

**CHEMICAL SPECIATION OF COMPLEXES OF MALEIC ACID
AND L-PHENYLALANINE WITH TOXIC METAL IONS IN
DIMETHYLFORMAMIDE-WATER MIXTURE**

By

WAKGARI BUNGULA NEGASSA, B.Ed



**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**

SEPTEMBER, 2018

ADAMA, ETHIOPIA

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DECLARATION

I hereby declare that this thesis entitled “**Chemical speciation of complexes of maleic acid and L-phenylalanine 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. Tegene Desalegn during the period of September 2017-September 2018. 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.

Adama, Ethiopia
Date 18/09/20108

Wakgari Bungula Negassa

ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

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CERTIFICATE

This is to certify that the thesis entitled “**Chemical speciation of complexes of maleic acid and L-phenylalanine with toxic metal ion in DMF-water mixtures**” submitted by **Wakgari Bungula Negassa** to the Department Chemistry, School of applied natural science, Adama Science and technology University, Adama for the award of the degree of **master of science in Chemistry** is a bona fide record of research work carried out by him 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.

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We, the undersigned, members of the board of examiners of the final open defense by **Wakgari Bungula Negassa** have read and evaluated his thesis entitled “**Chemical speciation of complexes of maleic acid and L-phenylalanine with toxic metal ions in Dimethylformamide - water mixture**” 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.

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

DEDICATED TO

ALMIGHTY GOD AND

MY FAMILY

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LIST OF ABBREVIATION

CNS	Central Nervous System
COST	Concentration of Solution by titration
CTAB	Cetyltrimethylammonium bromide
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetate
F	Phenylalanine
GI	Gastrointestinal
IUPAC	International Union of Pure and Applied Chemistry
MA	Maleic Acid
MeHg	MethylMercury
NAP	Number of associable protons
NDP	Number of dissociable protons
NMR	Nuclear Magnetic Resonance
NP	Number of experimental points
PAD	Peripheral arterial disease
SD	Standard deviation
SLS	Sodium lauryl sulphate
TL ₀	Total initial Concentration of ligand
TM ₀	Total initial Concentration of metal
VSEPR	Valence Shell Electron Pair Repulsion

ABSTRACT

The protonation equilibria of L-phenylalanine and maleic acid and the formation of binary complexes of Pb (II), Cd (II), and Hg (II) with L-phenylalanine and maleic acid were investigated pH metrically in Dimethyl formamide-water mixtures (0-50%v/v) at 303 K and ionic strength of 0.16 mol dm⁻³ separately. The protonation constants and stability constants have been calculated using the computer program MINQUAD75 and the best fit models are arrived at based on statistical grounds employing crystallographic R factor, χ^2 , skewness and kurtosis. The dominant species refined were ML₂, ML₂H, and ML₃ of F and MA complexes of Pb(II), Cd(II) and Hg(II) in DMF-water mixtures. The appropriateness of the experimental conditions was verified by introducing errors deliberately. The trend in variation of protonation constants and stability constant of the complexes with dielectric constant of the medium was attributed for the formation and possible structures of the complex species presented. The chemical speciation was explained based on the distribution diagrams drawn using HYSS HYPERQUAD suite program.

Keywords: *speciation, complex equilibria, Dimethyl formamide, L-phenylalanine, maleic acid, stability constant, protonation constant, HYSS HYPERQUAD, MINQUAD75*

CHAPTER 1

INTRODUCTION

1.1. BACK GROUND OF THE STUDY

Heavy metals are naturally occurring elements that have a high atomic weight and density at least five times greater than that of water. They are common in our environment and many of them are essential to living organisms but some of them are highly toxic or become toxic at a given sufficient exposure and accumulation in the body. Their toxicity depends on several factors including the dose, route of exposure and chemical species as well as the age, gender, genetics and nutritional status of exposed individuals. The mechanism of the toxicity of metals is resulted 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 [1].

Metals such as Hg, Cd, Pb, Sn, Cr and As are generally not required for metabolic activity and are toxic to living organisms even at low concentrations (0.03ppm). Human civilization and increasing in industrial activity have gradually redistributed many toxic metals from the earth's crust to the environment and increased the possibility of exposure [2]. Lead, Cadmium and Mercury are among the various toxic heavy metals especially prevalent in nature due to their high industrial use. These metals serve no biological function and their presence in tissues reflects contact of the organism with its environment [3].

The main sources of lead (Pb) pollution are, from the battery industry, leaded petrol and gasoline exhaust which affects every organ of the body, specially the bones and teeth, the kidneys, and the nervous, cardiovascular, immune and reproductive systems [4]. This lead pollution also interferes with the normal metabolism of calcium in cells and causes it to build up within them [5].

Cadmium (Cd) pollution in urban areas from metallurgical plants, Cd plating and battery fabricators. It can enter the environment through natural causes, such as volcanic activity and forest fires [6]. Human exposure to cadmium mainly occurs through cigarette smoking but exposure can also occur through contaminated food, water or air [7]. Cadmium is a known carcinogen metal to mammals [8].

Mercury (Hg) 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 man-made

activities [9]. Mercury is a highly toxic element because of its accumulative and persistent character in the environment and living organisms [10]. It affects the immune system, alters genetics and enzyme systems, and damages the nervous system, and the senses of touch, taste and vision [11].

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. L- Phenylalanine (F) is an α -amino acid with the formula $C_9H_{11}NO_2$. It can be viewed as a benzyl group substituted for the methyl group of alanine, or a phenyl group in place of terminal hydrogen of alanine. It is an essential aromatic amino acid in humans (provided by food) and plays a key role in the biosynthesis of other amino acids. This essential amino acid is classified as neutral, and nonpolar because of the inert and hydrophobic nature of the benzyl side chain. It is important in the structure and function of many proteins and enzymes [12].

Maleic acid (MA) is an organic compound that is a dicarboxylic acid and is the cis isomer of butenedioic acid. It is mainly used as a precursor to fumaric acid, and relative to its parent maleic anhydride. Industrially, maleic acid is derived by hydrolysis of maleic anhydride, the latter being produced by oxidation of benzene or butane. It is an industrial raw material for the production of glyoxylic acid by ozonolysis [13].

N, N-Dimethylformamide is a colorless liquid at room temperature with a faint amine odor. There are many synonyms for this compound, the most common being the acronym DMF with a molecular mass of 73.09 and molecular formula of (C_3H_7NO) . Commercially contains trace amounts of methanol, water, formic acid, and dimethylamine. It is miscible in all proportions with water and most organic solvents. It is also a powerful solvent for a variety of organic, inorganic, and resin products [14].

Speciation studies of toxic and essential 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 [15]. 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 [16].

Chemical speciation of metals is important for an understanding of their distribution, mobility, bioavailability, toxicity and for setting environmental quality standards [17]. Bioavailability of a particular metal depends on its complex chemical reactions of dissolution, binding and complexation with the constituents of the environmental aquatic media [18,19]. To reveal the solvent effects on equilibrium processes involving charged species in this work, the complex formation of L-phenylalanine and maleic acid with Pb(II), Cd(II) and Hg(II) as a good example in modeling of the bonding modes of peptides to toxic metal ions in aqueous media and N, N-dimethylformamide (DMF)-water mixture was investigated.

1.2. STATEMENT OF THE PROBLEM

For some heavy metals, toxic levels can be just above the background concentrations naturally found in nature. Therefore, it is important to inform about the toxic heavy metals and to take protective measures against excessive exposure. Living organisms exposed environmentally to high metal concentrations follow various mechanisms to counter potential toxicity [20].

However, the study of metal-protein interactions involves complications due to the complex nature of proteins. An exact model of the metal-protein system may be difficult to construct analytically in simple bio-molecules like amino acids and peptides but such models may give a tremendous amount of information about the structure of proteins and function of bio-molecules in biological systems by chemical speciation method. Most of the speciation studies are performed in aqueous media under conditions comparable to those existing in physiological systems. But bio-systems are associated with low dielectric media of different magnitude and metabolic reactions are carried out under strictly compartmentalized conditions. Some studies were performed in aqua-organic mixtures to mimic these conditions but no attempt was made to account for the DMF-water mixture for the selected ligands [21]. So in the present study L-phenylalanine and maleic acid were chosen as a model compounds to investigate the binding effect of bio-molecules with the toxic metal ions of Pb(II), Cd(II) and Hg(II) in aqueous media and DMF- water mixture under conditions comparable to those of biological systems [22].

1.3. OBJECTIVE OF THE STUDY

1.3.1. General objective

The overall objective of this study was:

To investigate the types of species refined, protonation equilibria of L-phenylalanine and maleic acid and their binary complexes with toxic metal ions in DMF-water mixtures of varying compositions (0-50%v/v) at an ionic strength of 0.16 mol dm^{-3} .

1.3.2. Specific objectives

The specific Objectives of this study were to:

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

1.4. SIGNIFICANCE OF STUDY

Knowledge of chemical speciation of metal ions is needed in human biology, nutrition, and toxicology and clinical practice because bioavailability, metabolism and excretion, toxicity, biological activity and detoxifying processes are dependent on the particular species considered. In addition, transport of the metal in the body and its final deposition/storing in target organ (e.g. brain, liver and bone) can be conditioned 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 [23].

The solution DMF-water mixture was chosen to maintain the dielectric constant of the medium and to mimic those of the bio-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].

In speciation study, the most important parameter to characterize proton-ligands and metal-ligand complexation is the stability constant, which is thermodynamically unique for an organic ligand and a complex formed with the metal ion. The species refined and their relative concentrations under the present experimental conditions represent the possible forms of ligand and metal-ligand in the biological fluids [25].

This type of study throws light on 1) the effect of electrostatic and non-electrostatic interactions on complex formation in vitro, 2) the nature of active site cavity of enzymes, 3) detection of specific solute-solvent interactions and 4) understanding the selectivity and sensitivity of various protonated complexes and relative abundance of free components.

CHAPTER 2

LITERATURE REVIEW

2.1. CHEMICAL SPECIATION

In all living systems, the biochemical functions of both essential and toxic metals are mediated through specific chemical species, or complexes, and the concentrations of these particular species are important for the biochemical reactions but not just the total concentration of the metal in the system [26]. For instance, some elements can be highly toxic to various life forms; others are considered essential, but can become toxic at higher doses. Many of these effects depend strongly on the particular form in which the element is present in the system. For example, Cr(VI) ions are considered far more toxic than Cr(III). On the other hand, 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 been accepted in such diverse fields as toxicology, clinical chemistry, geochemistry and environmental chemistry [27].

New developments in analytical instrumentation and methodology now often allow the identification and measurement of the species present in a particular system. Because of these possibilities, numerous publications have appeared in which the term “speciation” is employed. However, this term 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 [28].

In an attempt to end the confusion regarding the usage of the term speciation IUPAC commissioned an interdisciplinary committee to consider the issue and 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. This recommendation leads to the following definitions: [29]

- i. Chemical species: Specific form of an element defined as to isotopic composition, electronic or oxidation state, or complex or molecular structure.
- ii. Speciation analysis: Analytical activities of identifying and measuring the quantities of one or more individual chemical species in a sample.
- iii. Speciation of an element: Distribution of an element amongst defined chemical species in a system under specified conditions.

Various conformations, excited states, or transient forms of an element, its coordinated atoms, and the molecules of which they are part technically constitute unique species. Nevertheless, analytical methodology and practical considerations dictate that the speciation analysis of a system yields a profile of sufficiently different and measurable species for the desired level of understanding of the system's behavior. 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 [30]. Speciation scheme suggesting the possible distribution of metal ions in the environment is given in Fig.2.1.

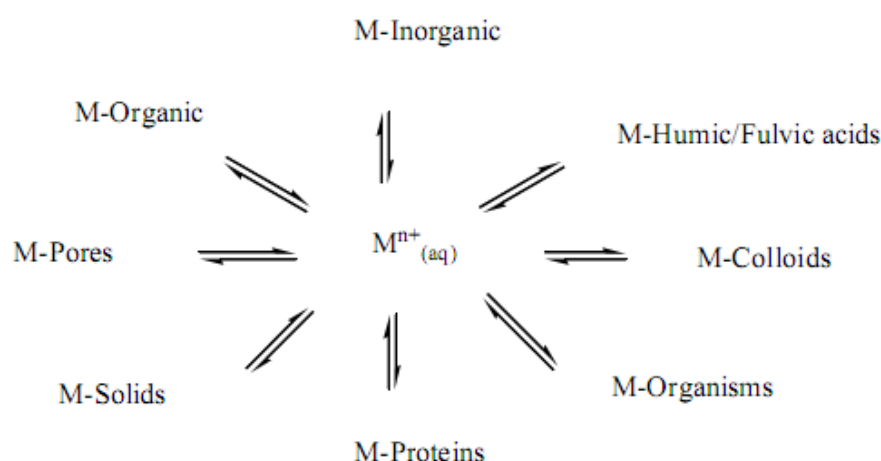


Fig.2.1: Speciation scheme suggesting the possible distribution of metal ions(M) in the environment.

2.2. BIOLOGICAL FUNCTIONS OF AMINO ACIDS

Amino acids are organic molecules containing amino group ($-\text{NH}_2$) and carboxylic acid group ($-\text{COOH}$) both attached to the same carbon atom called α carbon. They are the structural units that make up proteins. They join together to form short polymer chains called peptides or longer chains called either polypeptides or proteins. 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 [31].

2.3. PHENYLALANINE

Phenylalanine (F) is an α -amino acid with the formula $\text{C}_6\text{H}_5\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$. It is an essential amino acid and classified as nonpolar because of the hydrophobic nature of the benzyl side chain. It is an electrically neutral amino acid, one of the twenty common amino acids used to form proteins biochemically and naturally found in the breast milk of mammals. It is used in the manufacture of food and drink products and sold as a nutritional supplement for its reputed analgesic and antidepressant effects [32].

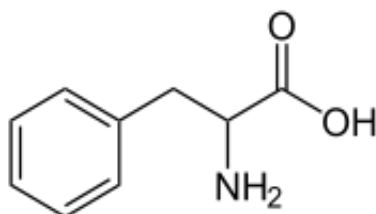


Fig.2.2: Structure of phenylalanine

2.4. MALEIC ACID

Maleic acid (MA) is an organic compound that is a dicarboxylic acid, a molecule with two carboxyl groups. It is the cis isomer of butenedioic acid, whereas fumaric acid is the trans isomer. It is mainly used as a precursor to fumaric acid, and relative to its parent maleic anhydride, has few applications [13].

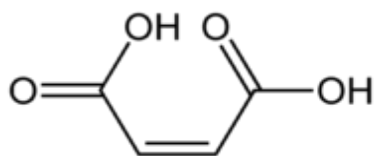


Fig.2.3: Structure of maleic acid

Maleic acid is a less stable molecule than fumaric acid. The difference in heat of combustion is $22.7 \text{ kJ}\cdot\text{mol}^{-1}$. The heat of combustion is -1355 kJ/mole . Maleic acid is more soluble in water, than fumaric acid. The melting point of maleic acid ($139\text{--}140^\circ\text{C}$) is also much lower than that of fumaric acid (287°C). Both properties of maleic acid can be explained on account of the intramolecular hydrogen bonding that takes place in maleic acid at the expense of intermolecular interactions and that are not possible in fumaric acid for geometric reasons [33].

2.5. TOXIC HEAVY METALS

Toxic heavy metal is any metallic chemical element that has high density and is toxic or poisonous at lower concentration. They may enter the body through food, water, air, or absorption through the skin when they come in contact with humans in agriculture and in manufacturing, pharmaceutical, industrial, or residential settings, however the mechanism of the intoxication of these metals is very complicated. The metal ion can get 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 get deactivated and they could not perform their biochemical functions. Metals such as Hg, Cd, Pb, Sn, Ni, Se, Cr and As which are generally not required for metabolic activity and are toxic to living organisms [34].

Living organisms exposed environmentally to high metal concentrations follow various mechanisms to counter potential toxicity. They reduce intake, enhance excretion and/or sequestration of the metals within tissues to render them nontoxic. In human and other animals' chelation therapy is the preferred medical treatment for reducing the toxic effects of metals. Chelating agents are capable of binding to toxic metal ions to form complex structures which are soluble, stable, non-toxic and easily excreted from the body removing them from intracellular or extracellular spaces [35].

2.5.1. Lead toxicity

Lead poisoning can cause a variety of symptoms and signs which vary depending on the individual and the duration of lead exposure [36]. The exposure of lead causes sweetish metallic taste, burning in mouth, severe thirst, inflammation of the GI tract with vomiting and diarrhea. Chronic anorexia, lead line on gum margin, nausea, vomiting, severe abdominal pain, paralysis, mental confusion, visual disturbances, anemia, convulsions. It is a risk factor for peripheral arterial disease (PAD) that affect thiol metabolism and may partly explain the association of homocysteine with PAD [37].

2.5.2. Cadmium toxicity

Cadmium is considered one of the most toxic substances in the environment due to its wide range of organ toxicity and long elimination half-life [38]. Inhalation of cadmium fumes or particles can be life threatening, and although acute pulmonary effects and deaths are uncommon, sporadic cases still occur. Acute exposure can manifest as dysuria, polyuria, dyspnea, chest pain, irritability, fatigue, headache, and dizziness [39]. From the known its cellular effect 50-60% of exposed populations has been shown to have chromosomal damage. It is known to bind to the mitochondria of the cell and is capable of inhibiting both cellular respirations by 75% and oxidative phosphorylation by 100% at low concentrations. This mitochondrial toxicity can completely inhibit the hydroxylation of Vitamin D in renal tissue [40].

Some of the specific changes that lead to tissue damage and death in chronic exposure have been related to oxidative stress and thiol depletion. Cellular damage results from cadmium binding to sulfhydryl groups in tissue, the production of lipid peroxides, and depletion of glutathione. It also has a very high affinity for glutathione and can form a complex with glutathione that is eliminated in bile. It also inhibits the activity of antioxidant enzymes, including catalase, manganese-superoxide dismutase and copper/zinc-superoxide dismutase. Cadmium-induced lipid peroxidation has been seen in animal studies in liver, kidney, brain, lung, heart, and testes [41].

2.5.3. Mercury toxicity

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. Mitochondrial damage, lipid peroxidation, microtubule destruction, and the neurotoxic accumulation of serotonin, aspartate, and glutamate are all mechanisms of methyl mercury neurotoxicity. The divalent mercury in the brain leads to strange symptoms, including erythema. 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 [42].

Mercury's 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. 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) [43].

The majority of toxicity from mercury is due to MeHg exposure (estimated 100 to 1000 times more toxic than elemental mercury in humans). MeHg seems to specifically target the central nervous system (CNS). It can cause demyelination, autonomic dysfunction, sensory nerve conduction delay, abnormal neuronal migration and abnormal central nervous system cell division. The key in understanding why MeHg is so toxic is to see that structural similarities in biochemical reactions can lead to active transport of toxins. In the case of organisms with a highly advanced CNS, this active transport can lead to accumulation of MeHg in the brain. Most of the effects involve complex biochemical reactions that are affected by the presence of mercury compounds. These include the immune system, particular organs, brain growth and development and behavioral patterns. This is part of the reason that there is so little known about chronic, low-level mercury exposure [44].

2. 6. N, N-DIMETHYLFORMAMIDE

N, N-Dimethylformamide is an organic compound with the formula $(\text{CH}_3)_2\text{NC}(\text{O})\text{H}$. Commonly abbreviated as DMF (although this initialism is sometimes used for dimethylfuran, or dimethyl fumarate) which is miscible with water and the majority of organic liquids [14].

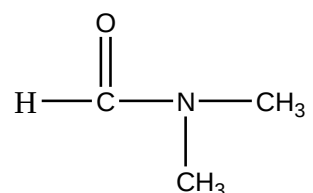


Fig.2.4: Structure of N, N-Dimethylformamide

It is a colorless liquid, used as a solvent in the synthesis of fine chemicals, high-boiling point, strongly polar(hydrophilic) aprotic solvent, hygroscopic liquid with a faint amine odor.

It is a flammable liquid with an acrid odor and infinitely miscible with water and with many hydrophilic solvents. It facilitates reactions that follow polar mechanisms [45].

2.7. PROTONATION CONSTANT

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. Two determinations, carried out within the same research group, have been corrected to zero ionic strength ('thermodynamic' constants) and have been reported at a range of temperatures between 0 and 40 °C. Enthalpy changes were also determined calorimetrically [46]. The quoted formation constants are consistent between the two determinations and may be accepted as tentative constants. A second pair of determinations has also been carried out by the same research group [47].

