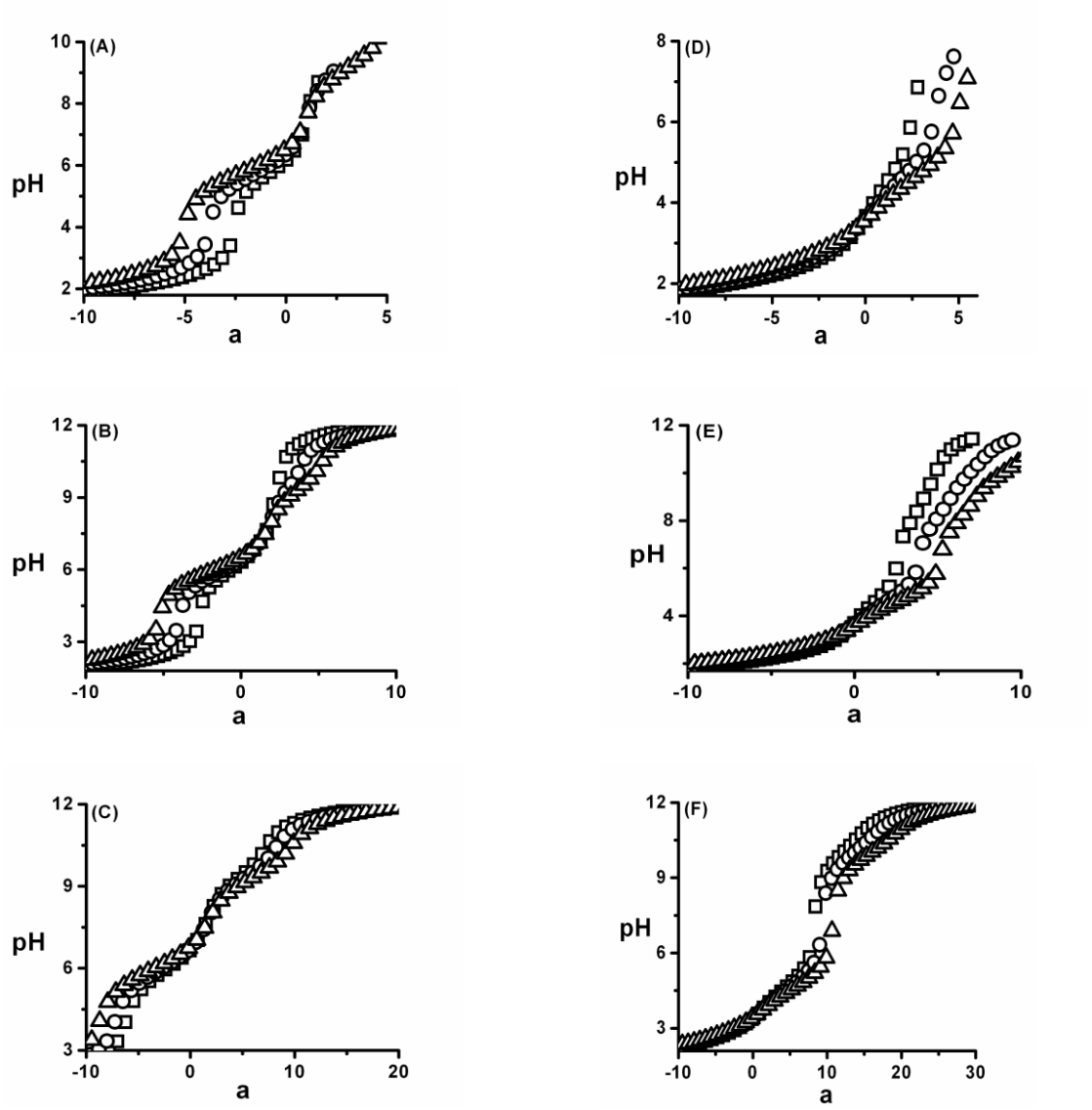


Figure 23: Number of moles of alkali (a) versus pH curves of H and E complexes in 30% v/v DMF –Water mixture; (A) Pb(II), (B) Cd(II) and (C) Hg(II) complexes of H and (D) Pb(II), (E) Cd(II) and (F) Hg(II) complexes of E. Ligand concentrations in mmols are 0.250(□-□), 0.375(O-O) and 0.5(∆ - ∆).

Although the stability constants calculated from the formation function data are vitiated in presence of protonated, hydroxylated or polymeric species, still a wealth of information is hidden in versus pH or pL plots which can have many applications. For example, PSEUDOPLOT [109] technique has become very popular in detecting species other than those invoked in the model and even in rejecting a species refined by a computer program. Detection of systematic errors in the concentrations of mineral acid and ligand and non-linear response

of glass electrode in extreme pH ranges etc. is another interesting application of this auxiliary function.

4.2.2 Selection of Species

Based on the earlier reports the maximum number of ligands bound to metal ion is restricted to three. In all the cases, the maximum observed $\bar{n}$ values were less than three, with no ascending slope [110], $\bar{n}$ greater than three or ascending slope, which indicates the presence of a complex species with higher ligand number. The preliminary screening of a few species based on several thumb rules [108] resulted in a short list of ML_2H_4, ML_2H_3, ML_2H_2, ML_2H and ML_2 for Pb(II) and Cd(II) and Hg(II) forms ML_2H_4, ML_2H_3, ML_2 and ML for complex of H. In the similar way, the models for E complexes were MX_2H_4, MX_2H_3, MX_2H_2, MX and MX_2 for Pb(II), Cd(II) and Hg(II).

4.2.3 Selection of Best fit Model

The models containing different number of species were tried from the primary alkalimetric titration data. Only a few species were refined while other species were rejected by MINIQUAD75 [92]. The models with combinations of different species at a time were also tested. After arriving at a valid model, the species rejected in the primary scrutiny were again tried. Some species were removed from the model if their percentage is less than 10.

As the number of species increased, the models gave better statistics denoting the better fit. This indicates the final model appropriately fits the experimental data. Such exhaustive modeling is performed for all the systems and the final models are given in Tables 13-14 for Pb(II), Cd(II) and Hg(II) complexes of H and E in DMF-water mixtures. The tables contain the stoichiometric coefficients and stability constants of the complex species, standard deviations in the stability constants and residual statistics of the best-fit models.

Table 13 : Parameters of best-fit chemical models of H complexes of Pb(II), Cd(II) and Hg(II) in DMF-water

DMF % v/v	**log β_{mlh}(SD)**						**NP**	**Ucorr x10^8**	**Skewness**	**Kurtosis**	**χ^2**	**R-Factor**	**pH-Range**
	110	120	121	122	123	124							
Pb(II)													
0.0	-----	09.36(0)	18.38(3)	25.09(3)	31.12(6)	36.92(6)	99	0.0001	0.64	3.11	12.10	0.006	3.4-9.1
10.0	-----	10.15(1)	18.51(1)	24.77(5)	30.36(8)	35.93(7)	67	0.0072	1.83	2.45	12.62	0.004	3.4-9.1
20.0	-----	10.39(1)	19.00(6)	25.15(6)	30.63(0)	36.00(5)	84	0.0030	0.07	2.36	12.35	0.002	3.4-9.1
30.0	-----	10.30(2)	19.63(7)	25.98(5)	31.34(3)	36.91(0)	60	0.0081	0.28	3.70	12.81	0.003	3.4-9.1
40.0	-----	11.60(1)	20.02(8)	25.26(7)	30.67(5)	35.16(9)	99	0.0034	0.07	2.50	12.13	0.007	3.4-8.9
50.0	-----	10.92(0)	19.75 (2)	25.60(5)	30.70(9)	35.83(7)	78	0.0090	0.65	4.61	12.83	0.002	3.4-9.1
Cd(II)													
0.0	-----	10.23(5)	17.83(2)	24.37(6)	30.57(9)	36.29(18)	77	0.0028	1.84	2.01	13.21	0.007	3.0-8.2
10.0	-----	10.76(6)	17.90(9)	24.13(12)	30.05(4)	35.35(14)	90	0.0078	1.13	3.26	12.51	0.006	3.0-8.2
20.0	-----	10.95(7)	18.17(7)	24.25(7)	30.05(4)	35.33(16)	70	0.0017	1.90	3.17	12.53	0.005	3.0-8.2
30.0	-----	11.12 (5)	18.25(9)	24.30(5)	29.66(4)	34.65(24)	88	0.0026	1.35	4.15	11.33	0.001	3.4-8.0
40.0	-----	12.30(4)	19.15(8)	24.95(6)	30.25(1)	34.57(33)	67	0.0044	0.27	5.43	14.17	0.007	3.0-8.0
50.0	-----	11.92(1)	18.76(7)	24.67(0)	29.60(8)	34.59((9)	87	0.0068	0.07	4.96	12.75	0.001	3.0-8.0
Hg(II)													
0.0	10.08(7)	13.19(5)	-----	-----	33.85(2)	35.23(4)	90	0.0081	-0.76	4.00	13.13	0.009	2.0-8.0
10.0	11.76(5)	15.65(9)	-----	-----	34.20(2)	35.42(5)	68	0.0043	-0.50	4.60	12.41	0.005	1.9-8.0
20.0	11.97(4)	15.93(4)	-----	-----	34.27(0)	36.03(7)	70	0.0069	-0.20	3.21	12.25	0.003	2.0-8.0
30.0	12.15(7)	15.72(5)	-----	-----	34.83(7)	36.51(6)	93	0.0057	-0.09	3.50	11.80	0.004	2.0-8.0
40.0	12.20(7)	16.65(9)	-----	-----	34.35(6)	36.15(5)	88	0.0014	-0.36	3.20	12.71	0.003	2.0-8.0
50.0	14.97(6)	20.45(6)	-----	-----	34.97(9)	37.50(8)	99	0.0043	0.23	4.41	12.70	0.003	2.0-8.0

Table 144: Parameters of the best-fit chemical models of E complexes of Pb(II), Cd(II) and Hg(II) in DMF- water mixtures

DMF % v/v	log β_{mlh}(SD)					pH-Range	NP	U_{corr} *10^8	χ^2	Skewness	Kurtosis	R-Factor
	MX	MX_2	MX_2H_2	MX_2H_3	MX_2H_4							
Pb(II)												
0.0	4.85(7)	8.86(8)	24.07(3)	29.16(4)	33.00(9)	3.9-8.0	55	0.0045	12.78	0.36	3.74	0.0031
10.0	5.34(1)	9.13(1)	24.26(4)	28.90(9)	32.52(0)	3.6-8.0	77	0.0077	12.36	0.44	3.94	0.0046
20.0	5.47(9)	9.67(1)	24.70(8)	29.51(8)	33.28(7)	2.7-8.0	99	0.0063	12.44	0.07	3.72	0.0047
30.0	5.48(2)	9.27(5)	24.90(6)	30.30(0)	34.23(8)	4.0-8.0	77	0.0023	12.99	0.22	3.90	0.0071
40.0	7.40(8)	12.17(1)	26.80(7)	31.36(9)	35.47(6)	3.0-8.0	88	0.0012	11.90	-0.37	7.14	0.0022
50.0	6.30(0)	10.18(5)	25.87(7)	31.17(7)	35.65(7)	4.0-8.0	90	0.0014	12.11	0.44	3.12	0.0044
Cd(II)												
0.0	3.96(6)	7.57(0)	23.77(1)	28.92(4)	33.17(7)	3.0-9.0	66	0.0055	12.90	1.07	4.24	0.0083
10.0	4.53(4)	8.63(6)	24.26(6)	29.00(6)	32.86(8)	3.4-9.0	47	0.0066	12.88	1.18	4.60	0.0089
20.0	4.90(9)	8.70(8)	24.57(1)	29.43(1)	33.38(9)	2.0-9.0	99	0.0077	12.67	0.15	4.82	0.0045
30.0	4.72(9)	8.16(7)	24.50(1)	29.87(1)	33.83(1)	2.0-9.5	107	0.0022	11.44	-0.56	4.60	0.0045
40.0	5.51(5)	9.72(5)	25.27(5)	30.06(9)	33.96(2)	2.4-9.0	88	0.0017	12.77	0.03	4.25	0.0031
50.0	5.23(5)	9.09(9)	24.77(9)	30.67(0)	35.47(5)	4.4-9.0	65	0.0010	12.80	0.18	3.26	0.0080
Hg(II)												
0.0	9.25(7)	12.26(7)	26.05(9)	30.08(5)	32.67(9)	2.0-9.0	99	0.0013	12.41	0.26	7.90	0.0034
10.0	9.67(6)	14.00(8)	26.49(7)	30.30(5)	33.25(6)	2.0-9.0	87	0.0012	12.45	1.95	5.12	0.0024
20.0	10.26(7)	13.75(4)	27.83(7)	31.95(4)	34.77(5)	2.0-9.0	66	0.0014	11.06	0.65	6.51	0.0023
30.0	12.63(0)	14.68(3)	30.35(4)	34.43(7)	37.56(8)	2.0-10.0	45	0.0019	12.13	-0.44	6.00	0.0033
40.0	11.63(5)	14.92(8)	30.36(7)	33.33(4)	36.55(3)	2.0-10.0	47	0.0111	12.73	-0.06	4.80	0.0020
50.0	12.62(6)	18.36(1)	30.44(9)	34.25(6)	37.35(4)	2.0-10.0	49	0.0213	12.44	-0.87	5.07	0.0021

Ucorr = U/(NP-m)X10^8, where m = number of species; NP=Number of experimental points; SD=Standard deviation

4.2.4 Residuals Analysis

The residuals differences between what is measured or observed (pH, volume, concentration of ingredients etc.) and that predicted by a model-measure the unexplained variation in the dependent variable by the regression equation. The goal of rigorous residual analysis is to conclude that the assumptions in regression analysis and of chemical laws (mass-balance equations, electrode response, equilibrium expression etc.) are valid for a data set on hand. The residuals should follow normal distribution for the best-fit model. The use of plots of residuals versus calculated volumes of dependent variables (EMF or volume) and independent variables (TM, TL) is popular.

