CHEMICAL SPECIATION OF ESSENTIAL METAL ION COMPLEXES OF L-PHENYLALANINE AND MALEIC ACID IN DIMETHYLFORMAMIDE-WATER MIXTURE

BY

WONDIMU TESFA TEFERA, B.Ed

THESIS SUBMITTED TO

DEPARTMENT OF CHEMISTRY

SCHOOL OF APPLIED NATURAL SCIENCE

PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT

FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

OFFICE OF GRADUATE STUDIES

ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

SEPT., 2018 E.C.

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DEDICATED TO

MY FAMILY

DECLARATION

The work incorporated in this thesis has been carried out in the research laboratories of Department of Chemistry, School of Applied natural Science, Adama Science and Technology University. The extent of information derived from the existing literature has been indicated in the body of the thesis at appropriate places, giving the references. The work embodied in this thesis is original and I declare that it has not been submitted in part or in full for any degree or diploma of any other University.

Adama, Ethiopia
Date: 18/09/2018 **Wondimu Tesfa Tefera**

ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

SCHOOL OF APPLIED NATURAL SCIENCE

DEPARTMENT OF CHEMISTRY

CERTIFICATE

This is to certify that the thesis entitled **"**Chemical speciation of essential metal ion complexes of L-phenylalanine and maleic acid in DMF-water mixture" submitted by **Wondimu Tesfa Tefera** to the Department of Chemistry, School of Applied Natural Science, Adama Science and Technology University, for the award of the degree of **Master of Science in Chemistry** is a bona fide record of research work carried out by 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.

Hadgu Hailekiros Belay (PhD)
Major Advisor
Date: 18/09/2018

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Co-Advisor
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Approval of Board of Examiners

Adama Science And Technology University

School Of Applied Natural Science

Department Of Chemistry

We, the undersigned, members of the board of examiners of the final open defense by **Wondimu Tesfa Tefera** have read and evaluated his thesis entitled "**Chemical speciation of essential metal ion complexes of L-phenylalanine and maleic acid in DMF-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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Chairperson	Signature	Date
_____	_____	_____
Internal Examiner	Signature	Date
_____	_____	_____
External Examiner	Signature	Date

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First and above all, I praise God, the almighty for providing me this opportunity and granting me the capability to proceed successfully. Next, I would like to forward heartfelt thanks for those who deserve it, for their assistance, guidance and realization of this work.

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Wondimu Tesfa Tefera

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LIST OF ABREVIATIONS

Co(II)	Cobalt(II) ion
COST	Concentration of solution by titration
CTAB	Cetyltrimethylammonium bromine
Cu(II)	Copper(II) ion
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
L-DOPA	L-Dihydroxyphenylalanine
MA	Maleic Acid
NAP	Number of associable protons
NDP	Number of dissociable protons
Ni(II)	Nickel(II) ion
NP	Number of experimental points
Phe	Phenylalanine
PKU	Phenylketonuria
SD	Standard Deviation
SLS	Sodium lauryl sulphate
TLo	Total initial concentration of ligand
TMo	Total initial concentration of metal
WHO	World Health Organization

ABSTRACT

Protonation equilibria and binary stability of metal complexes of Co(II), Ni(II) and Cu(II) with L-phenylalanine and maleic acid has been studied pH-metrically in the concentrations range of 0-50% v/v DMF-water mixtures maintaining an ionic strength of 0.16 mol L^{-1} at 303K. The selection of best fit chemical models is based on statistical parameters and residual analysis. The predominant species detected were ML, ML_2, and ML_2H_2 for Co(II), Ni(II) and Cu(II). The appropriateness of the experimental conditions were verified by introducing error deliberately. The models containing different numbers of species were refined by using the computer program MINIQUAD75. The chemical speciation was explained based on the distribution diagrams drawn using HYSS HYPERQUAD. The trend in variation of stability constants of the complexes with dielectric constant of the medium was attributed for the formation and possible structures of the complex species presented.

***Keywords**: Metal complexes, protonation equilibria, stability constants, phenylalanine maleic acid, DMF, MINIQUAD75 and HYSS HYPERQUAD.*

CHAPTER-1

INTRODUCTION

1. INTRODUCTION

1.1. Back Ground of the Study

Chemical speciation is an important aspect of environmental analysis and research. Bioavailability and toxicity of an element depend on its species. Metal ions are complexed in fresh water, sea water, polluted water and bio-system with naturally occurring ligands and chelating agents such as humic substances, sulfide and pesticides. For example; cobalt, nickel and copper are predominantly organically complexed in seawater and bio-system. It is estimated that the coordination compounds entering environment are about 5-10 million kinds, most of them being contained in aquatic environment, including water and sediment. These metal coordination interactions would affect the biogeochemical pathways taken by the metal, such as its bioavailability and toxicity to aquatic organisms, and adsorption/ desorption reactions on suspended materials [1].

International Union of Pure and Applied Chemistry (IUPAC) defined "speciation" as the distribution of an element amongst the defined chemical species in a system. The distribution of each species relies on the binding strength of the metal-ligand complexes which is quantitatively expressed as the stability constant. A chemical species is a specific form of an element which attributes to its isotopic composition, electronic or oxidation state, and/or complex or molecular structure. The speciation determines the behavior of trace elements in a system, and in the human organism speciation has a profound effect on bioavailability, distribution and toxicity. Speciation may also exploit certain issues in medical sciences such as therapeutic, diagnostic and investigative uses of trace metals [2].

Variety conformations, excited states, or transient forms of an element, its coordinated atoms, and the molecules of which they are part 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 [1,3].

Complexes are formed by some of the essential elements such as cobalt, nickel, copper, zinc, calcium, magnesium and heavier elements like arsenic, selenium, cadmium, indium, antimony, tellurium, mercury, thallium, lead, and bismuth. These elements and their complexes are mobilized and transported in air, surface waters, in sediments and in the soil, thereby significantly perturbing the environment. Moreover, their intakes by humans via food, water, and particulates in the air have caused health concerns [4].

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 [5]. Importantly, elucidation and understanding of the various phenomena in the biological systems requires the determination of the protonation constants of the bio-ligands along with their stability constants with various metal ions in media similar to those of biological systems [6,7].

L-phenylalanine (Phe) is non-polar α-amino acid because of the hydrophobic nature of the benzyl side chain with the formula $C_6H_5CH_2CH$ (NH_2) COOH. This is essential amino acid, one of the twenty common amino acids used to form proteins biochemically. It is converted to cinnamic acid by the action of enzyme phenylalanine ammonia-lyase [8].

Maleic acid (MA) is industrially derived by hydrolysis of maleic anhydride; maleic anhydride is produced by oxidation of benzene or butane. It is an industrial raw material for the production glyoxylic acid by ozonolysis. Maleic acid and fumaric acid do not spontaneously interconvert because rotation around a carbon-carbon double bond is not energetically favorable. However, conversion of the cis isomer into the trans-isomer is possible by photolysis in the presence of a small amount of bromine [9].

N,N'-Dimethylformamide (DMF) is a polar (hydrophilic) aprotic solvent with high boiling point. It facilitates reactions that follow polar mechanisms. It is able to form the H-bonded network structures with dipolar aprotic solvents [10]. Therefore, DMF-water mixture was selected as media to maintain the dielectric properties of the medium in comparable levels to those of the physiological fluids since the polarity at the active site cavities should generally

be applicable when it is possible to compare ligand binding to the metal ion as in protein residues and solvent environment [11].

In this study, speciation of binary complexes of Co(II), Ni(II) and Cu(II) with L-phenylalanine and maleic acid in aqueous and DMF-water mixtures was investigated.

1.2. Statement of the Problem

In all living systems, the biochemical functions of both essential and toxic metals are mediated through specific chemical species or complexes, and their concentrations are important for the biochemical reactions but not just the total concentration of the metal in the system [12]. 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 [13]. The bioavailability and toxicity of the complexes are critically dependent on their thermodynamic and kinetic stability. Identifying these species (i.e., speciation) and obtaining reasonable estimates of their thermodynamic and kinetic stabilities are the problems that continue to plague environmentalists and toxicologists [4]. Hence, extensive attention has been paid in recent years to the study of the chemical speciation of ligands with metal ions.

The elucidation and pathways of proteins are always fascinating, as these macromolecules form the vital ingredients in almost all parts of the body and thus maintain its proper functioning. Most of the metabolic reactions are catalyzed by metalloenzymes or metal-activated enzymes. The activity of these enzymes is believed to be due to the metal-enzyme-substrate complexes. The active site of metalloenzymes in hydrophobic environment has low polarity compared to the bio-fluids [14].

Most of the speciation studies are performed in aqueous medium under the conditions comparable to those existing in the biological systems. These are taken as models for the systems existing in bio-fluid and natural water [15]. The dielectric constant of the medium decreases with increasing concentration of the co-solvent. Hence, Dimethylformamide-water mixtures are chosen to study the acido-basic and complexation equilibria to mimic the physiological conditions where the concept of equivalent solution dielectric constant for protein cavities is applicable [16].

So in the present study, organic solvent (DMF) is chosen to mimic the permittivity of the biological fluids and L-phenylalanine (Phe) and maleic acid (MA) are chosen as model compounds to proteins and substrates.

1.3. Objectives of the Study

1.3.1. General Objective

The overall objective of this study is to investigate the protonation equilibria of L-phenylalanine and maleic acid and their binary complexes with biological essential metal ions in Dimethylformamide-water mixtures of varying compositions.

1.3.2. Specific Objectives

The specific objectives of the study were to:

i. Identify the protonation equilibria of the L-phenylalanine and maleic acid in aqueous and DMF-water mixtures.
ii. Investigate the effect of dielectric constant of solvent on protonation of Phe and MA.
iii. Identify the species distribution of L-phenylalanine and maleic acid in aqueous and DMF-water mixtures.
iv. Determine the binary stability constant of the complexes involving essential metal ions Co(II), Ni(II) and Cu(II) and ligands in vitro under mimicked physiological conditions.
v. Investigate the effect of dielectric constant on the complex equilibria of Phe and MA with the metal ions, Co(II), Ni(II) and Cu(II).
vi. Identify the species distribution of complexes of Cu(II), Ni(II) and Co(II) ions with L-phenylalanine and maleic acid in Dimethylformamide- water mixtures.
vii. Propose the structure of the complexes of Cu(II), Ni(II) and Co(II) ions with L-phenylalanine and maleic acid in Dimethylformamide- water mixtures.
viii. Refine the binary species for Phe and MA complexes of Cu(II), Co(II) and Ni(II).

1.4. Significance of the Study

Stability constants of the complex formation of biologically active ligands with metal ions in addition to concentration of each species at any pH are very important for the whole understanding of the physiochemical manner of such compounds require determination of their protonation constants [17]. Speciation study of essential metal ion complexes with amino acids is useful to understand (i) the role played by the active site cavities in biological molecules, (ii) the type of complex formed by the metal ion and (iii) the bonding behavior of the protein residues with the metal ion. The species refined and their relative concentrations under the present experimental conditions represent the possible forms of amino acids and their metal complexes in the bio-fluids [18]. Therefore, in recent years, researchers have shown interest in the study of metal ions interaction with various ligands under the presented experimental condition and to validate the models by statistical treatment of the data.

CHAPTER-2

LITERATURE REVIEW

2. LITERATURE REVIEW

2.1. Biological Functions of Amino Acids

Amino acids 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. The twenty standard amino acids, with a variety of carbon skeletons going into the composition of proteins, have twenty different pathways for degradation. All these pathways taken together, in human beings, account for 10-15% of the body's energy production [19].

2.2. Phenylalanine

Phenylalanine is an α-amino acid with the formula $C_6H_5CH_2CH(NH_2)COOH$. Its molecular mass is $165.19 gmol^{-1}$. It is an electrically neutral amino acid one of the twenty common amino acid used to biochemically form proteins, coded for by DNA (Deoxyribonucleic acid). It is found naturally in the breast milk of mammals. It is used in the manufacture of food and drink products and sold as nutritional supplement for its reputed analgesic and antidepressant effects. It is a direct precursor to the neuromodulator phenyl ethylamine a commonly used dietary supplement [20].

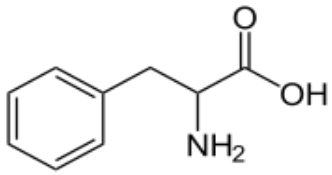


Fig 2.: Structure of phenylalanine

L-phenylalanine is biologically converted into L-tyrosine, one of the DNA-encode amino acids. L-tyrosine in turn is converted into L-DOPA which is further into dopamine, norpinephrine (noradrenaline), and epinephrine (adrenaline). The latter three are known as the catecholamines [20].

The genetic disorder phenylketonuria (PKU) is the inability to metabolize phenylalanine. Individuals with this disorder are known as "phenylketonurics" and must regulate their intake of phenylalanine.

Phenylalanine uses the same active transport channel as tryptophan to cross the blood-brain barrier and, in large quantities, interferes with the production of serotonin. Individuals who cannot metabolize phenylalanine must monitor their intake of protein to control the buildup of phenylalanine as their bodies convert protein into its component amino acids [21].

2.3. Maleic Acid (MA)

Maleic Acid (MA) is an organic compound that is a dicarboxylic acid, a molecule with two carboxyl groups. Its chemical formula is $HO_2CCH=CHCO_2H$. It is used to make artificial resins and anti-histamines, and to preserve fats and oils. Maleic acid is the cis-isomer of butene dioic 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, maleic acid has few applications [22].

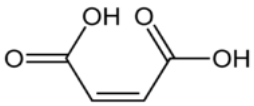


Fig 2.2: Structure of maleic acid

The major industrial use of maleic acid is its conversion to fumaric acid. This conversion, an isomerization, is catalyzed by a variety of reagents, such as mineral acids and thiourea. Maleic acid is a less stable molecule than fumaric acid. It is more soluble in water, than fumaric acid. The melting point of maleic acid (139–140°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 [22].

2.4. Trace Elements

Many mineral elements occur in plant and animal tissues in small quantities. A trace element is considered as essential for both man and animal life, if it meets the listed conditions: i) it is present in all healthy tissues, ii) its concentration from one species to the next is fairly constant, iii) depending on the species studied, the amount of each element has to be maintained within its required limit if the functional and structural integrity of the tissues is to be safeguarded and the growth, health and fertility to remain unimpaired, iv)

its withdrawal induces reproducibly the same physiological and or structural abnormalities and v) its addition to the diet either prevents or reverses the abnormalities.

Several trace elements are known to fulfill these criteria of which the most well-known are iron, zinc, manganese, chromium, copper, cobalt, nickel, molybdenum and iodine. Many of them act as catalysts in many enzymatic functions called as metalloenzymes [23].

2.4.1. Cobalt

The occurrence of cobalt in the earth crust is about 25ppm. The dietary intake of cobalt per day is 40-50µg. Concentration of cobalt in human blood is 1.043ppm. Cobalt is an essential element for life in minute amounts (10 mg/day), at higher levels of exposure it shows mutagenic and carcinogenic effects. It can be toxic to humans because it is not regulated at the point of absorption. Cobalt has toxic action on steroid producing cells in the adrenal gland in the testis. Excess cobalt can cause polycythemia (increased red blood cells), bone marrow hyperplasia, pancreatic failure or congestive heart failure. At large doses it also interferes with the absorption of iron [24]. Minot and Murphy discovered that pernicious anemia can be treated by feeding the patient with large amounts of liver which contains vitamin B_{12}. Trace amounts of vitamin B_{12} are essential for the synthesis of hemoglobin. Its deficiency causes anemia, lipid accumulation and lipid peroxidation [25].

2.4.2. Nickel

Nickel plays numerous roles in the biology of microorganisms and plants [26, 27]. Urease, an enzyme which assists in the hydrolysis of urea, contains nickel. The NiFe-hydrogenases contain nickel in addition to iron-sulfur clusters. The nickel center appears to undergo changes in oxidation state and evidence has been presented that the nickel center might be the active site of these enzymes [28]. Other nickel-containing enzymes include a class of superoxide dismutase and a glyoxalase [29]. There is no nickel requirement or allowance set for humans. However, because it is essential for several animal species, it seems reasonable to accept that it is required by humans. The dietary intakes of nickel in humans range between 60 and 260µg/day. Less than 10% of ingested nickel is normally absorbed by gastro intestinal tract. Unabsorbed nickel is excreted through feaces. The plasma nickel is excreted through urine which is complexed with histidine and aspartic acid [30].

Deficiency of nickel causes cellular level structures became disorganized and membrane properties changed, abnormal bone growth, poor absorption of ferric iron and it disrupts nitrogen metabolism. Excess intake of nickel and accumulation of nickel results in interference with normal protein-metal binding and catalysis, as well as regeneration of reactive oxygen species [31].

Toxicity has occurred in workers exposed to nickel dust or nickel carbonyl fumes in refining. Excessive nickel in tissue is pro-oxidant (damaging chromosomes and other cell components) and alters hormone and enzyme activities. Under some conditions, large amounts of nickel may precipitate magnesium deficiency or cause of accumulation of iron or zinc [32].

2.4.3. Copper

Copper is an essential element for life on earth. It is one of the transition elements found at the active site of proteins. Human body requires 2-3 mg/day copper (30 µg/kg body-weights according to WHO) through diet for healthy growth [33]. Copper is distributed widely in the body and occurs in liver, muscle and bone. It is transported in the blood stream a plasma protein called ceruloplasmin. When copper is first absorbed in the gut it is transported to the liver bound to albumin. Copper metabolism and excretion is controlled delivery of copper to the liver by ceruloplasmin, where it is excreted in bile. The recommended dietary allowance for copper in normal healthy adults is 0.9 mg/day [34]. Because of its role in facilitating iron uptake, copper deficiency can often produce anemia like symptoms, a decrease in certain white blood cells, loss of hair color and skin pallor.

Children with copper deficiencies may experience ruptured blood vessels, central nervous system abnormalities, growth retardation, and poor temperature regulation. Toxicity excessive doses of copper can cause diarrhea, epigastric pain and discomfort blood in the urine, liver damage, low blood pressure and vomiting. In addition to its enzymatic roles, copper is used for biological electron transport [35].

2.5. Solvent

Organic solvent-water binary mixtures are powerful solvent systems frequently used [36], because their chemical properties such as ionization power and hydrophobicity can be readily controlled by simply changing the mixing ratios. In these aqueous solvent mixtures, chemical reactions turn out to be greatly influenced by the solvent effect [37].