The first results covered the temperature range of 20 to 35 °C while the second was at 25 °C. Both refer to an ionic strength of 0.05 mol dm⁻³ (KCl) and they are in good agreement at 25 °C. They may therefore be accepted as tentative constants. The remaining determinations refer to 25 °C but one refers to an ionic strength of I = 3.0(NaClO₄) [48] one to I = 0.5(KNO₃) [49] and the others refer to 0.1 (KNO₃) [50]. The protonation constants of L-phenylalanine are determined in ethanol–water mixtures by potentiometrically at 25 °C with an ionic strength of 0.10 mol dm⁻³ (NaCl) [51]. The protonation constants of MA reported earlier by pH metrically and spectrophotometrically in 0.10 mol dm⁻³ micellar media [52].

Table 2.1: Protonation Constants of L-Phenylalanine reported in the literature [53,54].

S/No.	Log K		Ionic Strength (mol dm ⁻³) at 25°C
	log K ₁	Log K ₂	
1	2.28	9.95	1.0(NaClO ₄)
2	2.20	9.38	0
3	2.41	9.35	0.37(NaNO ₃)
4	2.09	9.20	0.05(KCl)
5	2.27	9.06	0.5(KNO ₃)
6	1.80	9.10	0.1(KCl)
7	2.20	9.11	0.05(KCl)
8	2.75	9.61	3.0(NaClO ₄)
9	2.39	9.25	0.1(KNO ₃)

Table 2.2: Protonation constants of L-phenylalanine at $25.0 \pm 0.1^\circ \text{C}$ for different dioxan-water mixtures ($\mu=0.10 \text{ M NaClO}_4$) reported in the literature [49].

S/NO	%(v/v) Dioxan	Mole fraction((x)	Log K_1	Log K_2
1	10	0.03	2.57	9.15
2	20	0.05	2.64	9.10
3	30	0.08	2.81	9.12
4	40	0.12	3.10	9.15
5	50	0.17	3.36	9.16
6	60	0.24	3.50	9.15

Table 2.3: Protonation constants of maleic acid reported in the literature at $25.0 \pm 0.1^\circ \text{C}$ [50].

S/NO	Medium	Instrumentation	Log K_1	Log K_2
1	0.01M CTAB	pH-metry	1.82	5.56
2	0.01M SDS	pH-metry	1.80	5.99
3	0.01M TX 100	pH-metry	1.86	6.24
4	0.01M CTAB	Spectrophotometry	1.83	5.54
5	0.01M SDS	Spectrophotometry	1.83	5.98
6	0.01M TX 100	Spectrophotometry	1.85	6.22

2.8. BINARY STABILITY CONSTANTS

Several literature reports have been devoted to the study of stability constants of metal-L-phenylalanine and maleic acid complexes. Ahmed et al. [55] have conducted a detailed study of the amino acid's complexes of certain metals in their unusual oxidation states by using pH-metric and Potentiometric methods. Stability constants were calculated by Bjerrum's method. They found that there seems to be no definite relationship between the nature of an amino acid and the stability constant of its resulting complex.

Barrio et al. [56] determined the stability constants of Zn(II) and Pb(II) complexes with several amino acids by Potentiometric method. This method gives not only the apparent stability constants of ML type complex but also the acid constants of MLH_x type complex. Perkins [57] determined the stability constants by using Potentiometric method in which ratio of amino acid to metal was 2:1 for bivalent and 1:1 for univalent metals. Group II metal ions form complexes with very low stability constants as compared to group 12 metals which form complexes with very high stability constants. The order of stability was found to be $\text{Hg(II)} > \text{Be(II)} > \text{Zn(II)} > \text{Cd(II)}$.

Kabiruddin and Zubaida [58] have used polarographic technique to study the complexing behavior of Cd(II) with some amino acids as the primary ligand and 2, 2-bipyridyl as the secondary ligand. Dissociation constants of the amino acids have been calculated and the stability constants of binary and ternary complexes have been determined.

Patil and Gurav [59] studied polarographically Pb(II) complexes of amino acids (asparagines, phenylalanine and tryptophan). Lead forms three complex species with asparagines and two complex species with phenylalanine and tryptophan. They have also investigated Cd(II) – phenylalanine complexes by polarographic studies at three different temperatures. The stability of (1:1), (1:2) and (1:3) – (metal: ligands) complexes were calculated. The thermodynamics of the coordination complexes were calculated from the temperature dependence and the dissociation constant of phenylalanine was determined by the method of Irving and Rassoti [60].

Bychkova et al [61] carried out potentiometric studies on the complex formation of MA with transition metals such as Zn(II), Ni(II), Co(II) and Cu(II) and alkaline earth metals such as Ca(II), Mg(II), Ba(II) and Sr(II). The stability constants of Ba(II) and Ca(II) with MA were studied by U. Klanning et al [62]

Table 2.4: Stability constants of metal- L-phenylalanine complexes reported in literature.

Metal	Ionic Strength	Temperature °C	Log β_{ML}	Log β_{ML2}
Cd(II)	2.0(KNO ₃)	25	4.06	6.92
Cd(II)	0.37(NaNO ₃)	20	3.90	6.73
Ce(III)	0.1(KCl)	20	3.5	-
Mg(II)	0.1(NaNO ₃)	25	12.4	19.6
Pb(II)	0.37(NaNO ₃)	20	4.02	8.86

Table 2.5: Stability constants of metal–maleic acid complexes reported in literature.

Metal	Log β_{mlh}		Ionic Strength mol dm ⁻³	Instrumental Method
	ML	ML ₂		
Ni(II)	2.78	4.62	0.1	Potentiometry
Ca(II)	1.91	---	0.1	„
Sr(II)	1.89	-	0.1	Potentiometry
Ba(II)	2.35	-	0.0	Spectrophotometry
Mn(II)	6.39	---	1.0	pH-metry
Fe(III)	5.15	----	1.0	„

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials & Apparatus

Burette, Funnel, mass balance, measuring cylinder, 25ml Volumetric pipette, PH meter, 250mL Erlenmeyer flask, test tube, stirrer, 50ml & 100ml Beakers

3.2. Chemicals & Reagents

All reagents and chemicals to be used are of analytical Grade, unless otherwise stated.

Triply distilled 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, DMF, L-phenylalanine, Maleic acid, Lead(II) Nitrate, Cadmium(II) Nitrate & Mercury(II) Nitrate

3.3. EXPERIMENTAL

3.3.1. Preparation of solutions

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

3.3.1.1. L-Phenylalanine and maleic acid solutions

0.05 mol dm⁻³ aqueous solution of L-phenylalanine (F) (GR grade, Loba, India) and maleic acid (MA) (GR grade, Loba, India) were prepared by dissolving samples in distilled water. To increase the solubility of ligands, 0.05 mol dm⁻³ nitric acid concentration was maintained in the solutions. The probable errors that may creep into the concentrations of the stock solutions of the ligands were determined by the computer program COSWT [63].

3.3.1.2. 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) solution using Eriochrome Black-T as indicator.

3.3.1.3. Metal ion solutions

0.1 mol dm⁻³ aqueous solutions of lead(II), cadmium(II), and mercury(II) nitrates were prepared by dissolving GR grade (E-Merck, Germany) salts in triply distilled water. The stock solutions were rendered slightly acidic to repress hydrolysis of the metal ions [64]. 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. The free hydrogen ion concentration in the stock solution was determined by Gran plot method [65].

3.3.1.4. 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 triply distilled water. The solution was further diluted to the required concentration of 0.4 mol dm⁻³. The strength of alkali was determined using the Gran plot method. The errors in the concentration of ligand, metal ions, alkali and mineral acid were subjected to analysis of variance ANOVA [66].

3.3.1.5. Nitric acid and Sodium nitrate

An approximately 2 mol dm⁻³ nitric acid solution was prepared and diluted further to 0.2 mol dm⁻³. Its strength was determined by titrating with a standard sodium hydroxide and borax solutions. Aqueous solution of 2 mol dm⁻³ sodium nitrate was prepared to maintain the ionic strength in the titrand.

3.3.1.6. N, N-Dimethylformamide

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

3.4. ELECTROMETRIC TITRATION ASSEMBLY

An ELICO (Model LI 120, India) pH meter of 0.01 readability (0-14 pH) in conjunction with a glass combination pH electrode was used to monitor changes in hydrogen ion concentration.

3.4.1. Calibration of glass combination pH electrode

In these studies, potassium hydrogen phthalate (0.05 mol dm^{-3}) and borax (0.01 mol dm^{-3}) solutions were used in order to preliminarily check the response of the glass electrode in acidic ($\text{pH}=4.01$) and basic ($\text{pH}=9.18$) regions. In electrometric studies, the glass electrode response is to be established over a wide range of pH. The acid-base titrations were analyzed by using the Gran plot method in which the functions ϕ and ϕ' were calculated by using the formulae [67].

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

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

A typical Gran plot is given in Fig. 3.1

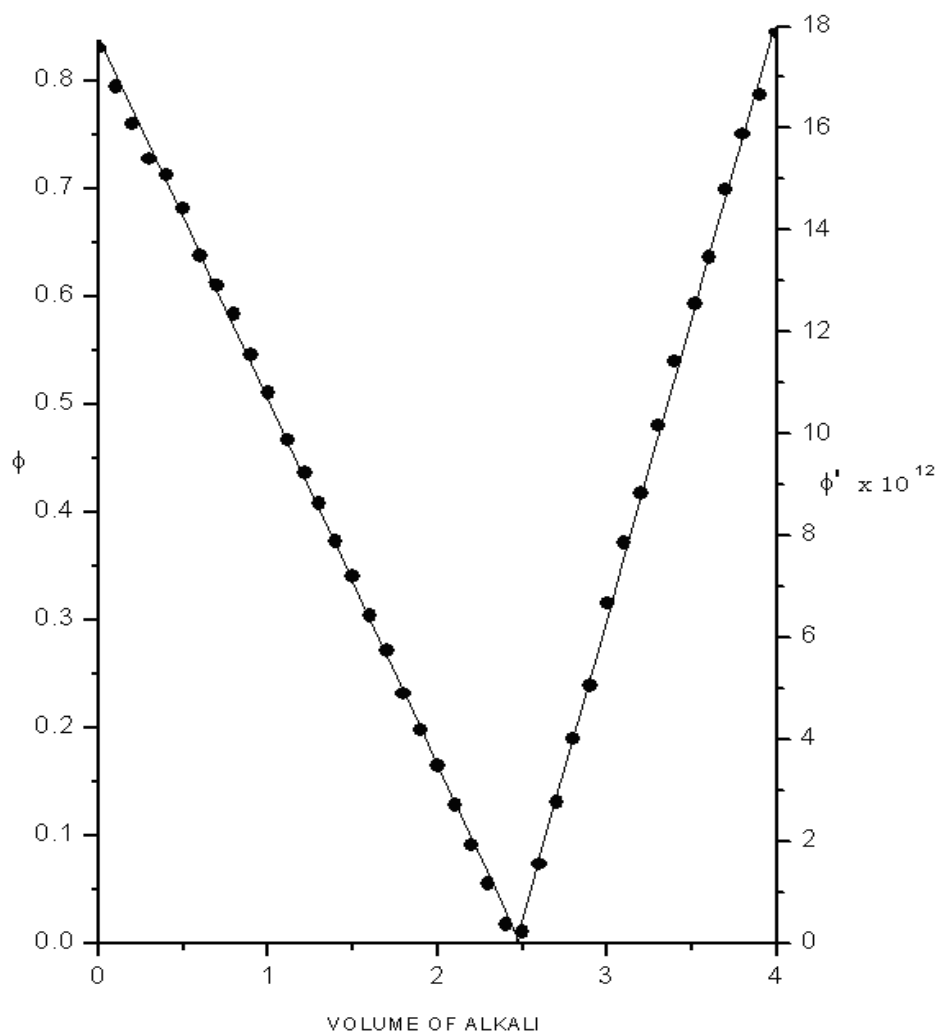


Fig.3.1: Gran plot for the titration of nitric acid with alkali.

3.4.2. Equilibration of electrode in 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 meter 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.

3.4.3. Determination of correction factor

The effect of variation in asymmetry, liquid junction potential, activity coefficient, sodium ion error on the response of glass electrode is to be considered for accurate determinations. Correction factor proposed by McBryde [68] and others [69] to account for the above parameters was used in the present electrometric titrations. The simulated acid-base titrations data (pH_{C_i}) calculated by SCPHD program was used to compute correction factor ($\log F$) for each of the solvent compositions. The $\log F$ value is used to convert pH meter dial reading into logarithm of reciprocal of hydrogen ion concentration (pH_{E_i}) according to the equation.

$$\log F = \text{pH}_{\text{C}_i} - \text{pH}_{\text{E}_i}$$

$\log F$ 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 [70].

Each point in the titration data past the equivalence point was corrected for sodium ion error ($\sim < 0.05$ units) using the data available for aqueous system. From the volume of alkali added, the values of pOH and subsequently the value of pK_w were calculated.

3.4.4. Titration procedure

The titrations were carried out in media containing varying amounts of DMF maintaining an ionic strength of 0.16 mol dm^{-3} with NaNO_3 at 303K. In these titrations, the titrand consisted of mineral acid and ligand, in the presence and absence of metal ion, in a total volume of 50 cm^3 . Titrations were performed by adding each time 0.1 cm^3 portions of sodium hydroxide

(0.4 mol dm⁻³) to the titrand. The pH meter reading was recorded only after a constant value was displayed. Typical duplicate titrations showed that equilibration is fast and titration data do not differ by more than 0.02 units.

3.4.5. Proton-ligand equilibria

Requisite amounts of sodium nitrate solution (to give overall concentration of 0.16 mol dm⁻³), water and 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 Tables 3.1.

Table 3.1: Total initial concentrations of ingredients (in mmol) in proton - ligand titrations in DMF-water mixtures. [NaOH] = 0.4 mol dm⁻³; V₀ = 50 cm³; Temperature = 303 K, Mineral acid = 1.0 mmol; μ = 0.16 mol dm⁻³

% of DMF	No of titration	F	MA
0.0	3	0.25002 0.37511 0.50022	0.25022 0.37542 0.50001
10.0	3	0.25031 0.37544 0.50045	0.25002 0.37532 0.50045
20.0	3	0.25042 0.37523 0.50011	0.25006 0.37511 0.50010
30.0	3	0.25023 0.37532 0.50040	0.25022 0.37532 0.50044
40.0	3	0.25050 0.37533 0.50043	0.25004 0.37506 0.50011
50.0	3	0.25021 0.37512 0.50033	0.25023 0.37532 0.50040

3.4.6. Metal-ligand complexes

In each of the titrations, the titrand consisted of nitric acid of approximately 1 mmol in a total volume of 50 cm³ 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 1.0:0.25 to 1.0:0.50) were titrated with 0.4 mol dm⁻³ sodium hydroxide. In all these 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 3.2. 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. The initiation of precipitation was also indicated by a downward drift of pH meter dial readings.

Table 3.2: Total initial concentrations of ingredients (in mmol) for metal - ligand titrations in DMF-water mixtures.

[NaOH] = 0.4 mol dm⁻³; V₀ = 50 cm³; Temperature = 303 K, Mineral acid = 1.0 mmol; μ = 0.16 mol dm⁻³.

% v/v DMF	N ₀ of titration	Pb(II)	Cd(II)	Hg(II)	F	MA	TL ₀ :TM ₀
0.0	3	0.099	0.099	0.102	0.25012	0.25011	2.50
					0.37561	0.37572	3.75
					0.50011	0.50011	5.00
10.0	3	0.099	0.099	0.102	0.25011	0.25025	2.50
					0.37561	0.37531	3.75
					0.50012	0.50002	5.00
20.0	3	0.099	0.099	0.102	0.25013	0.25001	2.50
					0.37560	0.37503	3.75
					0.50014	0.50004	5.00
30.0	3	0.099	0.099	0.102	0.25014	0.25002	2.50
					0.37565	0.37511	3.75
					0.50012	0.50044	5.00
40.0	3	0.099	0.099	0.102	0.25011	0.25023	2.50
					0.37560	0.37521	3.75
					0.50012	0.50021	5.00
50.0	3	0.099	0.099	0.102	0.25010	0.25021	2.50
					0.37561	0.37521	3.75
					0.50010	0.500	5.00

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 [71].

Thus, it is essential that the formation constants used in any chemical speciation modeling are 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). A perusal of the literature [72] revealed that potentiometry using glass electrode gained wide popularity in the determination of equilibrium constants in preference to Spectro-photometry, 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 [73] and automation in data acquisition systems [74], Kaden and Zuberbuhler advocated [75] hyphenated Spectro-pH meter but for this work the potentiometry using glass electrode have been used.

3.5.3. Formulation of mathematical model

For simplicity of mathematical treatment, the interaction of a metal ion with a ligand (LH_n) is considered and the same treatment can be extended to other species encountered in complex equilibria of mononuclear complexes summarized in Table 3.3.

Table 3.3: Types of species likely to be encountered in complex equilibria of mononuclear complexes.

Species	Stoichiometric coefficients		
	M	L	H
Metal hydrolysis	1	0	<0
Binary complexes			
(a) Unprotonated, unhydroxylated (ML _n)	1	>0	0
(b) Protonated	1	>0	>0
(c) Hydroxylated	1	>0	<0

m, l, h: Stoichiometric coefficients of metal, ligand and hydrogen, respectively.

The interaction of metal ion, with most anionic form of the ligand and proton(s) in solution phase results in NB complexes and the equilibrium for the formation of the jth species is represented by Eq. 3.1.



Then the formation constant for the jth species can be written as

$$\beta_{m(j)l(j)h(j)} = \frac{M_{m(j)}L_{l(j)}H_{h(j)}}{FM_i^{m(j)} * FL_i^{l(j)} * FH_i^{h(j)}} \\ = \frac{CS_{j,i}}{FM_i^{m(j)} * FL_i^{l(j)} * FH_i^{h(j)}} \quad \text{.....3.2}$$

Where, FM_i and FL_i are the free concentrations of metal ion and ligand and CS_{j,i} is the concentration of jth complex at ith experimental point: h(j) is negative for hydroxylated species. A titrand of total volume, V₀ containing an indifferent electrolyte, V_m cm³ of metal ion (C_M mol dm⁻³) and V_l cm³ 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 cm³ of alkali added, the total concentrations at ith experimental point are given by

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

Where TM₀ and TL₀ are the analytical concentrations of metal and ligand in V₀ cm³ of solution. The calculated total metal ion concentration is

$$\begin{aligned}
TMC_i &= FM_i + \sum_{j=1}^{NB} m(j) * \beta_m(j) l(j) h(j) * FM_i^{m(j)} * FL_i^{l(j)} * FH_i^{h(j)} \\
&= FM_i + \sum_{j=1}^{NB} m(j) * CS_{j,i}
\end{aligned}
\tag{3.4}$$

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

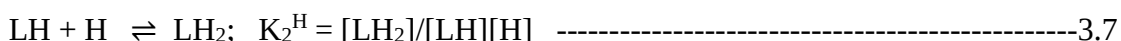
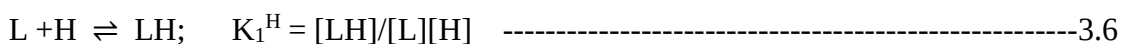
$$\left. \begin{aligned}
TLC_i &= FL_i + \sum_{j=1}^{NB} l(j) * CS_{j,i} \\
THC_i &= FH_i + \sum_{j=1}^{NB} h(j) * CS_{j,i} + OH_i
\end{aligned} \right\}
\tag{3.5}$$

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 [76] 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 [77]. 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 [78] to modeling strategies [79] 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 Eqs. 3.6 and 3.7.



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

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

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 [80] 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

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

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

or the generalized equation can be written as

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

The computer program, SCPHD [81] employs Eq. 3.12.

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

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 autoionization 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 $\log \beta_{011}$, $\log \beta_{012}$ 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.

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

Eq. 3.13 can be transformed into Eq. 3.14

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

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.

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

This algorithm was used in SCPHD to calculate the paired data ($\bar{n}_i$ Vs PL_i). Some thumb rules [82] about formation function are given in Table 3.4.