Sabatini et al. [111] applied sample standard deviation (SD) in weighted least squares analysis for the calculation of β's and suggested that any value less than three is satisfactory. The SD and confidence intervals in β are meaningful only when unweighted residuals follow χ^2 distribution, which measures the possibility of residuals forming a plot of standard normal distribution with zero mean and unit standard deviation. Higher χ^2 values than expected are due to 1) the inadequacy of the model although the experimental data are of high quality, 2) use of poor data even though the model is appropriate, and 3) invoking optimistic estimates of errors in primary data.

A persual of Tables 13-14 indicates that the χ^2 values range between 11.33-14.17 for DMF-water mixtures. The values of kurtosis and skewness range from 2.01-7.90 and -0.87-1.95 for DMF-water mixtures respectively. Most of the residuals form leptokurtic patterns and remaining form platykurtic patterns. Skewness values are very nearer to zero. This indicates the residuals form normal distribution. Deviation of the values of kurtosis and skewness from three and zero, respectively, show the tendency of these residuals to concentrate more to the left or right of the mean and broadening of the peak. However, the values of U_{corr} in all the three mass-balance equations, are very low confirming the adequacy of the chemical model to represent the experimental data.

4.2.5 Perturbation in Stability Constants due to Systematic Errors

In order to rely upon the best chemical model for critical evaluation and application under varied experimental conditions with different accuracies of data acquisition, an investigation was made by introducing pessimistic errors in the concentrations of alkali, mineral acid, ligand, metal and volume (Table 16). MINIQUAD75 has no provision to vary the influential parameters. Hence, some representative systems were studied in order to have a cognizance of the effect of errors in concentrations of ingredients on the stability constants of binary metal complexes. These results are given in Table 15. The data show that the order of affecting the magnitudes of stability constant is alkali > acid > ligand > metal. The increased standard deviation in stability constants and even rejection of some species on the introduction of errors confirms the correctness of the proposed models. This type of investigation is significant as the data acquisition was done under varied experimental conditions with different accuracies.

Table 15: Effect of errors in influential parameters on Pb(II)-H complex stability constants in 20% v/v DMF-water mixture

Ingredient	% of error	log β_{mlh}(SD)				
		ML	ML_2	ML_2H_2	ML_2H_3	ML_2H_4
	0	4.47(9)	9.67(1)	24.70(8)	29.51(8)	33.28(7)
Alkali	-5	3.18(44)	Rejected	Rejected	Rejected	33.27(45)
	-2	3.95(70)	7.36(55)	Rejected	28.97(55)	32.95(33)
	+2	6.95(22)	11.19(33)	25.85(27)	30.22(33)	34.00(26)
	+5	9.74(24)	Rejected	26.95(26)	31.34(21)	34.48(19)
Acid	-5	9.82(22)	14.57(23)	27.55(11)	31.75(19)	35.04(83)
	-2	6.67(23)	10.83(18)	26.00(17)	30.39(29)	34.25(51)
	+2	4.07(23)	7.63(12)	Rejected	28.57(42)	30.78(99)
	+5	3.17(96)	6.65(38)	Rejected	Rejected	Rejected
Ligand	-5	5.33(10)	9.38(29)	24.64(9)	29.86(25)	33.67(31)
	-2	5.09(19)	9.99(28)	24.58(9)	29.22(14)	33.16(12)
	+2	4.77(29)	9.49(29)	24.46(11)	29.56(10)	33.54(7)
	+5	4.52(10)	8.07(10)	24.28(14)	29.76(9)	33.77(6)
Metal	-5	4.44(10)	8.62(29)	24.77(10)	29.43(11)	33.20(19)
	-2	4.43(9)	8.68(28)	24.75(10)	29.41(11)	33.28(19)
	+2	4.41(9)	8.68(28)	24.72(19)	29.39(11)	33.26(19)
	+5	5.44(8)	8.66(38)	24.75(19)	29.19(13)	33.13(12)

** Standard deviation is very high

4.2.6 Effect of Solvent on Metal-ligand Equilibria

Co-solvent influences the equilibria in solution due to change in the dielectric constant (D) of the medium that varies the relative contribution of electrostatic and non-electrostatic interactions, which in turn vary the magnitude of stability constants. In this section the effect of DMF on the binary complexes of Pb(II), Cd(II) and Hg(II) with H and E are presented.

The DMF-water mixtures are the combination of aprotic and protic solvents with wide range of dielectric constants and with good solubility for polar as well as non-polar solutes. The co-solvent induced increased basicity [108] of DMF-water mixtures increases the stabilization of protons. At the same time the coordinating solvent (DMF) competes with the ligands for coordination with the metals. This decreases the stability of the complexes. Hence, the stability of the complex is expected to either increase or decrease.

The variation of overall stability constant values with co-solvent content depends upon two factors, viz., electrostatic and non-electrostatic. The trends of stability constant (log β) values of complexes of H and E with 1/D of DMF-water mixture are given in Figure 24. The almost linear trend indicates that the dielectric constant or long-range interactions are responsible for the stability trend. This linear increase indicates the dominance of the structure-forming nature of DMF over the complexing ability. The non-linearity observed in E complexes of Hg(II) in Figure 24F may be due to considerable contribution from non-electrostatic forces.

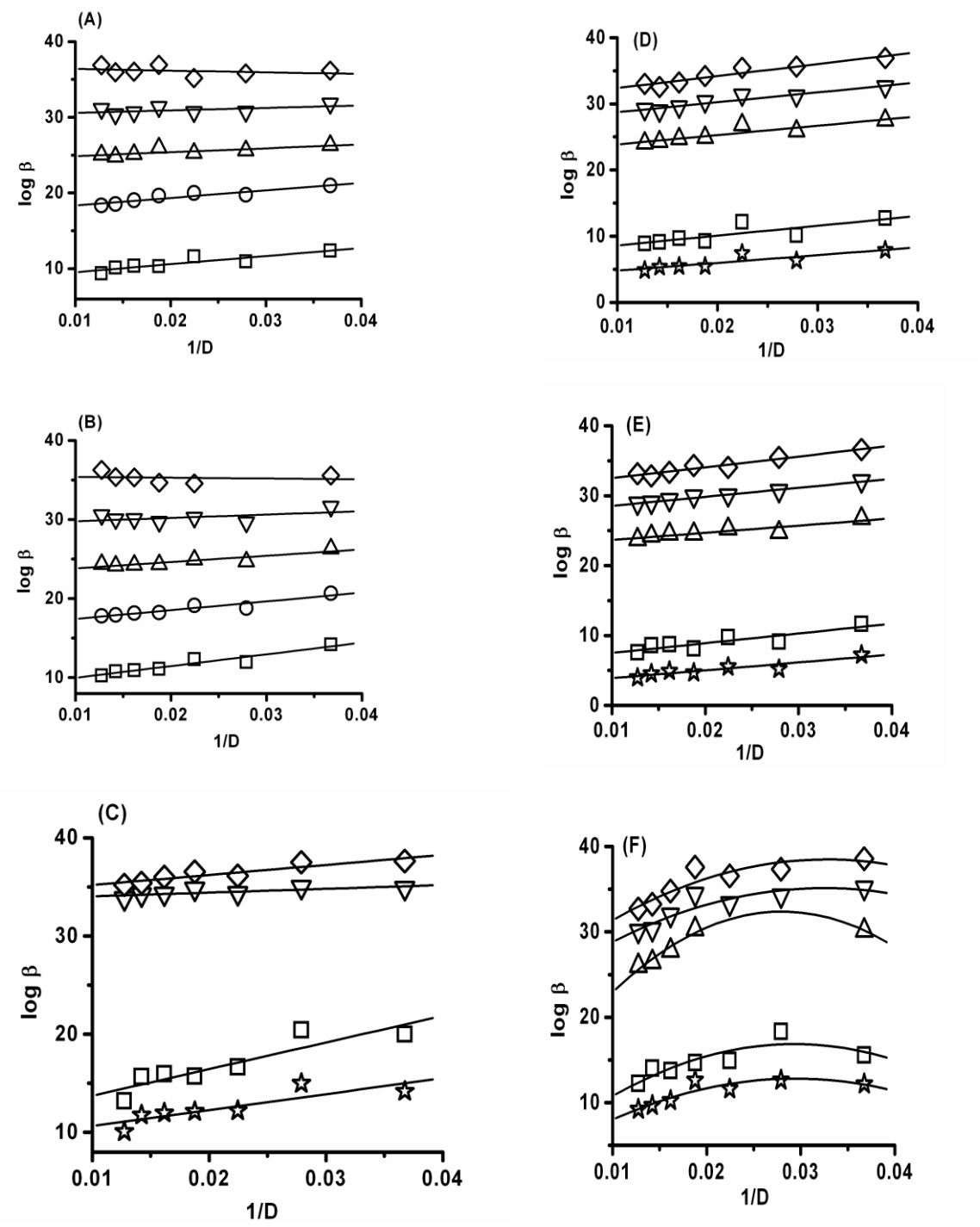


Figure 24: Variation of stability constant values of (A-C) H and (D-F) E complexes with reciprocal of dielectric constant (1/D) of DMF-water mixtures: (A and D) Pb(II); (B and E) Cd(II); (C and F) Hg(II); (☆)log $\beta_{ML;}$ (□)log β_{ML2}; (○)log β_{ML2H}; (Δ)log β_{ML2H2}; (∇)log β_{ML2H3}; (◊)log β_{ML2H4}.

4.2.7 Distribution Diagrams

The percentage of metal ion in the form of various complex species is given by Eq. 4.4.

$$PS = \frac{m(j) * \beta_{m(j)l(j)h(j)} * FL_i^{l(j)} * FH_i^{h(j)}}{\sum_{j=O}^{N} \beta_{m(j)l(j)h(j)} * FL_i^{l(j)} * FH_i^{h(j)}} * 100 \quad \text{----------4.4}$$

Using the formation constant in the best-fit model, distribution diagrams were obtained with the computer programs DISPLOT and SCPHD [94]. These programs can be used to output distribution contours in any required pH range and to study the effect of errors in stability constants or variation in the concentration of ingredients. This information is highly useful in choosing ingredient concentrations to increase or decrease the concentrations of selected species.

4.2.7.1 L-Histidine Complexes

The protonation of H is discussed in section 1. Hence, the plausible binary metal-ligand complexes can be predicted from these data. The present investigation reveals the existence of ML_2H_4, ML_2H_3, ML_2H_2, ML_2H and ML_2 for Pb(II) and Cd(II) and Hg(II) forms ML_2H_4, ML_2H_3, ML_2 and ML. The formation of various L-histidine complex species is shown in the following equilibria.

$M(II) + 2LH_3$	⇌	$ML_2H_4 + 2H^+$	(1)
$M(II) + 2LH_3$	⇌	$ML_2H_3 + 3H^+$	(2)
ML_2H_4	⇌	$ML_2H_3 + H^+$	(3)
$M(II) + 2LH_3$	⇌	$ML_2H_2 + 4H^+$	(4)
$M(II) + 2LH_2$	⇌	$ML_2H_2 + 2H^+$	(5)
ML_2H_4	⇌	$ML_2H_2 + 2H^+$	(6)
ML_2H_3	⇌	$ML_2H_2 + H^+$	(7)
$M(II) + 2LH_3$	⇌	$ML_2H + 5H^+$	(8)
$M(II) + 2LH_2$	⇌	$ML_2H + 3H^+$	(9)
ML_2H_4	⇌	$ML_2H + 3H^+$	(10)
ML_2H_3	⇌	$ML_2H + 2H^+$	(11)
ML_2H_2	⇌	$ML_2H + H^+$	(12)
$M(II) + LH_3$	⇌	$ML + 3H^+$	(13)
$M(II) + LH_2$	⇌	$ML + 2H^+$	(14)
$M(II) + 2LH_2$	⇌	$ML_2 + 4H^+$	(15)
$M(II) + 2LH$	⇌	$ML_2 + 2H^+$	(16)
$ML + LH$	⇌	$ML_2 + H^+$	(17)
ML_2H_4	⇌	$ML_2 + 4H^+$	(18)
ML_2H_3	⇌	$ML_2 + 3H^+$	(19)
ML_2H_2	⇌	$ML_2 + 2H^+$	(20)
ML_2H	⇌	$ML_2 + H^+$	(21)

The species distribution diagrams are shown in Figures 25-31. They indicate that H complexes of Pb(II), Cd(II) and Hg(II) are formed in the pH range 2.0-9.0. At lower pH, ML_2H_4 species is formed by the interaction of free metal ion with LH_3 form of the ligand (Equi.1). The species ML_2H_3 may be formed from M(II) and LH_3 or by the deprotonation of ML_2H_4 (Equi 2 and 3). In the same manner, the species ML_2H_2 may be formed either from free metal ion and LH_3/LH_2 (Equi. 4 and 5) or deprotonation of ML_2H_4 and ML_2H_3 (Equi.6 and 7). At lower pH, the interaction of free metal ion with LH_3 or LH_2 (Equi 4 and 5) and at higher pH, deprotonation of ML_2H_4 and ML_2H_3 (Equi 6 and 7) result in the formation of ML_2H_2 species. ML_2H is formed from M(II) and LH_3 or LH_2 at lower pH or deprotonation of ML_2H_4, ML_2H_3 and ML_2H_2 species at higher pH (Equilibria 8-12). The ML_2 species is formed from the interaction of free metal ion with LH_2 or LH (Equi. 15 and 16) or ML and LH (Equi. 17) or deprotonation of ML_2H_4, ML_2H_3, ML_2H_2 and ML_2H species (Equi. 18-21). In the case of Hg(II) (Figure 28) the ML species is formed from the interaction of free metal ion and LH_3 or LH_2 (Equi. 13 and 14).

