CHEMICAL SPECIATION OF COMPLEXES OF L-VALINE AND L-PROLINE WITH ESSENTIAL METAL IONS (Ca, Zn, Mn) IN SODIUM LAURYL SULFATE-WATER MIXTURE

By

Chaluma Sori Weyessa

A Thesis Submitted to Department of Applied Chemistry

School of Applied Natural Science

Office of Graduate Studies

Adama Science and Technology University

July, 2020

Adama, Ethiopia

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DECLARATION

I hereby declare that this thesis entitled "**Chemical speciation of complexes of L-Proline and L-Valine with essential metal ions in sodium lauryl sulfate-water mixture**" submitted to Adama Science and Technology University for the award of degree of master of science in Chemistry is the result of work carried out under the guidance of Dr. Hadgu Hailekiros and Dr. Rajalakshmanan Eswaramoorthy during the period of September 2019 –July 2020. It is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or currently submitted for any other degree or diploma in Adama Science and Technology University or other Institutions having the same title and content.

Name of student	Signature	Date

July, 2020

Adama, Ethiopia

Approval Sheet

As thesis research advisors, we hereby certify that we have read and evaluated this thesis entitled **"Chemical speciation of complexes of L-Proline and L-Valine with essential metal ions in Sodium lauryl sulfate -water mixtures" prepared under our guidance, by Chaluma Sori Weyessa.** We recommended that it can be submitted as fulfillment of the thesis requirement.

Advisor	Signature	Date
Co-advisor	Signature	Date

Approval of Board of Examiners

We, the undersigned, members of the board of examiners of the final open defense by **Chaluma Sori** have read and evaluated his thesis entitled **"Chemical speciation of complexes of L-Proline and Valine with essential metal ions in SLS-water mixtures"** and examined the candidate. This is therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the degree of Master of Science in chemistry (Inorganic Chemistry)

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ACKNOWLEDGMENT

First of all, I would like to thank the almighty of God, the most gracious and merciful who gave me the strength, health and patience with his handful blessing to complete this study. I can do nothing without Him. Second, I would like to express my deepest appreciation and sincere gratitude to my advisor, Dr. Hadgu H/Kiros for his kind encouragement, guidance, support and supervisions, starting from proposal writing to the completion of this thesis. He kindly read this thesis and offered invaluable detailed advices on organization and the theme of the thesis.

I would also like to thank my Co-Advisor Dr. Rajalakshmanan E., for his continuous technical, practical and moral support from the starting to the end of my works. My special thanks goes to my parents for their support, endless love and blessing that enable me to reach at this stage. I would like to appreciate my colleagues, Analytical Chemistry Laboratory Technicians and all individuals who supported me by sharing ideas or offering material support which is an input for this work. Last, but not least, I would like to thank the Adama Science and Technology University for their financial support and giving me the sponsorship opportunity to continue my post graduate study.

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Lists of acronyms and Abbrevations

AA……..Amino acid

COST……..Concentration of solution by titration

IUPAC ……International Union of Pure and Applied Chemistry

NAP……..Number Associable protons .