Table 3.4: Knowledge base of formation functions

If	Formation curves are overlapping for different ligand concentrations
Then	Protonated/hydroxylated/ligand species are absent and only ML_n species are present.
If	Formation curves are not overlapping for different ligand concentrations
Then	Protonated/hydroxylated complexes are present.
If	Formation curves exhibit ascending slope
Then	$Maxl = Maxn + 1.6$ and protonated/hydroxylated complexes are absent.
If	Formation curves of proton-ligand titrations are parallel for different ligand concentrations
Then	Polymeric species.
If	Systematic parallel spreading of $\bar{n}$ curves for different concentrations of ligands
Then	Existence of polynuclear species.
If	Slight rising of the lower points which do not reach $n=0$
Then	Protonated complexes are formed.

$Maxl$ = Maximum number of ligands per metal atom.

$Maxn$ = Maximum n value

CHAPTER 4

RESULTS AND DISCUSSIONS

SECTION-I

4.1. PROTONATION EQUILIBRIA OF L-PHENYLALANINE AND MALEIC ACID

L-Phenylalanine (F) actively participates in acido-basic equilibria especially in physiological pH conditions. F has two functional groups and one is protonated, amino group followed by one carboxylic group. Hence, F has bidentate behavior resulting in five membered ring structures. Nitrogen donor atoms can associate with hydrogen ions in physiological pH ranges [12].

Maleic acid (MA) is a dicarboxylic acid and acts as bidentate ligand resulting in seven membered ring structures. There is often significant competition between hydrogen and metal ion for the donor sites. This situation results in the simultaneous existence of a number of equilibria, producing an array of successively protonated complexes [13]. So, an understanding of the protonation-deprotonation equilibria of F and MA is necessary before an attempt is made to investigate the metal-ligand equilibria associated with F and MA.

4.1.1. Preprocessing of data

The alkalimetric titration curves for F and MA in aqueous medium and DMF- water mixtures are shown in Figs. 4.1-4.3. A perusal of the titration curves reveals that the acido-basic equilibria are active in the pH range 1.6-11.5 for F and 1.5-10.0 for MA. The computer program SCPHD was used to prune the data obtained in different experiments so that it contains more information than noise and the $\log \beta$ s obtained from the program are used as initial values for final refinement.

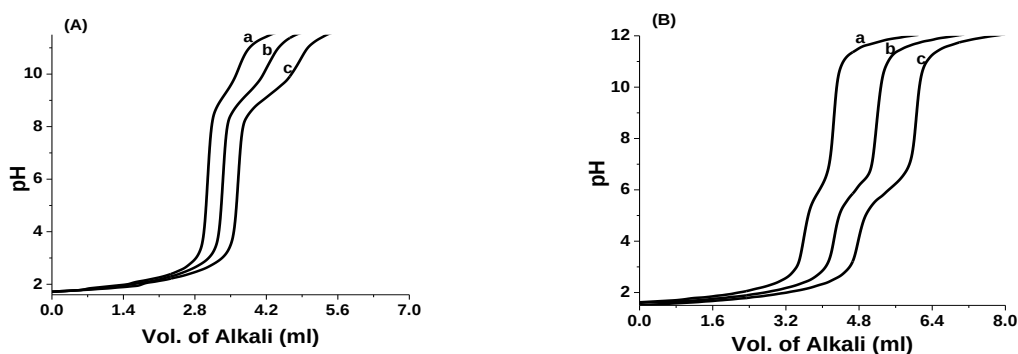


Fig.4.1: Alkalimetric titration curves for (A) F and (B) MA in aqueous medium

Ligand concentrations: (a) 0.25, (b) 0.375 and (c) 0.50 mmol.

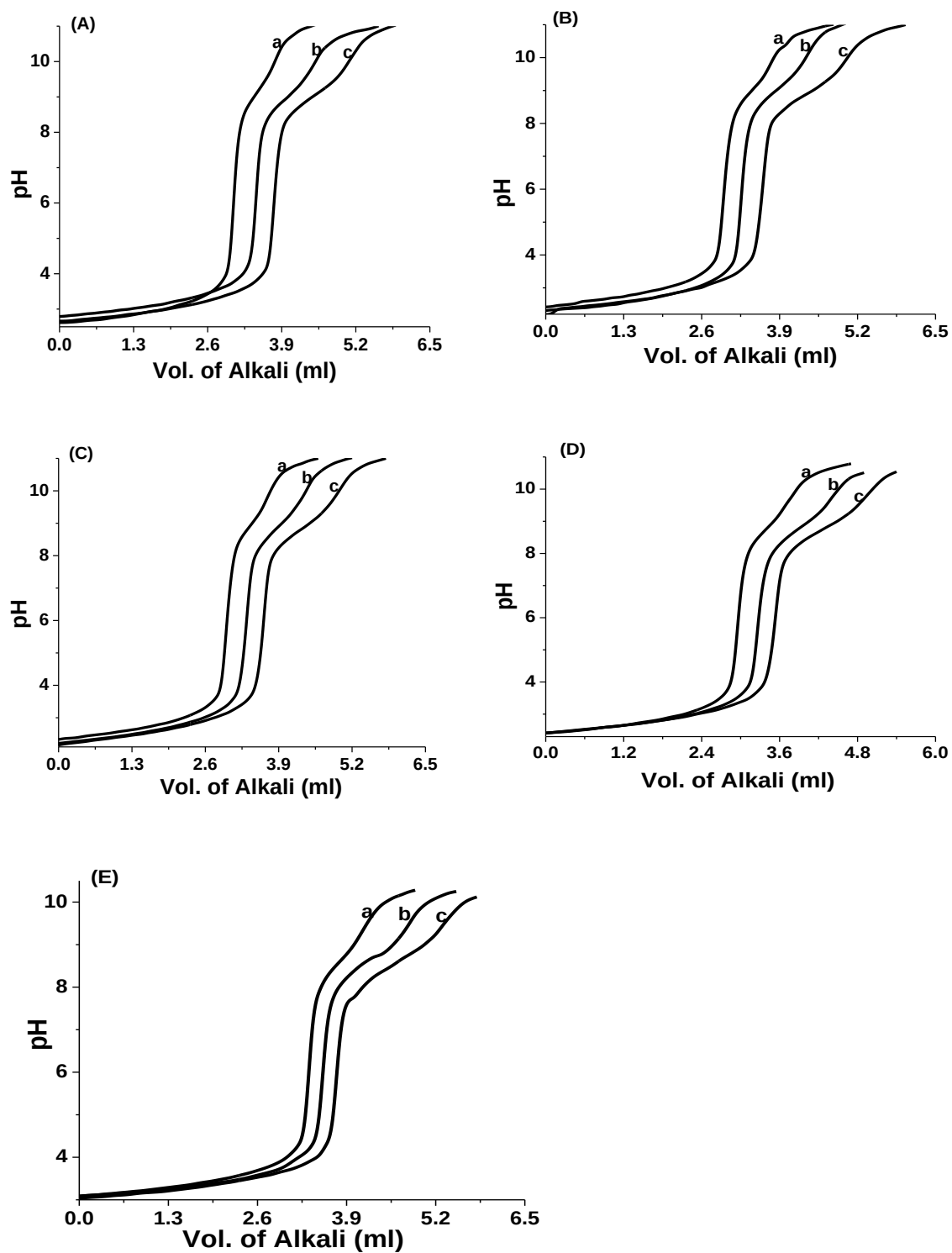


Fig.4.2: Alkalimetric titration curves for F in DMF-water mixtures % v/v.
 (A) 10% (B) 20% (C) 30% (D) 40% (E) 50%; Ligand concentrations:
 (a) 0.25, (b) 0.375 and (c) 0.50 mmol.

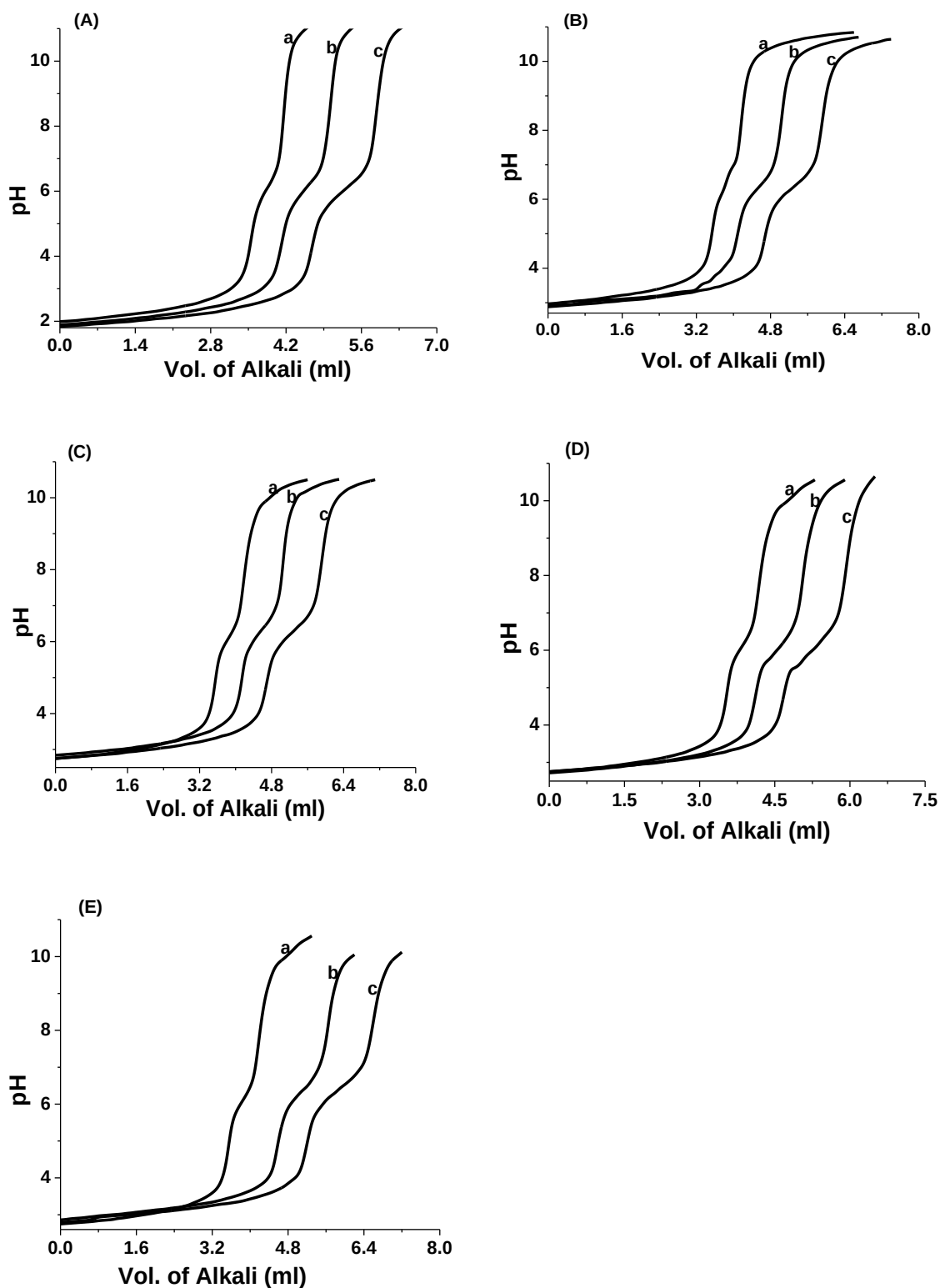


Fig.4.3: Alkalimetric titration curves for MA in DMF-water mixtures % v/v.
 (A) 10% (B) 20% (C) 30% (D) 40% (E) 50%; Ligand concentrations:
 (a) 0.25, (b) 0.375 and (c) 0.50 mmol.

Simultaneous neutralization and proton liberation due to auto-ionization of ligand and due to its complexation was recognized by many earlier investigators. Irving and Rossotti [62] systematized the formulae representing these two parallel reactions by introducing a function, formation function $\bar{n}_H$. It is average number of protons bound per mole of uncomplexed ligand. The graphical relation between $\bar{n}_H$ and pH is called formation curve. Formation function is a useful parameter for the detection of polymeric species (L_2H_x). Formation curves for different concentrations of ligands should overlap if there are no polymeric species. Any deviation indicates the presence of polymeric species. Figs. 4.4A and 4.4B show that there is no polymerization of F and MA.

Another secondary function relevant to protonation equilibria is the number of moles of alkali consumed per mole of ligand, **a**. one can detect whether the given ligand solution contains acid or not by using **a**. The negative values of **a** correspond to excess of mineral acid present in the titrand and the number of associable protons where as the positive values correspond to the dissociable protons. The maximum value of **a** in Fig. 4.5C is +1, which indicates that F has one dissociable (one carboxyl) proton. The corresponding value for MA (Fig. 4.5D) is +2, which clearly infers that MA has two dissociable (two carboxyl) protons.

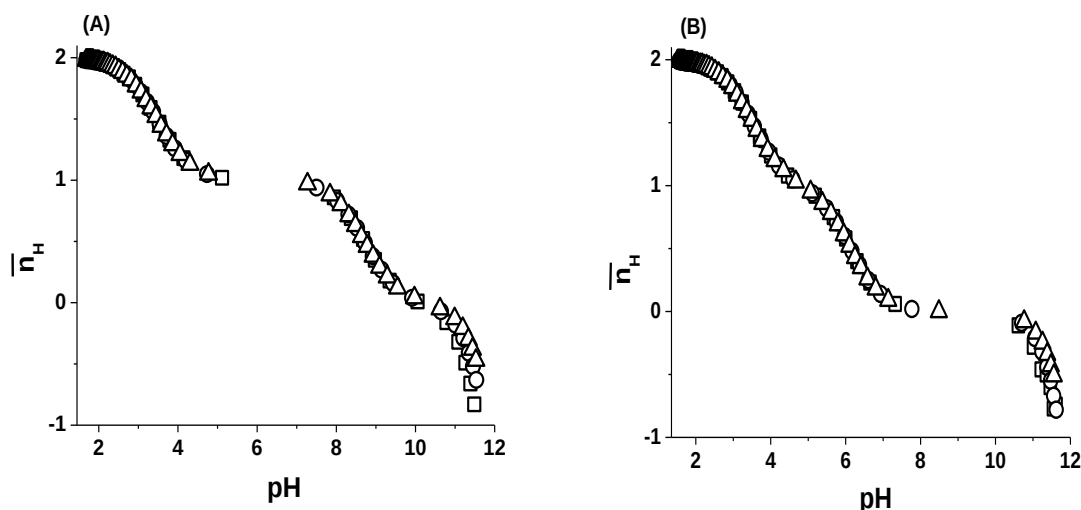


Fig.4.4: Formation curves of (A) F and (B) MA in 20% v/v DMF.

Ligand concentrations (in mmol) 0.25 ($\square$ - $\square$), 0.375($\circ$ - $\circ$) and 0.50 (Δ - Δ).

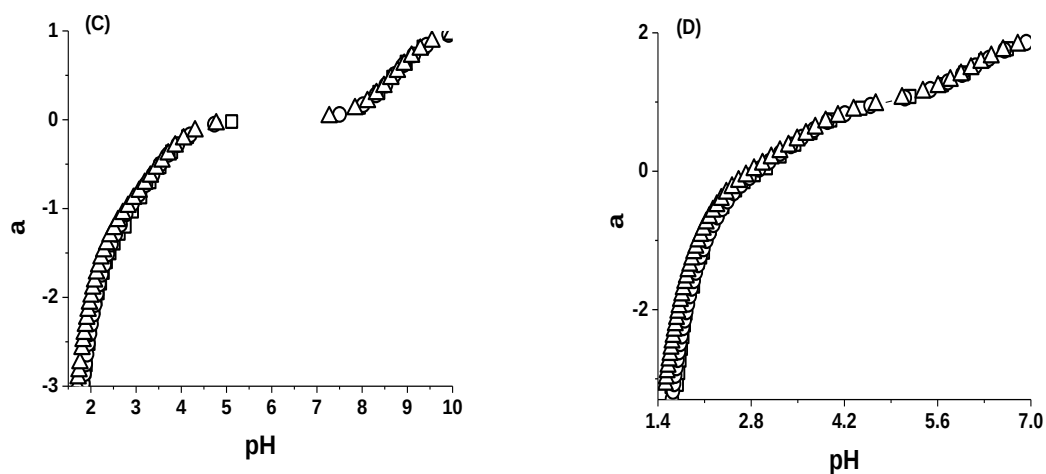


Fig.4.5: Number of moles of alkali versus pH curves of (C) F, (D) MA in 20% v/v DMF. Ligand concentrations (in mmol) 0.25 ($\square$ - $\square$), 0.375($\circ$ - $\circ$) and 0.50 (Δ - Δ).

4.1.2. Mathematical models for proton ligand equilibria of L-phenylalanine and maleic acid

The computer program MINQUAD75 [66] was used to refine protonation constants of the F and MA in aqueous and DMF- water mixtures. The results of the best fit model along with some of the important statistical parameters of F and MA are given in Table 4.1.

Table 4.1: Best fit chemical models of acido-basic equilibria of F and MA in aqueous and DMF-water mixture.

Temp= 303 K, Ionic strength=0.16 mol dm⁻³

L-PHENYLALANINE (pH range 1.6 -11.5)								
% of DMF	011	012	NP	U _{corr} ×10 ⁸	Skewness	Kurtosis	χ^2	R-factor
	Log β_1 (SD)	log β_2 (SD)						
0.0	9.27(1)	11.48(1)	72	1.857	-0.17	3.27	1.22	0.0096
10.0	8.80(2)	12.08(5)	25	10.43	0.24	2.88	2.16	0.0281
20.0	8.75(1)	11.86(2)	21	3.15	-0.05	2.87	2.95	0.0132
30.0	8.64(1)	11.59(3)	23	2.85	0.10	1.55	9.30	0.0142
40.0	8.71(2)	12.18(4)	47	15.11	0.59	3.57	5.40	0.0336
50.0	8.66(4)	12.85(8)	52	48.6	0.43	3.51	4.62	0.0582
MALEIC ACID (pH range 1.5 -10.00)								
0.0	6.15(1)	7.93(2)	102	6.8	-0.29	4.94	7.45	0.0151
10.0	6.73 (2)	10.07(4)	30	7.5	0.00	2.86	3.47	0.0227
20.0	6.48(2)	10.27(4)	48	1.30	0.43	3.09	8.17	0.0353
30.0	6.36(2)	9.91(3)	43	11.70	0.40	3.30	1.63	0.0306
40.0	6.13(1)	9.65 (3)	44	9.76	0.20	4.04	6.36	0.0293
50.0	6.13(3)	9.65(6)	48	3.5	0.66	2.99	1.33	0.0529

4.1.2.1. Statistical parameters of the best fit models

A very low standard deviation in $\log \beta$ values indicates the precision of these parameters. The small values of U_{corr} (the sum of the squares of deviations in concentration 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 [83].

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 [83].

Further, a model is considered adequate only if the residuals do not show any trend. Respecting the hypothesis of the least square analysis, the residuals are tested for normal distribution. Such tests are χ^2 , skewness, kurtosis and R-factor. These statistical parameters of the present data show that the best fit models portray the acido-basic equilibria of F and MA in DMF- water mixtures, as discussed below [83].

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 χ^2 calculated is less than the table value, the model is accepted [85]. The χ^2 values given in Table 4.1 indicate the sufficiency of the model.

Crystallographic R-test

Hamilton's R factor ratio test 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 [84]. The low crystallographic R-values given in Table 4.1 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 for F and MA recorded in Table 4.1 are between -0.17 and 0.59, -0.29 and 0.66 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). The kurtosis values in the present study indicate that the residuals form leptokurtic pattern [83].

4.1.3. Effect of systematic errors

In order to rely upon the best fit 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 influential parameters like concentrations of alkali, mineral acid and ligand. 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 Table 4.2 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 those in ligand. With the introduction of errors, the SD's are found to increase inferring the appropriateness of experimental condition and correctness of analytical concentration.

Table 4.2: Effect of errors in influential parameters on the protonation constants in 20% v/v DMF-water mixture.