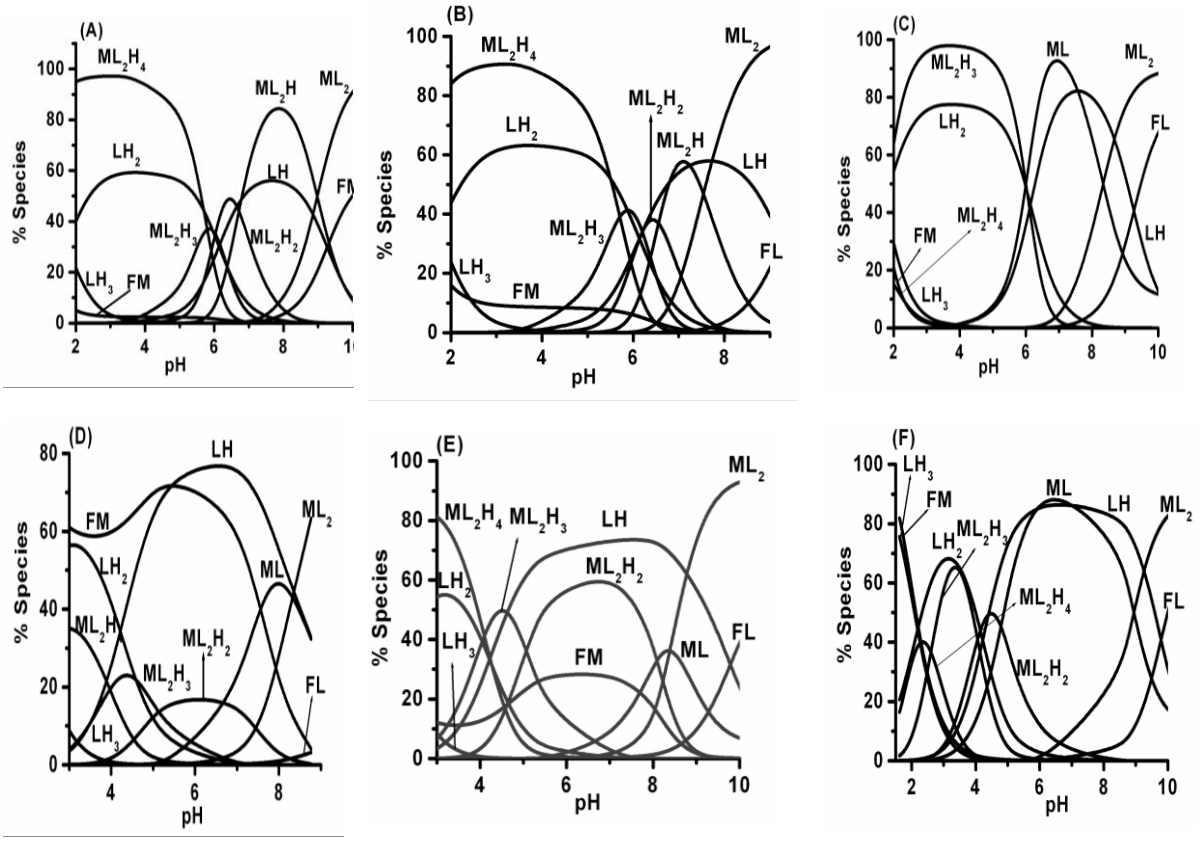


Figure 25: Distribution diagrams of H and E in aqueous medium: (A) Pb(II), (B) Cd(II), (C) Hg(II) with H and (D) Pb(II), (E) Cd(II), (F) Hg(II) with E. Ratio of Pb(II), and Cd(II) and H (or E) is 1.0:2.50 and for Hg(II) is 1.0:5.0

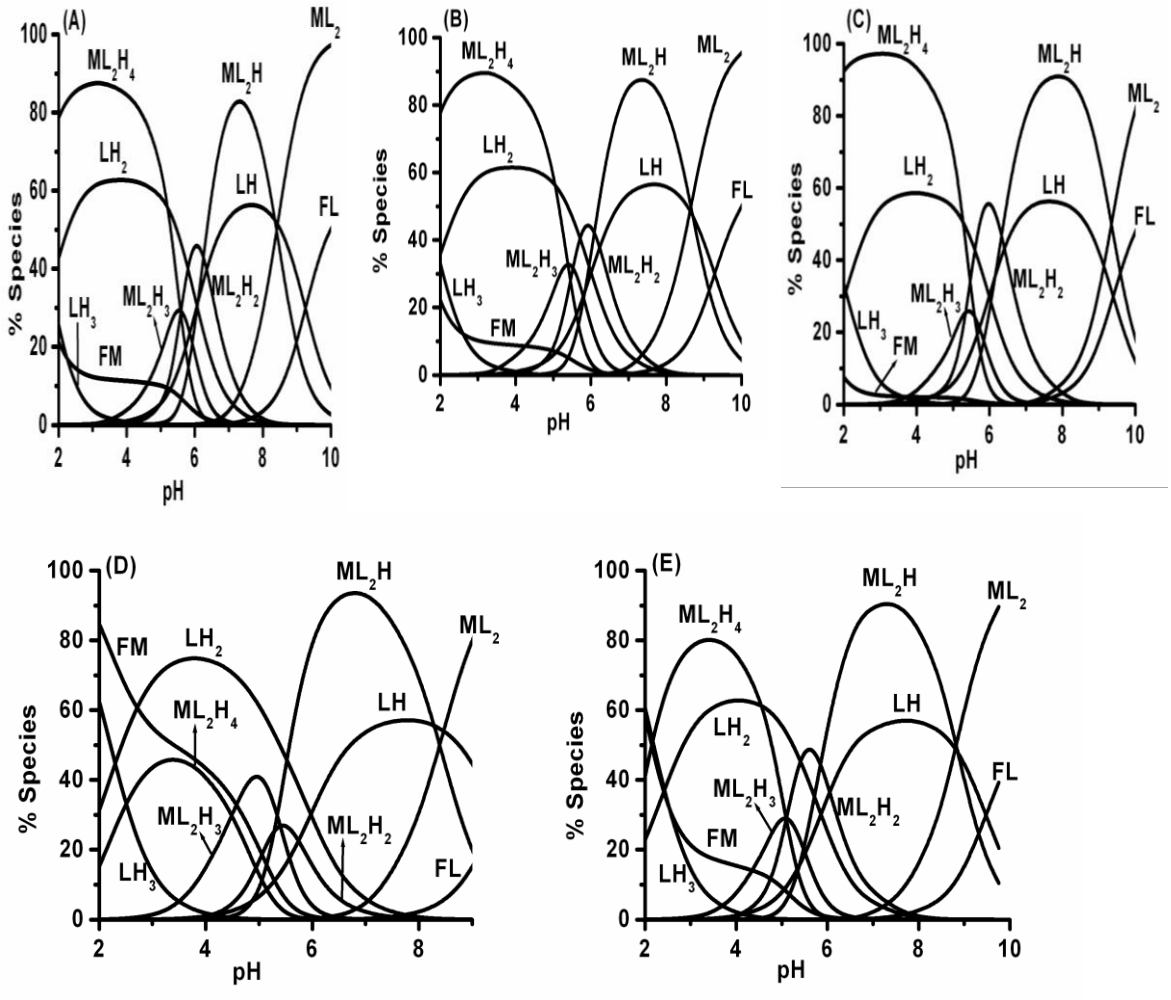


Figure 26: distribution diagrams of Pb(II)-H in DMF-water mixtures %v/v); (A) 10.0, (B) 20.0, (C) 30.0, (D) 40.0 and (E) 50.0. Ratio of Pb (II) and H is 1.0:2.50.

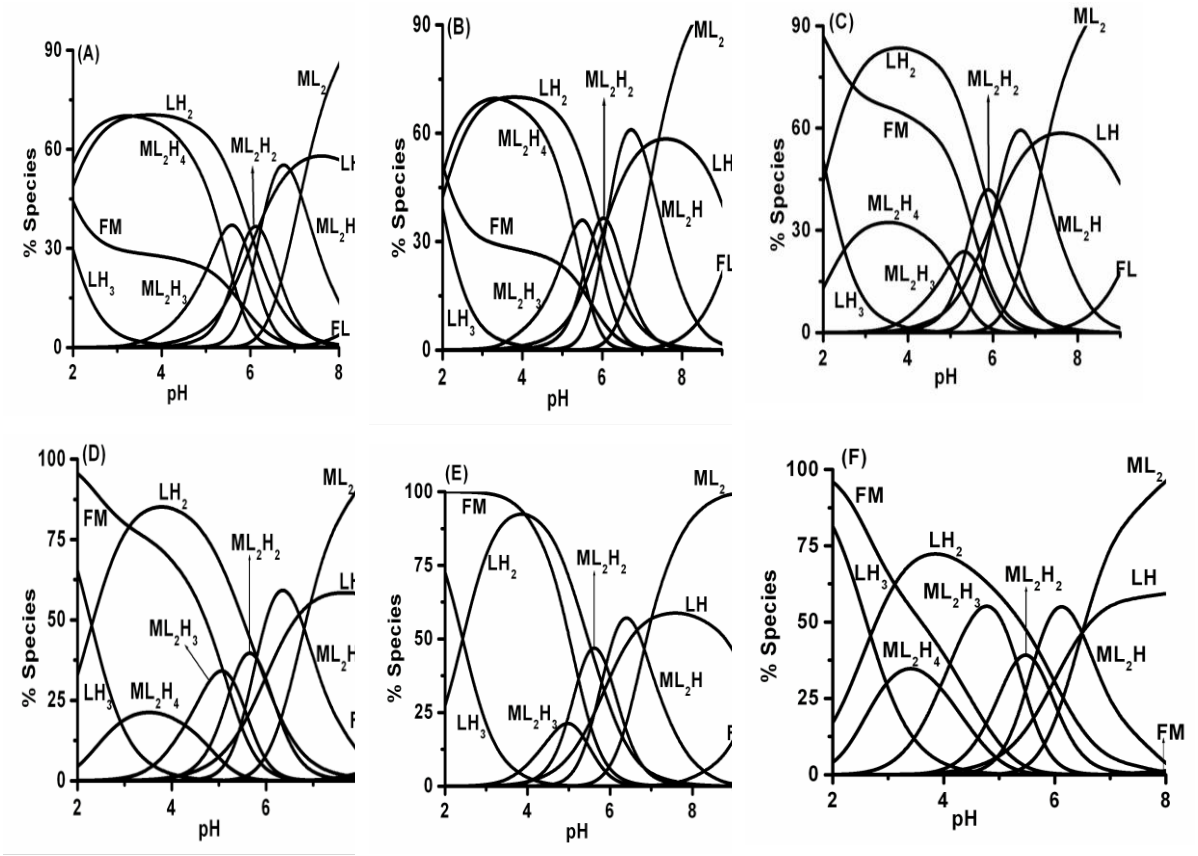


Figure 27: Distribution diagrams of Cd (II)-H in DMF-water mixtures (%v/v); (A) 10.0, (B) 20.0, (C) 30.0, (D) 40.0 and (E) 50.0. Ratio of Cd(II) and H is 1.0:5.0.

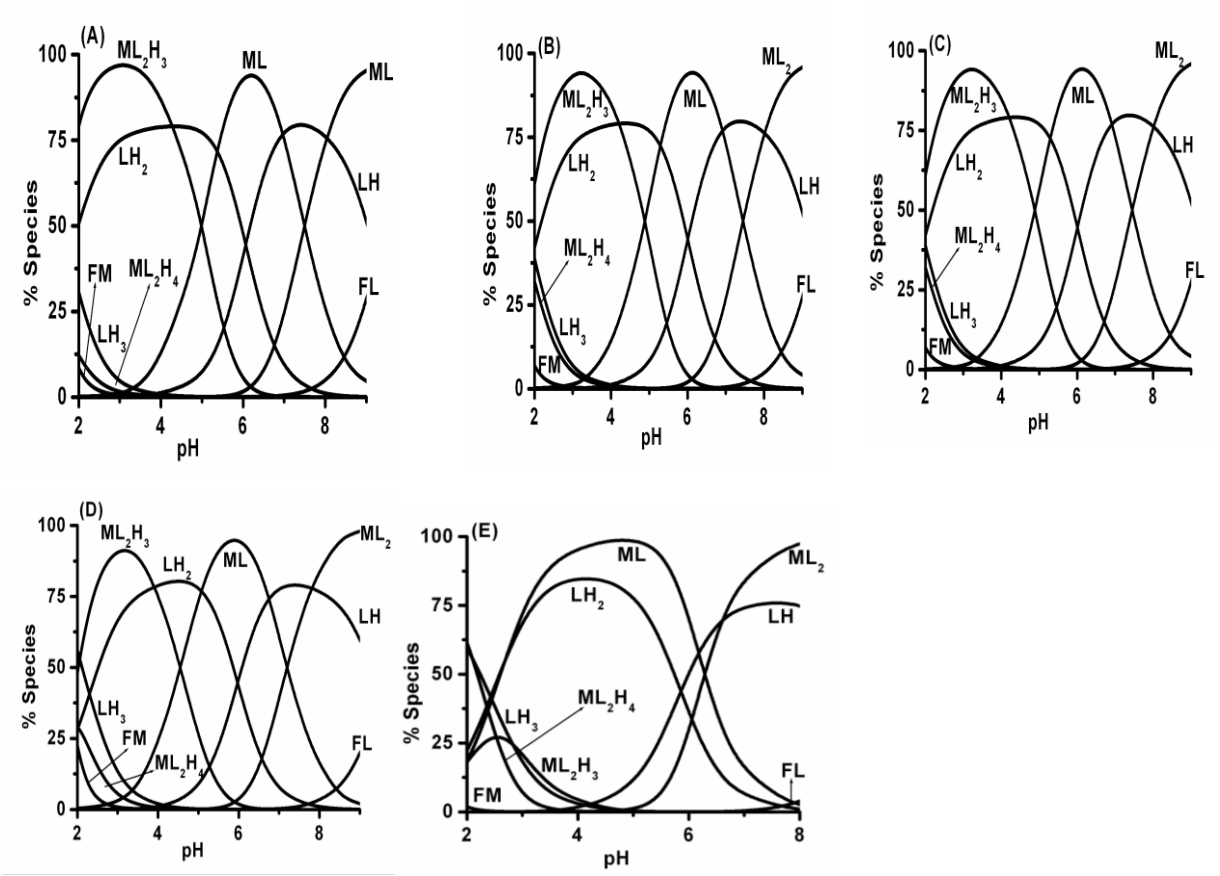


Figure 28: Distribution diagrams of Hg (II)-H in DMF-water mixtures (%v/v); (A) 10.0, (B) 20.0, (C) 30.0, (D) 40.0 and (E) 50.0. Ratio of Hg (II) and H is 1.0:5.0.