2.5.1. N,N-Dimethylformamide

Dimethylformamide is an organic compound with the formula $(CH_3)_2NC(O)H$ commonly abbreviated as DMF (although this initialism is sometimes used for dimethyl furan, or dimethyl fumarate), this colorless liquid is miscible with water and the majority of organic liquids [38].

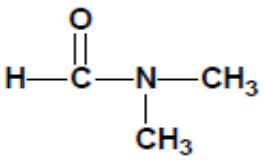


Fig 2.: Structure of N, N'-Dimethylformamide

It is prepared by combining methyl formate and dimethylamine or reaction of dimethylamine with carbon monoxide [39]. It is a common solvent for chemical reactions. As its name indicates, it is a derivative of formamide, the amide of formic acid. The primary use of DMF is as a solvent with low evaporation rate. It is also used as a solvent in peptide coupling for pharmaceuticals, in the development and production of pesticides, and in the manufacture of adhesives, synthetic leathers, fibers, films, and surface coatings. As a common and cheap reagent, it has many uses in the research laboratory [40].

2.6. Chemical Speciation

Chemical speciation refers to the distribution of an element amongst chemical species in a system. It is critical for understanding chemical toxicity, bioavailability and environmental fate and transport [41,]. 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 [42].

2.6.1. Chemical Speciation in Biological Systems

Of the 92 naturally occurring elements, about 30 are thought to be essential to animal or plant life at either bulk or trace levels [43]. The 30 elements believed to be essential to life forms can be divided into four major categories: (1) bulk elements (H, C, N, O, P, S); (2) macro minerals and ions (Na, K, Mg, Ca, Cl, PO_4^{3-}, SO_4^{2-}); (3) trace elements (Fe, Zn, Cu); and (4) ultra-trace elements (F, I, Se, Si, As, B, Mn, Mo, Co, Cr, V, Ni, Cd, Sn, Pb, Li).

A high degree of chemical speciation can exist for any trace element in biological systems. Due to the predominantly aqueous nature of biological systems biochemically active forms of all metallic elements tend to be ionic, these elements being complexed by molecules possessing electron-donating functional groups. Metal ions in biological systems tend to be distributed between four different states. First, there is the inert (non-labile) form of the metal complex which describes those molecules in which the metal ion is integrated into a molecular function, e.g. metal-activated enzymes or storage proteins. The labile protein-bound fraction is used to denote metal ions complexed to large molecules whose primary function is metal ion transport. In the majority of situations, the free aquated metal ion, $[M(H_2O)x]n^+$, is present at vanishingly low concentrations. The last two complexes and the free aquated metal ions are in equilibrium such that a rise in metal ion concentration present in the biological fluid will bring about across-the board increase in labile protein, low molecular mass and aquated metal ion concentrations [44].

2.6.2. Protonation Constants of L-phenylalanine and Maleic acid

Protonation constants of amino acids can be determined by analysis of acid-base titrations. The methods have been critically reviewed [45]. The protonation constants of Phe and MA are reported in the literature are given in Table 2.1 and 2.2 respectively. There is a considerable difference in the values of protonation constants reported under similar conditions. Hence, the author has investigated the protonation equilibria of Phe and MA in DMF-water media and the effect of mole fraction of the medium on protonation constant values.

Table 2.1: Protonation constants of L-phenylalanine reported in the literature.

S.No.	Log β		Ionic Strength(M)	Temperature (^oC)	Reference
	011	012			
1.	2.32	9.05	0.5(KNO_3)	25	46
2.	2.26	9.19	0.1(KNO_3)	25	47
3.	2.39	9.25	0.1(KNO_3)	20	48
	2.37	9.03	0.1(KNO_3)	30	48
4.	2.09	9.20	0.05(KCl)	20	49

Table 2.2: Protonation constants of maleic acid reported in the literature.

S.No.	Log β		Instrumentation	Medium	Reference
	011	012			
1.	1.83	5.44	pH metry	0.01M CTAB	50
2.	1.80	5.97	,,	0.01M SLS	50
3.	1.86	6.24	,,	0.01M TX100	50
4.	1.83	5.54	Spectrophotometry	0.01M CTAB	50
5.	1.83	5.98	,,	0.01M SLS	50
6.	1.85	6.22	,,	0.01M TX100	50

2.6.3. Determination of Binary Stability Constants of L-phenylalanine and Maleic acid

Stability constants are well known tools for solution chemists, biochemists and chemists in general to determine the properties of metal-ligand reactions in water and biological systems. They have important medicinal implication to measure the metal ligand selectivity in terms of relative strength of metal-ligand bonds [51, 52]. Metal coordination complexes have been extensively used in clinical applications as enzyme inhibitors [53]], anti-bacterial [54] antiviral [55] and as anti-cancer [56] drugs. Transition metal ion chelate complexes are also exploited by industry in the large-scale purification of amino acids and a wide range of drug and drug precursors containing an amino carboxylic acid moiety [57].

Several literature reports have been devoted to the study of stability constants of metal- Phe and metal-MA complexes. Bychkova et al [58,59] carried out potentiometric studies on the complex formation of MA with transition metals such as Zn(II), Ni(II), Co(II) and Cu(II). Stability constants of the complexes formed by MA with Cu(II) has been studied potentiometrically by M. Grasso et al [60] , G.Venkatnarayana et al [61] and H.Azab.[63]. Potentiometric study on Ni(II) with MA has been carried out by M.Yasada et al.[64]. The stability constants of Cu(II)- Phe complexes were studied by J.Koska, et al.[66] using pH metry and by A.Siham Lahsasni, et al.[67]. The stability constants of Co(II), Ni(II) and Cu(II) with MA were determined by A.Gergely, et al.[68].

The equilibrium studies involving various metals and Asp were not exhaustive and many of these studies were performed in limited concentration ranges of the ingredients.

Further, there are some differences in the nature of the reported species. There are several instances where in different authors reported different species for the same system under comparable conditions. Probably this may be attributed to the nature of probes, the errors associated with the probing techniques or the inadequacies associated with the modeling strategies [68,69].

The stability constants of metal-MA and metal- Phe complexes with different electrolytic background at 25^0C reported in literature are recorded in Table 2.3 and 2.4 respectively.

Hence, the author has investigated the complex equilibria of MA and Phe with Co(II), Ni(II), and Cu(II) in DMF-water media. This type of study throws light on 1) the effect of electrostatic and non-electrostatic interactions (denaturation) 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.

Table 2.3: Stability constants of metal-maleic acid complexes reported in literature.

Metal	Log β_{mlh}		Ionic strength mol dm^{-3}	Instrumental method	Ref.
	ML	ML_2			
Zn(II)	2.48	-	0.1	Potentiometry	58
Ni(II)	2.78	4.62	0.1	,,	58
	2.00	-	0.1	,,	63
	6.08	-	1.0	pH-metry	65
Co(II)	2.10	-	0.1	Potentiometry	58
Cu(II)	3.91	6.31	0.1	,,	58
	3.42	5.1	0.1	,,	60
	4.02	6.84	0.1	,,	61
	4.69	-	0.1	,,	62
	4.89	-	1.0	pH-metry	65
Mg(II)	1.79	-	0.1	,,	59
Ba(II)	1.94	-	0.1	,,	59
	2.35	-	0.0	Spectrophotometry	64
Mn(II)	6.39	-	1.0	pH-metry	65

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

Metal	Log β_{mlh}		Ionic strength mol dm^{-3}	Instrumental method	Ref.
	ML	ML_2			
Cu(II)	7.76	14.52	0.1	pH-metry	66
	8.16	16.44	0.1	Potentiometry	67
	7.78	14.77	0.05	-	68
	8.18	15.18	0.05	-	69
Co(II)	4.05	7.56	0.05	-	68
	4.03	7..47	0..05	-	69
Ni(II)	5.15	9.46	0.05	-	68
	5.11	9.43	0.05	-	69

CHAPTER-3

MATERIALS AND METHODS

3. MATERAILS AND METHODS

3.1. Materials and Apparatus

Test tubes, 25mL volumetric pipette, pH meter, burette, glass electrode, funnel, beakers, 125mL or 250mL Erlenmeyer flasks, mass balance, measuring cylinder, dropper and stirrers.

3.2. Chemicals and Reagents

All the chemicals used in this investigation were of analytical reagents grade purity. L-phenylalanine, maleic acid, HCl, HNO_3, methanol, acetone or diethyl ether, triple distilled water, NaOH pellet, hexamethylenetetramine powder, oxalic acid, potassium hydrogen phthalate, borax, DMF, EDTA, acetic acid, 2-amono ethanol, $ZnSO_4$,$CoCl_2$, $CuCl_2$, $NiCl_2$, NaCl, Eriochrome-black.T as indicators, xylenol orange, murexide and sulphone black-F as indicator.

3.3. Experimental Procedure

3.3.1. Preparation of Solutions

All solutions were prepared in boiled out triple 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 (GR grade, E-Merck, Germany) and maleic acid (GR grade, E-Merck, Germany) were prepared by dissolving samples in water. To increase the solubility of ligands, 0.05 mol dm^{-3} hydrochloric 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 [70]. The pessimistic errors in the preparation of the ligand solutions by weight method did not exceed 0.1%.

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 0.1 mol dm^{-3} solution was prepared and standardized complexmetrically with a 0.1mol 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 cobalt(II), nickel(II) and copper(II) chlorides were prepared by dissolving GR grade (E-Merck, India) salts in triple distilled water. The stock solutions were rendered slightly acidic to repress hydrolysis of the metal ions. The concentrations of the metal ions were determined complexometrically by titrating against a standard solution of EDTA using the xylenol orange, murexide and fast sulphon black-F as indicators and hexamethylenetetramine powder as buffer for cobalt to maintain the pH at 5-6. The free hydrogen ion concentration in the stock solution was determined by Gran plot method [71].

3.3.1.4. Sodium Hydroxide

A stock solution of 1 mol dm^{-3} sodium hydroxide was prepared by dissolving GR grade (E-Merck, India) sodium hydroxide pellets in triple distilled water. The solution was further diluted to the required concentration. The strength of the alkali was determined by titrating it against a standard solution of oxalic acid and potassium hydrogen phthalate, while the molarity of hydrochloric acid was determined with sodium hydroxide. So as to assess the errors that might have crept into the determination of the concentrations, the data were subjected to analysis of variance of one way classification (ANOVA) using the computer program COST(concentration of solution by titration). The strength of alkali was determined using the Gran plot method. The errors in the concentration of ligand, metal ions and alkali were subjected to analysis of variance ANOVA [72].

3.3.1.5. Hydrochloric Acid

The concentration of stock hydrochloric acid (GR grade, Merck, India) solution was calculated from its specifications (specific gravity, purity and molecular weight) and diluted to the required concentration by dissolving in triple distilled water. Its strength was determined by titrating with standard sodium hydroxide solution.

3.4. Solvent

GR grade N,N'-Dimethylformamide (Finar, India) of 99.5% purity was used as received.

3.5. 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. The pH metric titration assembly consisted of a double walled spout less Pyrex glass vessel of 100cm^3 capacity fitted with a Perspex lid through which the glass combination pH electrode and burette tip were admitted.

3.5.1. Calibration of Glass Combination pH Electrode

Calibration of glass electrode by buffer solution is still in practice. But 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 (pH=4.01) and basic (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 [73].

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

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

A typical Gran plot is given in Fig. 3.1

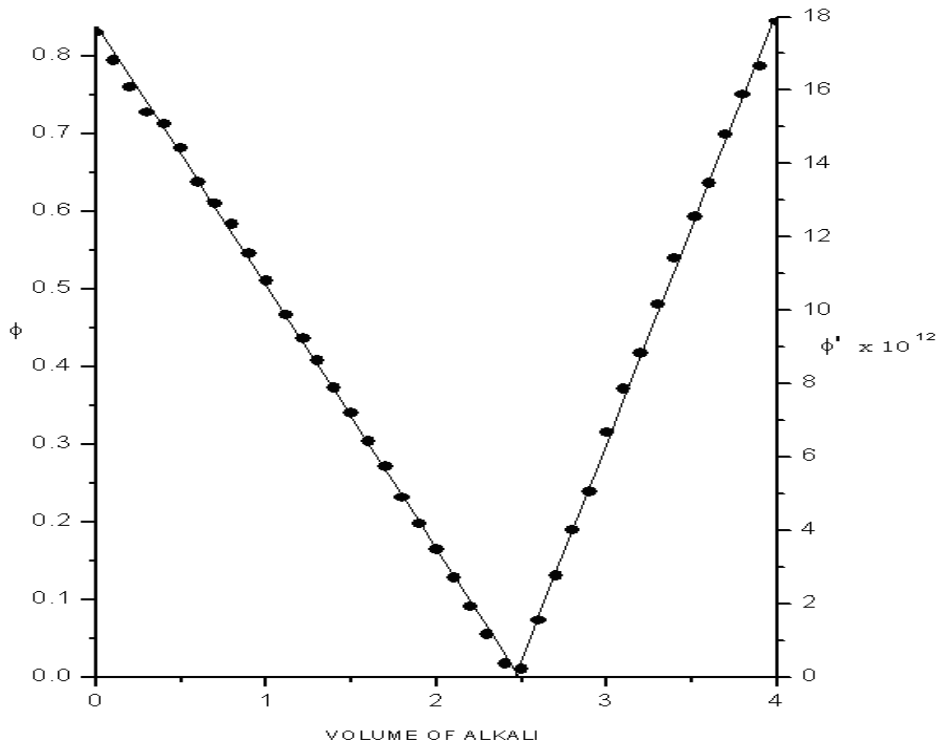


Fig 3.1: Gran plot for the titration of hydrochloric acid with alkali.

3.5.2. Equilibration of Electrode in Organic Solvent Mixtures

In the present study the glass combination pH electrode was immersed in a well stirred mixture of required composition of DMF-water (0-50% v/v) mixture for two days, before use according to the general procedure of electrode calibration and specific procedures provided with electrode. 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. The earlier experience in this laboratory showed that equilibrating the electrode from higher percentage to lower one was rapid and reproducible results were obtained.

Prior to each titration, 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.

3.5.3. 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 and others [74] to account for the above parameters was used in the present electrometric titrations. The simulated acid-base titrations data (pHC_i) calculated by SCPHD program was used to compute correction factor (log F) for each of the solvent compositions [75]. The log F value is used to convert pH meter dial reading into logarithm of reciprocal of hydrogen ion concentration (pHE_i) according to the equation.

$$\text{Log F} = pHC_i - pHE_i$$

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. From the volume of alkali added, the values of pOH and subsequently the value of pK_w were calculated.

3.5.4. Titration Procedure

The titrations were carried out in media containing varying amounts (0-50% v/v) of DMF maintaining an ionic strength (μ) of 0.16 mol L^{-1} with NaCl at 303.0 K. 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 mL. Titrations were performed by adding each time 0.1mL portions of 0.4 mol L^{-1} sodium hydroxide to the titrand. The pH meter reading was recorded only after a constant value was displayed [76]. Typical duplicate titrations showed that equilibration was fast and titration data did not differ by more than 0.02 units.

3.5.5. Proton-ligand Equilibria

Requisite amount of sodium chloride solution (to give overall concentration of 0.16 mol L^{-1}) water and DMF were added such that the overall percentages of media were in the range of (0-50% v/v). The amount of mineral acid maintained was 1 mmol, while the concentration of Phe and MA were of the order of 1:2.5, 1:3.75 and 1:5.0 mmol in different experiments. The initial concentrations of the ingredients used in the experiments are summarized in Table 3.1.

Table 3.: Total initial concentrations of ingredients (in mmol) in proton-ligand titrations in DMF-water mixtures.

[NaOH] = 0.4 mol L^{-1}; V_0 = 50 mL; Temperature = 303.0 K;

Mineral acid = 1.0 mmol; μ = 0.16 mol L^{-1}. TL0 = Total initial concentration of primary ligand.TX0 = Total initial concentration of secondary ligand.

% v/v DMF	No. of Titration curves	TL0 of Phe in DMF	TX0 of MA in DMF
0.0	3	0.25501 0.3750 0.5015	0.2506 0.3756 0.5035
10.0	3	0.2491 0.3743 0.4903	0.2475 0.3761 0.4951
20.0	3	0.2491 0.3743 0.4975	0.2556 0.3766 0.5191
30.0	3	0.2491 0.3633 0.4902	0.2452 0.3676 0.4965
40.0	3	0.2493 0.3741 0.4981	0.2506 0.3727 0.5031
50.0	3	0.2492 0.3736 0.4973	0.2507 0.3743 0.5199

3.5.6. Metal-ligand Equilibria

In each of the titrations, the titrand consisted of approximately 1mmol hydrochloric acid in a total volume of 50 mL and the ionic strength was adjusted to 0.16 mol L^{-1} with sodium chloride. Solutions containing metal ions and ligands (metal to ligand ratios being in the range of 1:2.5, 1:3.75 and 1:5.0) were titrated with 0.4 mol L^{-1} sodium hydroxide. Metal ion was the last component added to the titrand in all these titrations.

The actual concentrations of the constituents in different percentages of the DMF are given in Tables 3.2 and 3.3. In order to minimize local buildup 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 chosen 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. The conditional stability constants of binary species of metal complexes with Phe and MA were calculated from alkalimetric titrations obtained in the presence of different ratios of initial concentration of metal to ligand.

Table 3.: Total initial concentrations of ingredients (in mmol) for metal- Phe titrations in DMF-water mixtures

[NaOH] = 0.4 mol L^{-1}; V_0 = 50 mL; Temperature = 303.0 K;

Mineral acid = 1.0 mmol; μ = 0.16 mol L^{-1}.

DMF % v/v	Nº. of Titration curves	TM0			TL0	TL0/TM0
		Co(II)	Ni(II)	Cu(II)		
0.0	3	0.102	0.097	0.101	0.2503	2.50
					0.3743	3.75
					0.5004	5.00
10.0	3	0.102	0.097	0.101	0.2515	2.50
					0.3764	3.75
					0.5013	5.00
20.0	3	0.102	0.097	0.101	0.2516	2.50
					0.3772	3.75
30.0	3	0.102	0.097	0.101	0.2491	2.50
					0.3783	3.75
					0.4991	5.00
40.0	3	0.102	0.097	0.101	0.2495	2.50
					0.3761	3.75
					0.4993	5.00
50.0	3	0.102	0.097	0.101	0.2491	2.50
					0.3761	3.75
					0.4993	5.00

Table 3.: Total initial concentrations of ingredients (in mmol) for metal-MA titrations in DMF-water medium

[NaOH] = 0.4 mol dm^{-3}; V_0 = 50 cm^3; Temperature = 303 K

Mineral acid = 1.0 mmol; μ = 0.16 mol dm^{-3}.