Ingredient	% Error	Log β (SD)			
		F		MA	
		LH	LH ₂	XH	XH ₂
Acid	0	8.75(1)	11.86(2)	6.48(2)	10.27(4)
	-5	Rejected	11.52(16)	5.80(13)	8.95(18)
	-2	8.59(42)	11.93(14)	6.00(12)	9.38(14)
	+2	8.84(10)	12.45(15)	Rejected	Rejected
	+5	9.04(40)	12.86(18)	6.48(13)	10.35(15)
Alkali	-5	Rejected	12.86(18)	6.65(13)	10.60(16)
	-2	8.86(12)	12.45(15)	6.33(12)	10.01(14)
	+2	8.57(12)	11.93(14)	5.94(12)	Rejected
	+5	8.35(13)	Rejected	5.67(14)	Rejected
Ligand	-5	8.67(12)	12.19(15)	5.99(12)	9.44(15)
	-2	8.70(12)	12.19(14)	6.07(12)	9.57(14)
	+2	8.73(12)	12.19(14)	6.19(11)	9.73(13)
	+5	8.76(12)	12.19(14)	6.28(11)	9.85(13)

4.1.4. Effect of organic media 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 is either charged or has a dipole moment [85]. Seleem *et al.* [86] 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 logarithm of step-wise protonation constants (log K) can be calculated by

$$\log K_1 = \log \beta_2 - \log \beta_1 \quad 4.1$$

$$\log K_2 = \log \beta_1 \quad 4.2$$

for both F and MA, where β_1 and β_2 are overall protonation constants.

The values of step wise protonation constants are given in Table 4.3.

Table 4.3: Step-wise protonation constants of F and MA in aqua-organic mixtures.

% of DMF	F		MA	
	log K ₁	log K ₂	log K ₁	log K ₂
0.0	2.21	9.27	1.78	6.15
10.0	3.28	8.80	3.34	6.73
20.0	3.11	8.75	3.79	6.48
30.0	2.91	8.64	3.55	6.36
40.0	3.47	8.71	3.52	6.13
50.0	4.19	8.66	3.52	6.13

Different researchers have explained the effect of electrostatic and non-electrostatic forces of interaction on different cosolvent-water mixtures that resulted in variation in the values of protonation constants of ligands or change in free energy [87]. The variation of log K^M or change in free energy with cosolvent content depends upon two factors and can be represented as the sum of the changes in ΔG due to electrostatic and non-electrostatic factors.

$$\begin{aligned}\Delta G^t &= -RT (\ln K^M - \ln K^W) \\ &= \Delta G_{el} + \Delta G_{nel}\end{aligned}\quad (4.3)$$

Born's classical treatment [88] 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:

$$\Delta G_{el} = \frac{Ne^2}{Z} * \frac{1}{D} * \frac{1}{r} \quad (4.4)$$

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, log Ks of both F and MA increased linearly (Fig. 4.6) as a function of the reciprocal of the dielectric constant (1/D) of DMF-water mixtures.

In the case of some mono- and di- carboxylic acids and simple phenolic ligands, electrostatic (long-range, non-specific or universal) solute-solvent interactions are predominant in binary mixtures of water with methanol, ethanol, dioxan or acetone as cosolvent [89].

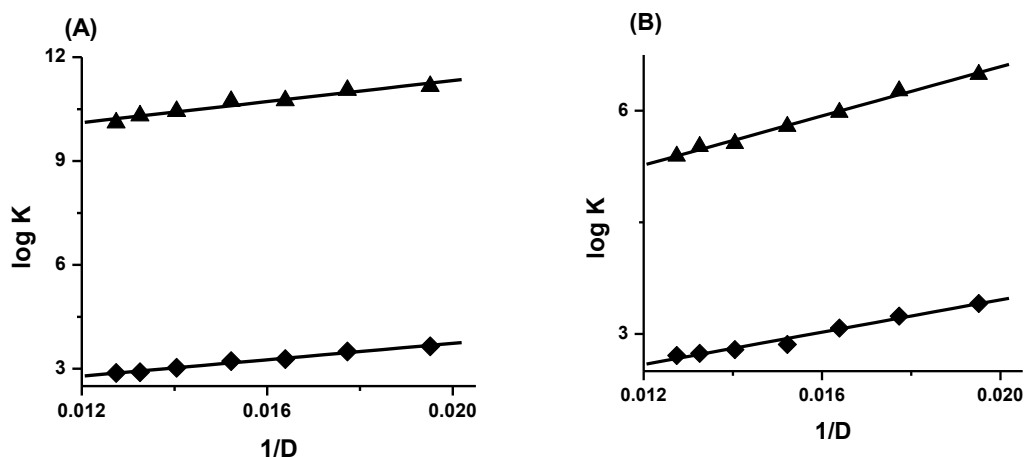


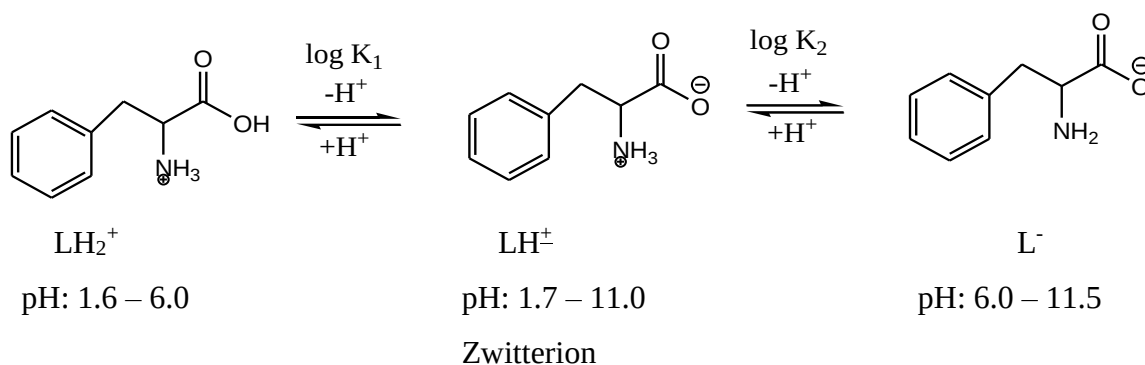
Fig.4.6: Variation of stepwise protonation constant ($\log K$) with reciprocal of dielectric constant ($1/D$) of solvent. (A) F and (B) MA in DMF-water mixtures ($\blacklozenge$) $\log K_1$ and ($\blacktriangle$) $\log K_2$.

Many workers [90] were of the opinion that both electrostatic and non-electrostatic effects should be considered even in the case of simple acido-basic equilibria; one dominates the other, depending upon the nature of solute and solvent [91]. The cation stabilizing nature of cosolvents, specific solvent-water interactions, solvation power, donor number, acceptor number [92], 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 $\log K$ with $1/D$.

The protonation-deprotonation equilibria of F and MA are shown with the pH ranges of existence of the species in Fig.4.7. In these equilibria F can exist in anionic, zwitterionic and cationic forms, whereas MA can exist in only anionic or neutral form at different pH ranges. F contains one carboxylic and one amino group. The different forms of F are LH_2^+ , LH and L^- in the pH ranges 1.6 – 6.0, 1.7 – 11.0 and 6.0 – 11.5 respectively. MA has two carboxyl functional groups and all are deprotonated as XH_2 , XH^- and X^{2-} in the pH ranges of 1.5-6.0, 1.5-9.0 and 4.0-10.0, respectively. The glass electrode used in this study is sensitive at 1.0 to 12.0 pH. In this range the number of dissociable and associable protons of F and the

number of dissociable protons of MA is only 2. Thus, only two log K values are considered for both F and MA in this present study.

(A)



(B)

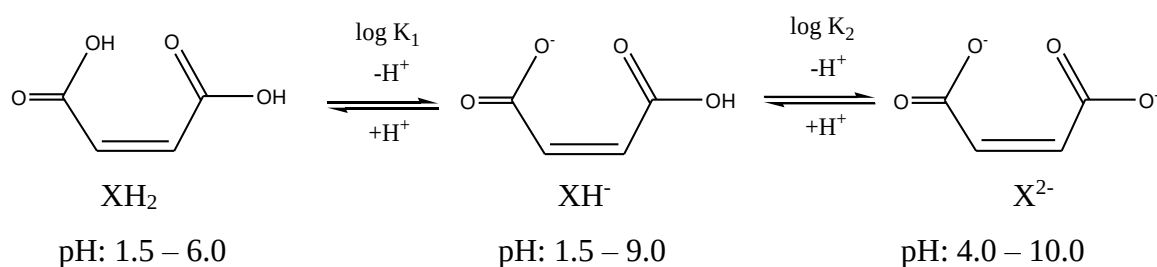
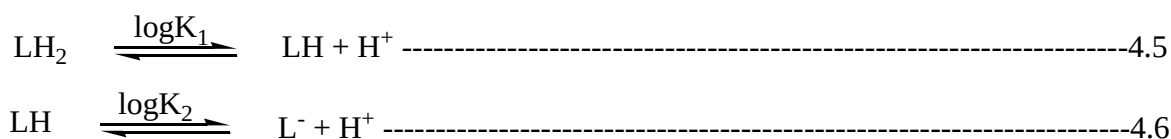
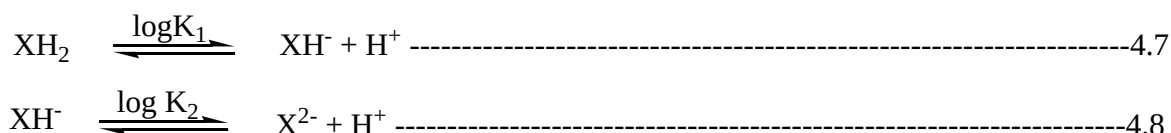


Fig.4.7: Protonation-deprotonation equilibria of (A) F and (B) MA

The deprotonation -protonation equilibria of F are shown as follows



The deprotonation -protonation equilibria of MA are:



The above observations can be explained as follows. When F (Equilibria 4.5 and 4.6) and MA (Equilibria 4.7 and 4.8) are successively protonated, the charge of the species is decreased and low dielectric medium favors the protonation reaction, due to dominant electrostatic interactions. Thus, decrease in dielectric constant of the medium increased the protonation constants.

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.

L-Phenylalanine has one dissociable proton and one amino group which can associate with a proton. It exists as LH_2^+ at low pH and gets deprotonated with the formation of LH and L^- successively with increase in pH. Maleic acid has two dissociable protons. It exists as XH_2 at low pH and gets deprotonated with the formation of XH^- and X^{2-} successively with increase in pH. Typical distribution plots in aqueous and DMF- water mixtures given in Figs. 4.8-4.10 show the existence of LH_2^+ , LH and L^- for F and XH_2 , XH^- and X^{2-} for MA. In the case of F, the zwitterionic form LH^+ is present to an extent of 80-90% in the pH ranges 1.7–11.0. The XH^- form MA is present to an extent of 80-90% in the pH range 1.5–9.0.

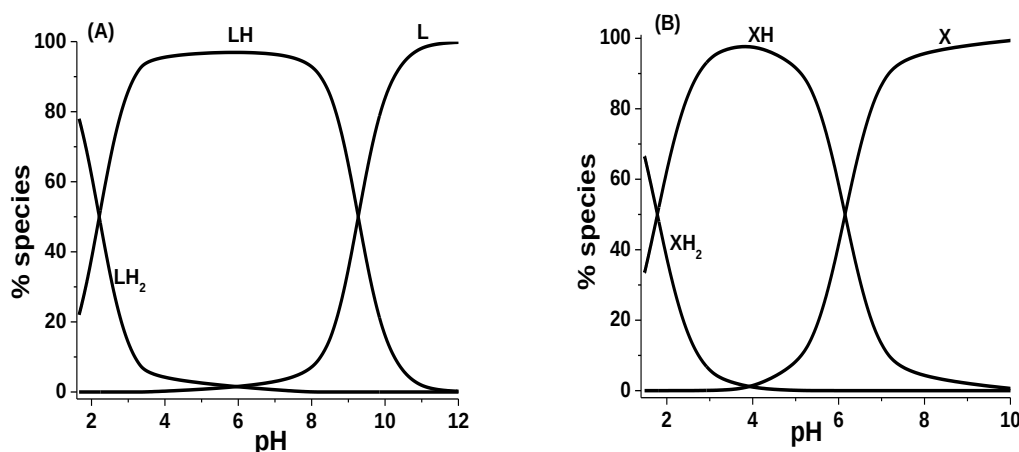


Fig.4.8: Distribution diagrams of (A) F and (B) MA in aqueous mixtures

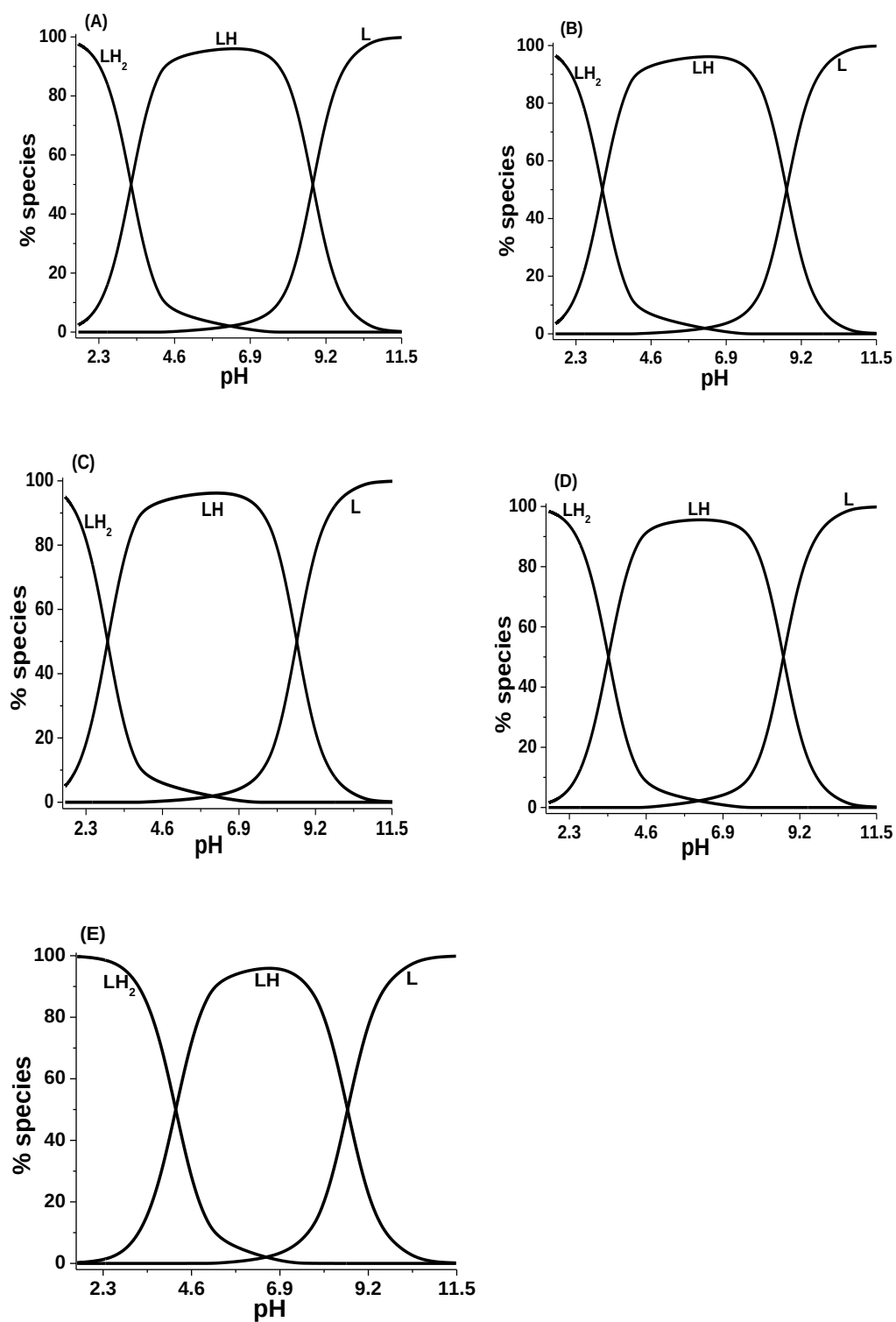


Fig.4.9: Distribution diagrams of F in DMF-water mixtures % v/v.
(A)10, (B) 20, (C) 30, (D), 40 and (E) 50

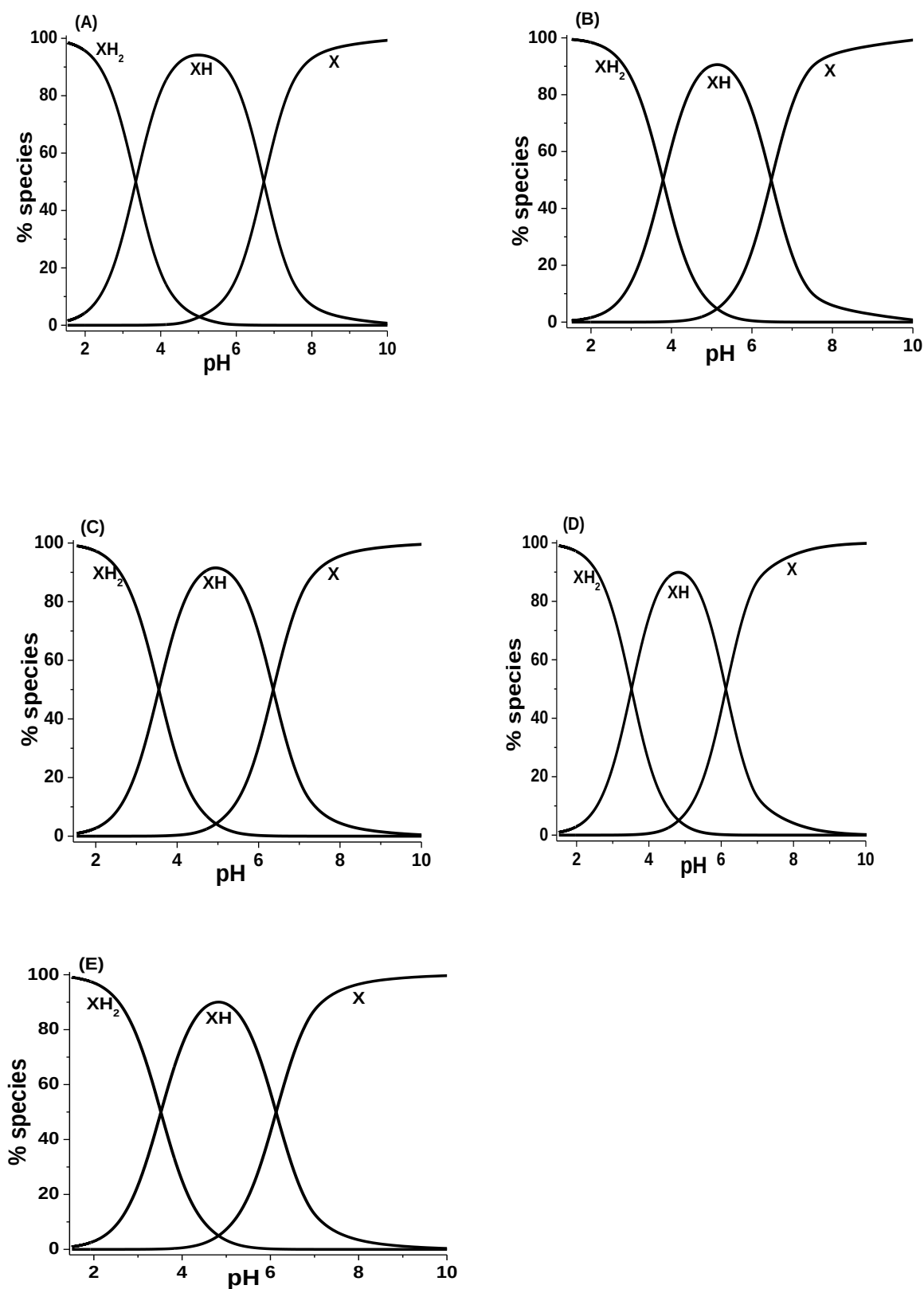


Fig.4.10: Distribution diagrams of MA in DMF-water mixtures % v/v.

(A)10, (B) 20, (C) 30, (D) 40, and (E) 50

SECTION -II

4.2. BINARY STABILITY CONSTANTS OF L-PHENYLALANINE AND MALEIC ACID

The earlier literature reports and the distribution patterns of different forms of F and MA in SLS, CTAB- and TX100-water mixtures reported in the literature indicate a high probability for the formation of various protonated metal complexes of different stoichiometries [54]. Hence, the author has investigated the complex equilibria of F and MA with Pb(II), Cd(II) and Hg(II) in DMF - water mixtures.

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. Further, different species proposed for the same metal-ligand system are many a time judged on the best fit criteria. Typical alkalimetric titration curves are given in Figs. 4.11 from which the stability constants of binary metal-ligand complexes were determined.