4.2.7.2 L-Glutamic Acid Complexes

L-Glutamic Acid has two dissociable carboxyl protons and an amino group that can associate with a proton. The different forms of E that exist in the pH regions 2.0-4.2, 3.5-5.5, 3.0-10.5 and 7.4-11.0 are XH_3^+, XH_2, XH^- and X^{2-}, respectively. The probable species were predicted from these data. The species formed in the present study for different metals as given in Table 15 are MX_2H_4, MX_2H_3, MX_2H_2, MX and MX_2 for Pb(II), Cd(II) and Hg(II) in DMF-water mixtures. The formation of various binary complex species is shown through the following equilibria. The charges of the species are omitted for simplicity.

$M(II) + 2XH_3$	⇌	$MX_2H_4 + 2H^+$	(1)
$M(II) + 2XH_3$	⇌	$MX_2H_3 + 3H^+$	(2)
$M(II) + 2XH_3$	⇌	$MX_2H_2 + 4H^+$	(3)
$M(II) + XH_3$	⇌	$MX + 3H^+$	(4)
MX_2H_4	⇌	$MX_2H_3 + H^+$	(5)
MX_2H_4	⇌	$MX_2H_2 + 2H^+$	(6)
MX_2H_4	⇌	$MX_2 + 4H^+$	(7)
$M(II) + 2XH_2$	⇌	$MX_2H_3 + H^+$	(8)
$M(II) + 2XH_2$	⇌	$MX_2H_2 + 2H^+$	(9)
$M(II) + 2XH_2$	⇌	$MX_2 + 2H^+$	(10)
$M(II) + XH_2$	⇌	$MX + 2H^+$	(11)
MX_2H_3	⇌	$MX_2H_2 + H^+$	(12)
MX_2H_3	⇌	$MX_2H + 2H^+$	(13)
MX_2H_3	⇌	$MX_2 + 3H^+$	(14)
MX_2H_2	⇌	$MX_2H + H^+$	(15)
MX_2H_2	⇌	$MX_2 + 2H^+$	(16)
MX_2H	⇌	$MX_2 + H^+$	(17)
$M(II) + 2XH$	⇌	$MX_2 + 2H^+$	(18)

Distribution diagrams were drawn for various complex species using the formation constants of the best-fit models as shown in Figures 29-31. At a pH below 4.0 MX_2H_4 species is formed for Pb(II), Cd(II) and Hg(II). With increasing pH deprotonation of MX_2H_4 takes place and gives MX_2H_3, MX_2H_2, MX_2H, and MX_2. Figure 29 represent the Pb-E binary complexes. MX_2H_3 species is formed from free metal and XH_3 (Equi.8). MX_2H_2 species concentration is very high and it is formed from Equi. 4.26, 4.29, 4.32 and 4.35. MX_2H_2 is deprotonated with increasing pH and gives MX_2 species (Equi.16). MX species is formed by the interaction of free metal ion with protonation ligand form of LH_3/LH_2 (Equi 4 and 11). Figure 30 represent the Cd-E complexes. MX_2H_2 is formed from deprotonation of MX_2H_4 and MX_2H_3 (Equi 6 and 12). The concentrations of MX_2H_4 and MX_2H_3 species are very low. Above a pH of 6.0 MX_2 species is formed (Equi.7, 10, 14 and 16). MX species is formed from Equi. 4 and 11. Figure 31 represent the Hg-E binary complexes. MX species is formed from free metal and XH_3 form of ligand (Equi. 4). MX_2 is formed by the deprotonation of MX_2H_4, MX_2H_3 and MX_2H_2.

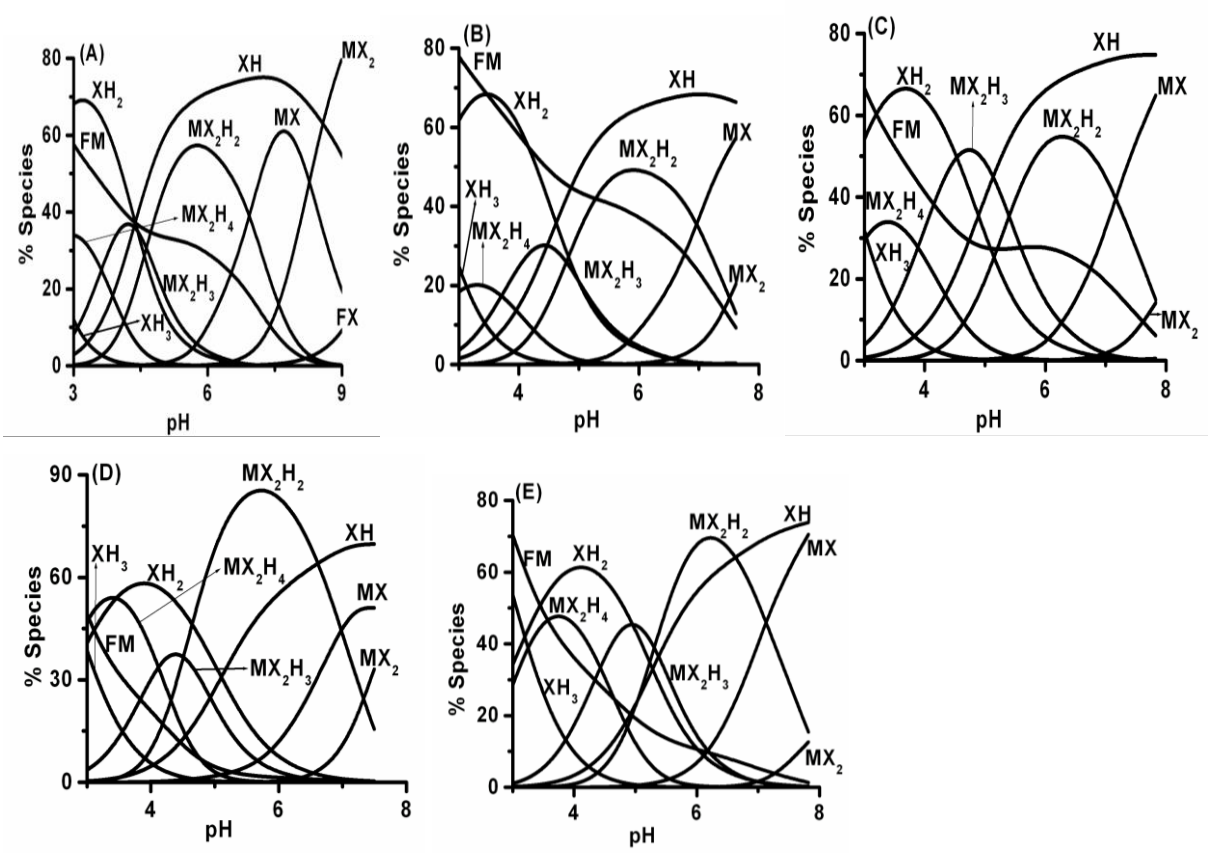


Figure 29: Distribution diagrams of Pb(II)-E in DMF-water mixtures (%v/v); (A) 10.0, (B) 20.0, (C) 30.0, (D) 40.0 and (E) 50.0. Ratio of Pb(II) and E is 1.0:5.0.

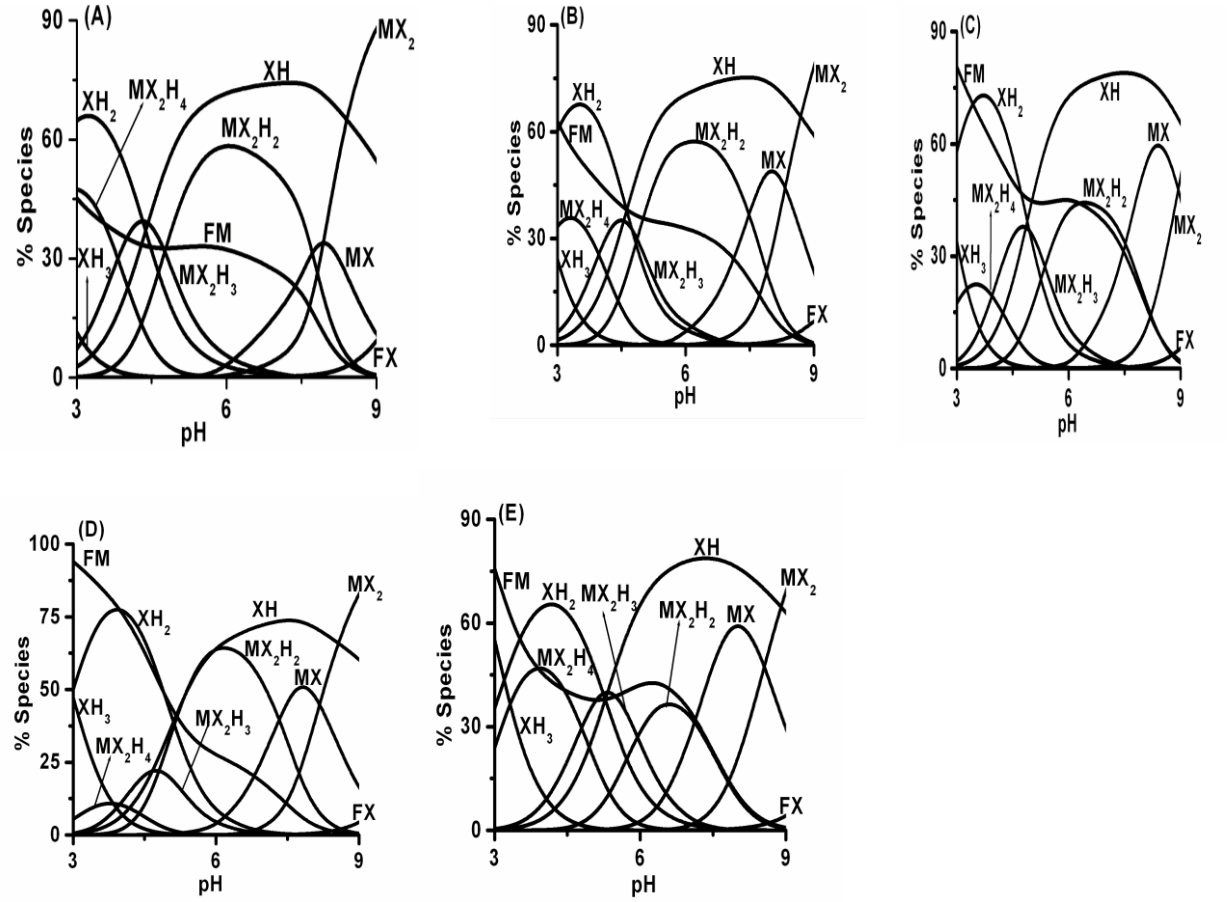


Figure 30: distribution diagrams of Cd(II)- E in DMF-water mixtures (%v/v); (A) 10.0, (B) 20.0, (C) 30.0, (D) 40.0 and (E) 50.0. Ratio of Cd(II) and E is 1.0:3.750.

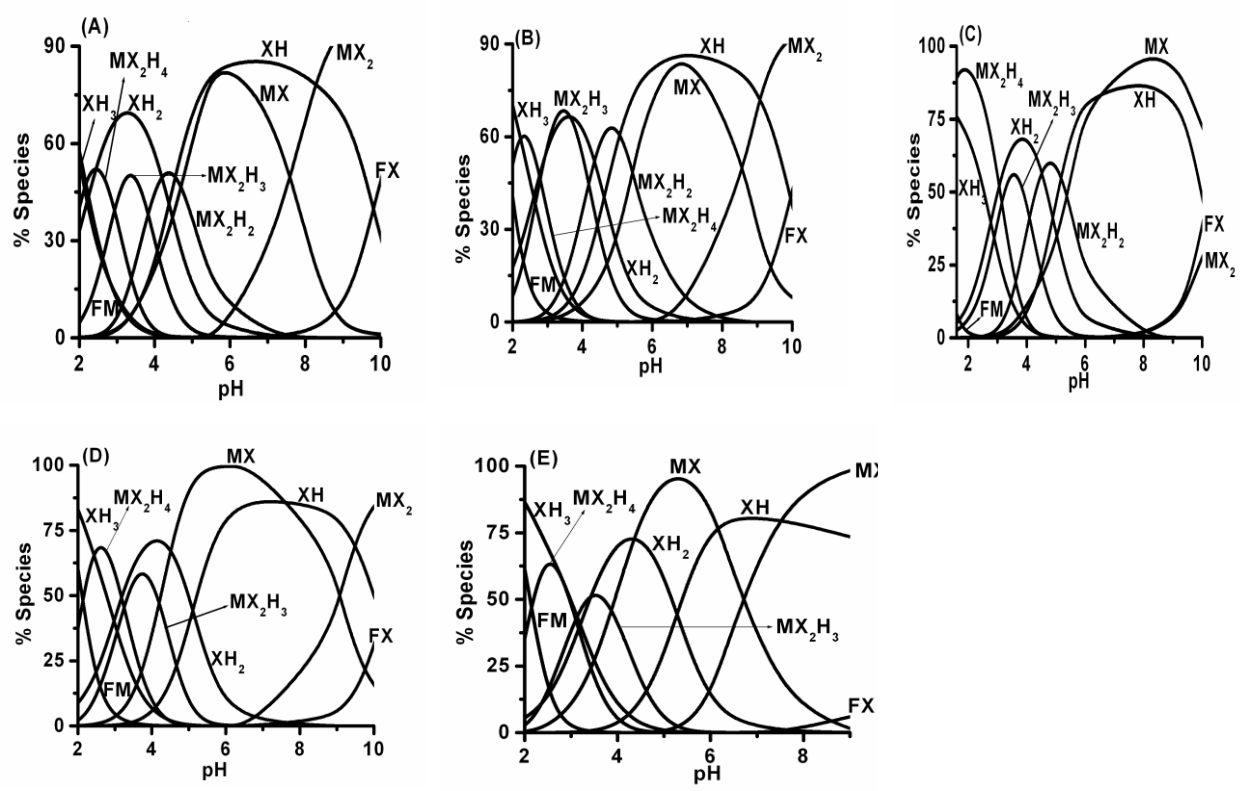


Figure 31: Distribution diagrams of Hg(II)-E in DMF-water mixtures (%v/v); (A) 10.0, (B) 20.0, (C) 30.0, (D) 40.0 and (E) 50.0. Ratio of Hg(II) and E is 1.0:5.0.