DMF % v/v	No. of Titration curves	TM0			TX0	TX0/TM0
		Co(II)	Ni(II)	Cu(II)		
0.0	3	0.102	0.097	0.101	0.2493 0.3761 0.4983	2.50 3.75 5.00
10.0	3	0.102	0.097	0.101	0.2512 0.3761 0.5009	2.50 3.75 5.00
20.0	3	0.102	0.097	0.101	0.2491 0.3761 0.4993	2.50 3.75 5.00
30.0	3	0.102	0.097	0.101	0.2494 0.3761 0.4993	2.50 3.75 5.00
40.0	3	0.102	0.097	0.101	0.2501 0.3761 0.5011	2.50 3.75 5.00
50.0	3	0.102	0.097	0.101	0.2512 0.3736 0.5007	2.50 3.75 5.00

3.6. Processing of Data and Modeling Strategy

3.6.1. Software for Modeling Complex Equilibria

Glass electrode measures protons associated with the solvent (including those in looser combinations). The appropriate protonation constants of Phe and MA were calculated with computer program SCPHD. The binary stability constants were calculated from pH metric titration data using the computer program MINIQUAD75. The best fit chemical model for each system was investigated by using non-linear least squares computer program MINIQUAD75. The chemical speciation, metal bioavailability and transportation were explained based on the distribution diagrams drawn using HYSS HYPERQUAD [77].

3.6.2. Formulation of Mathematical Model

For simplicity of mathematical treatment, the interaction of a metal ion with a ligand (LH_h) is considered and the same treatment can be extended to other species encountered in complex equilibria of mononuclear complexes summarized in Table 3.4.

Table 3.: Types of species likely to be encountered in complex equilibria of mononuclear.

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

m, l, x, h: stoichiometric coefficients of metal, primary ligand, secondary 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 j^{th} species is represented by Eq. 3.1.

$$m(j)\,M + l(j)L + h(j)H \rightleftharpoons M_{m(j)}L_{l(j)}H_{h(j)} \quad \text{……..3.1}$$

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

$$\beta_{m(j)l(j)h(j)} = \frac{M_{m(j)}L_{l(j)}H_{h(j)}}{FM_i^{m(j)} * FL_i^{l(j)} * FH_i^{h(j)}}$$

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

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

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

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

$$TMC_i = FM_i + \sum_{j=1}^{NB} m(j) * \beta_m(j)l(j)h(j) * FM_i^{m(j)} * FL_i^{l(j)} * FH_i^{h(j)}$$

$$= FM_i + \sum_{j=1}^{NB} m(j) * CS_{j,i} \quad \text{..... ..... 3.4}$$

Similarly for ligand and hydrogen ion, the corresponding equations are

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

If the free concentrations of all the three ingredients (ligand, metal and hydrogen ion) are monitored, it is sufficient to consider NB points. Solving of simultaneous linear equations 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 [78]. In many cases the determination of metal ion and free ligand

concentration becomes difficult. Then hydrogen ion concentration is the only parameter that can be monitored and hence, non-linear mass-balance equations have to be solved. A large number of computer programs are available ranging in application from calibration of glass electrode to modeling strategies applicable to quaternary complexes containing protonated, hydroxylated and poly nuclear species. All the programs adopt this mathematical model for the above tasks [79].

3.6.3. 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.

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

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

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.

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

Where, $\beta_{011} = K_1^H$ and $\beta_{012} = K_1^H * K_2^H$ and FH_i is the free hydrogen ion concentration. One of the several algebraic transformations useful for the determination of equilibrium constants is

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

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

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

The computer program, SCPHD [80] 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 auto ionization of water, respectively. From this secondary data the stepwise stability constants are calculated from Eq. 3.11.

The output of SCPHD in the present investigation was used (i) to prune the data in different experiments so that it contains more information than noise and (ii) to utilize log β_{011}, log β_{012} etc. as approximate (or initial) values for the refinement.

Similar to $\bar{n}_H$ for proton-ligand complexes, secondary formation function $\bar{n}_i$ (average number of ligands bound per mole of metal ion) can be calculated by Eq. 3.13.

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

Eq. 3.13 can be transformed into Eq. 3.14

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

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.

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

This algorithm was used in SCPHD to calculate the paired data ($\bar{n}_i$ Vs PL_i). Some thumb rules [81] about formation function are given in table 3.5.

Table.3.5: Knowledge base of formation functions

If	Formation curves are overlapping for different ligand concentrations
Then	Protonated/hydroxylated/mixed 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

4. RESULTS AND DISCUSSIONS

SECTION-I

4.1. Protonation Equilibria of L-phenylalanine and Maleic Acid

L-Phenylalanine (Phe) actively participates in acido-basic equilibria especially in physiological pH conditions. Phe has two functional groups and one is protonated, amino group followed by one carboxylic group. Hence, phenylalanine has bidentate behavior resulting in five membered ring structures. Nitrogen donor atoms can associate with hydrogen ions in physiological pH ranges [59].

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. So, an understanding of the protonation-deprotonation equilibria of Phe and MA is necessary before an attempt is made to investigate the metal-ligand equilibria associated with Phe and MA [58].

4.1.1. Preprocessing of Data

The alkalimetric titration curves of maleic acid and L-phenylalanine 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 2.0-11.0. The computer program SCPHD [80] was used to prone the data obtained in different experiments so that it contains more information than noise and the logβs given by the program are used as initial values for final refinement.

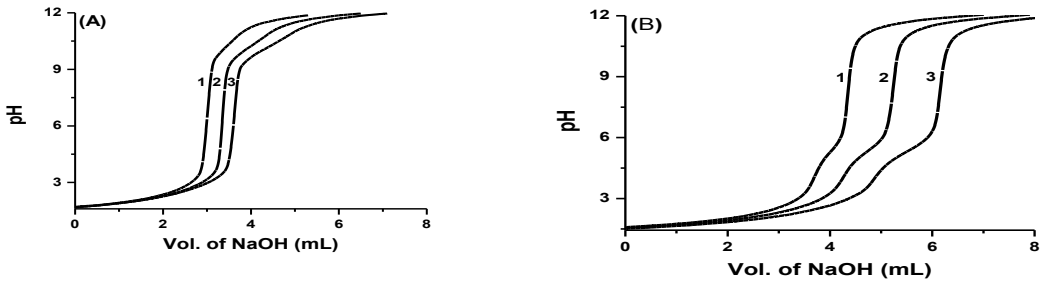


Fig 4.1: Alkalimetric titration curves for (A) Phe (B) MA in aqueous medium;.
Amount of ligands: (1) 0.25, (2) 0.375 and (3) 0.50 mmol.

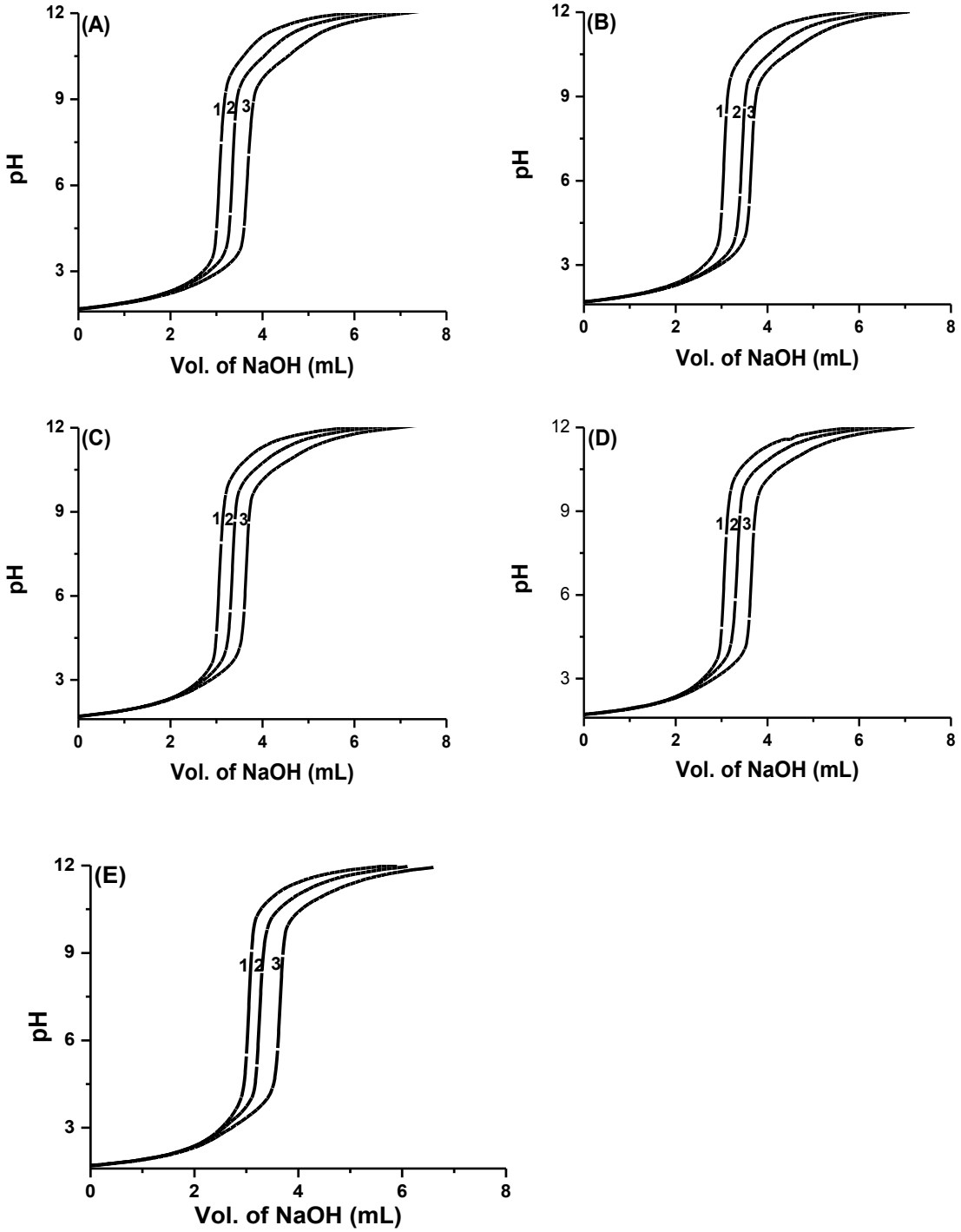


Fig 4.2: Alkalimetric titration curves for Phe in DMF-water mixtures % v/v.

A) 10.0 (B) 20.0 (C) 30.0 (D) 40.0 (E) 50.0 Amount of ligands: (1) 0.25, (2) 0.375 and (3) 0.50 mmol.

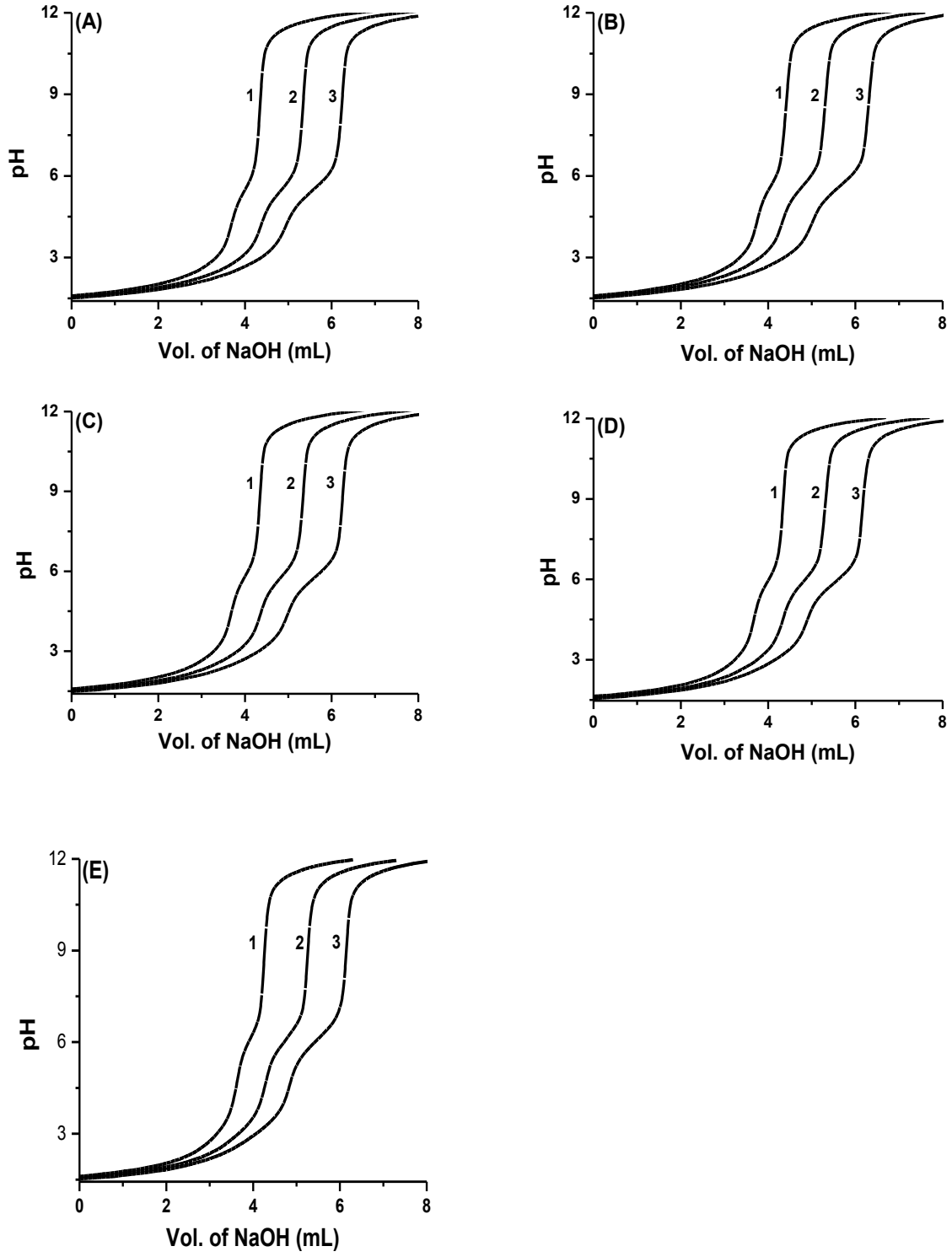


Fig 4.3: Alkalimetric titration curves for MA in DMF-water mixtures % v/v.
(A) 10.0 (B) 20.0 (C) 30.0 (D) 40.0 (E) 50.0 Amount of ligands: (1) 0.25, (2) 0.375 and (3) 0.50 mmol.

The formation function, $\overline{n}_H$ (average number of moles of protons bound per mole of ligand) is a useful parameter for the detection of polymeric species (L_2H_x). If there are no polymeric species a plot of $\overline{n}_H$ versus pH should overlap. Any deviation indicates the presence of polymeric species. Fig 4.5A and B shows that there is no polymerization of Phe and MA.

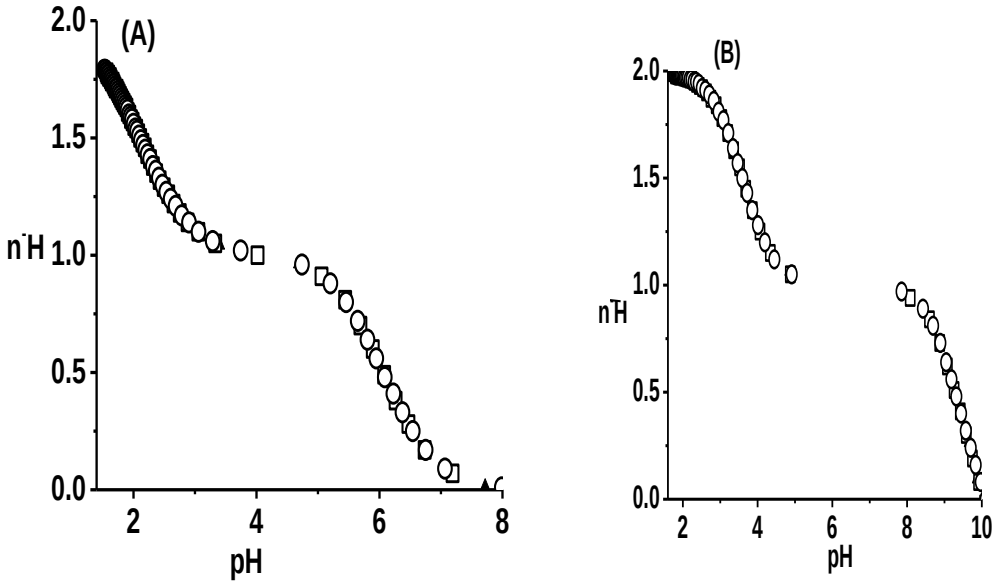


Fig 4.4: formation curves of (A) MA and (B) Phe in 20% v/v DMF- water medium for three amounts (0.25 (□), 0.375(○) and 0.5(Δ) mmols.)

The number of moles of alkali consumed per mole of the ligand denoted by **a**, is another secondary function like $\overline{n}_H$. A curve of **a** versus pH gives the number of equivalents of alkali consumed per mole of ligand. The negative values of **a** correspond to the excess of mineral acid present in the titrand and the number of associable protons, whereas the positive values correspond to the dissociable protons. The maximum value of **a** is 2 for MA and 1 for Phe which indicates that maleic acid has two dissociable protons and phenylalanine has 1 dissociable proton.