NB……..Number of bonded

NDP……..Number of dissociable protons

P**….**…….L-Proline

SD ……. Standard deviation

SLS ……Sodium Lauryl Sulfate

SOD…….Superoxide dismutase

TLO ……Total initial Concentration of ligand

TLC ……Total metal concentration

THC……Total hydrogen concentration

TMC……Total metal concentration

TMO …….Total initial Concentration of metal

V…….L-Valine

μ……Ionic strength

Abstract

The chemical speciation of an element, either essential or toxic is relevant, because the chemical form in which an element enters the body, mostly determines its absorption and transport properties, and hence its biological and physiological activities. In this study, Chemical speciation of Protonation equilibria of L-Proline and L-Valine, binary and ternary complexes of Ca(II), Zn(II) and Mn(II) with L-Proline and L-Valine have been studied pH-metrically in the varying concentrations (0.0–2.5% w/v) of SLS-water mixtures maintaining an ionic strength of 0.16 mol L^{-1}*(NaCl) at 310K. The Protonation constant was calculated with the computer program SCPHD. Stability constants of binary and ternary have been calculated with MINIQUAD75. The best-fit chemical models were selected based on statistical parameters and residual analysis. In the present investigation, the protonation constants from the best fit models show the formation of* LH_2^+*, LH and* L^- *for both L-proline and L-valine. The binary species that are refined are* ML^+*,* MLH^{2+} *and* ML_2H^+ *for Ca(II), Zn(II) and Mn(II) with L-proline and L- valine. In mixed ligand speciation, alkalimetric titrations were carried out with different relative concentrations (M: L: X = 1:2.5:2.5, 1:2.5:5.0, 1:5.0:2.5) of metal (M) to L-proline (L) to L-valine (X). The species detected for mixed ligand complexes are protonated and unprotonated ternary species like MLX,* ML_2XH *and* $MLXH_2^{2+}$ *for Ca(II), MLX,* ML_2X^-*,* $MLXH_2^{2+}$ *for Zn(II) and MLX,* ML_2XH *and* $MLXH_2^{2+}$ *for Mn(II). The trend in the variation of step -wise protonation constant and stability constants with change in mole fraction of the medium were explained on the basis of electrostatic and non-electrostatic forces. The species distribution diagrams and the plausible equilibria for the formation of the species are also presented. The chemical speciation, metal bioavailability and transportation have been explained based on stability constant and distribution diagrams drawn using HYSS HYPERQUAD. The ternary complexes are more amenable for metal transport because of their extra stability while the binary complexes make the metal bioavailable due to their decreased stability.*

Key words: *Chemical speciation**,** Protonation Constant, Stability Constant, SLS, HYSS HYPERQUAD, MINIQUAD75*

CHAPTER 1

INTRODUCTION

1.1. Back ground of the study

Chemical speciation refers to the distribution of an element amongst chemical species in a system under specified conditions. The chemical speciation of an element, either essential or toxic is relevant, because the chemical form in which an element enters the body, mostly determines its absorption and transport properties, and hence its biological and physiological activities**.** Speciation study of essential metal ion complexes is useful to understand the role played by the active site cavities in biological molecules and the bonding behavior of protein residues with the metal ion [1]. Chemical speciation is an important aspect of environmental analysis and research. Chemical species is specific form of an element defined as to isotopic composition, electronic or oxidation state, or complex or molecular structure. Speciation analysis is analytical activities of identifying and measuring the quantities of one or more individual chemical species in a sample [2].

Metals in water, sediment and soil occur both as dissolved, colloidal and particulate species depending on the particular environmental conditions. Metal ions are complexed in fresh water, sea water, polluted water and biosystem with naturally occurring ligands and chelating agents such as humic substance, sulfides and pesticides. It is estimated that the 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 [3].

Chemical speciation of metals is important for understanding of their distribution, mobility, bioavailability, toxicity and for setting environmental quality standards [4]. The coordination behaviors of essential metal ions have great interest in the area of analytical, environmental, electrochemical and biological chemistry. The interaction of metals with amino acids, catechols, carboxylic acids and peptides has been the subject of much interest due to the importance of metals in many biochemical processes such as respiration,

metabolism and nerve transmission [5]. The essential metal ions like Ca(II), Mn(II), Zn(II), have significant role in complexation with amino acid and peptides in living system which act as model for many complexes metal-amino acid equilibria occurring in enzymatic process [6].

Amino acids are organic compounds that contain amine ($-NH_2$) and carboxyl (-COOH) functional groups, along with a side chain (R group) specific to each amino acid [7]. They are building blocks of protein. Amino acids have special importance compared to other chemical compounds in the sense that they are regarded as the foundation stones of living organisms. They are key precursors for syntheses of hormones and low-molecular weight nitrogenous substances, which have enormous biological importance. Besides their role as building blocks of proteins and polypeptides, some amino acid regulates key metabolic pathways that are necessary for maintenance, growth, reproduction and immunity [8]. They are called functional amino acid, which include arginine, cysteine, glutamine, leucine, proline and tryptophan. 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 [9].

L-Proline (P) is a non-essential imino acid containing pyrrole type of nitrogen rather than the amino nitrogen of the amino acids [10]. It acts as a bidentate ligand and actively participates in acido-basic equilibria especially in physiological pH conditions. Proline is one of the main components of the collagen. Since proline is necessary to make collagen, it helps strengthen cardiac muscle, improve skin texture and reduce the loss of collagen through the aging process. It promotes the formation of bone, skin and cartilage. It is extremely important for the proper functioning of joints and tendons. It has several significant applications in biological systems [11].