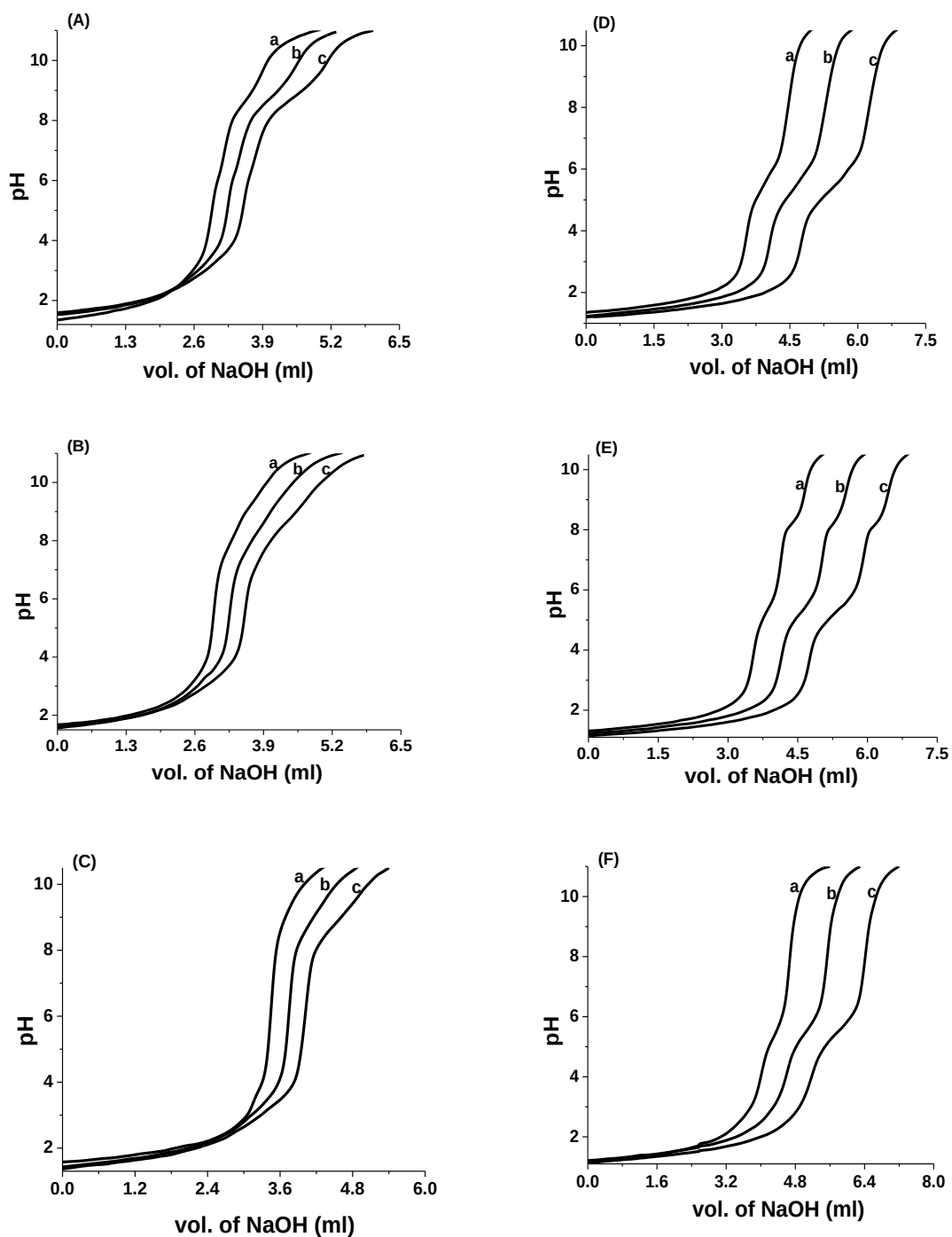


Fig.4.11: Alkalimetric titration curves for F and MA complexes

(A) Pb(II), (B) Cd(II) and (C) Hg(II) in 40% v/v DMF-water mixture for F complexes and (D) Pb(II), (E) Cd(II) and (F) Hg(II) in 40% v/v DMF-water mixture for MA complexes; Ligand concentration: (a) 0.25, (b) 0.375 and (c) 0.50 mmol.

Since the ratio of TL_O to TM_O is greater than unity in all the experiments, as clear from the data in Table 3.2 of Chapter 3, formation of poly nuclear complexes is not considered. The formation of polymeric species is possible if $TL_O:TM_O \leq 2$. Also, dimeric and trimeric species are ruled out since the experimental conditions are not favorable for their formation. Hydroxylated species are not considered in the present study.

The formation function ($\bar{n}$ – 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 $\bar{n}$ vs pH at all the ligand concentrations, with fixed metal ion concentration should overlap and any deviation indicates the presence of the protonated species. Some typical plots given in Figs. 4.12 show spread in some pH regions, indicating the presence of protonated metal complexes.

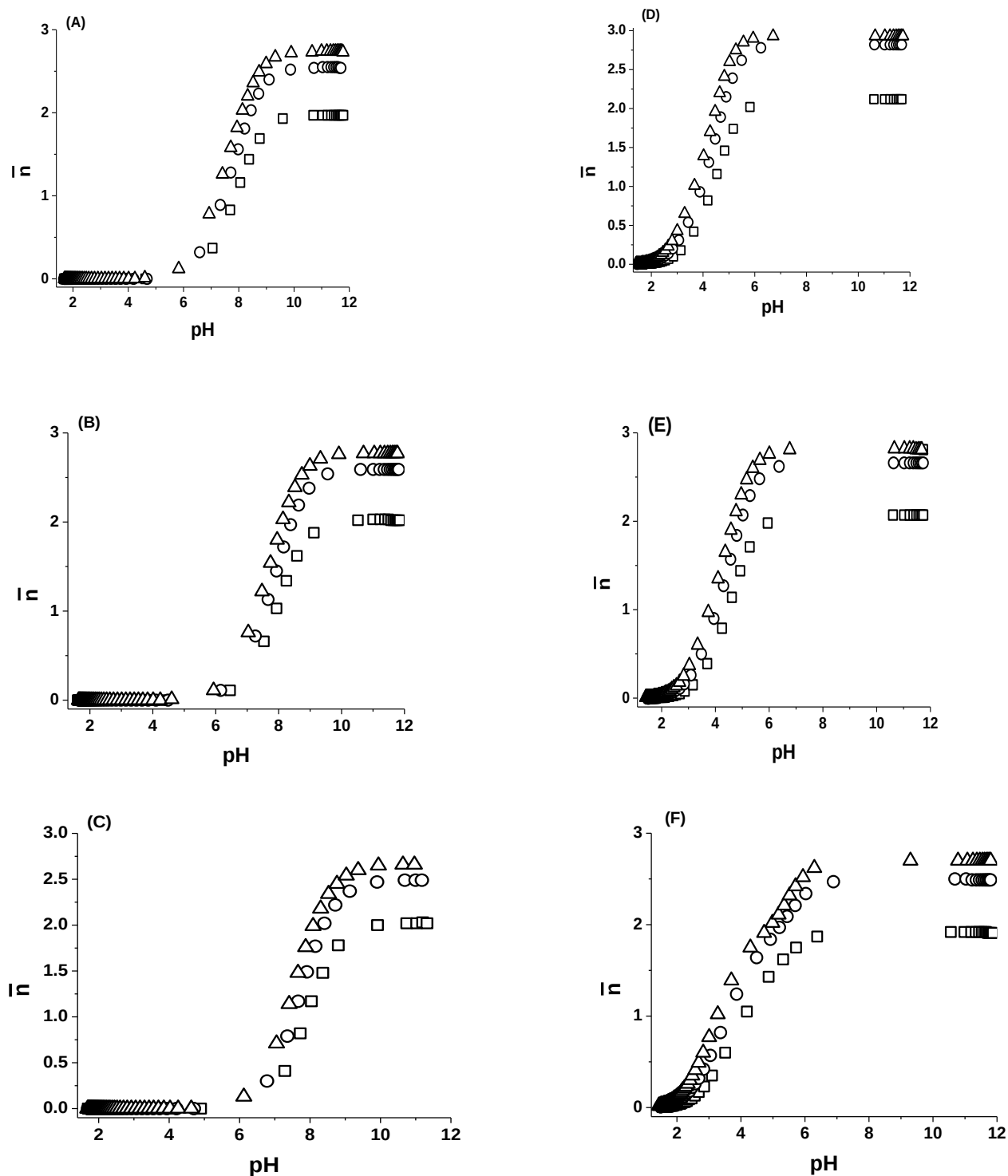


Fig.4.12: Formation curves of F and MA complexes.

(A) Pb(II), (B) Cd(II), (C) Hg(II) for F complexes and (D) Pb(II) (E) Cd(II)

(F) Hg(II) for MA complexes in 40% v/v DMF- water mixture;

Ligand concentration: ($\square$) 0.25, ($\circ$) 0.375 and (Δ) 0.50 mmol.

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 $\bar{n}$ versus pH plots which can have many applications. For example, PSEUDOPLOT [93] 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. Fig. 4.13 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.

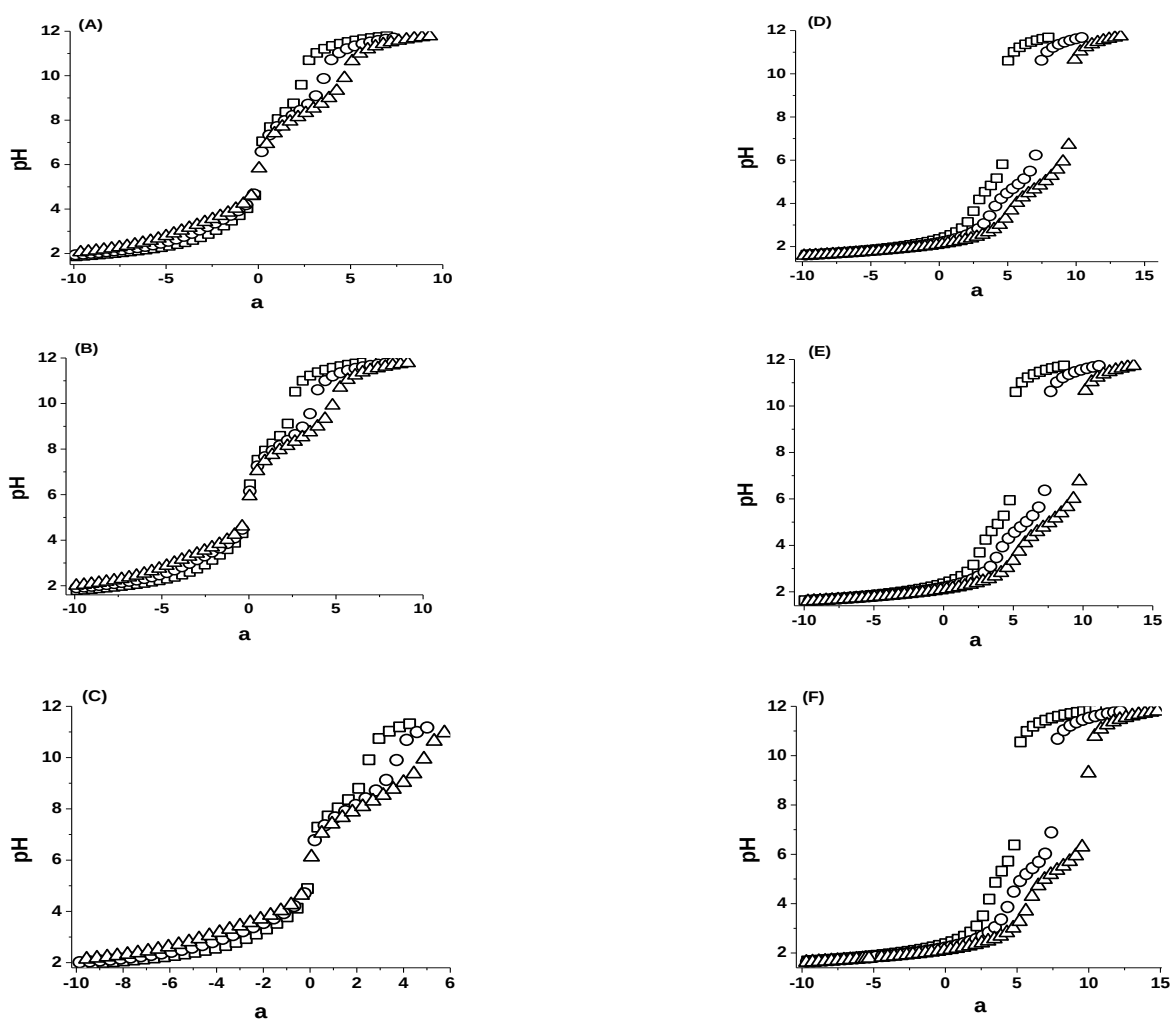


Fig.4.13: Number of moles of alkali versus pH curves of F and MA complexes. (A) Pb(II), (B) Cd(II), (C) Hg(II) for F complexes and (D) Pb(II), (E) Cd(II) and (F) Hg(II) for MA complexes in 40% v/v DMF-water mixture, Ligand concentration: ($\square$) 0.25, ($\circ$) 0.375 and (Δ) 0.50 mmol.

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 $\bar{n}$ values observed were less than three, with no ascending slope [94] the $\bar{n}$ values greater than three or ascending slope indicates the presence of a complex species with higher ligand number.

The preliminary screening of a number of species based on several thumb rules [82] resulted in a short list of ML_2H , ML_2 and ML_3 .

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 MINQUAD75. 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.

The models were evaluated assuming the simultaneous existence of different combinations of species. Models containing various number and combinations of species were generated using an expert system package CEES [95] and these models were refined using MINQUAD75. As the number of species was increased, the models gave better statistics denoting better fit. This indicates that the final model appropriately fits the experimental data. Such exhaustive modeling was performed for all the systems and the final models are given in Tables 4.4-4.5 for Pb(II), Cd(II) and Hg(II) complexes of F and MA 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 4.4: Parameters of best fit chemical models of Pb(II), Cd(II) and Hg(II)- F complexes in DMF- water mixtures.

% of DMF	log β_{mlh} (SD)			pH-Range	NP	U _{corr} ×10 ⁸	χ^2	Skewness	Kurtosis	R-factor
	ML ₂	ML ₂ H	ML ₃							
Pb(II)										
0.0	6.38(39)	15.03(06)	9.25(18)	7.0-9.1	30	9.44	1.52	-0.02	4.92	0.0162
10	6.90(92)	14.82(88)	9.97(04)	7.3-8.3	27	9.28	2.18	-0.01	2.90	0.0322
20	6.64(66)	14.80(83)	9.73(25)	7.7-8.9	31	6.67	3.92	1.23	5.02	0.0623
30	6.80(83)	14.94(95)	9.63(25)	7.6-8.9	30	2.61	2.93	-0.24	3.17	0.0491
40	6.79(74)	14.89(88)	9.72(21)	7.6-8.9	37	10.00	6.25	0.03	2.65	0.0372
50	6.79(76)	14.93(94)	10.15(21)	7.0-8.9	30	3.53	5.87	0.63	3.79	0.0223
Cd(II)										
0.0	6.27(28)	14.65(67)	9.06 (09)	7.0-9.1	41	3.33	5.95	-0.52	3.00	0.0352
10	6.64(67)	14.62(63)	9.10 (17)	7.3-9.1	43	1.00	1.35	3.42	5.49	0.0511
20	6.52(55)	14.58(59)	9.42(05)	7.3-9.3	43	1.5	6.16	2.01	2.78	0.0302
30	6.55(56)	14.33(36)	9.30(21)	7.3-9.3	41	7.78	7.13	0.62	3.01	0.0301
40	6.55(57)	14.65(64)	9.53(07)	7.3-9.5	40	1.76	1.33	0.23	7.96	0.0141
50	6.45(47)	14.60(62)	9.13(24)	7.3-9.5	47	2.86	2.80	-0.61	4.23	0.0161
Hg(II)										
0.0	6.88(89)	15.34(35)	9.42(07)	6.0-9.4	44	5.71	1.33	0.21	4.41	0.0242
10	6.37(39)	14.80(82)	9.52(21)	6.0-8.4	34	5.45	7.52	-0.59	4.41	0.0232
20	6.71(73)	14.93(91)	9.45(07)	6.0-8.4	26	1.54	2.00	0.13	2.53	0.0132
30	6.97(98)	14.45(46)	9.76(29)	6.0-8.4	21	4.44	9.76	-0.53	4.12	0.0223
40	7.05(07)	14.70(72)	9.85(03)	6.0-8.4	27	1.42	8.14	-0.31	4.12	0.0141
50	6.59(62)	15.06(07)	9.49(03)	6.0-8.4	22	1.11	8.85	-0.54	3.63	0.0052

Table 4.5: Parameters of best fit chemical models of Pb(II), Cd(II) and Hg(II)-MA complexes in DMF - water mixtures.

% of DMF	log β_{mlh} (SD)			pH-Range	NP	$U_{\text{cor4r}} \times 10^8$	χ^2	Skewness	Kurtosis	R-factor
	MX ₂	MX ₂ H	MX ₃							
Pb(II)										
0.0	6.65(66)	11.61(62)	10.15(16)	2.8-7.8	44	2.11	16.41	4.11	2.90	0.0493
10	6.83(84)	11.53(54)	9.50(51)	3.6-5.8	48	0.66	5.85	0.05	4.66	0.0083
20	6.73(74)	11.30(31)	9.60(62)	3.6-5.2	48	6.66	10.59	0.60	3.18	0.0244
30	7.10(12)	12.10(12)	10.50(51)	3.9-5.1	44	2.00	3.46	-0.14	2.62	0.0132
40	6.71(73)	11.65(66)	10.33(34)	4.1-5.2	46	0.66	9.41	0.76	5.95	0.0292
50	7.08(09)	11.91(92)	10.80(82)	3.8-5.1	77	6.00	6.58	-0.26	3.69	0.0243
Cd(II)										
0.0	6.76(77)	11.73(72)	10.32(33)	4.0-7.2	72	3.18	5.11	0.26	3.14	0.0154
10	6.65(66)	11.63(65)	9.34(35)	4.0-7.5	73	6.087	1.69	3.61	4.04	0.0265
20	6.41(43)	11.55(54)	9.33(36)	4.2-7.4	44	4.58	5.02	1.37	5.78	0.0226
30	6.33(35)	11.38(40)	9.43(44)	4.2-7.3	88	3.04	1.64	1.52	7.43	0.0197
40	6.76(77)	11.53(54)	9.84 (85)	4.2-7.4	88	6.84	9.64	0.25	5.39	0.0299
50	6.55(56)	11.32(33)	9.83(82)	4.2-7.4	90	4.21	3.82	-0.03	2.72	0.0221
Hg(II)										
0.0	6.93(94)	12.75(76)	9.35(36)	4.2-6.4	100	7.22	3.92	1.14	5.11	0.0292
10	6.58(61)	12.27(29)	9.22(23)	4.4-6.4	22	24.54	23.67	0.05	5.82	0.0583
20	6.36(37)	12.18(19)	9.36(35)	4.2-6.5	34	8.50	8.33	1.34	6.36	0.0344
30	6.69(70)	11.68(67)	9.39(40)	4.2-6.4	36	10.52	3.82	1.23	3.21	0.0375
40	6.39(40)	12.14(15)	9.22 (23)	4.2-6.4	77	5.26	38.00	1.95	2.12	0.0456
50	6.25(31)	12.46(47)	9.12(13)	4.5-6.4	65	8.23	4.00	1.16	3.45	0.0323

4.2.4. Residuals analysis

The residuals-difference between what is actually 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 residuals should follow normal distribution for the best fit model. The use of plots of residuals versus calculated volumes of dependent variables (volume) and independent variables (TM, TL) is popular.

Gans et al. applied sample SD in weighted least squares analysis for the calculation of β 's and suggested that any value less than three is satisfactory. of course, the ideal value of one was observed only in a single titration curve (unlike pooled data) inferring that the data have been correctly weighted. 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 part of standard normal distribution with zero mean and unit standard deviation [96].

A persual of Table 4.4 indicates that the χ^2 values range between 1.33 – 9.76 for DMF-water mixtures. The values of kurtosis and skewness range from 2.53 – 7.96 and -0.59 -3.42 for DMF-water mixtures. In table 4.5 the χ^2 values range between 1.64 – 38.00 for DMF-water mixtures. The values of kurtosis and skewness range from 2.12 – 7.43 and -0.26 – 4.11 for DMF-water mixtures. 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.