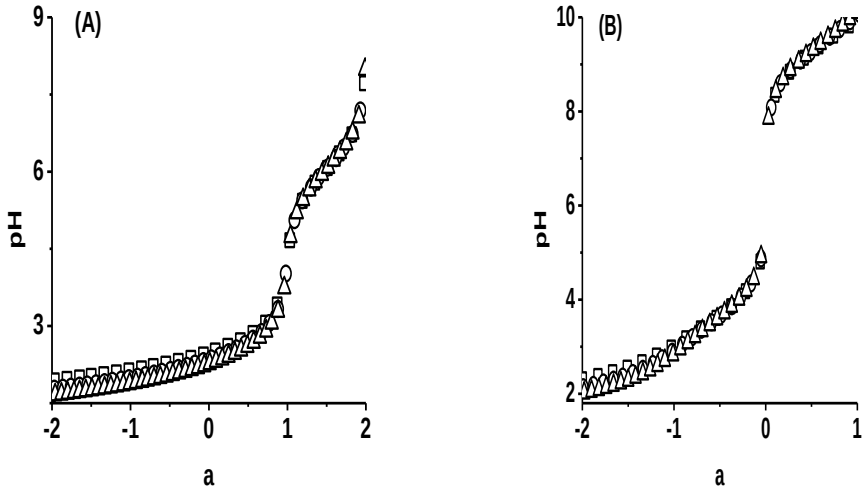


Fig 4.5**:** a versus pH curves of (A) MA and (B) Phe in 20% v/v DMF-water medium. For three amounts (0.25(□), 0.375(○) and 0.5(∆)mmol)

4.1.2. Mathematical models for proton-ligand equilibria of Phe and MA.

The computer program MINIQUAD75[74] was used to refine the protonation constants of the Phe and MA in aqueous and DMF-water medium. The results of the best fit model along with some of the important statistical parameters are given in Table 4.1.

Table 4.1: Best fit chemical models of protonation equilibria of MA and Phe in DMF-water mixtures.

Temp = 303 K, Ionic strength = 0.16 mol dm^{-3}.

%v/v DMF	logβ1(SD) 011	logβ2(SD) 012	NP	Ucorr	Skewness	Kurtosis	χ2	R-Factor	pH range
MALEIC ACID									
0.0	5.89(01)	7.69(01)	127	0.99	0.45	5.08	8.36	0.0050	1.7-10.0
10.0	5.88(04)	7.55(05)	44	9.82	0.49	4.47	6.73	0.0159	1.7-10.0
20.0	6.13(03)	8.42(04)	133	17.67	0.38	3.59	12.38	0.0190	1.7-10.0
30.0	6.06(02)	8.16(03)	86	9.89	-0.63	4.17	11.81	0.0207	1.95-9.5
40.0	5.96(05)	8.61(08)	117	61.47	1.05	5.09	26.38	0.0373	1.8-10.0
50.0	6.02(03)	8.13(04)	115	16.76	-0.48	3.91	3.95	0.0198	1.7-10.0
L-PHENYLALANINE									
0.0	9.13(01)	11.39(02)	43	1.23	-0.02	3.43	4.98	0.0097	2.5-9.5
10.0	9.26(02)	12.38(03)	63	7.70	-0.61	3.89	9.75	0.0219	2.5-10.0
20.0	9.34(02)	12.84(04)	62	8.3	-0.37	3.30	9.68	0.0220	2.5-10.0
30.0	9.30(02)	12.89(03)	64	6.46	-0.85	5.28	5.75	0.0191	2.5-10.0
40.0	9.34(03)	13.11(05)	58	12.39	-0.13	2.43	6.90	0.0256	2.5-10.0
50.0	9.41(03)	13.38(07)	60	45.41	0.03	2.90	0 .40	0.0336	2.5-10.0

$U_{corr}=U/(NP-m)X10^{8}$, where m=number of species; NP=Number of experimental points; SD=standard deviation

4.1.2.1. Statistical Parameters for the Best fit Models

Avery low standard deviations (SD) in logβ values indicate their precision. Small values of U_{corr} (sum of the squares of deviations in concentrations of ligand and hydrogen ion at all experimental points corrected for degrees of freedom) indicate that the experimental data can be represented by the models. In data analysis with least squares methods, the residuals (differences between the experimental data and the data simulated based on 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. Further, a model is considered adequate only if the residuals do not show any trend. Respecting the hypothesis that the errors are random, the residuals are tested for normal distribution. Such tests are χ^2, skewness, kurtosis and R-factor [82].

These statistical parameters show that the best fit models portray the proton-ligand equilibria of Phe and MA in aqueous and DMF-water medium, as discussed below.

χ^2 test

χ^2 is a special case of gamma distribution whose probability density function is an unsymmetrical 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 [82].

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 different values are obtained for models containing different numbers of species, models whose values are greater than R-table are rejected. The low crystallographic R values given in Table 4.3 indicate the sufficiency of the model [83].

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 mean and the peak is to the right of the mean if skewness is less than zero. The values of skewness recorded in Table 4.1 are between -0.63 and 1.05 for maleic acid and -0.85 and 0.03 for L-phenylalanine in DMF-water medium. This data evince that the residuals form a part of normal distribution; hence, least-squares method can be applied to the present data [82].

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 [84]. The alkalimetric titration data are simulated using the model parameters given in Table 4.1. These data are compared with the experimental alkalimetric titration data, to verify the sufficiency of the models. The simulated primary data curve generated by using the stability constants of the best fit model overlapped with the experimental ones, which supports the validity of models.

4.1.3. Effect of Systematic Errors on Best fit Model

In order to rely upon the best fit chemical model for critical evaluation and application an investigation was made by introducing pessimistic (maximum possible) errors in the concentrations 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 typical systems given in Tables 4.2 & 4.3 emphasize that protonation constants associated with carboxyl proton are more affected than the amino protons. This is probably due to lower magnitude of the former than that for amino proton.

The SD's are found to increase with the introduction of errors inferring the appropriateness of the experimental conditions and correctness of the analytical concentrations.

Table 4.2**:** Effect of errors in influential parameters on the protonation constants of MA in 30% v/v DMF-water medium.

Ingredient	% Error	$\log\beta_{mlh}$(SD)	
		011	012
	0	6.06(02)	8.16(03)
Alkali	-5	6.65(09)	9.14(12)
	-2	6.28(05)	8.53(07)
	+2	5.85(02)	7.81(03)
	+5	Rejected	Rejected
Acid	-5	5.71(01)	7.43(02)
	-2	5.92(02)	7.88(03)
	+2	6.20(05)	8.45(06)
	+5	Rejected	8.91(09)
Ligand	-5	5.89(02)	7.92(03)
	-2	5.99(03)	8.07(04)
	+2	6.13(04)	8.26(05)
	+5	6.22(05)	8.39(06)

Table 4.3: Effect of errors in influential parameters on the protonation constants of Phe in 20% v/v DMF- water medium.

Ingredient	% Error	$\log\beta_{mlh}$ (SD)	
		011	012
	0	9.34(02)	12.84(04)
Alkali	-5	9.85(15)	13.81(19)
	-2	9.53(13)	13.26(16)
	+2	Rejected	12.61(13)
	+5	Rejected	12.18(12)
Acid	-5	8.94(12)	12.15(13)
	-2	9.17(12)	12.61(13)
	+2	9.49(13)	13.25(16)
	+5	9.75(15)	Rejected
Ligand	-5	9.26(12)	12.93(14)
	-2	9.30(12)	12.93(14)
	+2	9.35(13)	12.93(15)
	+5	9.39(13)	12.92(15)

4.1.4. Effect of Dielectric Constant of Solvent on Protonation Equilibria

Ligand protonation constants are strongly affected by the dielectric constant of the medium because of the fact that at least one of the constituents is charged and other is either charged or has a dipole moment [85]. Seleem *et al.* [86] and others [87] 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

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

$$\log K_2 = \log \beta_1 \text{ for both Phe and MA}$$

where β_1 and β_2 are overall protonation constants. The values of step wise protonation constants are given in Table 4.4.

Table 4.4**:** step wise protonation constants of L-phenylalanine and maleic acid

% v/v DMF	Phe		MA	
	$\log K_1$	$\log K_2$	$\log K_1$	$\log K_2$
0.0	2.26	9.13	1.80	5.89
10.0	3.12	9.26	1.67	5.88
20.0	3.50	9.34	2.29	6.13
30.0	3.59	9.30	2.10	6.06
40.0	3.77	9.34	2.65	5.96
50.0	3.97	9.41	2.11	6.02

Phe contains one carboxylic and one amino group. The different forms of Phe are LH_2^+, LH^- and L^- in the pH ranges 1.80-6.50, 1.80-11.0, 6.0-11.50 respectively. The glass electrode used in this study is sensitive at 1.0 to 12.0 pH. In this range the number of dissociable protons is only 2. Thus only two log K values are considered for Phe in the present study and the corresponding forms of Phe are LH_2^+, LH^- and L^-. MA has two carboxyl functional groups and all are deprotonated as XH_2, XH^- and X^{2-} in the pH ranges of 1.70-4.30, 1.70-9.0 and 3.60-10.0, respectively.

Different researchers have explained the effect of electrostatic and non-electrostatic forces of interaction on different co-solvent-water mixtures that resulted in variation in the values of protonation constants of ligands or change in free energy.

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

$$\Delta G^t = -RT (\ln K^M - \ln K^W) \quad (4.1)$$
$$= \Delta G_{el} + \Delta G_{nel}$$

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

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

Hence, Equation 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 Phe 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 co-solvent [89].

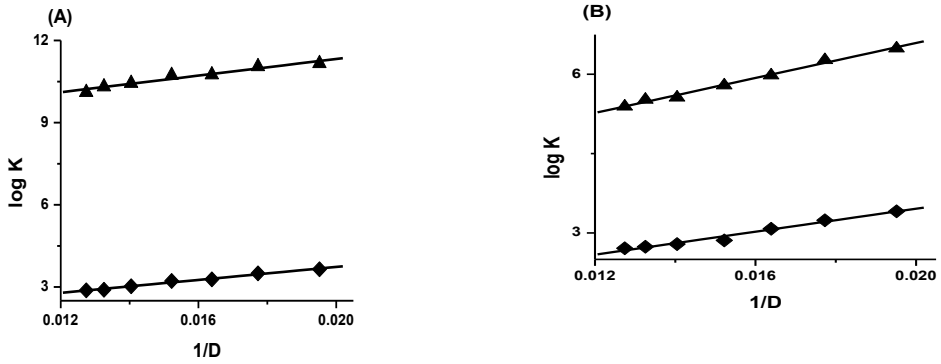
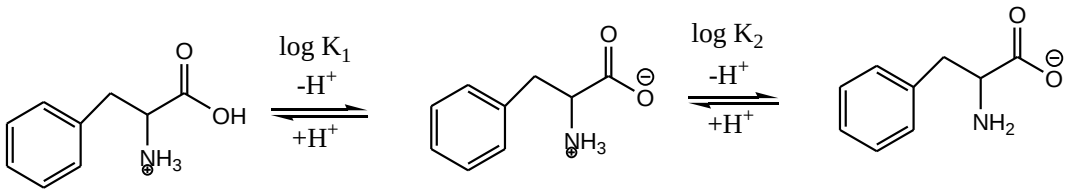


Fig 4.6: Variation of stepwise protonation constant(logK) with (1/D) of solvent.
(A) Phe and (B) MA in DMF-water mixtures; (◆) log K_1 and (▲) log K_2.

Many workers [90, 91] 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. The cation stabilizing nature of co-solvents, specific solvent-water interactions, salvation power, donor number, acceptor number [86, 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 Phe and MA are shown with the pH ranges of existence of the species in Fig. 4.7. In these equilibria Phe can exist in anionic, zwitterionic and cationic forms, whereas MA can exist in only anionic or neutral form at different pH ranges. These observations can be explained as follows. When Phe (Equilibria 4.3 and 4.4) and MA (Equilibria 4.5 and 4.6) 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.

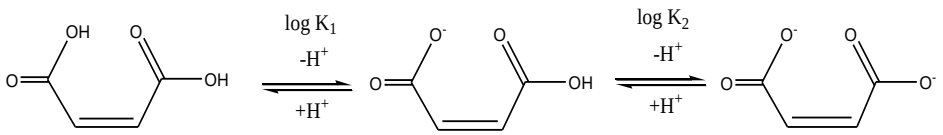
A)



LH_2^+ LH (Zwetterion) L^-

pH 1.8 -6.50 pH 1.8 - 11.0 pH 6.0 - 11.5

B)



pH 1.7-4.30 pH 1.7-9.0 pH 3.6 - 10.0

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

The deprotonation -protonation equilibria of F are shown as follows

$LH_2 \rightleftharpoons[\log K_1] LH + H^+$ ----------4.3

$LH \rightleftharpoons[\log K_2] L^- + H^+$ ----------4.4

The deprotonation -protonation equilibria of MA are:

$XH_2 \rightleftharpoons[\log K_1] XH^- + H^+$ ----------4.5

$XH^- \rightleftharpoons[\log K_2] X^{2-} + H^+$ ----------4.6

4.1.5 Distribution Diagrams

Distribution diagram-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 [93]. The two functional groups of Phe participate in protonation equilibria. Their distribution plots in DMF-water medium given in Figs.4.9 & 4.10 show the existence of LH_2^+, LH and L^- forms of Phe and XH_2, XH^- and X^{2-} forms of MA. The XH^- form of maleic acid is present to an extent of 80-90% in the pH range 1.7-9.0 and the LH form of Phe is present to an extent of 80-90% in the pH range 1.8-11.0. It is increased with the increase in organic medium concentration.

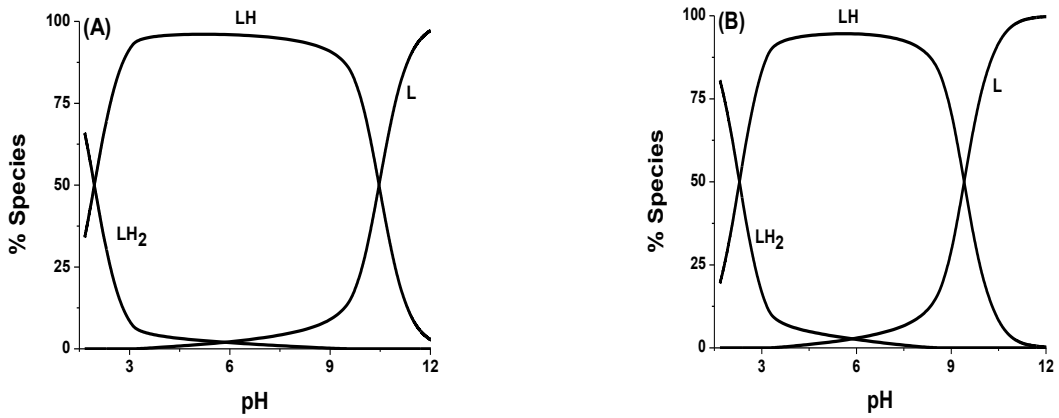


Fig 4.8: Distribution diagrams of (A) Phe and (B) MA in aqueous medium.

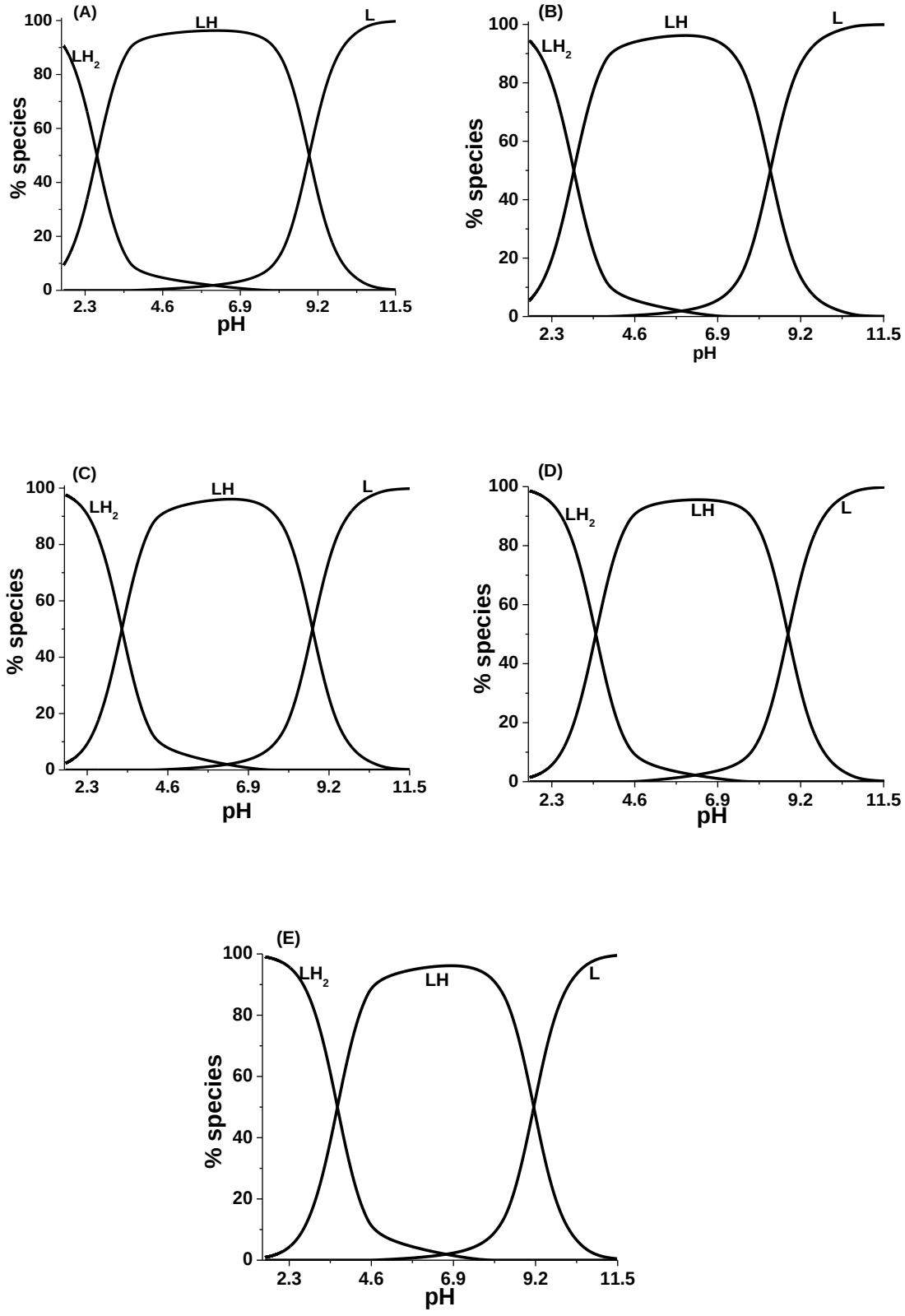


Fig 4.9: Distribution diagrams of Phe in DMF-water mixtures % v/v.