4.2.8 Structures of Binary Complexes

When the third donor site of the amino acid is a nitrogen atom, marked tridentate behavior is frequently found, more so when the additional chelation results in a five or six membered ring. Octahedral structures are proposed to the complexes of all the metal ions. The VSEPR theory suggests that Pb(II), Cd(II) and Hg(II) complexes shall be octahedral because there are six outer electron pairs. Amino nitrogen atoms can associate with hydrogen ions in physiological pH ranges. Hence, there is often significant competition between hydrogen and metal ions for this third donor site. This situation results in the simultaneous existence of a number of equilibria, producing an array of successively protonated complexes. Hence, protonated complex species are detected in the present study. Amino nitrogen and α-carboxyl oxygen of E participate in bonding with metal ions. The γ-carboxylic group is protonated at pH less than 5.0 because its protonation constant is around 4.0. Similarly, amino group is protonated at pH less than 10.0, since its protonation constant is around 9.0. Since the protonated metal complexes exist in the pH range 5.0-9.0, and the maximum concentration of the protonated complex is at a pH more than 5.0 it appears that amino group is protonated in the protonated complexes but not γ-

carboxyl group. This argument supports the structures of complexes proposed in Figures 32 and 33.

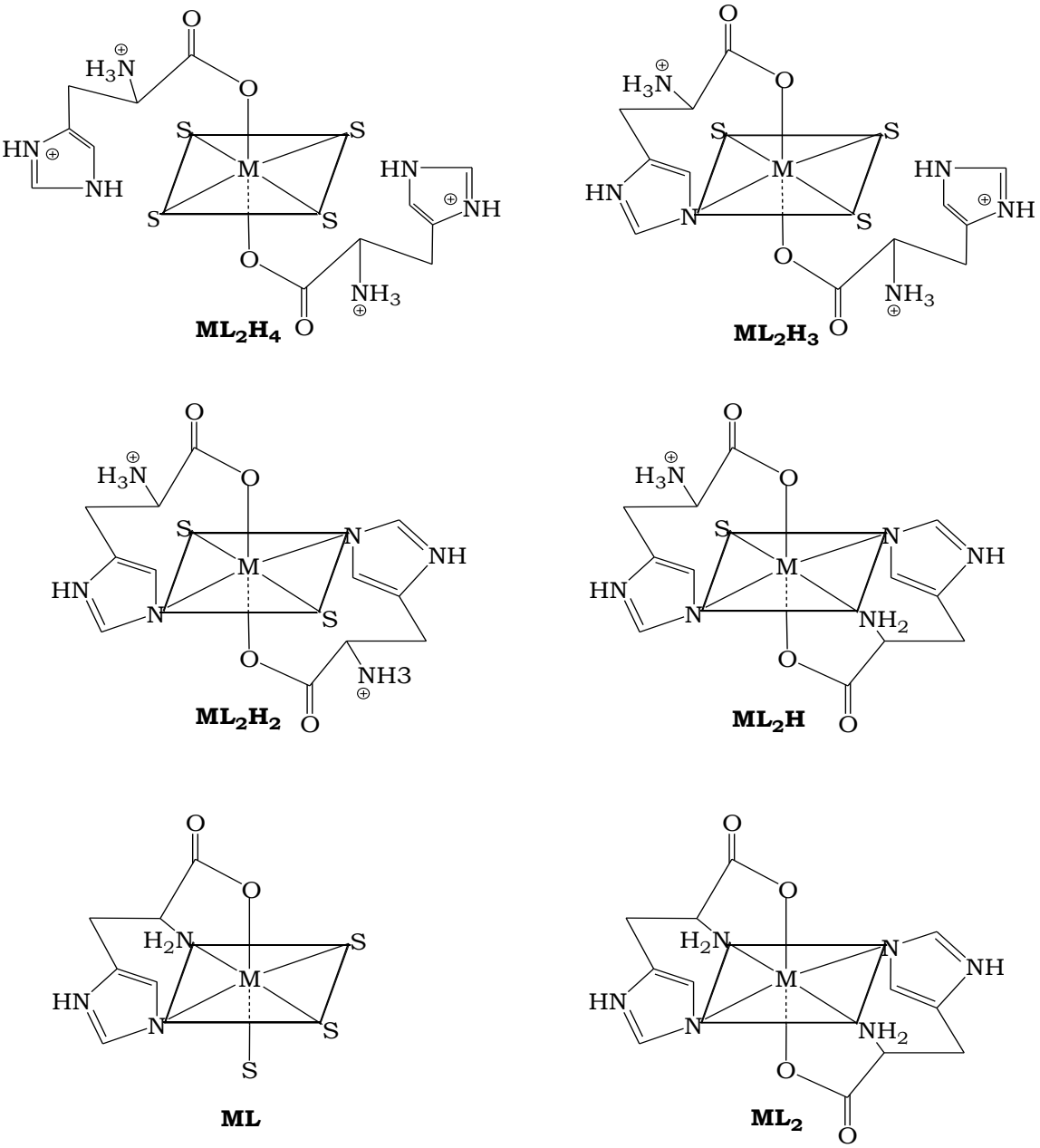


Figure 32: Speculated structures of Histidine complexes with Pb(II), Cd(II) and Hg(II); Where S is either solvent or water molecules.

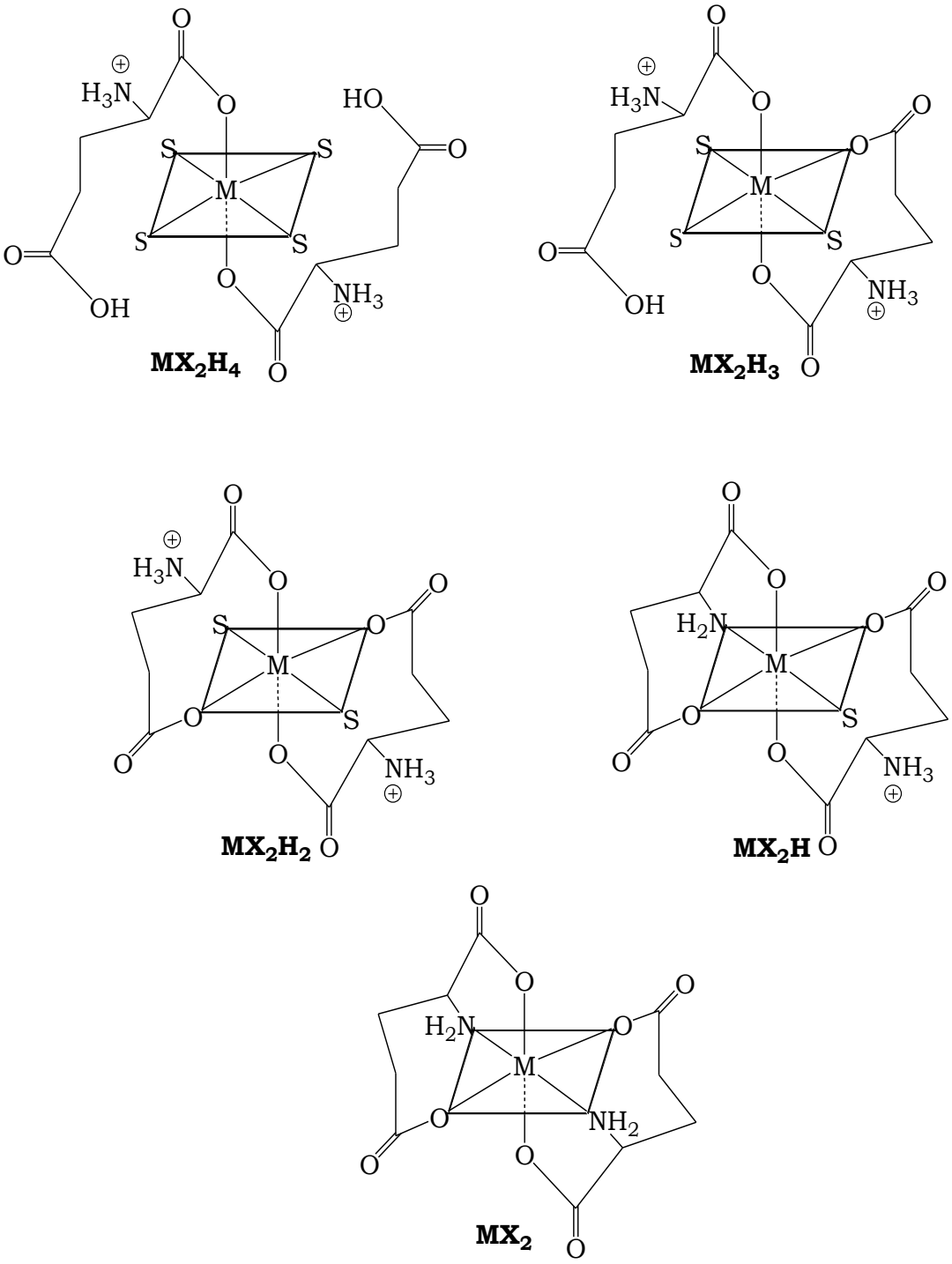


Figure 33: Speculated structures of Glutamic acid complexes with Pb(II), Cd(II) and Hg(II), Where S is either solvent or water molecules.

SECTION-III

4.3 DETERMINATION OF TERNARY STABILITY CONSTANTS

The ternary complexes containing a metal ion and two different ligands have been studied previously [81]. Stability constant of ternary complexes of some essential divalent metal ions with L-histidine and L-glutamic acid in DMSO–water mixtures [112] were reported. Hence, the stability constant of ternary complex of H and E with toxic heavy metal ion in DMF- water mixture were discussed in this section.

4.3.1 Alkalimetric Titration Curves

Titrations of solutions containing different ratios of metal to H and E (M: L: X=1.0:2.5:2.5, 1.0:2.5:5.0, 1.0:5.0:2.5 in the case of Pb (II) and Cd(II) and 1.0:5.0:5.0, 1.0:5.0:10.0, 1.0:10.0:5.0 in the case of Hg(II)) were carried out with 0.4 mol dm^{-3} NaOH as titrant in DMF-water mixtures. The actual concentrations of the metal ions, ligands and the mineral acid are given in Tables 8 Chapter 3. Some typical alkalimetric titration curves of mixtures containing different mole ratios of H and E in the presence of mineral acid and inert electrolyte are given in Figure 34.

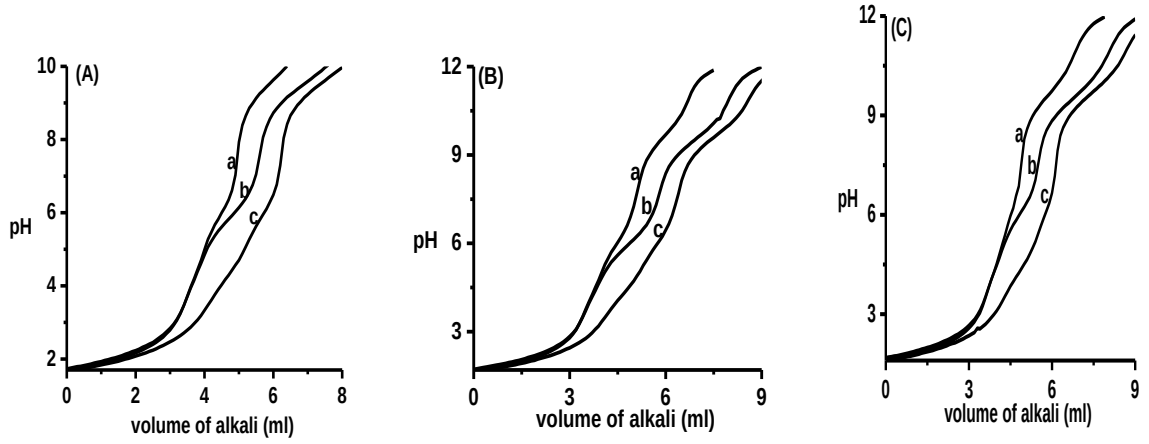


Figure 34: Alkalimetric titration curves for H and E complexes in 20 %(v/v) DMF water mixture. Where: (A) Pb(II), (B) Cd(II) and (C) Hg(II) and number of mmols of the ligands: a) 0.25, 0.25 b) 0.25, 0.50 c) 0.50, 0.25.

4.3.2 Selection of Best-Fit Model

The formation constants for acido-basic equilibria of both primary and secondary ligands and those for binary metal complexes were fixed in testing various chemical models using MINIQUAD75. The base model assumed in this case contained the species MLX and MLXH and all other species cited in Table 16 were used to generate different models. Existence of species was determined by performing exhaustive modeling. The models were evaluated assuming the simultaneous existence of different combinations of species. As the number of species increased, the models gave better statistics denoting better fit. The program MINIQUAD75 rejects all the metal complexes of the form ML_2XHh or MLX_2Hh. This may be because of the instability of these complexes due to the inability of the metal ions to accommodate three bulky ligands. The reasons for the existence of different species are ascribed under the head distribution diagrams.