4.2.5. Effect of influential parameters

The computer programs refine the stability constants by minimizing the random errors in the data. But in the presence of considerable systemic errors, not only the β 's is in errors; even some species may be rejected. MINQUAD75 [68] has no provision to vary the influential parameters. Hence, some representative systems were studied in order to have a cognizance if the effects of errors in concentrations of ingredients on the stability constants of binary metal complexes are given in Table 4.6. 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 4.6: Effect of errors in influential parameters on M(II)- F/MA complex stability constants in DMF-water medium.

Ingredient	% Error	Log $\beta_{\text{mlh}}(\text{SD})$		
		120	121	130
Pb(II)-F 50 % v/v DMF-water mixture				
Acid	0	6.79(16)	14.93(22)	10.15(21)
	-5	8.67(44)	16.99(30)	12.86(27)
	-2	7.28(37)	15.75(25)	11.11(21)
	+2	6.44(22)	Rejected	9.22(29)
	+5	5.99(31)	Rejected	Rejected
Alkali	-5	5.83(31)	Rejected	Rejected
	-2	6.43(33)	Rejected	9.08(21)
	+2	7.24(46)	15.76(27)	11.19(22)
	+5	8.39(29)	Rejected	Rejected
Ligand	-5	6.74(45)	15.06(38)	10.46(24)
	-2	6.74(36)	15.03(36)	10.35(23)
	+2	6.74(29)	14.98(33)	10.21(21)
	+5	6.73(24)	14.95(31)	10.10(21)
Metal	-5	6.74(32)	15.00(34)	10.28(22)
	-2	6.77(27)	14.96(35)	10.20(23)
	+2	6.80(22)	14.91(35)	10.10(24)
	+5	6.81(20)	14.87(36)	10.02(25)
Pb(II)-MA 30 % v/v DMF -water mixture				
Acid	0	7.10(25)	12.10(15)	10.50(24)
	-5	Rejected	13.82(29)	12.88(28)
	-2	7.05(60)	12.90(28)	11.50(30)
	+2	6.78(16)	Rejected	9.56(39)
	+5	6.16(21)	Rejected	Rejected
Alkali	-5	5.69(40)	Rejected	Rejected
	-2	6.63(19)	Rejected	9.38(48)
	+2	Rejected	13.04(26)	11.75(20)
	+5	Rejected	14.00(24)	13.33(22)
Ligand	-5	6.82(86)	12.62(22)	11.24(23)
	-2	7.06(28)	12.33(25)	10.81(15)
	+2	7.05(15)	11.85(25)	10.19(22)
	+5	6.98(22)	11.21(73)	9.59(45)
Metal	-5	7.09(20)	12.12(18)	10.61(26)
	-2	7.10(28)	12.11(28)	10.54(26)
	+2	7.10(26)	12.09(27)	10.46(27)
	+5	7.10(25)	12.07(27)	10.40(88)

4.2.6. Effect of solvent on metal-ligand equilibria

The presence of organic solvents influences the equilibria in solution due to change in the dielectric constant (D) of the medium that influences the contribution from electrostatic and non-electrostatic interactions that have a bearing on the magnitude of stability constants [97].

The variation of overall stability constant values or change in free energy with co-solvent content depends upon two factors, which is electrostatic and non-electrostatic. Born's classical conditions hold 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 constants. Hence, the $\log \beta$ values should vary linearly as a function of the reciprocal of the dielectric constant ($1/D$) of the medium [97].

(DMF) is a versatile polar aprotic and non-coordinating solvent capable of acting as hydrogen bond acceptor with random structure. 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 [45].

The increase in organic solvent content decreases the dielectric constant of the medium.

The change in $\log \beta$ values of Pb(II), Cd(II) and Hg(II) complexes of F and MA with $1/D$ (Fig. 4.14) is almost linear which indicates that the dielectric constant or long range interactions are responsible for the stability trend in both DMF-water media. The deviation from linearity may be due to some contributions from non-electrostatic forces.

Since complex formation can be viewed as a competition between the pure and solvated forms of the ligand and the metal ion, the solute-solvent interactions, relative thermodynamic stabilities and kinetic labilities are also expected to play an important role. Different types of electrostatic forces dominate in different ranges of the composition of DMF-water media.

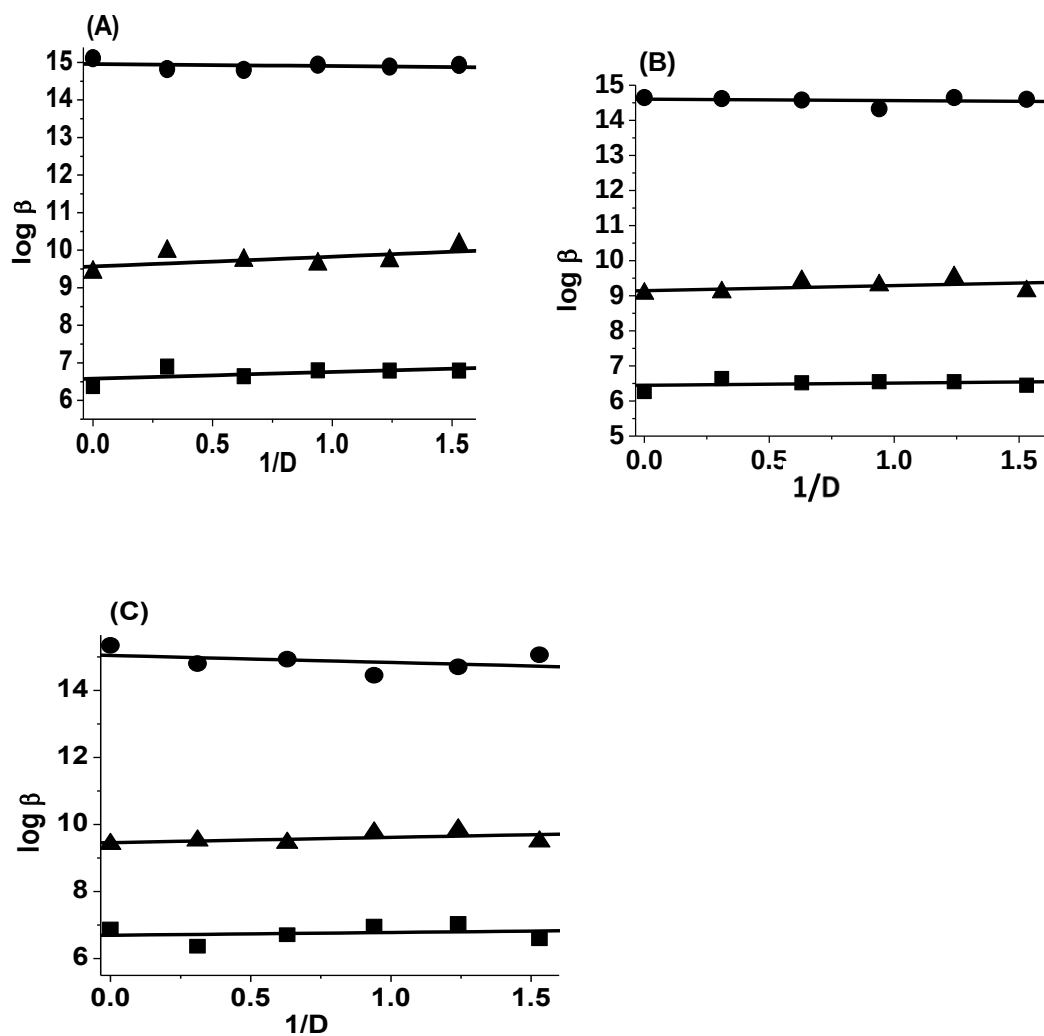


Fig.4.14: Variation of overall stability constant values of metal-F complexes with reciprocal of dielectric constant ($1/D$) of DMF-water mixtures (A) Pb(II); (B) Cd(II); (C) Hg(II); (■) $\log \beta_{ML2}$; (▲) $\log \beta_{ML3}$; (●) $\log \beta_{ML2H}$.

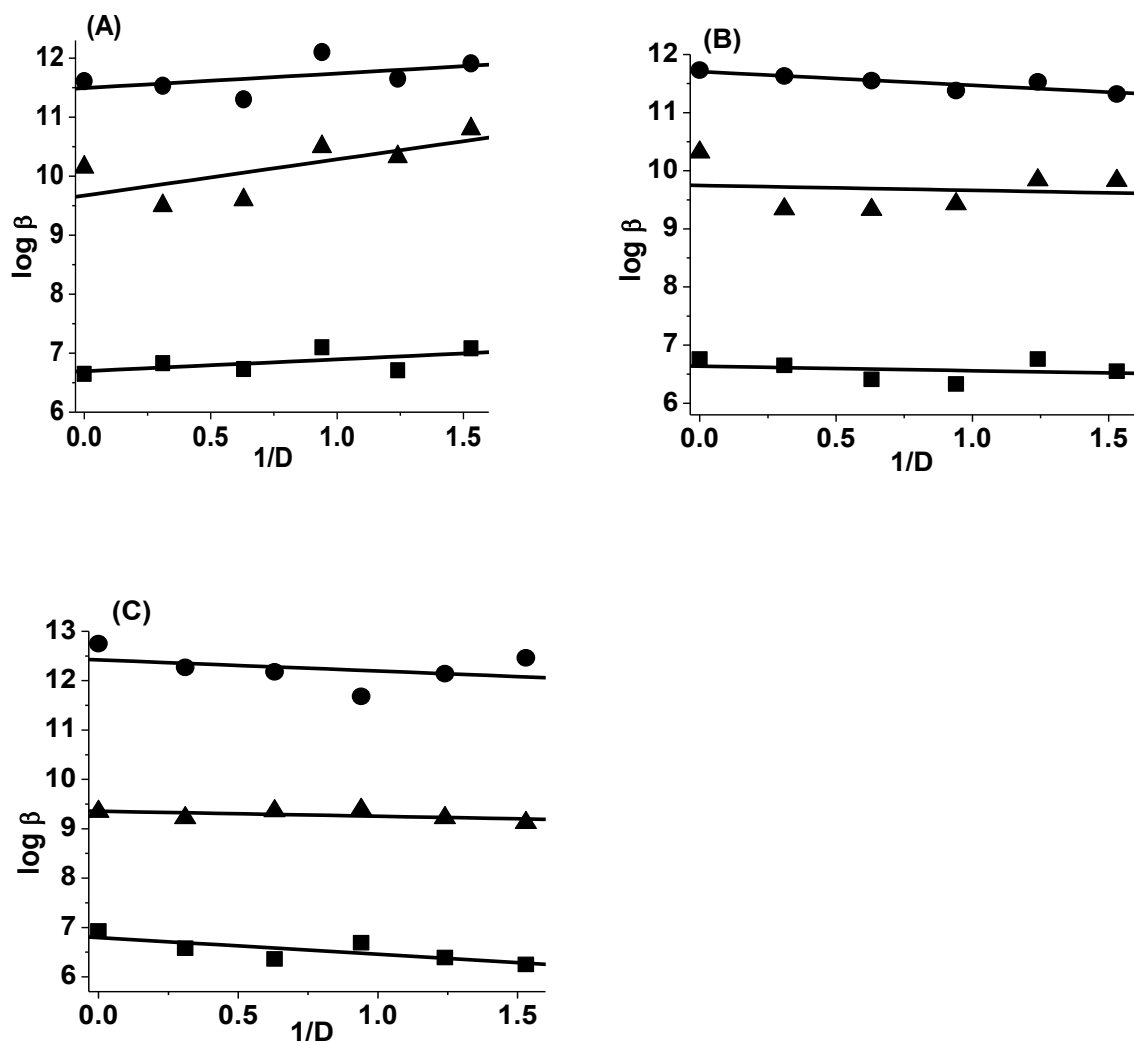


Fig.4.15: Variation of overall stability constant values of metal-MA complexes with reciprocal of dielectric constant ($1/D$) of DMF-water mixtures (A) Pb(II); (B) Cd(II); (C) Hg(II); (■) $\log \beta_{ML2}$; (▲) $\log \beta_{ML3}$; (●) $\log \beta_{ML2H}$.

4.2.7. Distribution diagrams of binary complexes

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

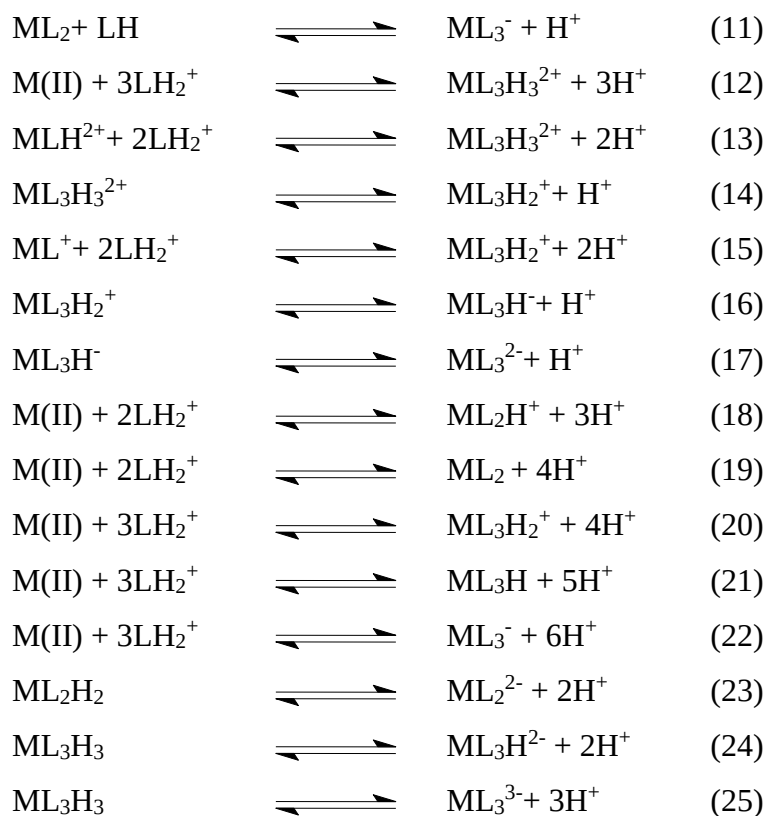
$$PS = \frac{m(j) \cdot \beta_{m(j)l(j)h(j)} \cdot FL_i^{l(j)} \cdot FH_i^{h(j)}}{\sum_{j=0}^N \beta_{m(j)l(j)h(j)} \cdot FL_i^{l(j)} \cdot FH_i^{h(j)}} \cdot 100 \quad (4.9)$$

Using the formation constant in the best fit model, distribution diagrams were obtained with the computer programs DISPLOT and SCPHD [98]. 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 in DMF- water mixtures.

The variation of species concentration with pH is shown in Figs. 4.16 - 4.22 for metal- F and metal-MA complexes in DMF- water mixtures. The patterns of the distribution of species with pH show that the concentrations of species are affected by organic media.

L-phenylalanine is a bidentate ligand that has one dissociable (carboxyl group) and one associable (amino) protons. The binary complexes of F with metal ions Pb(II), Cd(II) and Hg(II)) are refined and presented as ML_2 , ML_2H , ML_3 . The present study is confined to pH ranges 4.0-10.3 for Pb(II), 4.3-9.9 for Cd(II) and 6.0-9.8 for Hg(II). In this pH range the ligand is expected to be present in the form of LH_2^+ , LH , and L^- . Hence the possible metal-ligand species are ML_2 , ML_2H , and ML_3 which are confirmed by MINQUAD75. The equilibria can be represented as follows:





The species $\text{ML}_2\text{H}_2^{2+}$, $\text{ML}_3\text{H}_3^{2+}$, ML_3H_2^+ could not be detected in the present study probably because they are formed at very low pH. Distribution diagrams of F in DMF-water mixtures are shown in Figs. 4.17-4.19. They indicate that the binary complexes of Pb(II), Cd(II) and Hg(II) are formed in the pH range 5.0-10.0. The species ML_2H , ML_2 , ML_3 are formed in the pH range of 6.0-9.6. ML_2H is formed at lower pH with higher percentage [Equilibria (6), (7), (8) and (18)]. ML_2 , ML_3 are simultaneously formed with the increasing pH. ML_2 , ML_3 species percentage successively increases with increasing pH. Successive deprotonation of ML_2H forms ML_2 beyond a pH 9.0. The concentration of ML_2H species decreased, while the concentration of ML_2 and ML_3 increased in the pH range 7.5-9.5 [Equilibria (9), (10) and (11)]. ML_3 formed at higher pH with high percentage [Equilibria (11), (17), (22) and (25)].

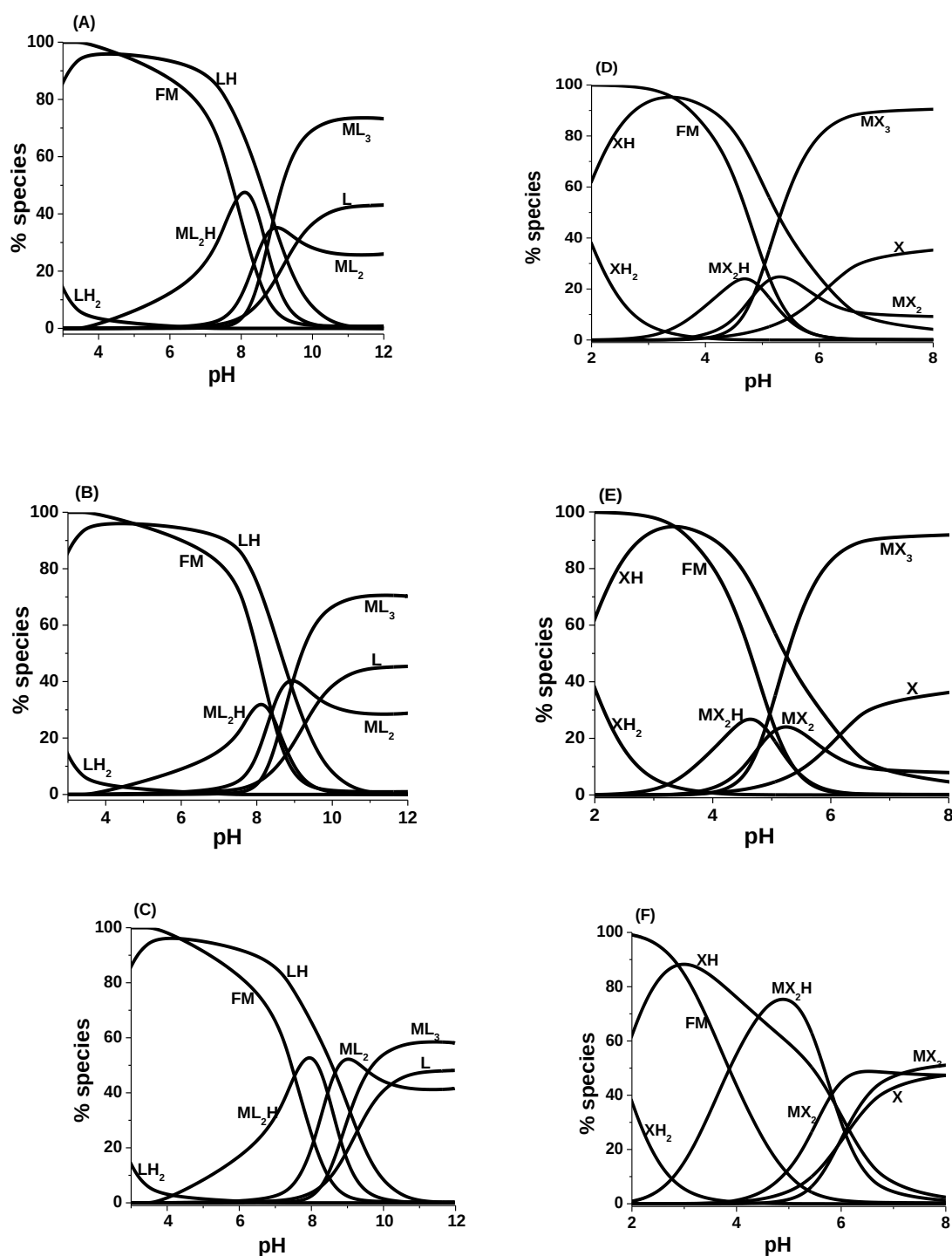


Fig.4.16: Distribution diagrams of M(II)- F /MA in aqueous media.