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

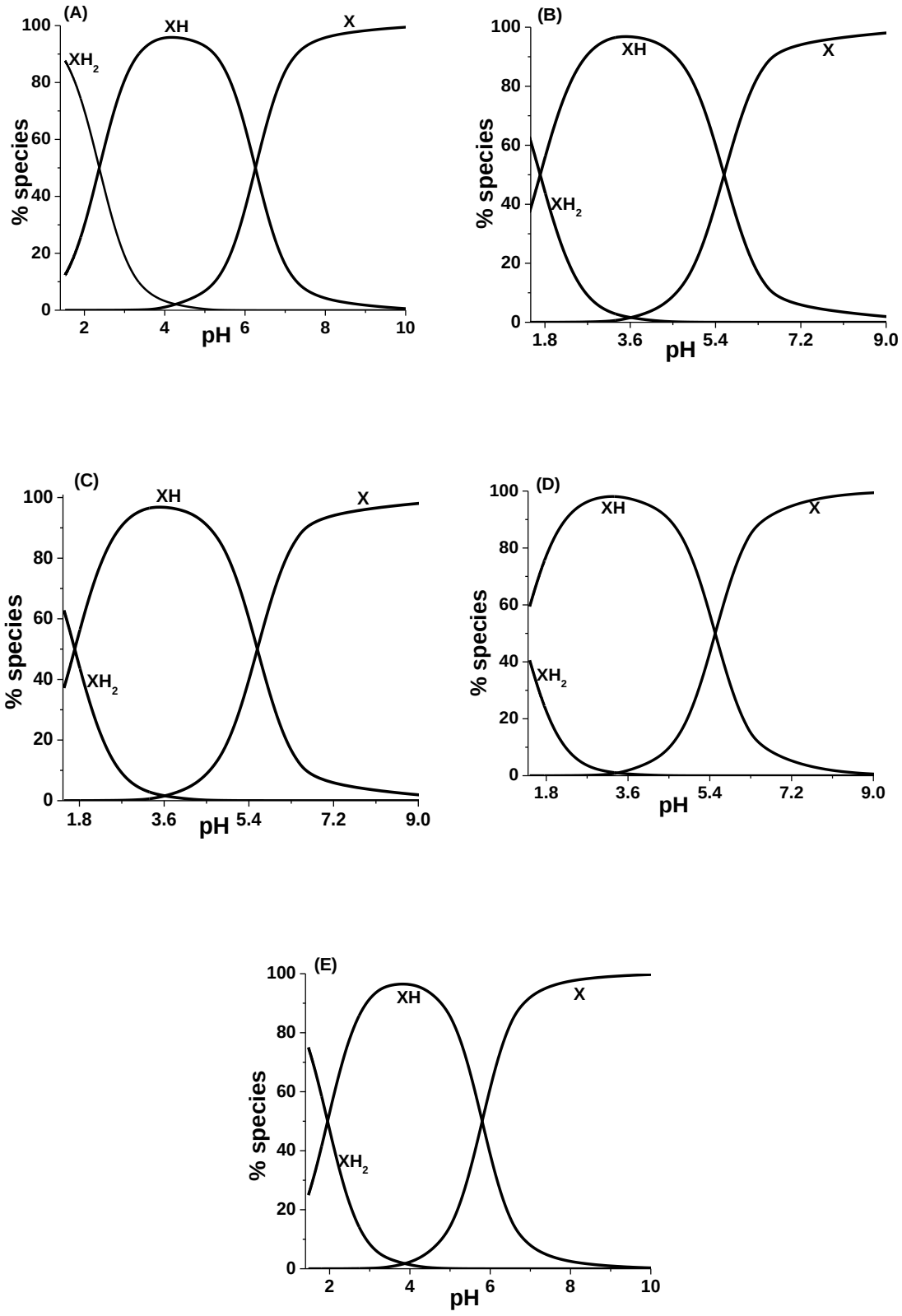


Fig 4.10: Distribution diagrams of MA in DMF-water mixtures % v/v.

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

SECTION-II

4.2. Determination of Binary Stability Constants

L-Phenylalanine (Phe) is an important essential α-amino acid and maleic acid is biologically important dicarboxylic acid. They form complexes with almost all metal ions. The study of their complexation with different metal ions opens their new potentialities in the synthesis of complexes with target properties. Both Phe and MA are bidentate ligands. They form chelate rings with metal atoms [58,59].

4.2.1. Formation of Species

In order to rationalize the contradictory models reported by different authors, greater details 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, are required. Further, different species proposed for the same metal-ligand system are many a time judged on the best fit criteria. It is very difficult to say a final word about it, as it is unequivocally proved that different algorithms (or same algorithm with different weighting schemes) produce different species [58-61].

In many chemical systems it was observed that, the predicted species based on the pH range and the form of the ligand were in fact not detected. This unexpected behavior could be attributed to i) the hard and soft acid base theory of the metal ion and ligand, ii) coordination number of the metal ion, and iii) stabilities of the complexes formed [68,69].

Both MA (two donor O atoms) and Phe (one N and one O donor atom) are bidentate ligands. If the ligand contains one or more donor atoms than those participating in complexation, there is possibility for the formation of protonated species, ML_1H_h in addition to ML_1 type of complexes. But no protonated species were reported in the literature. With progressively increasing pH, the protonated species lose protons to form unprotonated complexes, and if the complex is not broken, it may undergo hydrolysis resulting in $ML_1(OH)_k$ [58,59].

Typical alkalimetric titration curves are given in Figs. 4.11 and 4.12 from which the stability constants of binary complexes were calculated.

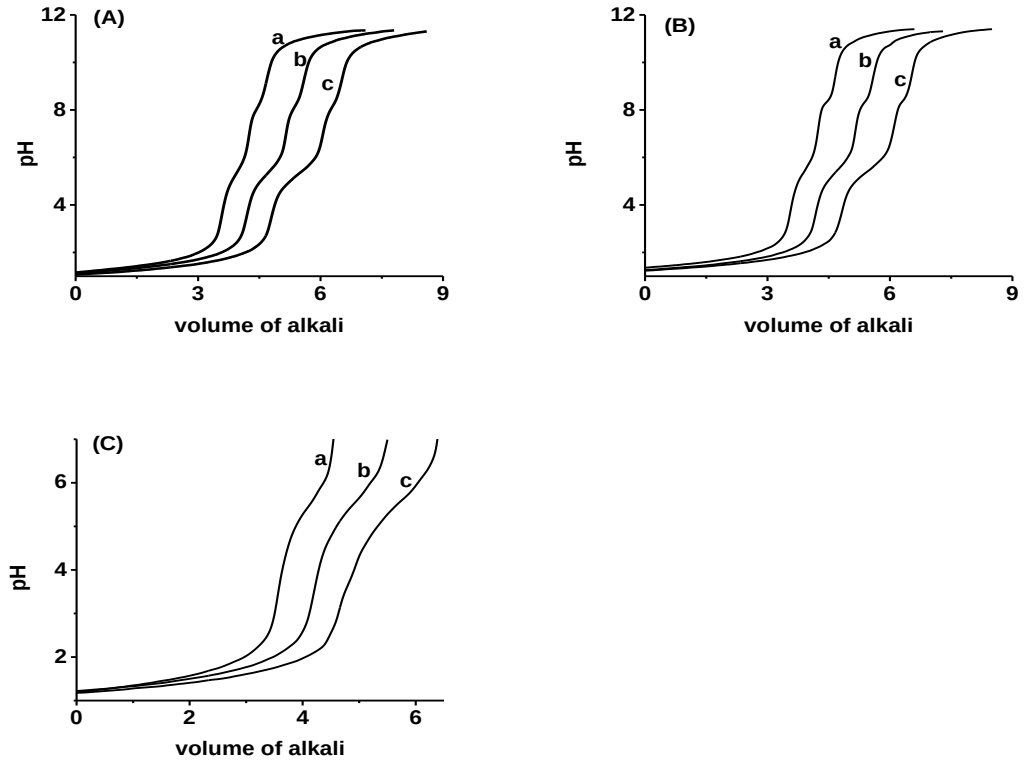


Fig 4.11: Alkalimetric titration curves of MA complexes with (A) Co(II), (B) Ni(II) and (C) Cu(II) in 30%v/v DMF-water medium. Amount of MA: (a) 2.5 (b) 3.5 (c) 5.0mmol.

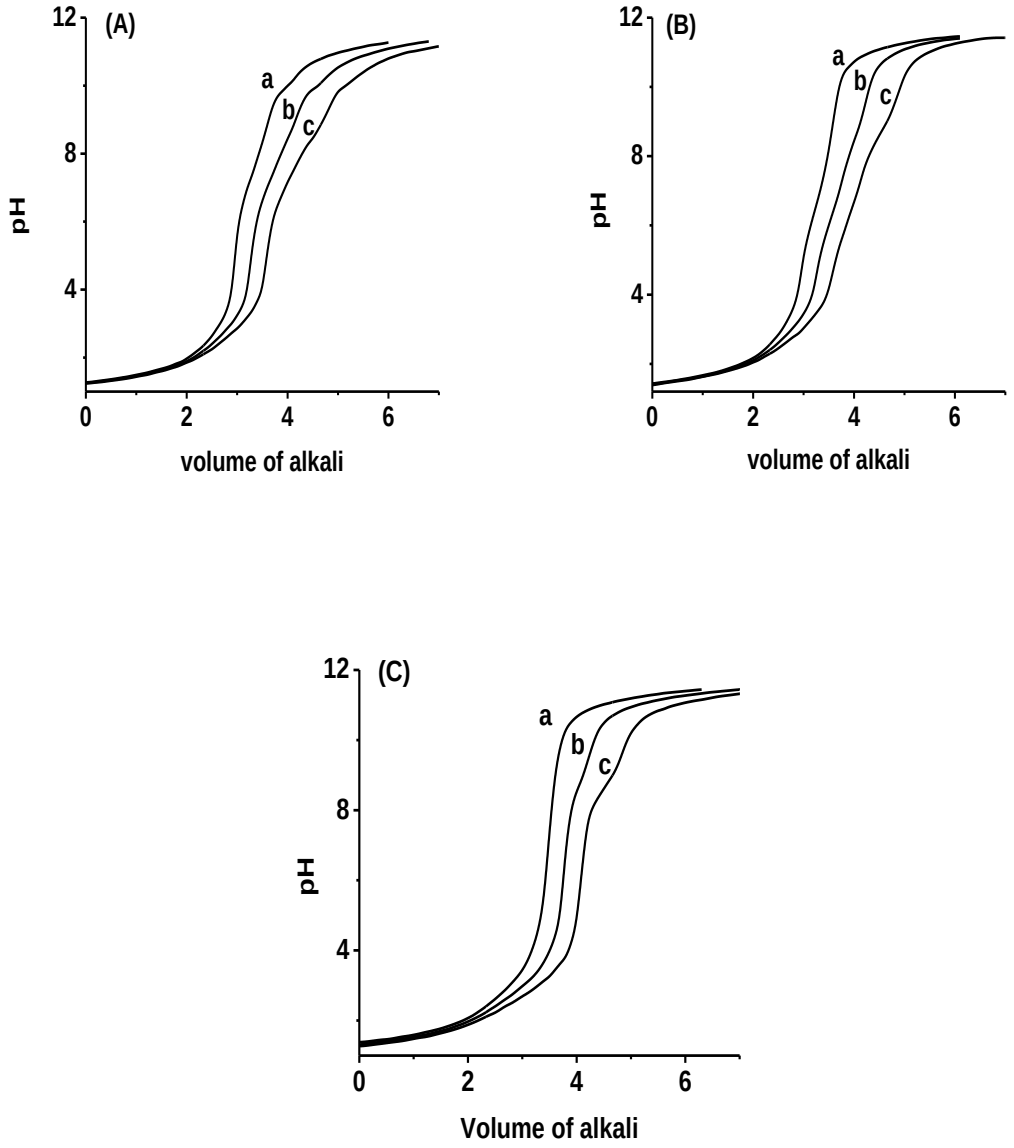


Fig 4.12: Alkalimetric titration curves of Phe complexes with (A) Co(II), (B) Ni(II and (C) Cu(II) in 30%v/v DMF-water medium. Amount of Phe: (a) 2.5 (b) 3.5 (c) 5.0mmol.

Since the ratio of TL0 to TM0 is greater than unity in all the experiments, as is clear from the data in Tables 3.2 and 3.3 of Chapter 3, formation of polynuclear complexes is not considered. If TL0:TM0$\leq$ 2 the formation of polymeric species is possible. 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 $\bar{n}$ versus pL at all the ligand concentrations, with fixed metal ion concentration should overlap. Any deviation indicates the presence of the protonated species.

Some typical plots given in Fig. 4.13 and 4.14 shows spread in some pH regions, indicating the presence of protonated metal complexes.

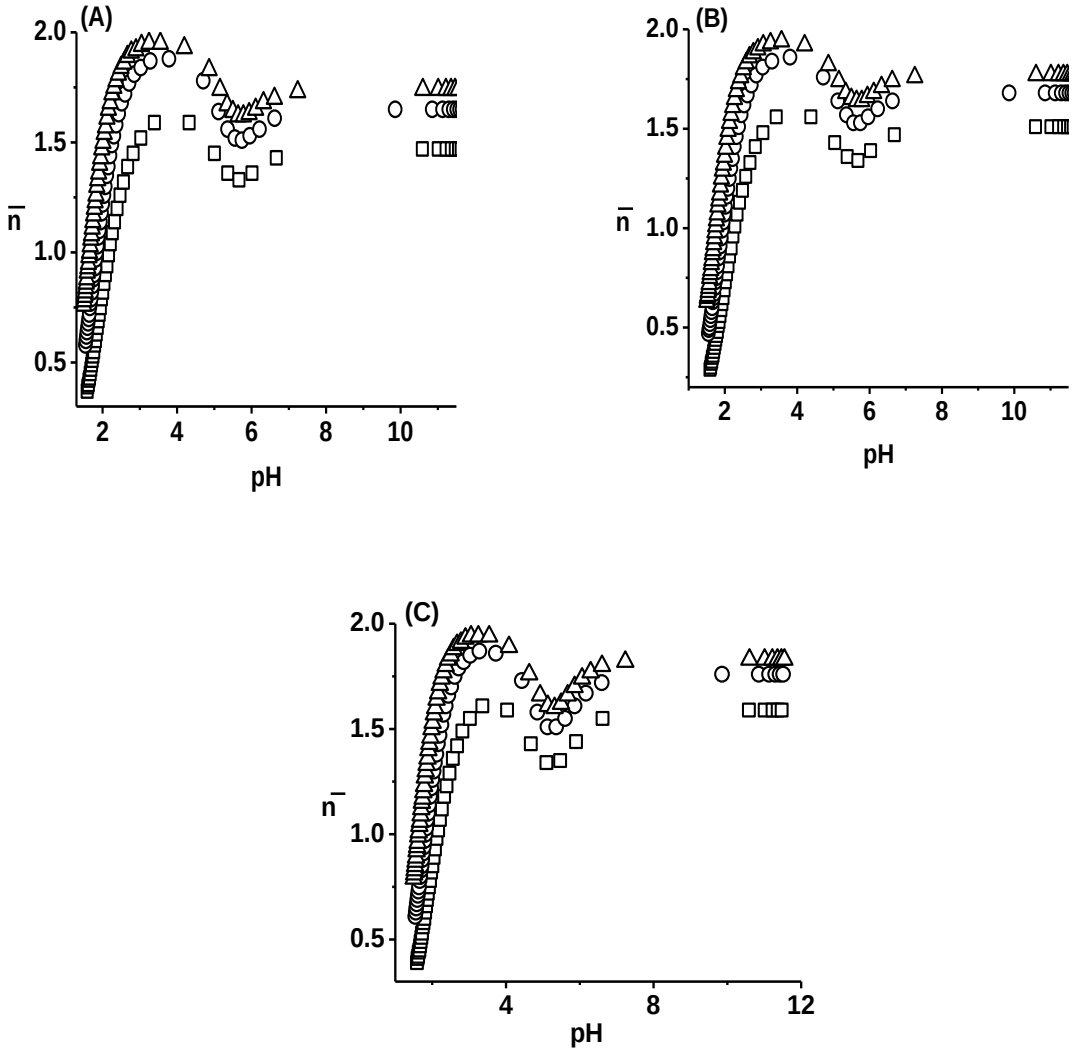


Fig 4.13: Formation curves of Phe Complexes with (A) Co(II) (B) Ni(II) (C) Cu(II in 40% v/v DMF-water medium. Amounts of Ligand in mmol are 0.25(□-□) 0.375(O-O) and 0.50(Δ-Δ).