Table 16: Parameters of best-fit chemical models of H and E complexes of Pb(II), Cd(II) and Hg(II) in DMF-water mixtures

%v/v DMF	log β_{mlxh}(SD)					NP	Ucorr $\times 10^8$	Skewness	Kurtosis	χ^2	R-Factor	pH-Range
	ML_2X	MLX_2H_2	MLXH	$MLXH_2$	$MLXH_3$							
Pb(II)												
00.0	22.35(9)	---	25.06(7)	31.15(3)	36.32(5)	67	0.32	0.07	5.60	12.50	0.003	4.5-9.6
10.0	23.82(8)	---	29.87(1)	35.40(0)	41.41(9)	99	1.77	1.80	10.0	12.51	0.006	4.5-9.6
20.0	23.82(0)	---	30.30(3)	36.17(4)	41.90(2)	54	3.22	1.05	5.61	11.82	0.005	4.5-9.6
30.0	22.70(1)	---	29.70(7)	36.00(7)	41.96(6)	77	4.33	1.01	3.11	12.31	0.006	4.5-9.6
40.0	23.86(7)	---	30.16(6)	34.95(0)	40.61(7)	95	0.63	0.41	4.90	12.62	0.002	4.5-9.6
50.0	22.70(7)	---	29.50(9)	35.71(8)	41.42(3)	85	1.21	0.95	3.20	13.63	0.007	4.5-9.6
Cd(II)												
00.0	13.55(4)	29.90(0)	---	---	---	67	8.66	-0.90	5.40	14.64	0.009	3.0-9.6
10.0	14.87(1)	30.46(8)	---	---	---	99	4.45	0.45	5.00	12.30	0.010	3.0-9.6
20.0	14.02(6)	30.60(1)	---	---	---	90	8.63	0.43	3.30	12.26	0.004	3.0-9.6
30.0	11.80(7)	30.25(4)	---	---	---	90	9.70	1.24	3.82	12.80	0.020	3.0-9.6
40.0	13.27(9)	30.75(3)	---	---	---	77	1.48	1.21	3.46	12.91	0.009	3.0-9.6
50.0	11.80(5)	30.93(5)	---	---	---	98	3.68	1.70	5.33	11.90	0.008	3.0-9.6
Hg(II)												
00.0	---	---	---	28.34(0)	32.76(7)	100	1.34	-1.13	4.25	12.30	0.003	2.0-9.0
10.0	---	---	---	28.35(4)	33.19(9)	39	0.18	0.03	2.80	13.76	0.009	4.0-8.0
20.0	---	---	---	29.90(5)	35.21(4)	45	3.96	-1.01	4.80	11.02	0.006	4.0-9.0
30.0	---	---	---	29.62(6)	35.07(5)	80	4.06	-0.53	5.70	12.70	0.006	4.0-9.0
40.0	---	---	---	29.90(17)	35.90(1)	55	2.78	-0.80	4.51	12.33	0.013	5.0-9.0
50.0	---	---	---	33.28(0)	39.27(4)	30	5.01	-0.57	3.60	12.57	0.021	5.0-9.0

Ucorr = U/ (NP-m); where m = number of species; NP = number of experimental points; SD=standard deviation

4.3.3 Residual Analysis

A very low standard deviation in log β values indicates the precision of the parameters. The small values of U_{corr} indicate the consistency of the model with the experimental data. For most of the systems the kurtosis values are around 3 hence the residuals form mesokurtic pattern. For some systems kurtosis values are more than 3 hence they form a leptokurtic pattern. The values of skewness between -1.13 and 1.80 in DMF-water mixtures show that the residuals form a part of normal distribution and hence a least squares method can be applied to the present data. The sufficiency of the model is further evident from the low crystallographic R factor values, which indicate the need for inclusion of additional species in the model. χ^2 is a special case of γ distribution which measures the probability of residuals forming a part of standard normal distribution [89].

4.4.4 Effect of Systematic Errors

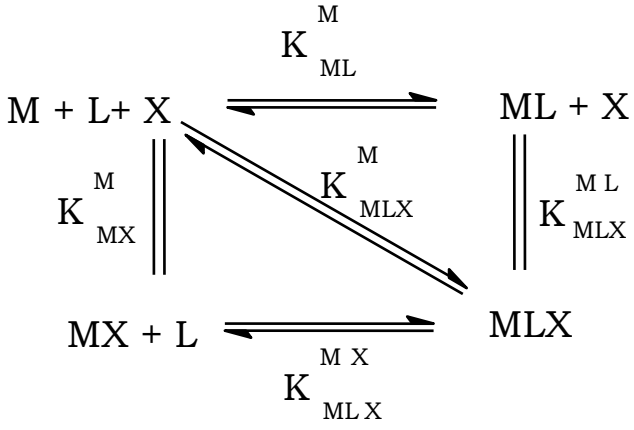
The results of effect of errors in the concentrations of alkali, mineral acid, ligands, metal and volume are given in Table 17, which emphasizes that the errors in alkali and acid affect stability constants more than those of the ligands, metal and volume.

Table 17: Effect of errors in influential parameters on H-Pb(II)-E complexes stability constants in 40% v/v DMF-water mixture.

Ingredient	% of error	log β_{mlxh}(SD)			
		ML_2X	MLXH	$MLXH_2$	$MLXH_3$
	0	23.86(7)	30.16(6)	34.95(0)	40.61(7)
Alkali	-5	Rejected	32.05(19)	39.50(22)	45.20(22)
	-2	Rejected	Rejected	35.68(28)	41.06(23)
	+2	23.80(18)	27.64(31)	32.16(55)	37.70(33)
	+5	25.09(30)	26.37(44)	31.30(66)	Rejected
Acid	-5	24.62(32)	26.40(32)	31.27(29)	35.80(50)
	-2	23.62(19)	Rejected	32.37(68)	37.90(35)
	+2	Rejected	29.73(29)	35.35(37)	40.83(20)
	+5	Rejected	30.80(29)	38.26(23)	43.74(23)
Ligand(L)	-5	24.12(19)	30.23(18)	35.08(26)	41.00(19)
	-2	23.96(17)	30.19(12)	35.05(20)	40.80(18)
	+2	23.80(14)	30.15(19)	34.93(19)	40.51(17)
	+5	23.7315)	30.09(15)	34.82(21)	40.30(19)
Ligand(X)	-5	23.80(10)	29.75(19)	34.14(17)	40.15(10)
	-2	23.87(19)	30.04(12)	34.70(8)	40.43(12)
	+2	23.90(17)	30.27(13)	35.27(9)	40.80(9)
	+5	24.00(11)	30.40(10)	35.60(19)	41.09(15)
Metal	-5	23.86(9)	29.20(8)	34.59(8)	40.39(19)
	-2	23.88(8)	30.06(7)	34.83(19)	40.59(18)
	+2	23.90(7)	30.18(7)	35.89(16)	40.70(18)
	+5	23.94(7)	30.29(6)	35.90(13)	40.68(9)
Volume	-5	23.87(9)	30.14(7)	35.00(22)	40.67(8)
	-2	23.88(9)	30.14(7)	35.00(21)	40.66(8)
	+2	23.90(9)	30.14(7)	34.99(18)	40.65(8)
	+5	23.92(8)	30.14(6)	35.00(10)	40.66(8)

4.4.5 Stability of Ternary Complexes

The formation of mononuclear unprotonated binary and ternary complexes from a mixture of metal ion (M) and primary (L) and secondary (X) ligands can be shown as the equilibria given below.



The change in stability of the ternary complexes as compared to their binary analogues was quantified [113] based on the disproportionation constant (log X) given by Equation 4.5

$$\log X = 2\log K^{M}_{MLX} - \log K^{M}_{ML_2} - \log K^{M}_{MX_2} \quad \text{----------4.5}$$

Which corresponds to the equilibrium

$$ML_2 + MX_2 \rightleftharpoons 2\,MLX$$

Under these equilibrium conditions, one can expect 50% ternary complex and 25% each of the binary complexes to be formed and the value of log X was reported [114] to be 0.6. A value greater than this account for the extra stability of MLX.

Another approach [115] to quantify the stability of ternary complexes was based on the difference in stability (Δlog K) for the reactions ML with X and $M_{(aq)}$ with L and X, where L is primary ligand (H) and X is the secondary ligand (E). It is compared with that calculated purely on statistical grounds. Equation 4.6 can be formulated based on the properties of the cyclic systems, from which it is clear that both the ligands in the ternary complex influence mutually to the same extent.

$$\Delta \log K = \log K^{M}_{MLX} - \log K^{M}_{ML} - \log K^{M}_{MX} \quad \text{---------- 4.6}$$

The electrostatic theory of binary complex formation and statistical arguments suggest the additional coordination positions of given multivalent hydrated metal ion available for the first ligand than for the second. Hence, the usual order of stability $K^{M}_{ML} > K^{ML}_{MLX}$ applies. This suggests that Δ log K should be negative, although several exceptions [116] have been found. The statistical values of Δ log K for bidentate L and X are -0.4, -0.6 and between -0.9 and -0.3 for octahedral, square planar and distorted octahedral complexes, respectively. Negative values of Δ log K can be understood as the secondary ligand forms a more stable complex with hydrated metal ion than with ML.

The log X and Δ log K values calculated from binary and ternary complexes are included in Tables 19. These values could not be calculated for some systems due to the absence of relevant binary species. In the present study, the Δ log K values range from 1.071 to 1.377 for DMF-water mixtures. The log X values range from 2.797 to 3.245 for DMF-water mixtures. Some Δ log K values and log X values are found to be more than the theoretical values, which account for the extra stability of the ternary complexes. The reason for the extra stability of these complexes may be due to interactions outside the coordination sphere such as the formation of

hydrogen bonds between the coordinated ligands, charge neutralization, chelate effect and stacking interactions

Table 18: Δ log K and log X values of ternary complexes of Pb(II), Cd(II) and Hg(II)-H and E in DMF-water mixtures

% v/v DMF	Δ log K 1210	log X 1111	1112	1113
Pb(II)		Pb(II)		
00.0	1.349	2.797	2.986	3.120
10.0	1.376	2.950	3.098	3.234
20.0	1.376	2.962	3.116	3.244
30.0	1.356	2.945	3.112	3.245
40.0	1.377	2.958	3.086	3.217
50.0	1.356	2.939	3.105	3.234
Cd(II)		Hg(II)		
00.0	1.131	--	2.904	3.030
10.0	1.172	--	2.905	3.042
20.0	1.146	--	2.951	3.093
30.0	1.071	--	2.943	3.089
40.0	1.122	--	2.951	3.110
50.0	1.071	--	3.044	3.188

4.3.6 Effect of Co-Solvent on Mixed-ligand Equilibria

Variations of logarithmic values of stability constants (log β) with 1/D of the medium are shown for both DMF- water mixtures in figure 35. These plots indicate the nature of electrostatic and non-electrostatic forces operating in the equilibria.

DMF is protophilic and aprotic solvent. DMF is a dipolar, non-aqueous, coordinating and structure former solvent. The addition of DMF to water decreases the dielectric constant of medium. The stability of ternary complexes may decrease/increase linearly with the decrease in dielectric constant of medium (with increase in DMF content). The trend indicates that the dielectric constant or long-range interactions alone are not responsible for the stability of the complexes. The nature of DMF species (neutral, anionic or cationic), cation stabilizing nature of co-solvents, specific DMF-water interactions, charge dispersion and specific interactions of DMF with solute, stacking interactions, charge neutralization, chelate effect and hydrogen bonding account for the deviation from the linear relationship. Different types of electrostatic and non-electrostatic forces dominate in different ranges of concentration of DMF-water

mixtures. The cumulative effect of both electrostatic and non-electrostatic interactions causes the non-linearity of stability constants of different species.

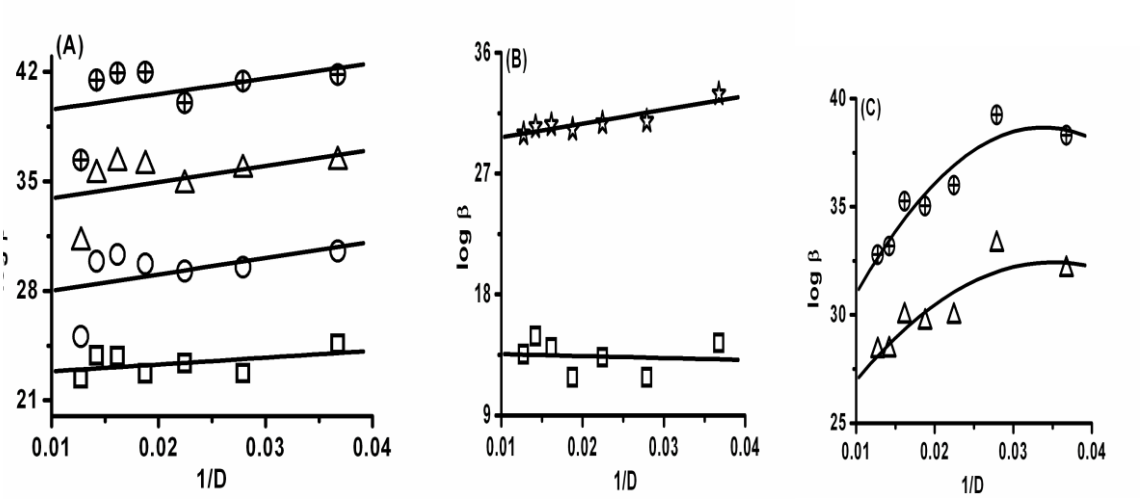


Figure 35: Variation of stability constant values of H and E complexes with reciprocal of dielectric constant (1/D) of Pb(II) (A); Cd(II) (B) and (C);Hg(II) in DMF-water mixtures. (□)log β_{ML2X}; (☆)log β_{MLX2H2}; (○)log β_{MLXH}; (Δ)log β_{MLXH2}; (⊕)log β_{MLXH3}

4.3.7 Distribution Diagrams

Distribution diagrams were drawn using the formation constants of the best-fit model and are discussed in this section. The species detected are $MLXH_3$, $MLXH_2$, MLXH and ML_2X for Pb(II), MLX_2H_2 and ML_2X for Cd(II) and $MLXH_3$ and $MLXH_2$ for Hg(II) in DMF-water mixtures. A perusal of the distribution diagrams (Figures. 36-39) reveals that at very low pH the concentration of mixed ligand complexes is less than those of protonated ligands. As the pH increased the concentrations of the ternary, species increased. The protonated ternary species, $MLXH_3$, $MLXH_2$ and MLXH are distributed at lower pH than that of the unprotonated ternary species, ML_2X. The concentrations of binary species are less compared to those of the ternary species, due to extra stability of ternary complexes. The ternary species exist in the pH ranges 2.0-9.9, 2.0-10.0 and 2.0-9.1 for Pb(II), Cd(II) and Hg(II), respectively. The formation of the complex species can be represented by the following equilibria.