(A) Pb(II)-F, (B) Cd(II)-F, (C) Hg(II)-F (D) Pb(II)-MA, (E) Cd(II)-MA and (F) Hg(II)-MA.

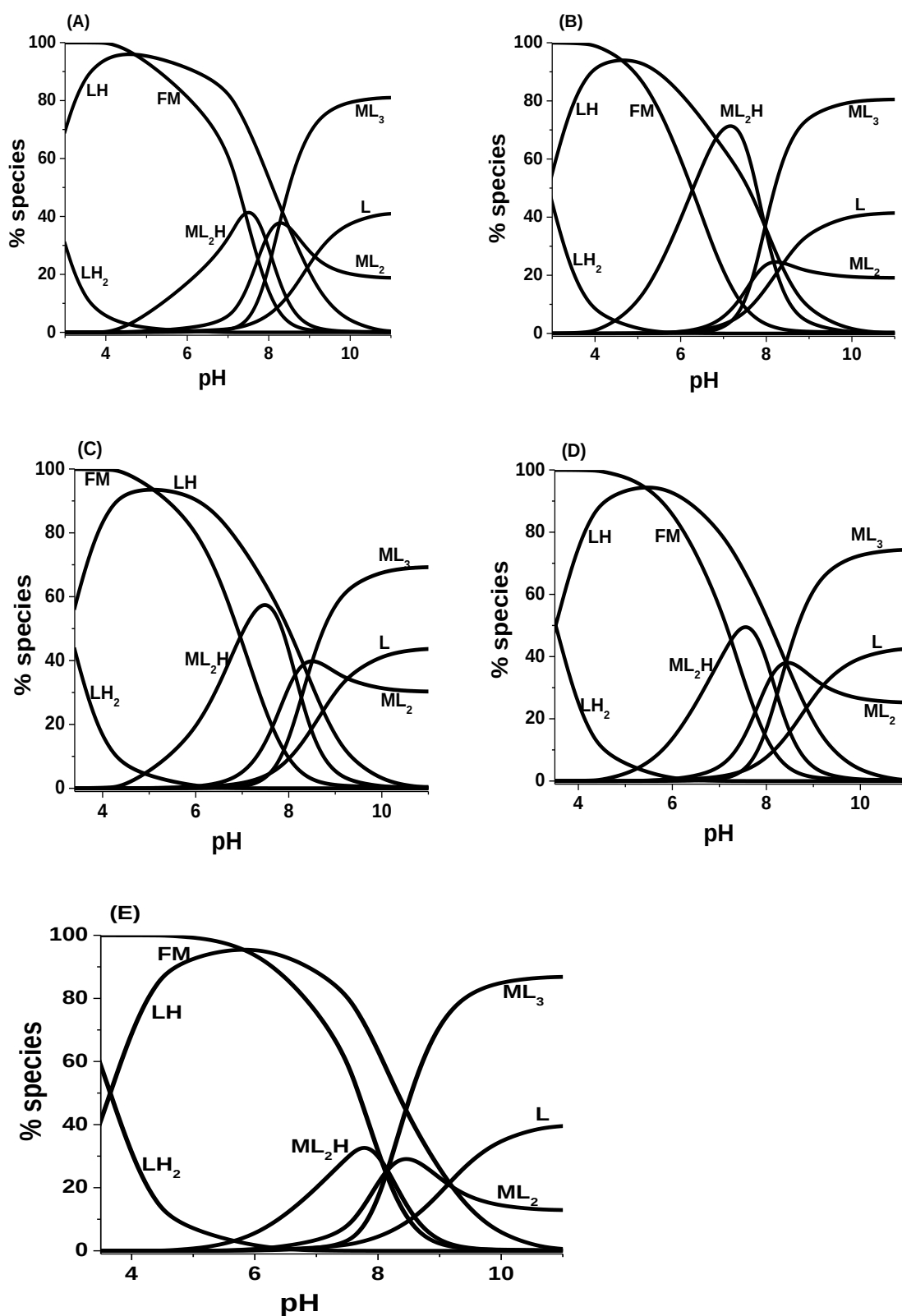


Fig.4.17: Distribution diagrams of Pb- F in DMF-water mixtures % v/v.

(A) 10, (B) 20, (C) 30, (D) 40, and (E) 50.

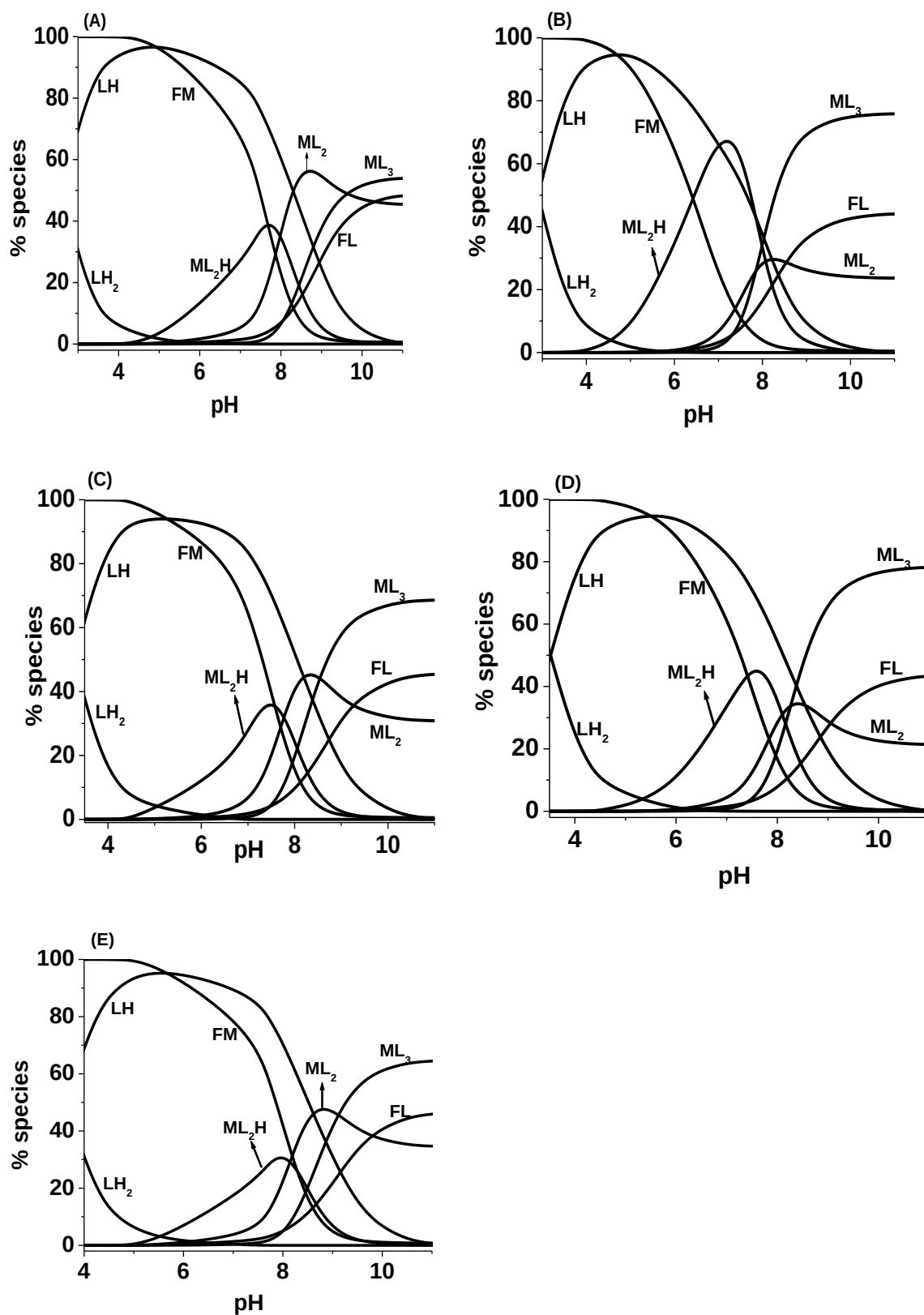


Fig.4.18: Distribution diagrams of Cd- F in DMF-water mixtures % v/v.

(A) 10, (B) 10, (C) 30, (D) 40, and (E) 50.

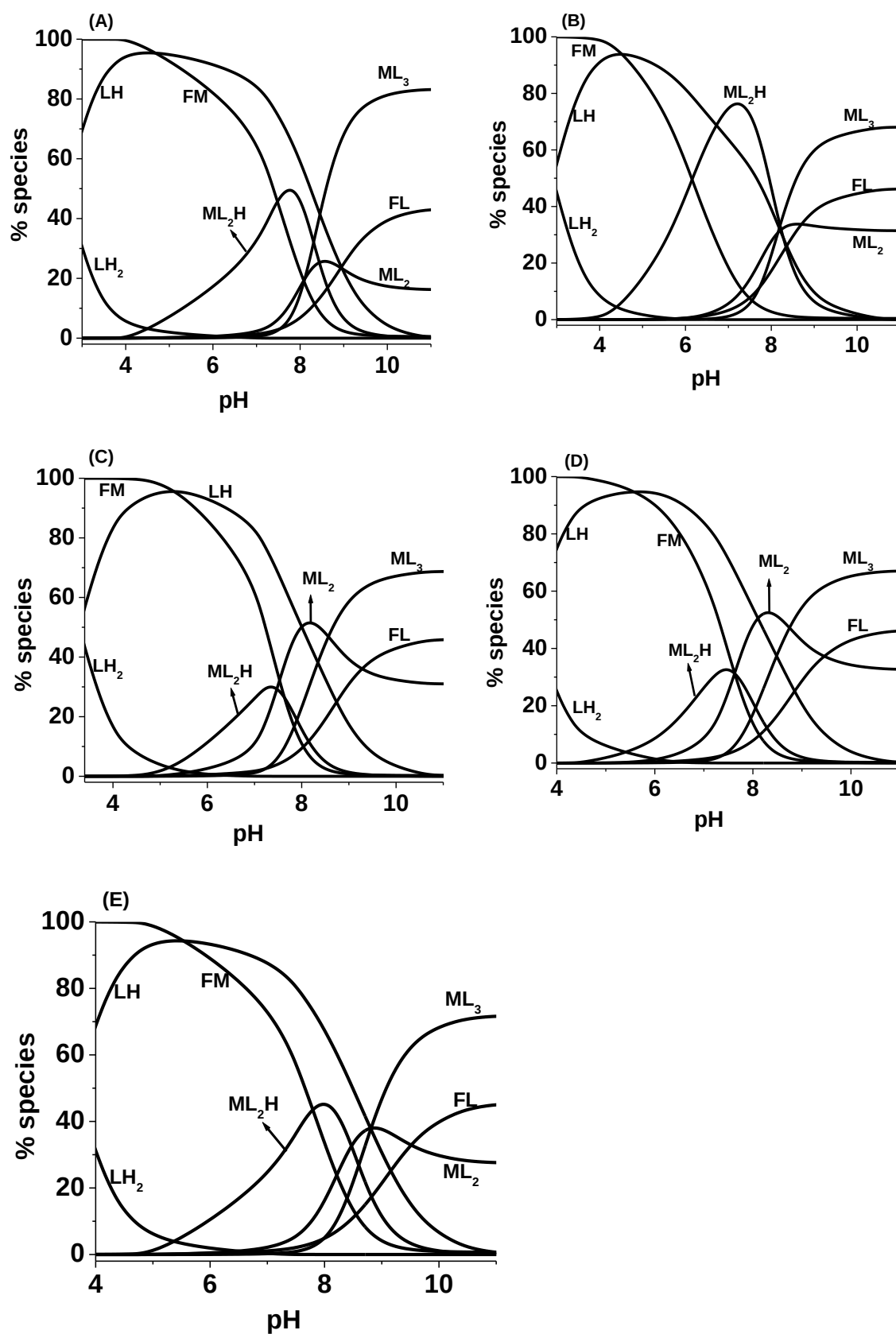
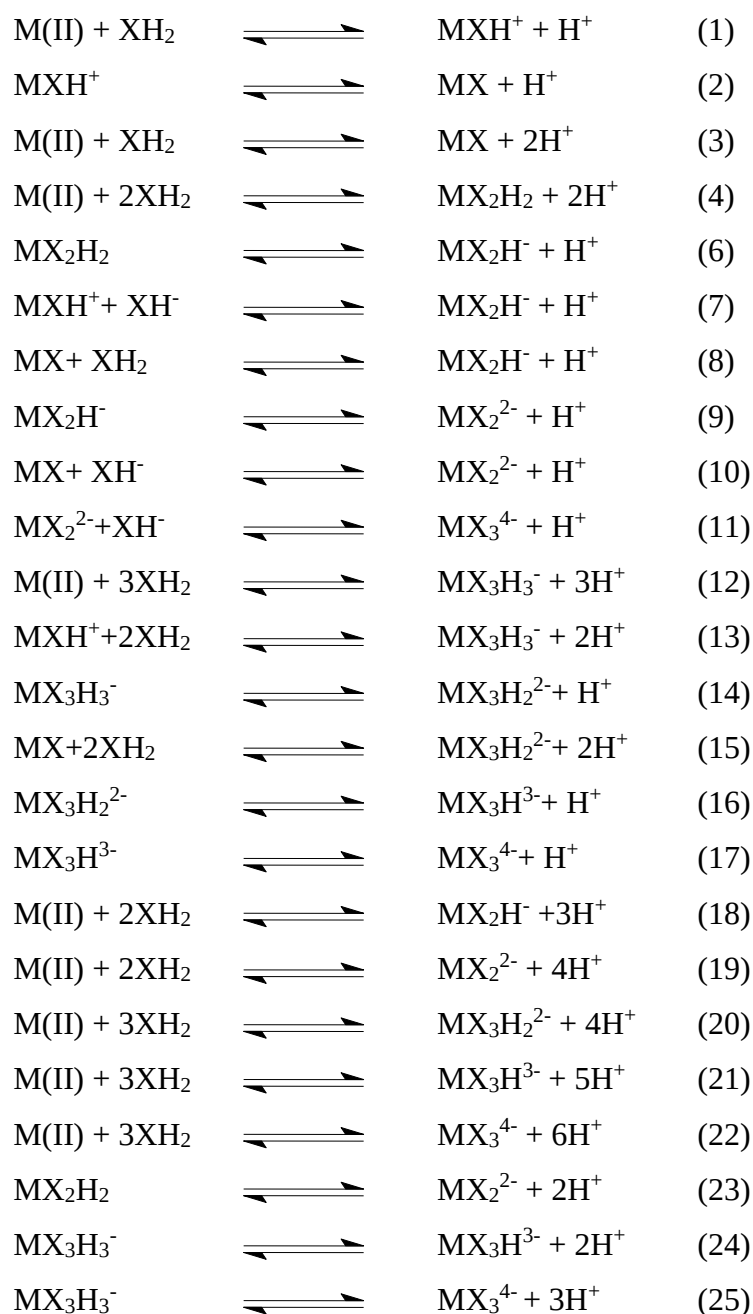


Fig.4.19: Distribution diagrams of Hg- F in DMF-water mixtures % v/v.

(A) 10, (B) 20, (C) 30, (D) 40, and (E) 50.

Maleic acid is a bidentate ligand that has two dissociable (carboxyl groups) protons. The different forms of maleic acid are XH_2 , XH^- , and X^{2-} in the pH range < 4.0 , $2.0\text{--}7.0$ and > 5.0 respectively. Hence, the plausible binary metal-ligand complexes can be predicted from these data. The present investigation reveals the existence of MX_2 , MX_2H , MX_3 for Pb(II) , Cd(II) and Hg(II) .

The present study is confined to pH ranges $2.8\text{--}7.7$ for Pb(II) , $4.0\text{--}7.5$ for Cd(II) and $4.2\text{--}7.0$ for Hg(II) . The formation of various maleic acid complex species is shown in the following equilibria.



Distribution diagrams of MA in DMF-water mixtures are shown in Figs.4.20-4.22. They indicate that the binary complexes of Pb(II), Cd(II) and Hg(II) are formed in the pH range 3.0-7.5. The species MX_2H , MX_2 , MX_3 are formed in the pH range of 3.0-7.0. MX_2H is formed at lower pH with higher percentage in case of lead and mercury [Equilibria (6), (7), (8) and (18)]. MX_2 , MX_3 are simultaneously formed with the increasing pH. Successive deprotonation of MX_2H forms MX_2 beyond a pH 6.0. The concentration of MX_2H species decreased, while the concentration of MX_2 and MX_3 increased in the pH range 4.0– 6.0 [Equilibria (9), (10) and (11)]. MX_3 formed at higher pH with high percentage [Equilibria (11), (17), (22) and (25)].

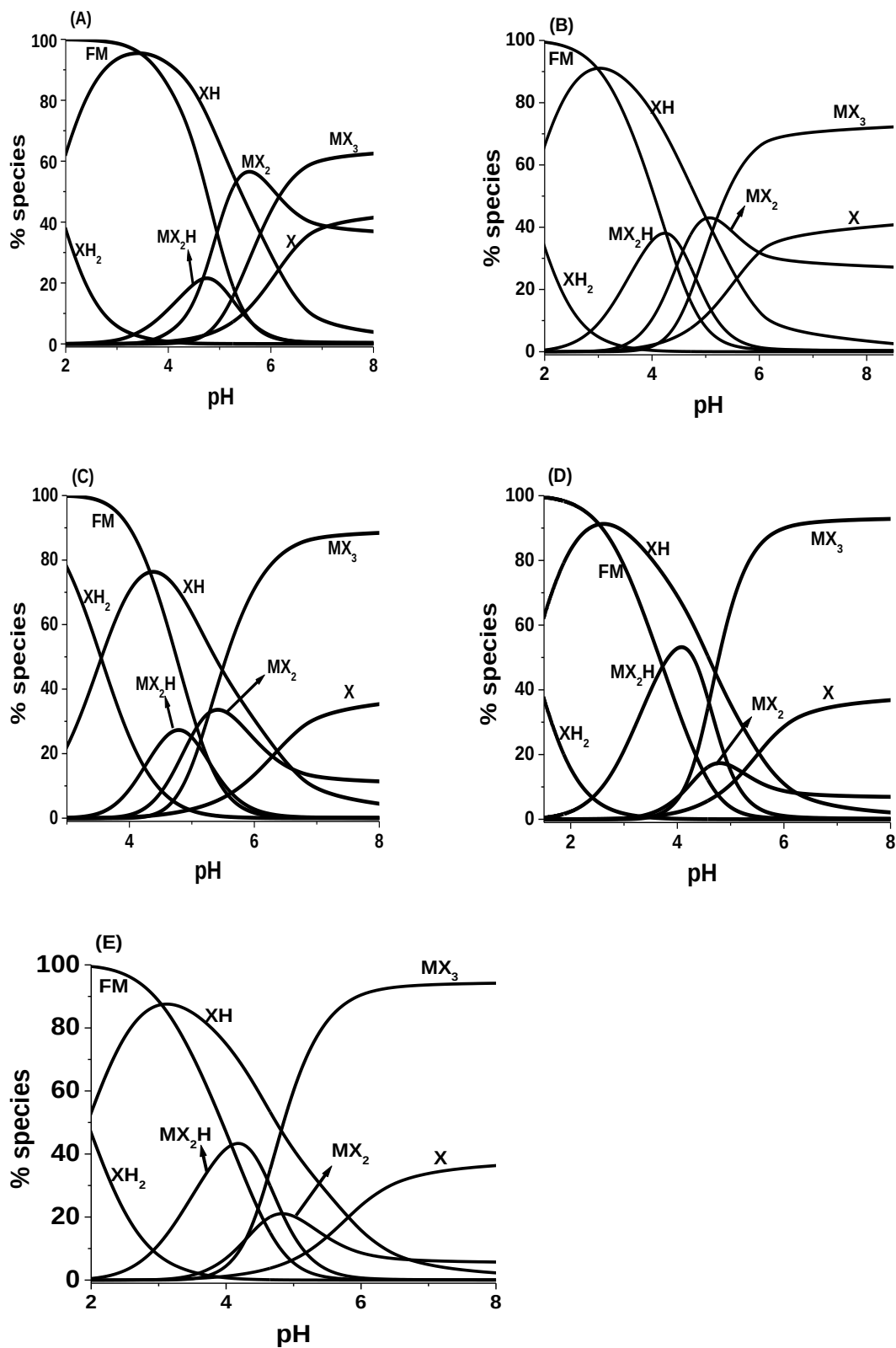


Fig.4.20: Distribution diagrams of Pb-MA in DMF-water mixtures % v/v.