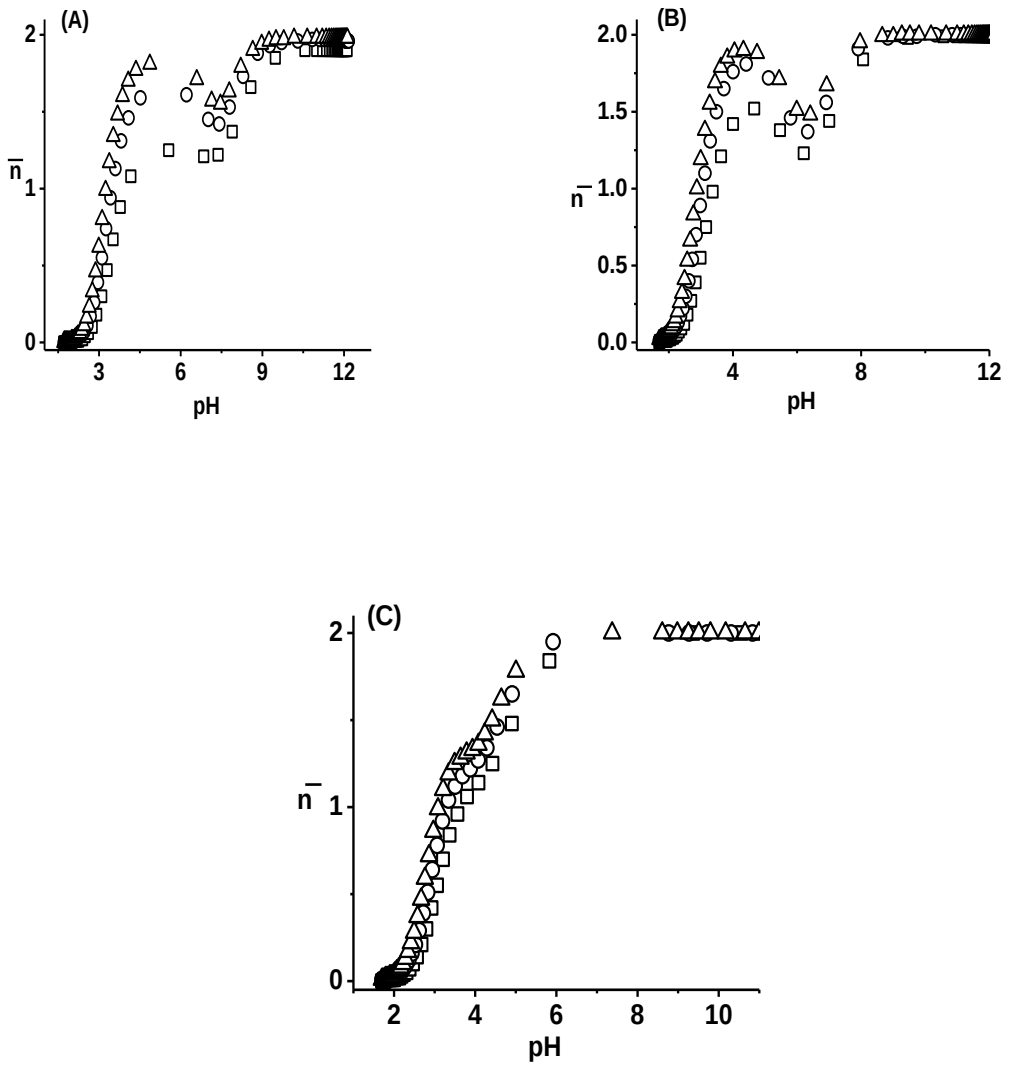


Fig 4.14: Formation curves of Phe Complexes with (A) Co(II) (B) Ni(II) (C) Cu(II) in DMF 20%v/v DMF-water medium.
Amounts of Ligand in mmol are: 0.25(□-□) 0.375(O-O) and 0.50(Δ-Δ).

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 or pL plots which can have many applications. For example, PSEUDOPLOT [94] 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.

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 [81]. $\bar{n}$ 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 [95] resulted in a short list of ML, MLH, ML_2, ML_2H and ML_2H_2.

4.2.3. Selection of Best fit Model

The models containing different number of species were tried from the primary alkalimetric titration data. Only a few species were refined while other species were rejected by MINIQUAD75 [95]. 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.

Parameters of the best fit chemical models are given in Tables 4.5 and 4.6 for Co(II), Ni(II) and Cu(II) complexes of MA and Phe respectively in DMF-water media. 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 4.5: Parameters of best fit chemical models of M(II)-MA complexes in DMF-water medium.

% v/v DMF	logβmlh (SD)			NP	Ucorr	Skew-ness	χ2	R-Factor	Kurtosis	pH-Range
	ML	**ML_2**	**ML_2H_2**							
Co(II)										
0.0	4.57(05)	6.89(05)	17.77(07)	50	1.06	1.34	14.57	0.0092	3.43	1.5-8.2
10.0	4.23(33)	6.09(08)	17.89(05)	67	1.48	0.13	12.09	0.0103	3.71	2.2-8.2
20.0	3.47(09)	6.30(06)	17.46(05)	69	1.11	0.12	19.54	0.0083	3.72	2.2-8.2
30.0	3.30(08)	5.90(05)	17.05(05)	72	1.86	0.33	13.70	0.0072	3.32	2.2-8.2
40.0	4.39(03)	7.09(06)	18.24(11)	75	1.09	-0.12	12.07	0.0182	3.49	2.0-8.2
50.0	3.12(03)	6.47(00)	17.35(09)	44	1.98	1.15	13.31	0.0111	6.51	2.0-8.2
Ni(II)										
0.0	5.46(08)	7.43(06)	16.39(02)	100	1.06	1.53	16.26	0.0131	4.92	1.8-6.6
10.0	3.77(02)	5.68(04)	17.31(02)	55	1.16	-0.14	9.59	0.0080	3.38	2.1-6.6
20.0	4.39(06)	7.30(03)	18.36(01)	56	1.77	1.51	10.53	0.0112	3.44	2.1-6.6
30.0	4.32(08)	7.47(03)	18.36(03)	77	1.73	-0.14	11.97	0.0151	2.64	2.1-6.6
40.0	4.23(01)	6.96(01)	18.04(10)	89	1.68	1.22	6.68	0.0202	3.26	1.9-6.6
50.0	3.40(07)	6.44(02)	17.63(06)	44	2.22	-0.22	10.44	0.0222	2.72	1.9-6.6
Cu(II)										
0.0	4.97(05)	7.80(09)	15.80(14)	63	1.59	0.01	8.84	0.0082	3.43	2.0-5.8
10.0	3.86(08)	6.24(08)	16.76(07)	69	1.57	-0.11	12.56	0.0042	3.34	2.0-5.8
20.0	4.77(08)	7.90(03)	18.44(12)	70	1.81	-0.13	9.36	0.0082	2.99	2.0-5.8
30.0	3.72(06)	6.95(09)	17.32(09)	88	1.68	0.62	6.69	0.0102	5.00	2.1-5.8
40.0	4.90(07)	7.85(01)	18.25(13)	448	1.58	-0.13	12.60	0.0203	3.90	2.1-5.8
50.0	3.30(06)	7.20(08)	17.38(34)	49	1.32	0.01	4.67	0.0185	3.33	2.5-5.8

$U_{corr} = U/(NP-m)X10^{8}$, where m = number of species; NP=Number of experimental points; SD=Standard deviation.

Table 4.6: Parameters of best fit chemical models of M(II)- Phe complexes in DMF-water medium.

% v/v DMF	logβmlh (SD)			NP	Ucorr	Skew-ness	$\chi 2$	R-Factor	Kurtosis	pH-Range
	ML	ML_2	ML_2H_2							
Co(II)										
0.0	5.59(09)	9.42(07)	23.85(03)	55	0.78	0.14	5.01	0.0043	5.02	1.8-8.6
10.0	5.70(01)	9.63(11)	24.35(06)	44	0.32	-0.13	5.47	0.0102	4.12	2.5-8.6
20.0	6.60(01)	10.83(10)	25.22(05)	66	0.98	0.07	7.62	0.0091	3.50	2.5-8.6
30.0	6.51(03)	10.80(10)	24.80(05)	75	0.47	0.71	4.68	0.0081	4.32	2.5-8.6
40.0	5.40(09)	9.39(08)	24.22(05)	44	0.81	0.74	5.30	0.0072	4.01	2.5-8.6
50.0	5.82(03)	9.90(10)	24.85(06)	46	0.23	0.11	8.05	0.0100	4.63	2.5-8.6
Ni(II)										
0.0	8.56(19)	13.35(04)	23.51(04)	90	0.70	0.72	62.28	0.0072	7.32	1.7-8.6
10.0	7.33(04)	12.19(02)	25.02(03)	100	0.91	0.64	31.73	0.0074	4.43	1.7-8.6
20.0	8.77(09)	13.83(08)	25.95(09)	62	0.19	0.01	3.08	0.0151	2.63	2.3-7.6
30.0	7.72(04)	12.78(03)	24.98(05)	67	0.94	0.10	2.87	0.0083	3.12	2.3-7.6
40.0	7.43(07)	12.31(07)	24.84(07)	99	0.88	-0.01	4.97	0.0133	2.62	2.3-7.6
50.0	6.42(07)	10.99(08)	24.72(08)	64	0.78	0.23	5.13	0.0122	3.21	2.3-7.6
Cu(II)										
0.0	9.19(05)	16.56(09)	23.66(07)	68	0.62	0.14	5.85	0.0081	4.71	2.0-8.3
10.0	9.68(05)	16.53(08)	24.48(05)	56	0.84	0.00	11.24	0.0051	2.83	2.0-8.3
20.0	10.89(08)	18.70(08)	25.92(04)	99	0.54	-0.02	4.89	0.0172	2.92	2.0-8.3
30.0	8.82(06)	15.88(11)	23.86(01)	77	1.61	0.33	3.33	0.0113	2.12	2.6-8.3
40.0	9.25(05)	16.38(09)	24.46(20)	44	0.39	-1.34	8.67	0.0053	9.01	2.3-8.3
50.0	9.73(08)	17.00(06)	25.59(02)	88	18.78	-1.62	12.63	0.0293	5.3	1.6-8.3

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

4.2.4. Residuals Analysis

The residuals differences between what is actually measured or observed (EMF, pH, volume, concentration of ingredients etc.) and that predicted by a model measure the unexplained variation in the dependent variable by the regression equation. The goal of rigorous residual analysis is to conclude that the assumptions in regression analysis and of chemical laws (mass-balance equations, electrode response, equilibrium expression etc.) are valid for a data set on hand. The residuals should follow normal distribution for the best fit model. The use of plots of residuals versus calculated volumes of dependent variables (EMF or volume) and independent variables (TM, TL) is popular [96].

Gans et al [97] 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 1 was observed only in a single titration curve (unlike pooled data) inferring that the data have been correctly weighted. Only when unweighted residuals follow χ^2 distribution, which measures the possibility of residuals forming a plot of standard normal distribution with zero mean and unit standard deviation, the SD and confidence intervals in β are meaningful.

Higher χ^2 values than expected are due to 1) the inadequacy of the model although the experimental data are of high quality, 2) use of poor data even though the model is appropriate, and 3) invoking optimistic estimates of errors in primary data [97].

A perusal of Tables 4.5 and 4.6 indicates that the χ^2 values range between 2.07 and 19.54 for M(II)-MA complexes and 3.08 and 12.63 for M(II)- Phe complexes. The values of skewness range from -0.22 to 1.53 for MA complexes and -1.62 to 0.74 for Phe complexes. Deviation of the values of kurtosis and skewness from three and zero, respectively, show the tendency of these residuals to concentrate more to the left or right of the mean and broadening of the peak. However, the value of U_{corr} in all the three mass-balance equations, are very low confirming the adequacy of the chemical model to represent the experimental data.

4.2.5. Perturbation in Stability Constants due to Systematic Errors

The computer programs refine the stability constants by minimizing the random errors in the data. But in the presence of considerable systematic errors, not only the βs are in errors; even some species may be rejected. MINIQUAD75 [95] has no provision to vary the influential parameters. Hence, some representative systems were studied in order to have a cognizance of the effect of errors in concentrations of ingredients on the stability constants of binary metal complexes. These results are given in Tables 4.7 and 4.8. The data show that the order of affecting the magnitudes of stability constants 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 4.7: Effect of errors in influential parameters on the stability constants of Cu(II)-MA complexes in 30% v/v DMF-water medium.

Ingredient	%Error	logβ(SD)		
		ML	ML_2	ML_2H_2
	0	3.72(06)	6.95(09)	17.32(09)
Alkali	-5	Rejected	Rejected	Rejected
	-2	Rejected	5.81(12)	16.12(24)
	+2	5.55(38)	9.02(37)	18.78(36)
	+5	7.04(33)	11.14(45)	Rejected
Acid	-5	Rejected	Rejected	Rejected
	-2	5.41(34)	8.80(32)	18.81(32)
	+2	2.32(79)	6.01(44)	16.19(38)
	+5	Rejected	5.06(36)	16.20(44)
Ligand	-5	4.29(36)	7.76(34)	17.84(33)
	-2	3.93(37)	7.26(32)	17.53(22)
	+2	3..50(47)	6.67(39)	17.15(44)
	+5	3.20(32)	6.27(40)	16..87(36)
Metal	-5	3.73(38)	7.07(44)	17.39(27)
	-2	3.72(39)	7.00(38)	17.06(28)
	+2	3.70(46)	6.92(33)	17.31(29)
	+5	3.69(45)	6.86(32)	17.08(30)

Table 4.8: Effect of errors in influential parameters on the stability constants of Co(II)- Phe complexes in 40% v/v DMF-water medium.

Ingredient	% Error	logβ(SD)		
		ML	ML_2	ML_2H_2
	0	5.40(09)	9.39(08)	24.22(05)
Alkali	-5	3.79(33)	Rejected	Rejected
	-2	4.65(44)	8.24(24)	23.58(33)
	+2	6.73(28)	10.89(22)	Rejected
	+5	8.90(26)	13.76(26)	24.93(33)
Acid	-5	9.12(27)	13.88(27)	25.20(41)
	-2	6.85(40)	10.97(29)	24.77(27)
	+2	4.57(30)	8.23(33)	23.44(28)
	+5	3.89(33)	Rejected	Rejected
Ligand	-5	5.24(43)	9.26(22)	24.00(29)
	-2	5.34(20)	9.33(19)	24.13(25)
	+2	5.51(28)	9.44(27)	24.30(24)
	+5	5.63(28)	9.53(28)	24.42(24)
Metal	-5	5.46(26)	9.52(28)	24.45(25)
	-2	5.44(20)	9.44(28)	24.23(35)
	+2	5.41(20)	9.33(38)	24.20(36)
	+5	5.38(20)	9.24(38)	24.18(37)

4.2.6. Effect of Solvent on Metal-ligand Equilibria

Co-solvent influences the equilibria in solution due to change in the dielectric constant (D) of the medium that varies the relative contribution of electrostatic and non-electrostatic interactions which in turn vary the magnitude of stability constants [88].

DMF is a dipolar aprotic solvent. It is a structure former and enhances the water structure in DMF-water mixtures; hence, it removes water from the coordination sphere of metal ions, making them more reactive towards the ligands. As a result, the stability of the complexes is expected to increase. At the same time, it is a coordinating solvent, and it competes with the ligands for coordinating the metals [10]. This decreases the stability of the complexes. Hence, the stability of the complexes is expected to either increase or decrease. The variation of overall stability constants with co-solvent content depends upon electrostatic and non-electrostatic factors.

The co-solvent induced increased basicity of DMF-water mixtures increases the stabilization of protons. The trends of stability constant (log β) values of complexes of MA and Phe with 1/D of DMF-water mixture are given in Figs. 4.15 and 4.16, respectively. The trend is almost linear which indicates that the dielectric constant or long range interactions are responsible for the stability trend. This linear increase indicates the dominance of the structure-forming nature of DMF over the complexing ability. Since complex formation can be viewed as a competition between the pure and solvated forms of 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 mixtures.

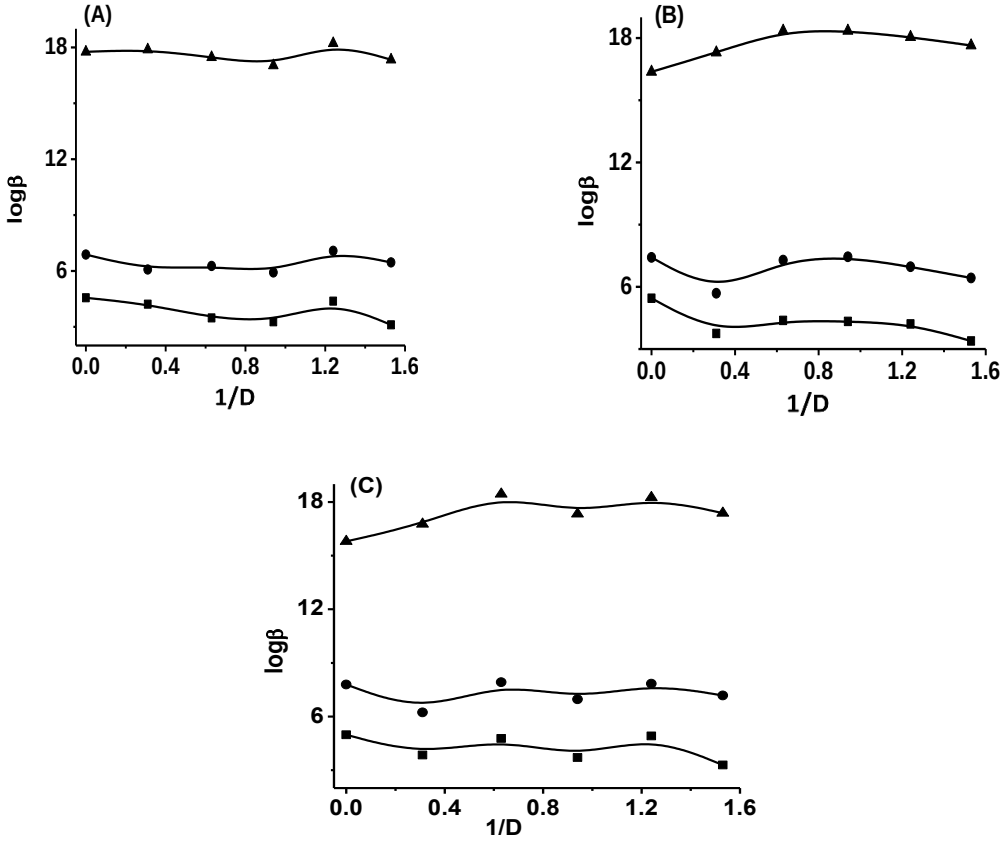


Fig 4.15: Variation of stability constant values of metal-maleic acid complexes with mole fraction of DMF-water medium. (A) Co(II), (B) Ni(II), (C) Cu(II) (■) $\log\beta_{ML,}$ (●)$\log\beta_{ML_2}$,(▲)$\log\beta_{ML_2H_2}$.