$M(II) + LH_3 + XH_3$	⇌	$MLXH_3 + 3H^+$	-------(1)
$M(II) + LH_3 + XH_3$	⇌	$MLXH_2 + 4H^+$	-------(2)
$MLXH_3$	⇌	$MLXH_2 + H^+$	-------(3)
$M(II) + LH_3 + XH_3$	⇌	$MLXH + 5H^+$	-------(4)
$M(II) + LH_2 + XH_2$	⇌	$MLXH + 3H^+$	-------(5)
$MLXH_3$	⇌	$MLXH + 2H^+$	-------(6)
$M(II) + LH_3 + 2XH_3$	⇌	$MLX_2H_2 + 7H^+$	-------(7)
$MX_2H_4 + LH_3$	⇌	$MLX_2H_2 + 5H^+$	-------(8)
$M(II) + 2LH_2 + XH_2$	⇌	$ML_2X + 6H^+$	-------(9)
$MLXH_2 + LH_2$	⇌	$ML_2X + 4H^+$	-------(10)
$MLXH + LH$	⇌	$ML_2X + 2H^+$	-------(11)
$ML_2H_3 + XH_2$	⇌	$ML_2X + 5H^+$	-------(12)
$ML_2H_2 + XH_2$	⇌	$ML_2X + 4H^+$	-------(13)
$ML_2H + XH$	⇌	$ML_2X + 2H^+$	-------(14)

The predominant species $MLXH_3$ of H-Pb(II)-E system is formed at low pH by the interaction of free metal (FM) ion with protonated ligands (Equi 1). $MLXH_2$ species is formed by the reaction of FM with LH_3 and XH_3 or by the deprotonation of $MLXH_3$ with increasing pH (Equi 2 and 3). MLXH is formed by the interaction of FM with LH_3 and XH_3 or LH_2 and XH_2 or by the deprotonation of $MLXH_3$ (Equi 4-6). Reaction of $MLXH_2$ with LH_2 or MLXH with LH forms ML_2X (Equi. 10 and 11). For H-Cd(II)-E system, ML_2XH_2 is formed by the interaction of FM with protonated ligands or MX_2H_4 with LH_3 (Equi. 7 and 8). ML_2X species is formed by the interaction of FM with LH_2 and XH_2 or reaction of ML_2H_3, ML_2H_2 and ML_2H with protonated ligand species (Equi 12-14). In the case of H-Hg(II)-E system, $MLXH_3$ species is formed by the interaction of FM with protonated species (Equi. 1). $MLXH_2$ is formed by the interaction of FM with protonated ligands or deprotonation of $MLXH_3$ with increasing pH (Equi 2 and 3).

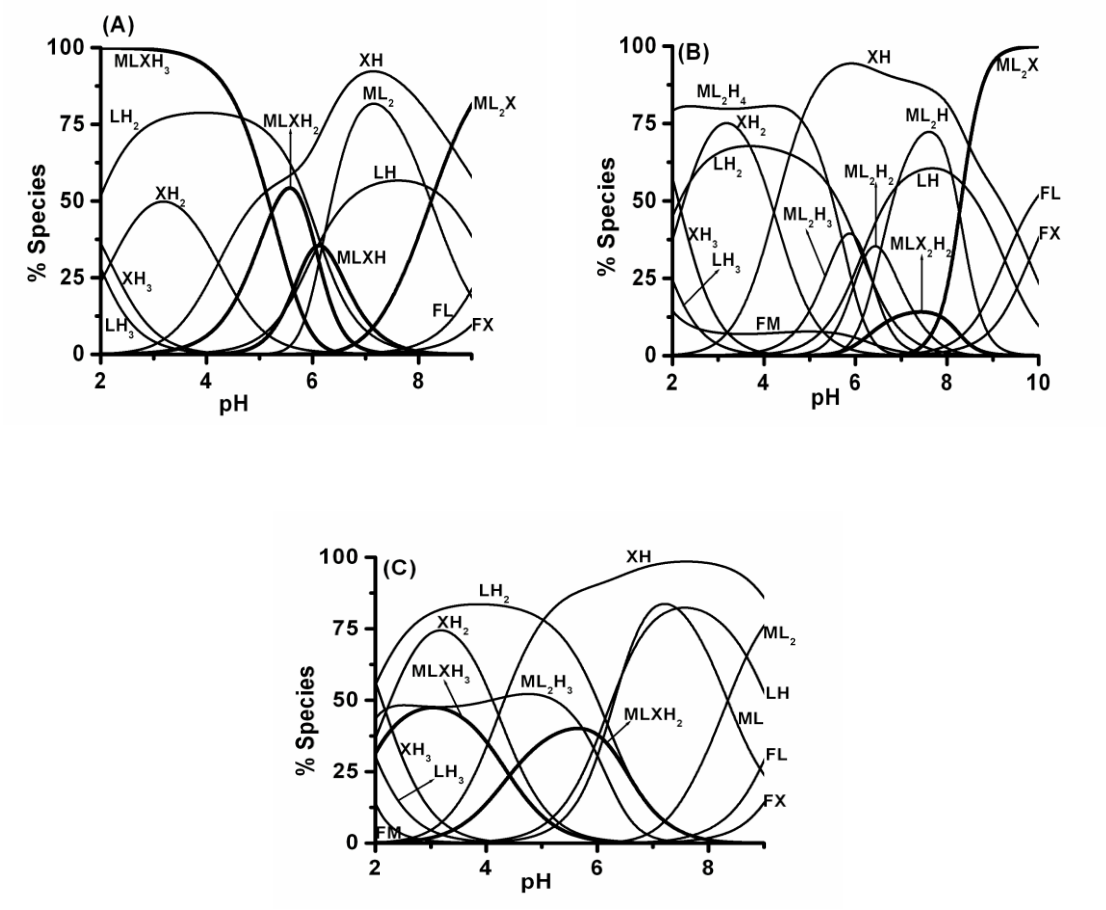


Figure 36: Distribution diagrams of H-E complexes of (A) Pb(II), (B) Cd(II) and (C) Hg(II) in aqueous medium; Where: Amounts of mixed ligand complexes of Pb(II) and Cd(II) 1.0:5.0:2.5 and Hg(II) 1.0:10.0:5.0 mmols

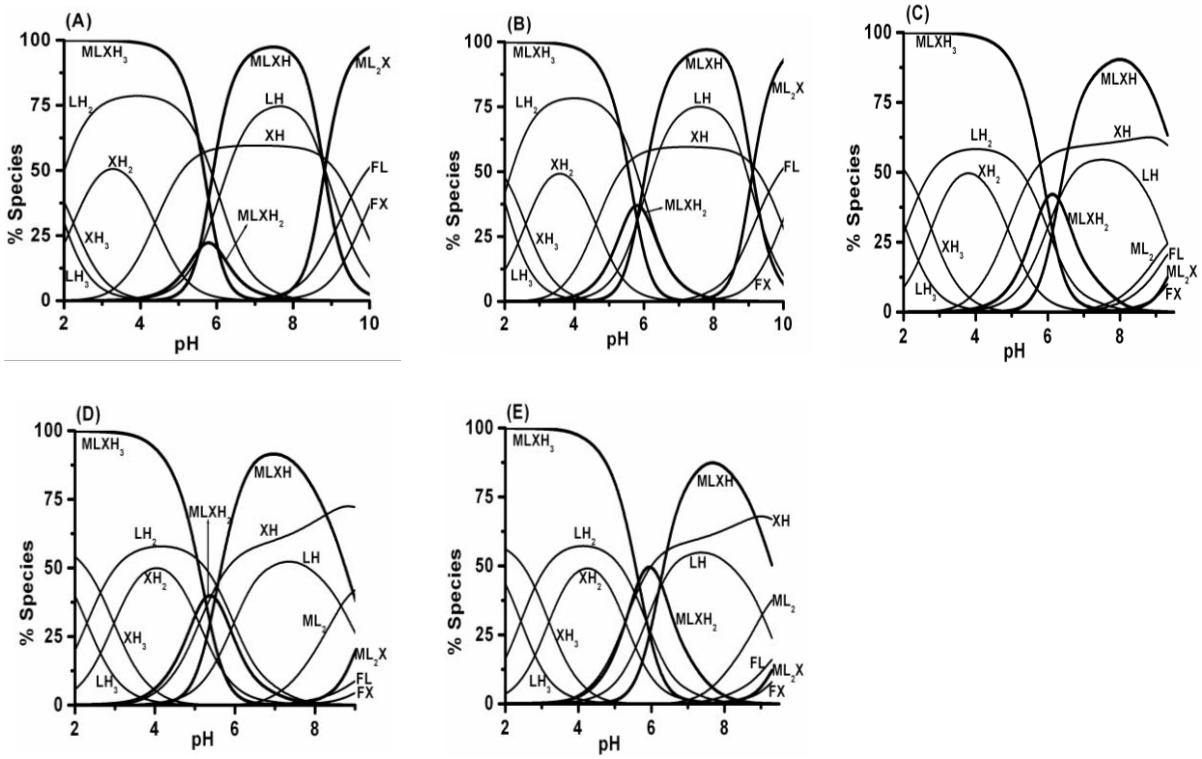


Figure 37: Distribution diagrams of H-Pb(II)-E complexes in DMF-water mixtures; Where: (A) 10.0%, (B) 20.0%, (C) 30.0%, (D) 40.0% and (E) 50.0%. Ratio of Pb(II), H and E is 1.0:5.0:2.5.

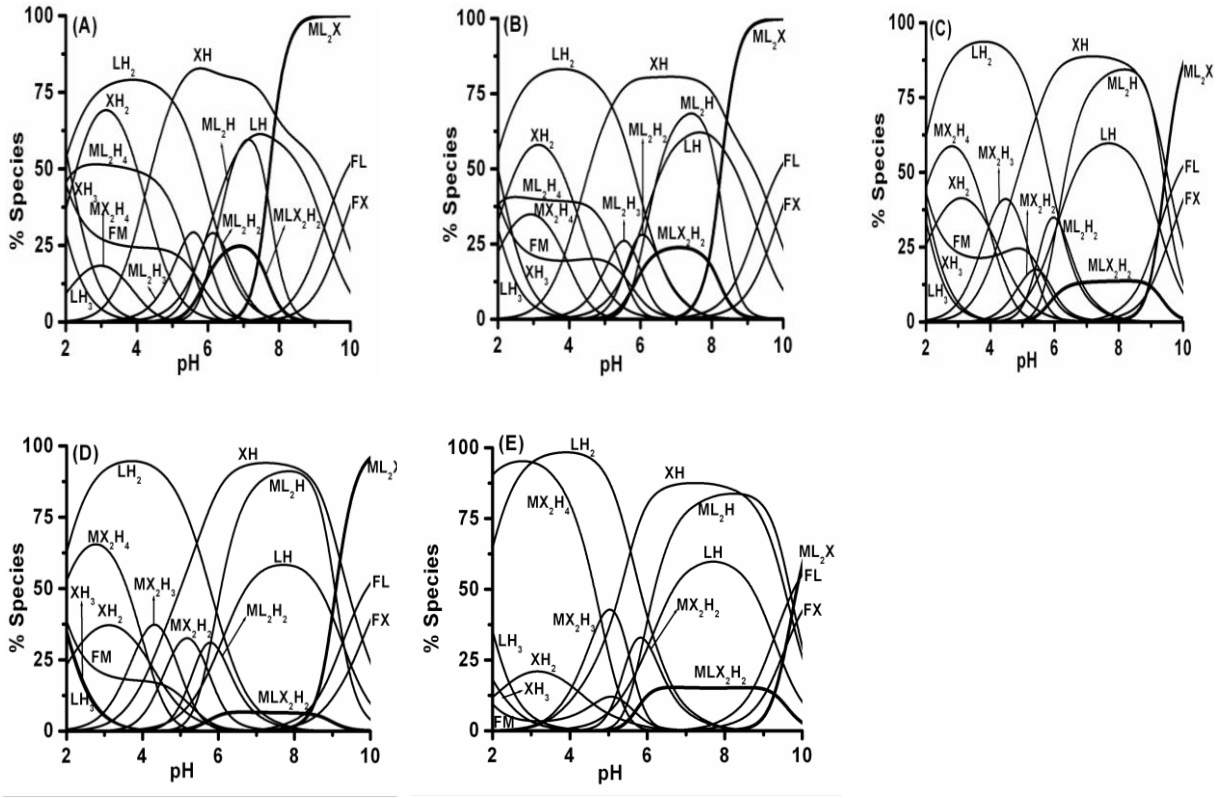


Figure 38 Distribution diagrams of H-Cd(II)-E complexes in DMF-water mixtures: Where: (A) 10.0%, (B) 20.0%, (C) 30.0%, (D) 40.0% and (E) 50.0% . Ratio of Cd(II), H and E is 1.0:5.0:2.5