L-Valine (V) is an essential proteinogenic amino acid and not synthesized in mammals. It acts as a bidentate ligand with one carboxylic group and one amino group. It is essential in humans, meaning the body cannot synthesize it: it must be obtained from the diet. Valine participates in the detoxification of ammonia and works along with α-ketoglutarate. It is an

important amino acid in the prevention of muscle wasting in diabetes. Valine promotes muscle growth and tissue repair. It is a precursor in the penicillin biosynthetic pathway. It also lowers elevated blood sugar levels and increases growth hormone production. It has wide applications in pharmaceutical and food industries [12].

Sodium lauryl sulfate (SLS) is an anionic surface active agent used as an emulsifier in many pharmaceutical vehicles, cosmetics, foaming dentifrices, and even foods and it is the sodium salt of lauryl sulfate that conforms to the formula: $CH_3(CH_2)10CH_2OSO_3Na$ [13]. It is an anionic surfactant and profoundly influences the bulk properties of physiological systems. It can solubilise, concentrate and compartmentalize ions and molecules. Sodium lauryl sulfate–water mixtures are chosen in these studies to maintain the dielectric constants of the medium comparable to those of the physiological fluids since the polarity of the active site cavities should generally be applicable, to compare ligand binding to the metal ion in protein and mixed solvent environments. In this study, protonation equilibria and speciation of binary and ternary complexes Ca(II), Zn(II) and Mn(II) with L- Proline and L-Valine had investigated in SLS-water mixtures under conditions comparable to those of biological systems.

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 [14]. For instance, some elements can be highly toxic to various life forms; others are considered essential, but can become toxic at higher doses. Bioavailability of a particular metal depends on its complex chemical reactions of dissolution, binding and complexation with the constituents of the environmental aquatic media. 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. Essential metal ions such as Zn(II) and Mn(II) are associated with several enzymes and any variation in their concentrations leads to metabolic disorders [15]. Hence, extensive attention has been paid in recent years to the study of the chemical speciation of

ligands with metal ions. An exact model of the metal-protein system may be difficult to construct analytically in simple bio-molecules like amino acids and peptides but such models may give a tremendous amount of information about the structure of proteins and function of biomolecules in biological systems by chemical speciation method. Most of the speciation studies are performed in aqueous media under conditions comparable to those existing in physiological systems. These are taken as models for the systems existing in biofluids and natural water. But the active site of the biosystems have very low dielectric constant of different magnitude and metabolic reactions are carried out under strictly compartmentalized conditions. Some studies were performed in aqua-organic mixtures to mimic these conditions but no attempt was made to account for the compartmentalization**.** Previously`no report has appeared on studies speciation of L-Proline and L-Valine with essential metal ions in SLS-water mixture of various concentrations. Hence, the speciation of the essential metal ions (Ca, Zn and Mn) complexes of P and V has been studied in surfactant media where micellisation leads to compartmentalization.

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-Proline (P) and L-Valine (V) and stability constants of their binary and ternary complexes with biological essential metal ions (Ca, Zn, Mn) in SLS -water mixtures of varying compositions.

1.3.2. Specific Objectives

The specific objectives of the study are to:

i. Determine the protonation constants of P and V in SLS-water mixture
ii. Investigate the effect of dielectric constant of solvent on protonation equilibria of P and V in SLS-water mixture.
iii. Predict the distribution diagrams of P and V in SLS- water mixture
iv. Determine the binary and ternary stability constant of the complexes involving essential metal ions Ca(II), Zn(II),and Mn(II) with P and V in vitro under mimicked physiological conditions.
v. Investigate the effect of dielectric constant of solvent on the binary and ternary complexes of P and V with Ca (II), Zn (II) and Mn (II) ions in SLS-water mixture
vi. Predict the types of species of binary and ternary complexes of P and V with Ca (II), Zn (II) and Mn (II) in SLS-water mixture.
vii. Propose the structure of binary and ternary complexes of P and V with Ca (II), Zn (II) and Mn (II) ions.

1.4. Significance of the Study

Knowledge of chemical speciation of metal ions is needed in human biology, nutrition, toxicology, in biomedical applications, and in clinical practice. Because bioavailability, metabolism and excretion, toxicity, biological activity and detoxifying processes are dependent on the particular species considered [16]. In addition, transport of the metal in the body and its final deposition/storing in target organ (e.g. brain, liver and bone) also determined by the speciation of the element because of the possible ligand-exchange reactions with several potential metal ion-binding bio-molecules in the bio-fluids or tissues. 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. It can arise invaluable information about physiological stability in biological system. Furthermore, pharmaceutical industries, researchers and individuals who are concerned to work on a correlated project will for most beneficials of this study. Hence, the speciation studies on the protonation equilibria of L-Proline and L-Valine and their binary and ternary complexes with essential metals in varying compositions of SLS-water mixtures have been carried out.