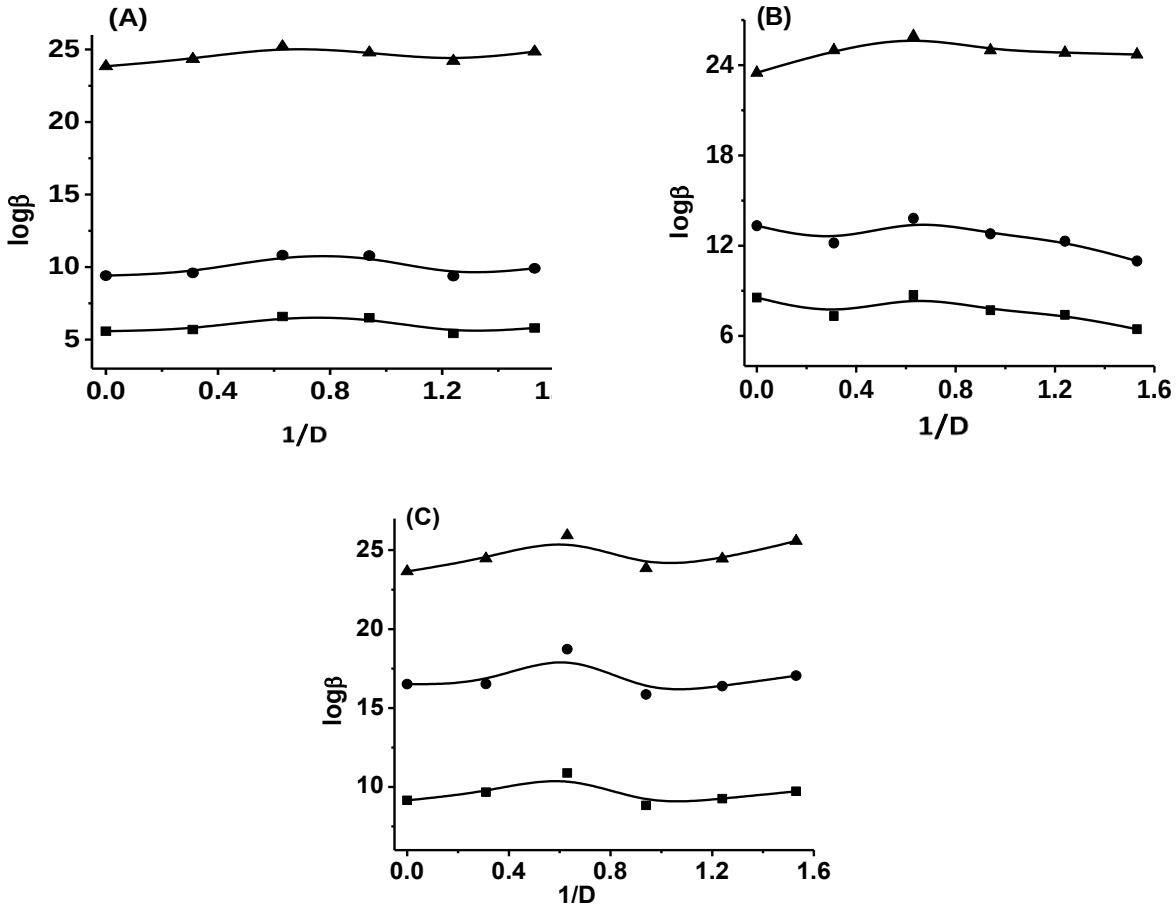


Fig 4.16: Variation of stability constant values of metal-phenylalanine complexes with mole fraction of DMF-water medium (A) Co(II), (B) Ni(II), (C) Cu(II) (■) $\log\beta_{ML}$, (●)$\log\beta_{ML_2}$,(▲)$\log\beta_{ML_2H_2}$.

4.2.7. Distribution Diagrams

The percentage of metal ion in the form of various complex species is given by Eq. 4.7.

$$PS = \frac{m(j) * \beta m(i)1(j)h(j) * FL_1^{1(j)} * FH_i^{h(j)}}{\sum_{J=0}^{N} \sum \beta m(j)1(j)h(j) * FL_1^{1(j)} * FH_i^{h(j)}} * 100 \qquad (4.7)$$

Using the formation constants 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 media.

The different forms of Phe are LH_2^+, LH and L^- in the pH range of 1.8 – 6.5, 1.8 – 11.0 and 6.0 – 11.5 respectively and for MA are XH_2, XH^- and X^{2-} in the pH-range of 1.7-4.30, 1.7-9.0 and 3.6–10.0. The plausible binary metal-ligand species in different systems can be predicted from these data. The present investigation reveals the existence of ML, ML_2 and ML_2H_2 for Co(II), Ni(II) and Cu(II). The ML_2 species is the predominant species (Figs. 4.17 & 4.18) at higher pH and ML_2H_2 is the predominant species at lower pH among all the binary complexes. Low concentration of free metal ion (FM) indicates the strong complexing nature of maleic acid and L-phenylalanine. The formation of various binary complex species is shown in the following equilibria.

$$M(II) + LH_2 \rightleftharpoons ML + 2H^+ \quad \text{….....(1)}$$

$$M(II) + LH \rightleftharpoons ML + H^+ \quad \text{…….....(2)}$$

$$M(II) + 2LH_2 \rightleftharpoons ML_2H_2 + 2H^+ \quad \text{.....(3)}$$

$$ML_2H_2 \rightleftharpoons ML_2 + 2H^+ \quad \text{.....(4)}$$

$$ML + LH_2 \rightleftharpoons ML_2 + 2H^+ \quad \text{……(5)}$$

$$ML + LH \rightleftharpoons ML_2 + H^+ \quad \text{…….....(6)}$$

Equilibria (1), (2) and (3) represent the formation of complexes from metal ion and the ligand. In alkalimetric titrations, protons are removed successively from the complexes by the addition of aliquots of the alkali. Equilibrium (4) represents the successive deprotonation of the complexes with increasing pH of the solution during alkalimetric titrations. Formation of ML_2 through the equilibria (4), (5) and (6) is proved by the increase in concentration of ML_2 by the decrease in concentration of ML and ML_2H_2.

Some typical distribution diagrams of aqueous and DMF-water media are shown in Fig. 4.17 to 4.22.

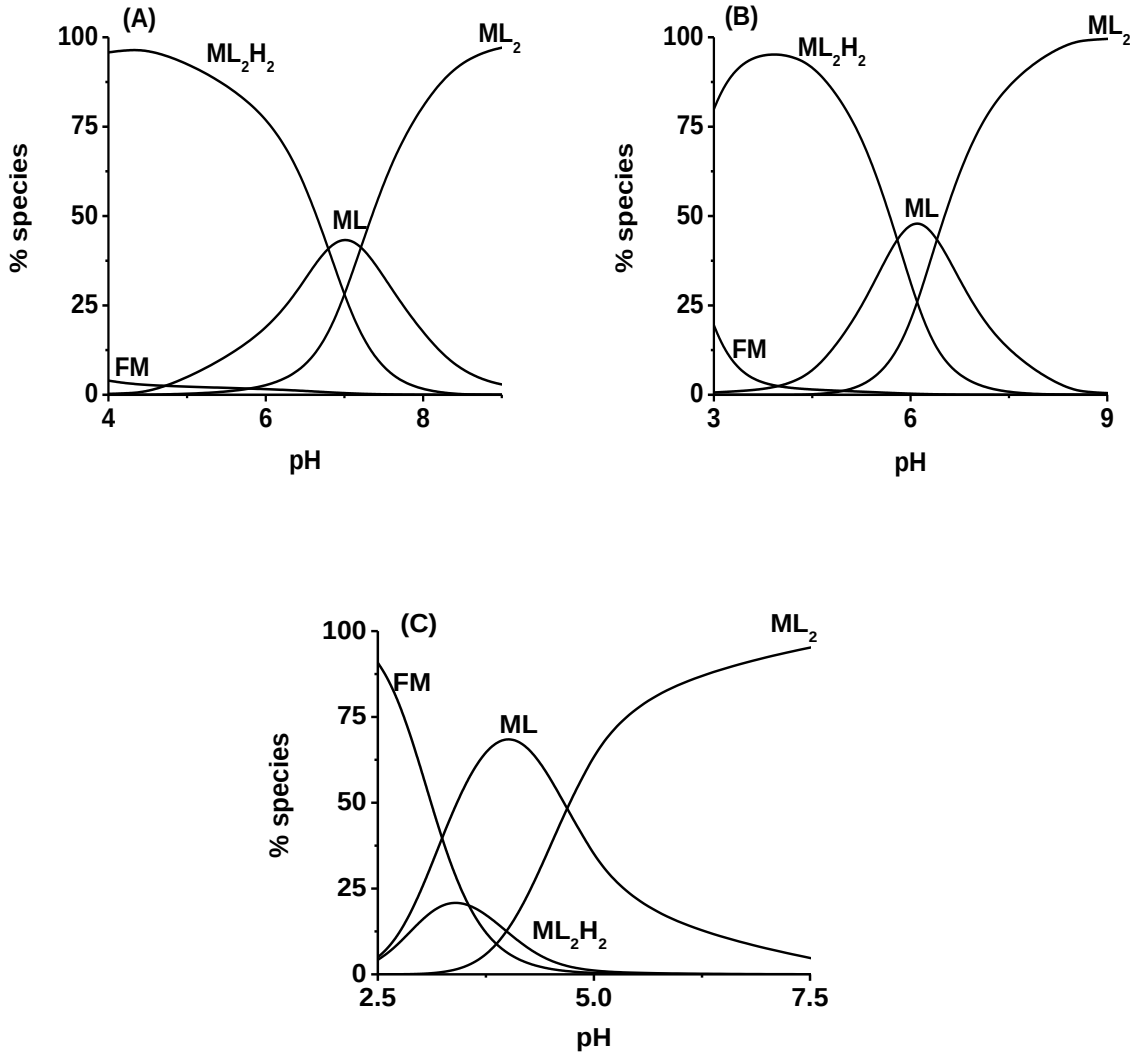


Fig 4.17: Distribution diagrams of Phe complexes in aqueous medium.
Co(II) (B) Ni(II) and (C) Cu(II).

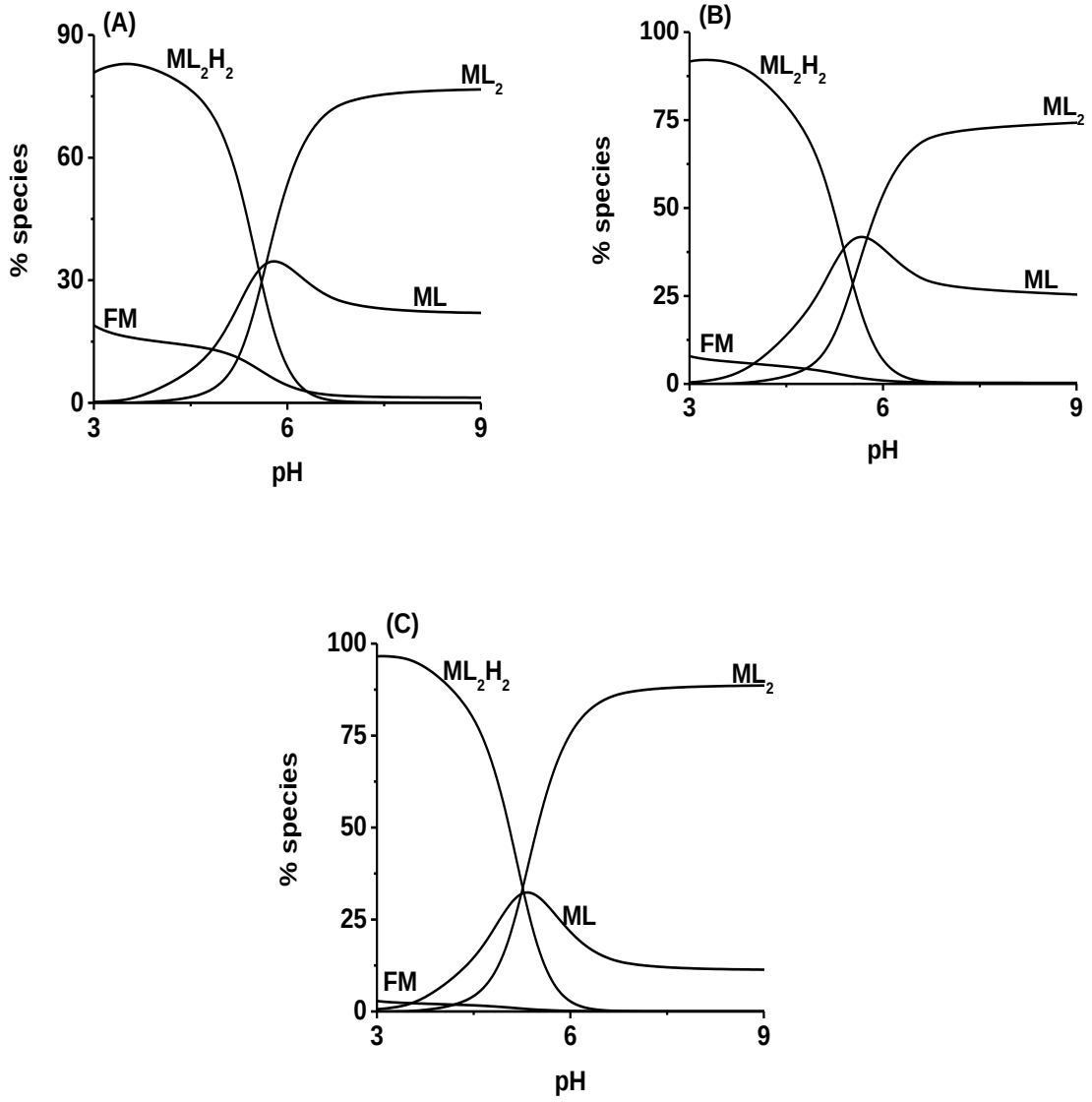


Fig 4.18: Distribution diagrams of MA complexes in aqueous medium.
(A) Co(II) (B) Ni(II) and (C) Cu(II)).

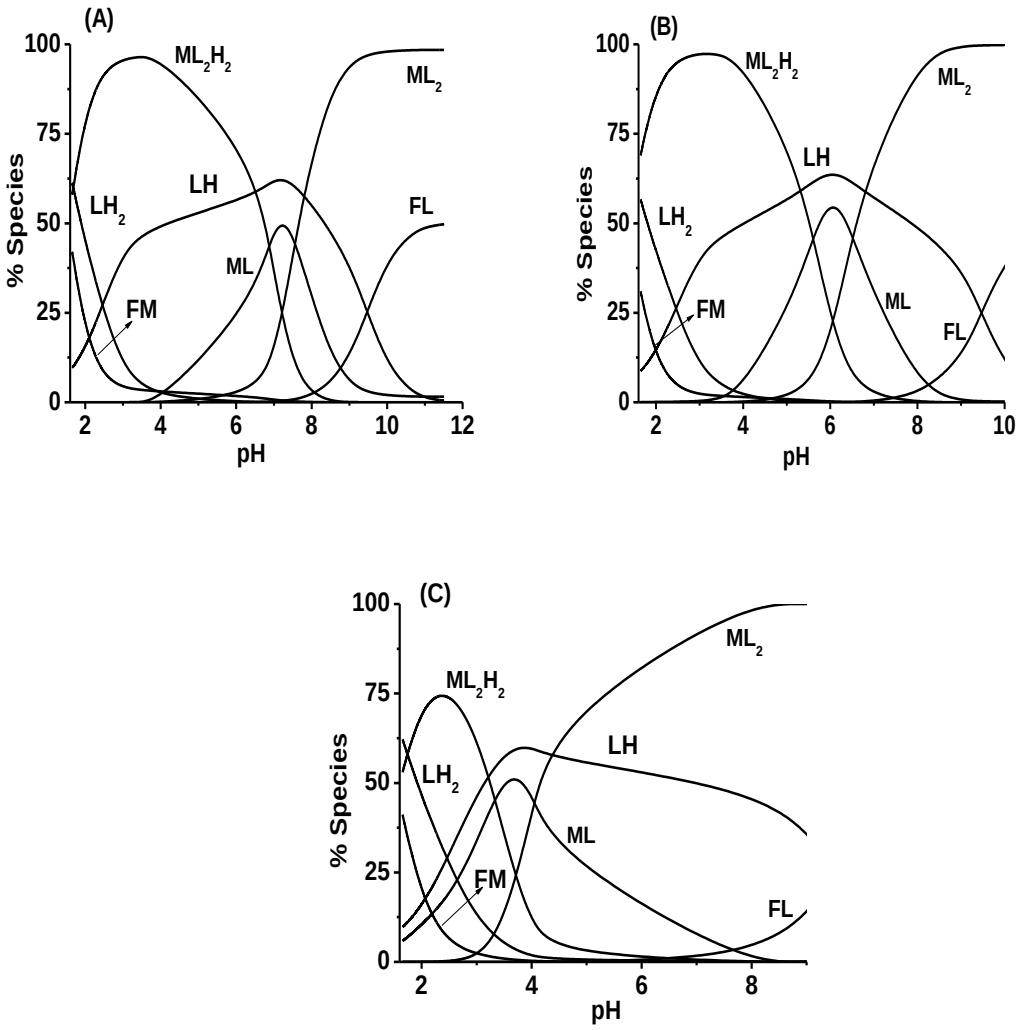


Fig 4.19: Distribution diagrams of Phe complexes in 10% v/v DMF-water medium.
(A) Co(II) (B) Ni(II) and (C) Cu(II)

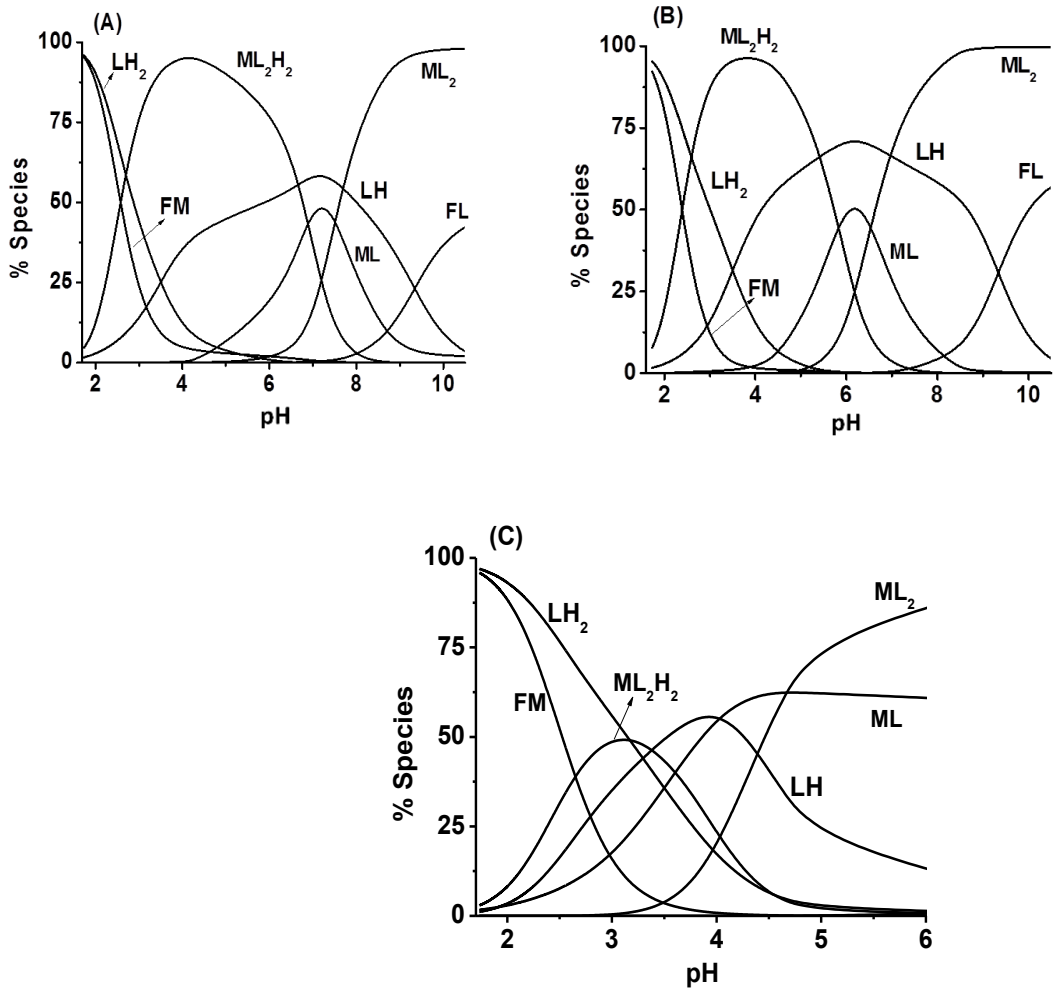


Fig 4.20: Distribution diagrams of Phe complexes in 20% v/v DMF-medium.