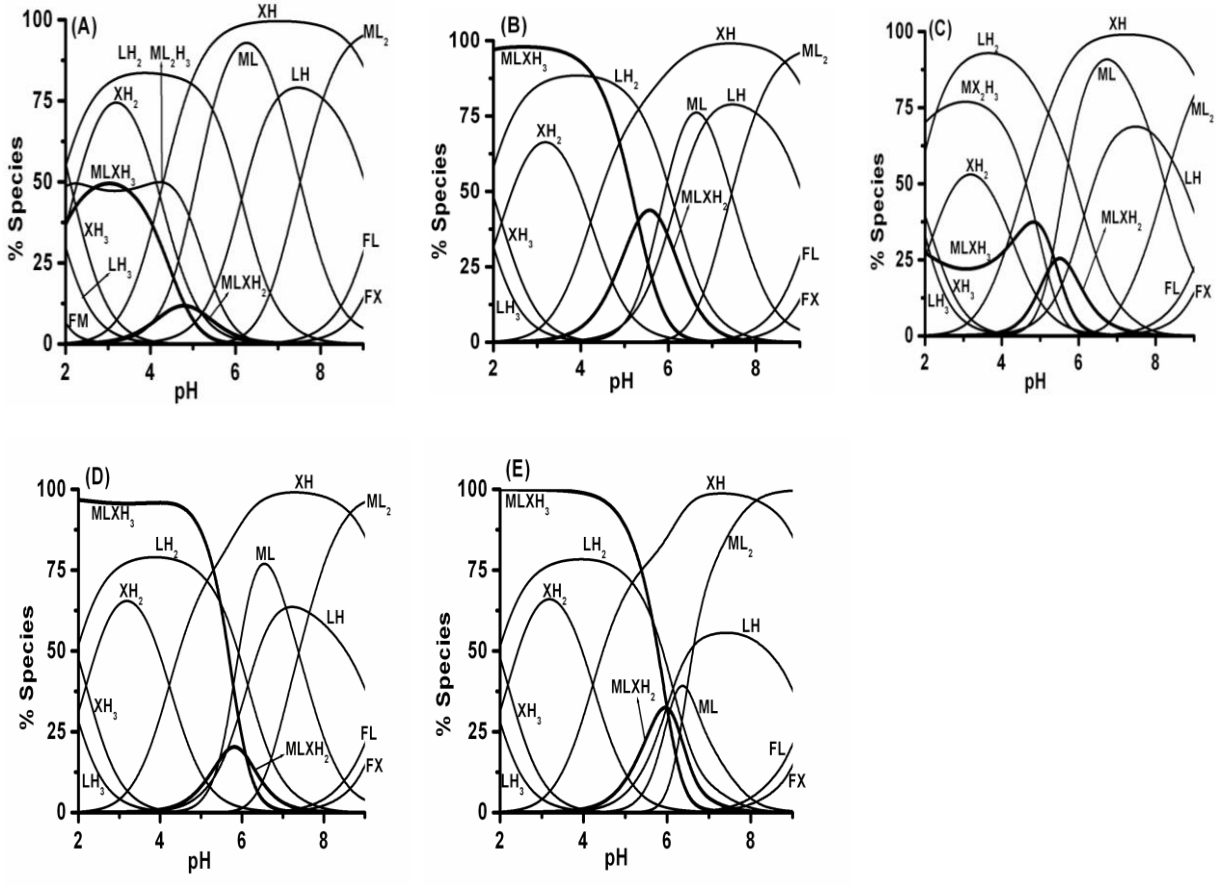


Figure 39: Distribution diagrams of H-Hg(II)-E complexes in DMF-water mixtures;(A) 10.0%, (B) 20.0%, (C) 30.0%, (D) 40.0% and (E) 50.0%. Ratio of Hg(II), H and E is 1.0:10.0:5.0.

4.3.8 Structures of Ternary Complexes

Depending upon the nature of the ligands, metal ions and based on the chemical knowledge the structures of ternary complexes were proposed as shown in Figure 40. These structures indicate that H and E act as bidentate or tridentate ligands depending upon the pH conditions. At physiological pH, the tridentate chelation of H ligand has been established for *bis*-metal complexes of Co(II), Ni(II), Zn(II) and Cd(II) [117].

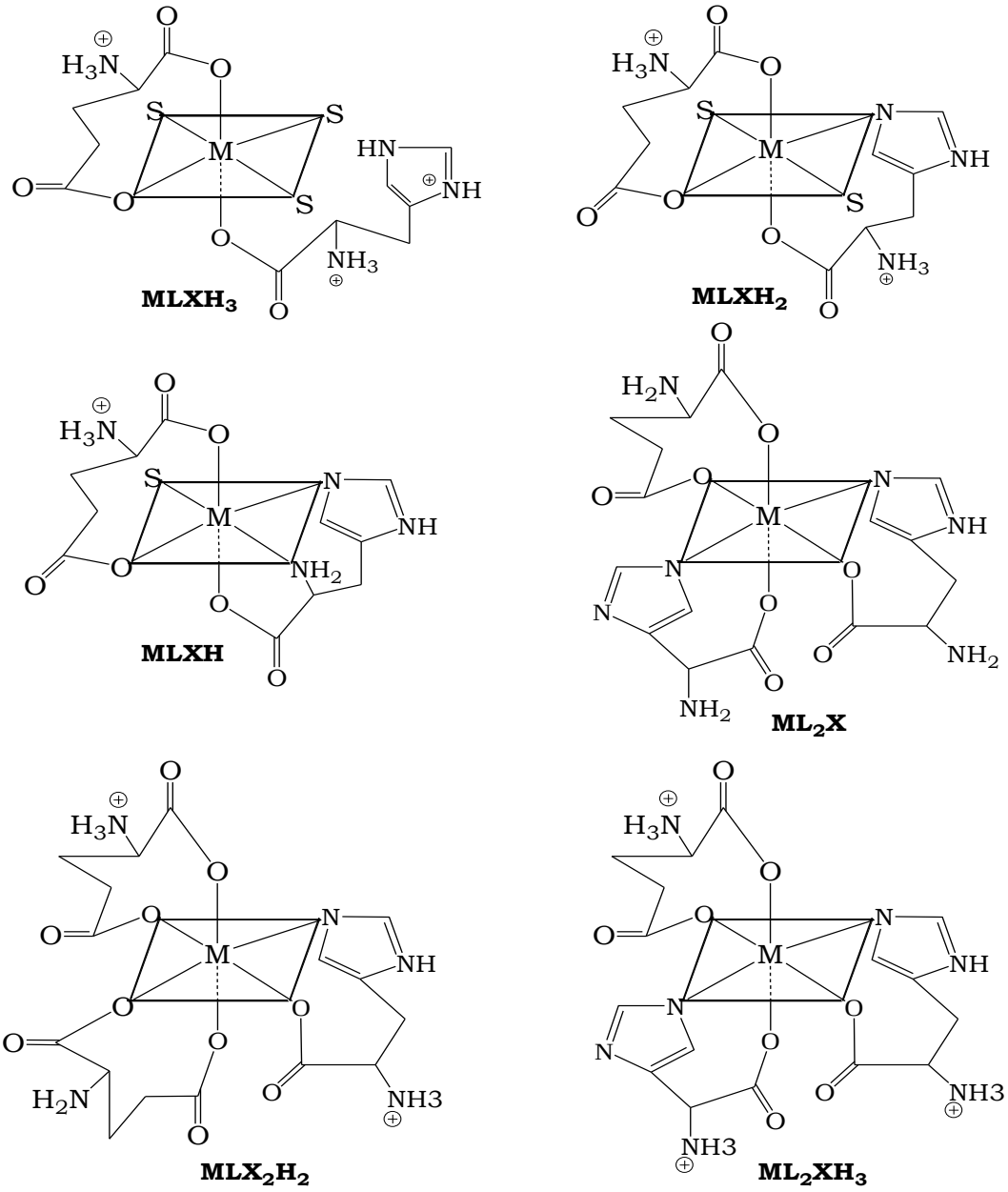


Figure 40: Speculated structures of ternary complexes of Pb(II), Cd(II) and Hg(II), Where S is either solvent or water molecules.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATION

5.1 CONCLUSIONS

Pollution and toxicity of heavy metal ions are of the latest health concern. Studies of interaction of toxic metal ions with bioavailable ligands throw light in understanding the mechanism of their adverse effects. These studies involve in determining different forms of the toxic metal ions and their speciation. Speciation includes oxidation state, concentration and composition of each of the species present in a chemical sample. Speciation has implications for various aspects of medicine and toxicology like dental care and total parenteral nutrition. Better computing and analytical methods led to an understanding of the speciation underlying many chemical reactions, with interesting conclusions in medicine, industry and in the environmental studies. Toxic metal ions inhibit the metabolic processes, sometimes fatal to organism. Cells can prevent toxic metal ions by inducing the formation of metal binding proteins. Hence, toxic metal ions Pb (II), Cd (II) and Hg (II) were selected for the study by considering L-Histidine and L-Glutamic acid in place of apoenzymes and substrates. The study is carried out in Dimethylformamide (DMF)-water mixtures to mimic the nature of the active sites of the biological molecules. The following conclusions are drawn based on the experimental results.

Protonation Equilibria

Different forms of H were LH_3^{2+}, LH_2^{+}, LH and L^{-} in the pH ranges 1.8-4.0, 1.8-6.0, 6.0-7.5 and 6.5-11.0, respectively. In the case of E contains two carboxylic and amino protons. The different forms of E are XH_3^{+}, XH_2, XH^{-} and X^{2-} in the pH ranges 2.0-4.2, 3.5-5.5, 3.0-10.5 and 7.4-11.0, respectively. The protonation constants increased with decreasing dielectric constants of the medium.

1. Histidine formed LH_3^{2+} at low pH and deprotonated takes place with the formation of LH_2^{+}, LH and L^{-} with the increase in pH and L-glutamic acid formed XH_3^{+} at low pH and deprotonated to form XH_2, XH^{-} and X^{2-} successively with the increase in pH.
2. Overlapping of formation function verses pH curves for different concentrations of the ligand indicates non-polymerization of this ligand under the pH condition employed in the presented study.

3. Linear increase is the log values of stability constants with decreasing dielectric constant of DMF-water mixtures indicated the dominance of electrostatic forces in the protonation-deprotonation equilibria.
4. Effect of errors in the ingredients indicated that the protonation constants associated with carboxyl proton were more affected than the amino proton. This is probably due to lower magnitude of carboxyl protons than amino proton. The errors in concentrations of alkali and acid affected the protonation constants more than that of ligand.
5. Overlapping of $\bar{n}_H$ versus pH curves for different concentrations of the ligand indicated non-polymerization of these ligands under the pH condition employed in the present study.
6. The presence of co-solvent in the medium influence the protonation deprotonation equilibrium in solution due to change in dielectric constant of the media

Metal-Ligand Equilibria

1. From the exhaustive modeling studies, it was concluded that as the number of species was increased, the models gave better statistics, which denoted the adequacy of the models. This indicates that the final models appropriately portray the experimental data.
2. None overlapping of the formation curves evinced the formation of protonated complexes.
3. The binary species refined for the H complexes of Pb(II) and Cd(II) were ML_2, ML_2H, ML_2H_2, ML_2H_3 and ML_2H_4. In the case of Hg(II), ML, ML_2, ML_2H_3 and ML_2H_4 were refined in the DMF media.
4. For E complexes of Pb(II), Cd(II) and Hg(II) the species MX, MX_2, MX_2H_2, MX_2H_3 and MX_2H_4 are refined in DMF-water mixtures.
5. Errors introduced in the concentrations of ingredients affected the magnitudes of stability constants of metal complexes, which were in the order: alkali > acid > ligand > metal. It was also reflected in the statistical parameters like standard deviation, skewness, kurtosis etc. Even some species were rejected as the introduction of errors. This supports the necessity of the accuracy of the experimental results.
6. Formation of same species in both media by ligand indicates that the solvent affects the stability of complexes only but not the formation of species.

Mixed-Ligand Equilibria

1. The interaction of metal ions (M) with H (L) and E (X) in DMF- water mixtures inferred the formation of (i) ML_2X, MLXH, $MLXH_2$ and $MLXH_3$ with Pb(II), (ii) ML_2X and MLX_2H_2 with Cd(II), and (iii) $MLXH_2$ and $MLXH_3$ with Hg(II).

2. The linear and non-linear increase in the stabilities of the ternary complexes with decrease in the dielectric constants was due to the dominance of the electrostatic forces and non-electrostatic forces.
3. The values of Δ log K indicated that the ternary species had extra stability compared to their binary species, may be due to the interactions outside the coordination sphere, such as the formation of hydrogen bonds between the coordinated ligands, charge neutralization, chelate effect, stacking interactions and the electrostatic interaction between non-coordinated charge groups of the ligands.
4. The protonation constant associated with carboxyl proteins are more affected than amino protein, The order of the ingredients that influence the magnitude of stability constants due to incorporation of errors was alkali > acid > E > H > metal ion.
5. The study gave an insight into the metal ion availability/metal ion transport in bio fluids, which may help in assessing the etiology of these metal ions. The ternary complexes are more amenable for 'metal ion transport' because of their extra stability and the binary complexes make the 'metal ion bioavailable' in bio systems due to their decreased stability.

5.2 RECOMMENDATION

From the outcomes of the study, the following recommendations for future work can be made:

- In our country Ethiopia, the study of chemical speciation of elements is understudied regarding the chemical speciation of elements to ensure the environmental quality, bioavailability and toxicity of chemical species. Hence the author recommend that researchers focus more on this study to ensure the concentration and composition of heavy metals in medicine/drugs, beverages, food complexes etc. manufacturing to safeguard public health and for environmental quality.
- Using distribution diagram of binary stability constant, the maximum yield of solid complexes optimum condition for the synthesis of solid complexes of a definite composition at fixed pH value decided the stability of metal ligand complex and its transportation. Thus, those who are interested to proceed the next investigation should use the solid complex that synthesized in the present study as the baseline for the further investigation.

- ❖ The structure of the solid complexes in the present study were proposed potentiometrically. Therefore, NMR and FTIR Spectroscopy should further confirm it.

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LIST OF PUBLICATIONS

1) Meti Mengistu, Rajalakshmanan Eswaramoorthy and Hadgu Hailekiros Belay, A Chemometric Speciation Study of Binary Complexes of L-Histidine and L-Glutamic Acid with Pb (II), Cd (II) and Hg (II) In Organic Media- Water Mixture

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2) Meti Mengistu, Rajalakshmanan Eswaramoorthy and Hadgu Hailekiros Belay, Solution Equilibrium Study of Complexation of Pb(II), Cd(II) and Hg(II) With L-histidine and L-glutamic acid: Bio-mimicked Study

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3) Meti Mengistu, Rajalakshmanan Eswaramoorthy, Meriama Kufa and Hadgu Hailekiros Belay, Shift in pKa of L-Histidine and L-Glutamic Acid in Organic Media: A Potentiometric Study

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