(A) 10, (B) 20, (C) 30, (D) 40, and (E) 50.

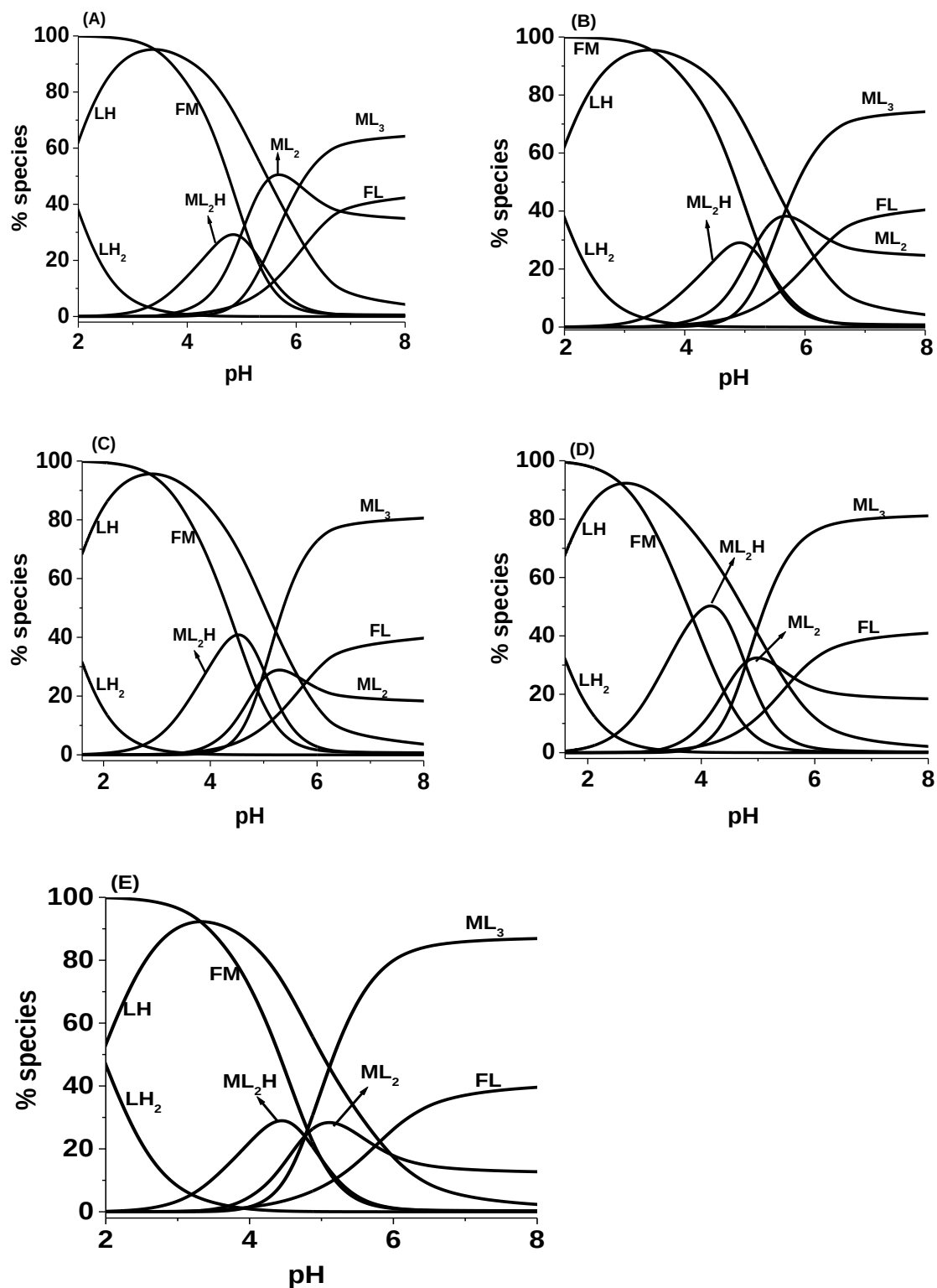


Fig.4.21: Distribution diagrams of Cd-MA in DMF-water mixtures % v/v.

(A) 10, (B) 20, (C) 30, (D) 40, and (E) 50.

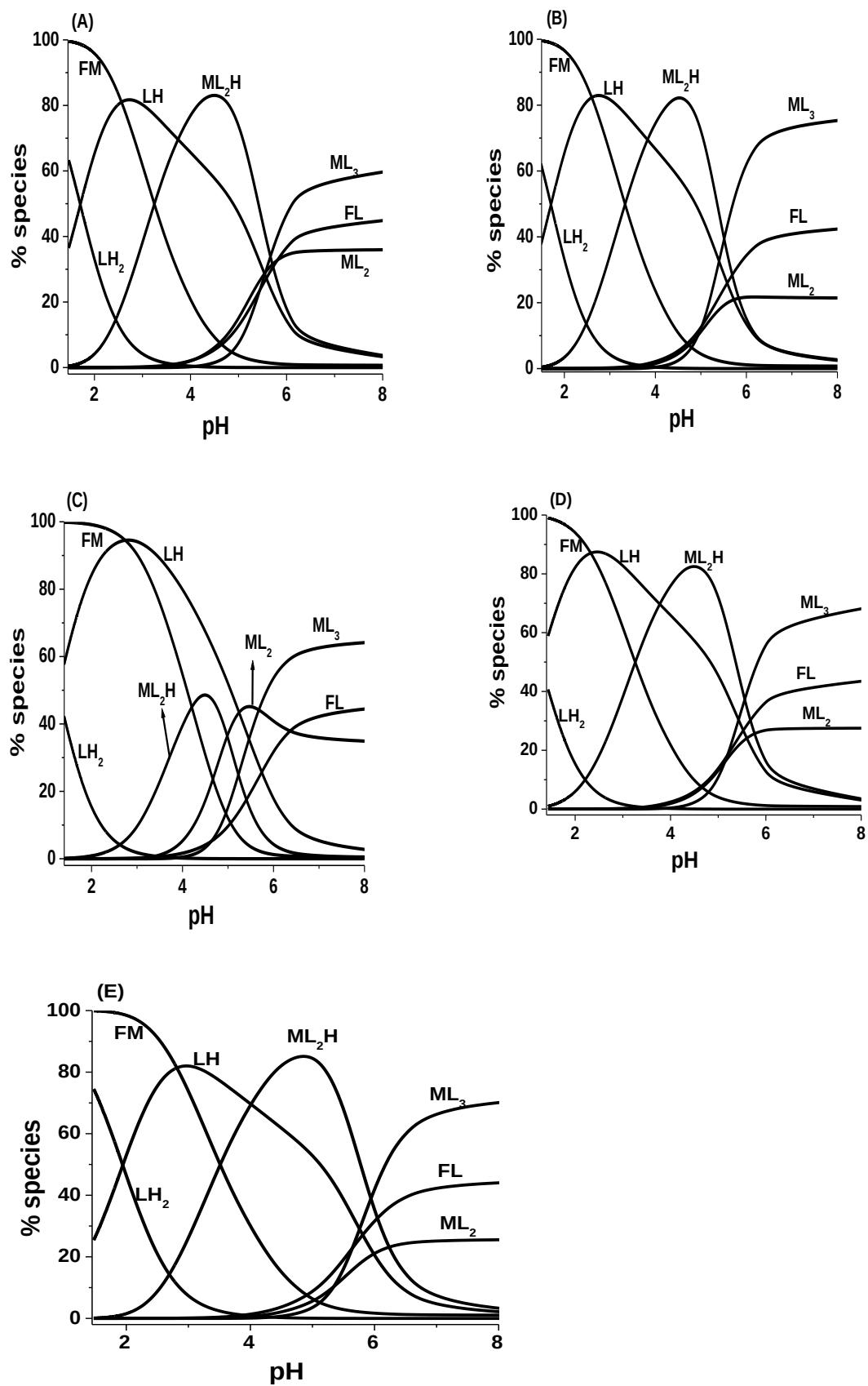


Fig.4.22: Distribution diagrams of Hg-MA in DMF-water mixtures % v/v.

(A) 10, (B) 20, (C) 30, (D) 40, and (E) 50.

4.2.8. Structures of binary complexes

When the second donor site of L-phenylalanine is a nitrogen atom, marked bidentate behavior is frequently found, more so when the additional chelation results in a five membered ring (Fig.4.23). 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 ion for this second 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 carboxyl oxygen of L-phenylalanine participate in bonding with metal ions. This argument supports the structures of complexes proposed in Fig.4.23.

Maleic acid acts as a bidentate ligand by using its two oxygen donor sites and the chelation results in highly stable seven membered rings (Fig.4.24). 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. This argument supports the structures of complexes proposed in Fig.4.24.

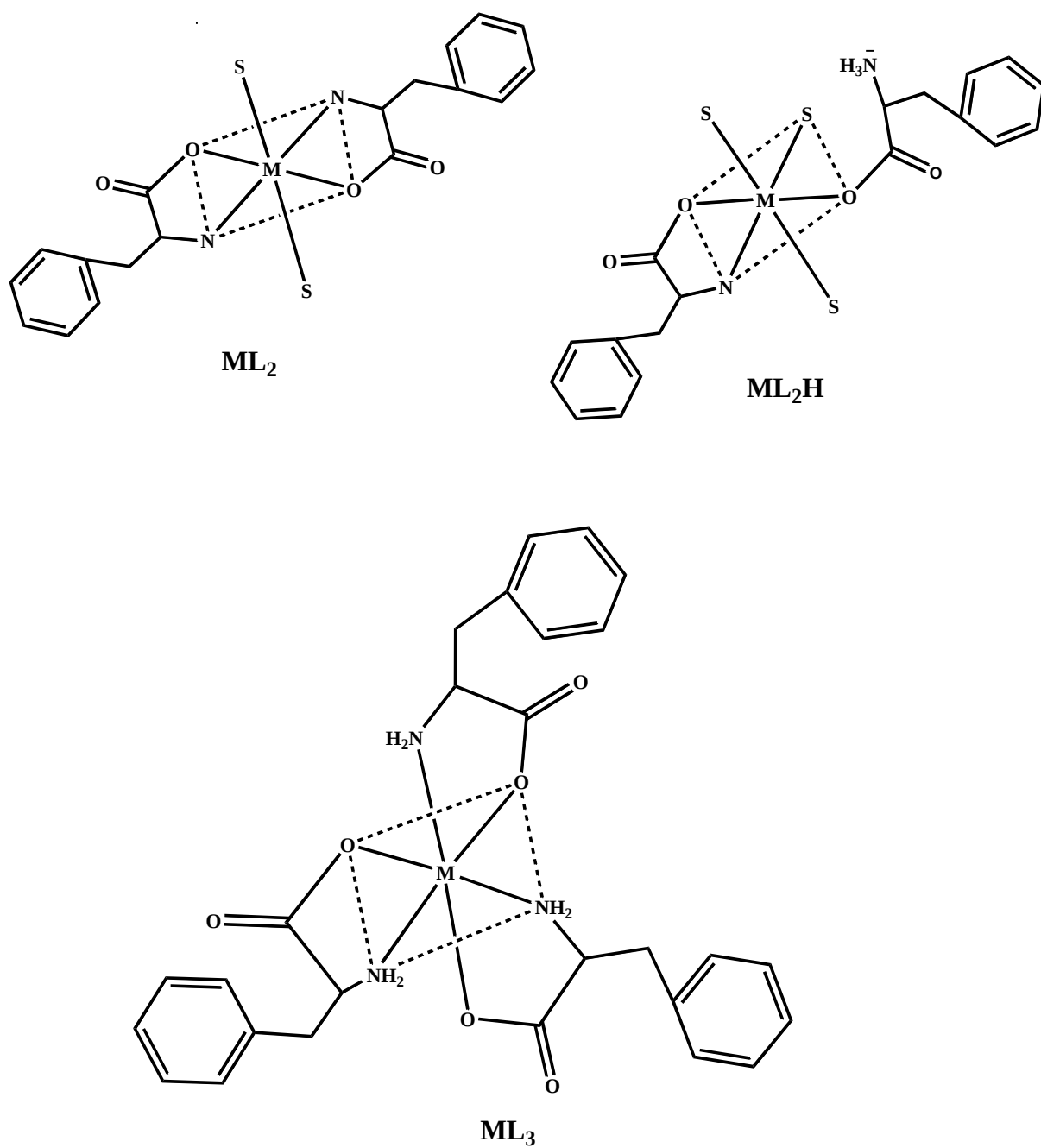


Fig.4.23: Speculative structures of F complexes with Pb(II), Cd(II) and Hg(II).
where S is either solvent or water molecules.

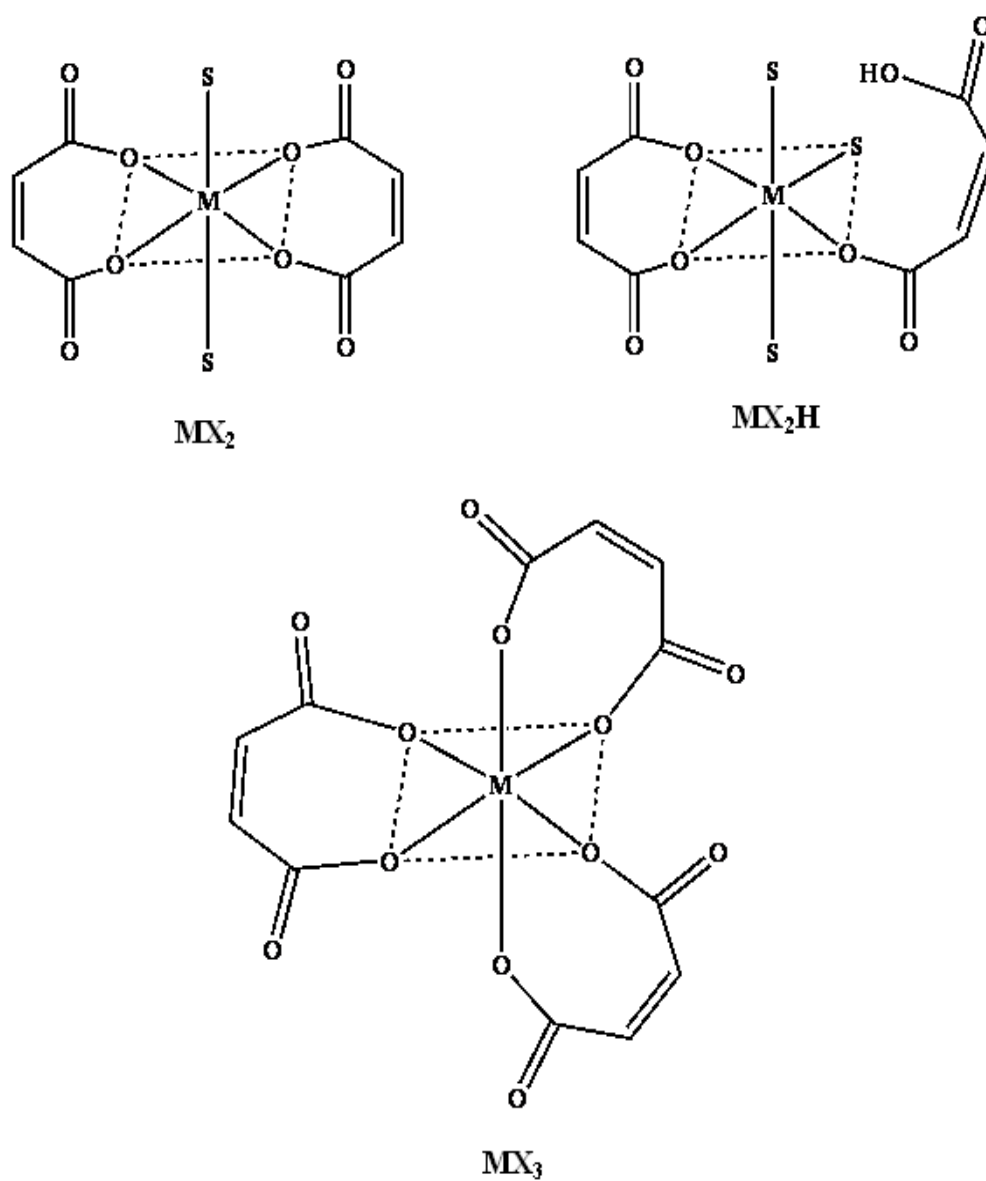


Fig.4.24: Speculative structures of MA complexes with Pb(II), Cd(II) and Hg(II) where S is either solvent or water molecules.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1. CONCLUSIONS

Pollution and toxicity of heavy metal ions are of the latest 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. Knowledge of speciation 1) saves a great deal of time, money and usage of equipment, 2) helps in understanding the complex interactions between dissolved, particulate, sedimentary and biological component, and 3) makes one to understand the absorption of metal ions from dietary materials which is greatly influenced by the presence of other metal ions and complexing agents.

The justification for measurement of speciation is to identify those species of metals that are likely to have adverse effects on biota. This includes measurement of both bioavailability and toxicity. 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. Varying amounts of toxic metals cause different changes in the biological system. 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-phenylalanine (F) and maleic acid(MA) in place of apoenzymes and substrates. The study was 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.

5.1.1. Protonation equilibria

Different forms of F were LH_2^+ , LH and L^- in the pH ranges 1.6 – 11.5 and those of MA were XH_2 , XH^- and X^{2-} in the pH ranges 1.5 – 10.0. The protonation constants increased with decreasing dielectric constants of the medium.

1. F formed LH_2^+ at low pH and deprotonated takes place with the formation of LH_2^+ , LH and L^- with the increase in pH. MA formed XH_2 at low pH and deprotonated to form XH^- and X^{2-} successively with the increase in pH.
2. Linear increase in the log values of protonation constants with decreasing dielectric constant of DMF-water mixtures indicated the dominance of electrostatic forces in the protonation-deprotonation equilibria.
3. Effect of errors in the ingredients indicated that the protonation constants associated with carboxyl proton were more affected than the amino proton. The errors in concentrations of alkali and acid affected the protonation constants more than that of ligand.
4. Overlapping of $\bar{n}_\text{H}$ versus pH curves for different concentrations of the ligand indicated non-polymerization of these ligands under the pH condition employed in the present study.

5.1.2. Metal-ligand equilibria

1. The possibility of the formation of poly-nuclear complexes was excluded as the ratio of TL_O to TM_O was greater than unity. Similarly, the presence of the dimeric and trimeric species was ruled out since the solutions used in all these experiments were very dilute and also the experimental conditions are not favorable for their formation.
2. The protonation constants determined from proton-ligand titration data were close to those retrieved from metal-ligand titration data confirming the existence of the same species.
3. Non-overlapping of the formation curves evinced the formation of protonated complexes.
4. The binary species refined for the F complexes of Pb(II) Cd(II) and Hg(II) were ML_2 , ML_2H , and ML_3 in the DMF-water mixture.
5. For MA complexes of Pb(II), Cd(II) and Hg(II) the species MX_2 , MX_2H and MX_3 are refined in DMF-water mixtures
6. 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.

5.2. RECOMMENDATIONS

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

- ✓ Using the present findings of protonation and binary stability constants, the author recommends to carry-out the chemical speciation of ternary complex containing maleic acid and L-phenylalanine with toxic metal ions in DMF-water mixture.
- ✓ Using the present finding data as a baseline, further investigation can be conducted to synthesize the maximum yield of solid complexes at a fixed pH value.

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LIST OF PUBLICATIONS

1. Wondimu Tesfa Tefera, Wakgari Bungula Negassa, Tegene Desalegn Zeleke, Hadgu Hailekiros Belay, Protolytic equilibria of L-phenylalanine and maleic acid in DMF-water mixture.

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2. Wakgari Bungula Negassa, Wondimu Tesfa Tefera, Tegene Desalegn Zeleke, Tajedin Alansi and Hadgu Hailekiros Belay, Chemical speciation of complexes of L- phenylalanine and maleic acid with toxic metal ions in Dimethylformamide-water mixtures.

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3. Wondimu Tesfa Tefera, Wakgari Bungula Negassa, Tegene Desalegn Zeleke, Tajedin Alansi, Hadgu Hailekiros Belay, Chemical Speciation of Binary Complexes of L-phenylalanine and Maleic Acid with Some Metal ions in DMF-Water Mixtures.

Int.J.Adv.Res. (accepted)

REPRINTS