(A) Co(II) (B) Ni(II) and (C) Cu(II)

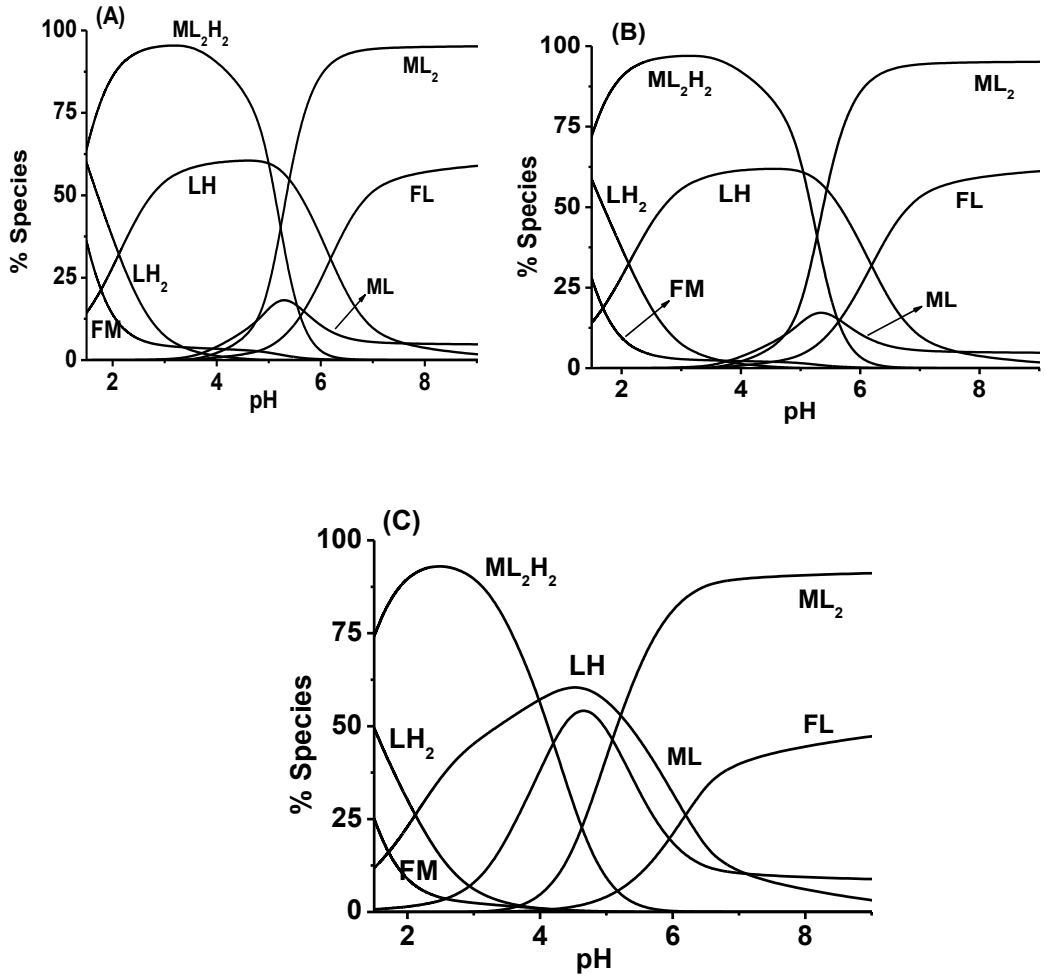


Fig 4.21: Distribution diagrams of binary complexes of MA in 10% DMF-water mixtures.
(A) Co(II), (B) Ni(II), and (C) Cu(II)

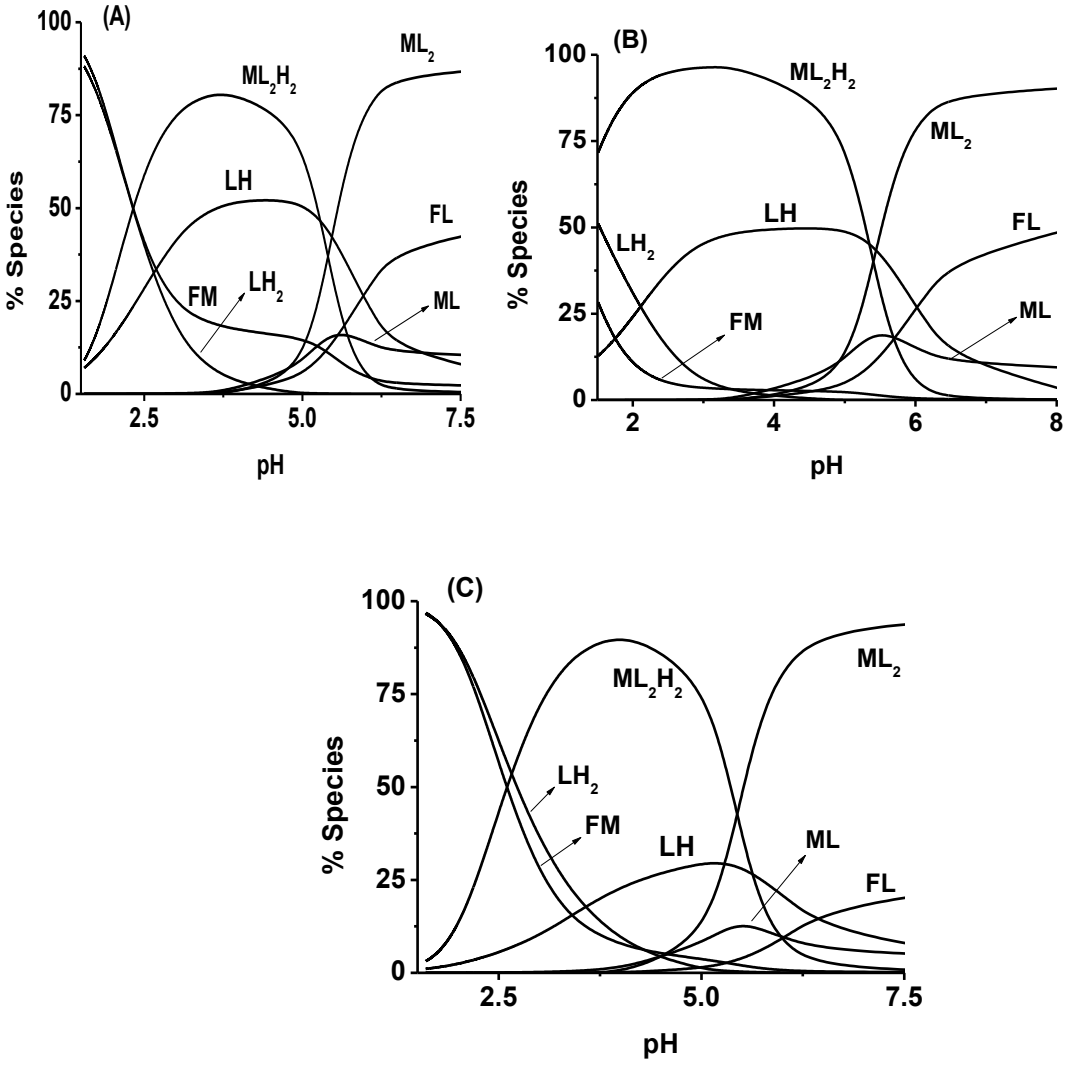
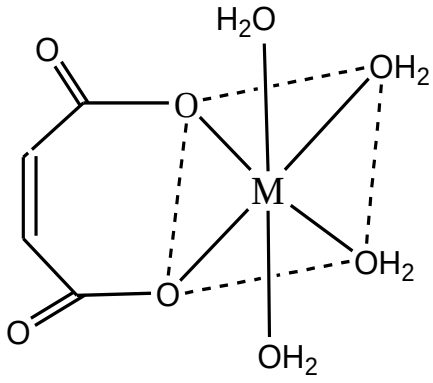


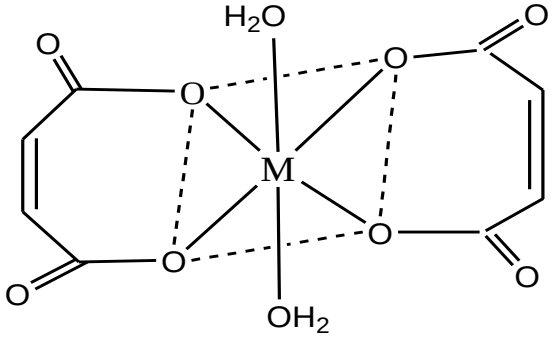
Fig 4.22**:** Distribution diagrams of MA complexes in 20% v/v DMF-water medium.
(A) Co(II) (B) Ni(II) and (C) Cu(II).

4.2.5. Structures of Binary Complexes

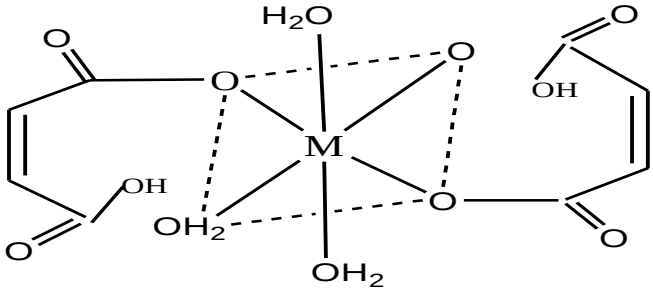
Although it is not possible to elucidate or confirm the structures of complex species pH metrically, they can be proposed based on the literature reports and chemical knowledge. In aqueous solutions, metal ions are coordinated by six water molecules. Amino acids replace water molecules and form metal-amino acid complexes [99]. Depending upon the nature of the ligands and metal ions and based on the basic chemical knowledge speculative structures of the complexes are proposed as shown in Figs. 4.23 and 4.24 for Phe and MA complexes. Carboxyl oxygen and amino nitrogen of ligands are bonded to the metal ions. The literature [100] suggests that Co(II) and Ni(II) complexes shall be octahedral and the Cu(II) ion forms distorted octahedral or square planar complexes due to Jahn-Teller effect.



ML

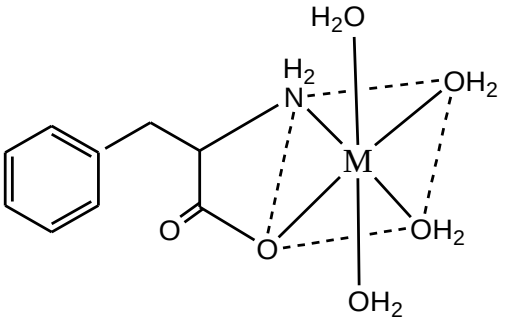


ML_2

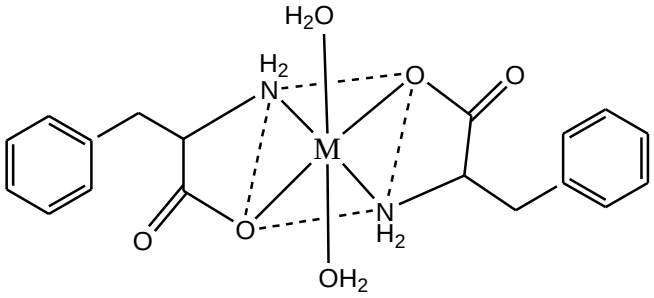


ML_2H_2

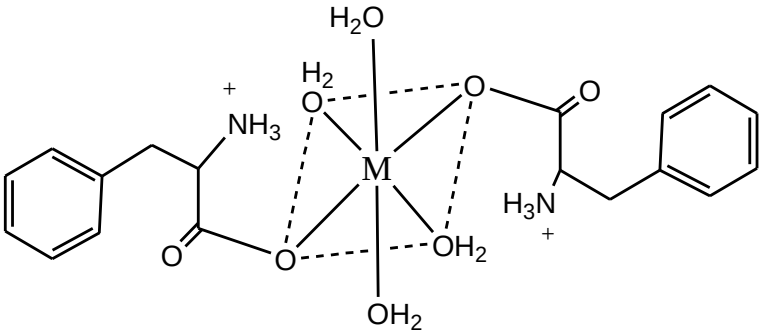
Fig 4.23: Speculative structures of binary complexes of MA with M(II).



ML



ML_2



ML_2H_2

Fig 4.24: Speculative structures of binary complexes of Phe with M(II).

CHAPTER-5

CONCLUSIONS AND RECOMMENDATIONS

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

The speciation studies of essential metal ion complexes are useful to identify those metal species that are likely to have adverse effect on biota. Better computing and analytical methods lead to an understanding of the speciation underlying many chemical reactions, with interesting conclusions, in industry, medicine and in the environmental studies.

The author has investigated the chemical speciation of L-phenylalanine and maleic acid complexes of Co(II), Ni(II) and Cu(II) in aqueous and DMF-water mixture. The following conclusions can be drawn based on the speciation studies performed on these systems.

5.1.1. Protonation Equilibria of L-phenylalanine and Maleic Acid

1. The investigation by the biomimetic studies indicates the pH ranges of protonation equilibria of Phe and MA in aqueous and DMF-water media as 1.8-11.5 and 1.7-10.0, respectively.
2. The log values of protonation constants of Phe and MA linearly vary with decreasing dielectric constant of DMF-water mixtures, indicating the dominance of electrostatic forces in the protonation-deprotonation equilibria.
3. In the present study overlapping formation curves for various concentrations of the ligands indicate non-polymerization of Phe and MA under the pH conditions employed.
4. The negative values of **a** (number of equivalents of alkali consumed per mole of ligand) correspond to the excess mineral acid present in the titrand and the associable protons of the ligands. The positive values of **a** correspond to the dissociable protons of the ligands. The pH values at half integrals of $\bar{n}_{H}$ values correspond to the pKa values of Phe and MA. Secondary formation functions indicate the number of protonation equilibria to be two for MA and Phe.
5. The effect of systematic errors in the influential parameters shows that the errors in the concentrations of alkali and mineral acid affect the protonation constants more than that of the ligand.

6. Phe has one dissociable carboxyl proton and one amino group which can associate with protons. It exists as LH_2^+ at low pH and gets deprotonated with the formation of LH and L^- successively with increasing pH.
7. MA has no amino groups with the formation of XH^-, X^{2-} exists up to a pH of 10.0. The mono protonated species XH^- exists in the pH range 1.70 - 9.0. Successive deprotonation of XH_2 with increasing pH forms ultimately X above a pH of 9.0 in DMF-water mixture.

5.1.2. Metal-ligand Complex Equilibria

1. The possibility of formation of poly nuclear complexes is excluded as the ratio of TL0 to TM0 is greater than 2. The presence of dimeric and trimeric species is ruled out since the experimental conditions are not favorable for their formation.
2. The ingredients that affect the magnitudes of stability constants of metal complexes were found to be: alkali > acid > ligand > metal. Introduction of errors worsened statistical parameters like standard deviation, skewness, kurtosis etc. This indicates that the experimental data gave best fit model than the data with errors, supporting the accuracy of the experimental results.
3. The binary species refined due to the interaction of Phe and MA with metals are ML, ML_2 and ML_2H_2 for Co(II), Ni(II) and Cu(II). These models are validated by statistical treatment of the data.
4. The stability constants of Phe and MA (log β) vary linearly with decreasing dielectric constant of DMF-water mixtures that indicates the dominance of electrostatic forces over non-electrostatic forces in the speciation of binary complexes.
5. The stabilities of the complexes followed the Irving-Williams order, i.e., Co(II) < Ni(II) < Cu(II).
6. The study also gave an insight into the metal ion availability/metal ion transport in bio-fluids which may help in assessing the toxicity of these metal ions if found to be in excess. The binary complexes with higher stability were more amenable for "metal transport" because of their extra stability and the binary complexes with less stability constants make the 'metal ion to be available' in biological systems due to their decreased stability.

5.2. Recommendations

In light of the finding the following recommendations were forwarded.

- Using the present findings of protonation and binary stability constants, the author recommends to carry out the chemical speciation of the ternary complexes containing L-phenylalanine and maleic acid with some essential metal ions in DMF-water mixture.
- Using the present findings data as a base line, the maximum yield of solid complexes can be synthesized at optimum conditions.

REFERENCE

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LIST OF PUBLICATIONS

1) Wondimu Tesfa Tefera, Wakgari Bungula Nagessa, Tegene Desalegn Zeleke, Hadgu Hailekiros Belay, Protoletic Equilibiria of L-phenylalanine and Maleic acid in Dimethylformamide-water mixture.

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2) Wondimu Tesfa Tefera, Wakgari Bungula Nagessa, 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).

3) Wakgari Bungula Nagessa, Wondimu Tesfa Tefera, Tegene Desalegn Zeleke, Tajedin Alansi, Hadgu Hailekiros Belay, Chemical Speciation of Binary Complexes of L-phenylalanine and Maleic Acid with toxic metal ions in Dimethylformamide-water mixtures.

Int.J.Pharm.drug. Anal. (accepted)

REPRINTS