CHAPTER 2

LETERETURE REVIEW

2.1. Chemical Speciation

Speciation studies of toxic and essential metal ion complexes are useful in order to understand the role played by the active site cavities in biological molecules and the bonding behavior of their residues with metal ions [17]. 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. Depending on the particular form in which the element is present in the system, some elements can be highly toxic to various life forms; others are considered essential, but can become toxic at higher doses. For example, Cr(VI) ions are considered far more toxic than Cr(III) [18]. Often these different chemical forms of a particular element or its compounds are referred to as 'species'.

Recently, speciation analysis plays a unique role in the studies of biogeochemical cycles of chemical compounds, determination of toxicity and Eco toxicity of selected elements, quality control of food products, control of medicines and pharmaceutical products, technological process control, research on the impact of technological installation on the environment, examination of occupational exposure and clinical analysis [19].

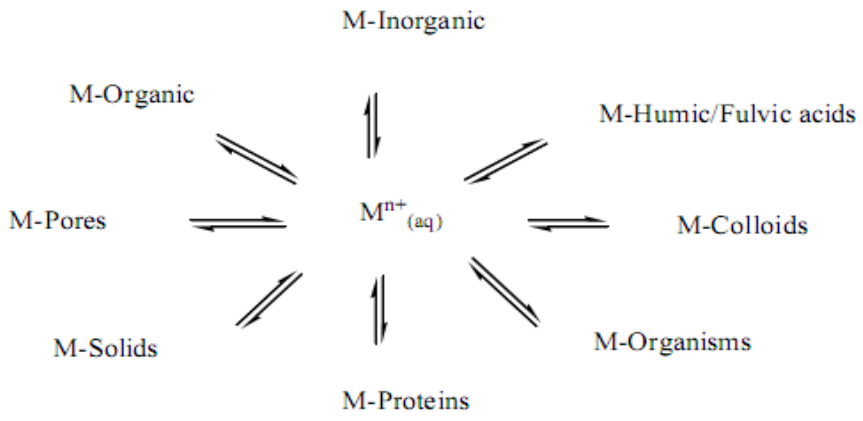


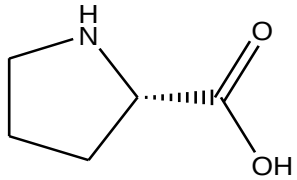
Figure 1: Speciation scheme suggesting the possible distribution of metal ions (M^{n+}) in the environment

2.1. Biological functions of amino acids

Amino acids are building blocks of protein. They join to form short polymer chains called peptides or longer chains called either polypeptides or proteins [20]. Amino acids are important low molecular weight ligands in humans and other biosystems. Physiological concentrations of AA and their metabolites (e.g., nitric oxide, polyamines, glutathione, taurine, thyroid hormones, and serotonin) are required for the biological functions. The oxidation of amino acids makes a significant contribution towards generation of metabolic energy. This energy varies greatly with the type of organism and with the metabolic situation in which an organism finds itself. The functional group of amino acid have potential to interactions with divalent metal ions which play an important role in promoting and maintaining their functionalities [21].

2.2.1. L-Proline

L-Proline (2-pyrrolidine carboxylic acid) is an imino acid containing pyrrole type nitrogen rather than the amino nitrogen of the amino acids. The molecular formula of L-proline is $C_5H_9NO_2$. Proline is one of the 20 DNA encoded amino acids. It is unique in that the α-amino group is secondary [22]. It is a bidentate ligand. It has one dissociable proton (carboxyl) and one imino group which can associate with a proton.



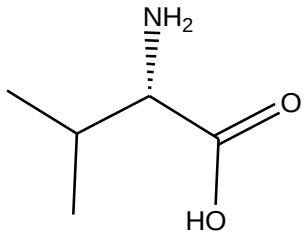
L-proline

Figure 2: Structure of L-proline

It can be derived from animal products such as red meat, fish, dairy products, eggs, beef and poultry. It is synthesized by the body through the breakdown of L-glutamate.

2.2.2. L-Valine

L-Valine is a non-polar, hydrophobic, branched chain, essential α-amino acid with aliphatic R-group. It is chemically neutral in nature. The chemical formula of valine is $HO_2CCH(NH_2)CH(CH_3)_2$. Valine is an essential amino acid important for smooth nervous system and cognitive functioning. Lack of L-valine may influence the growth of body, cause neuropathic obstacle and anemia. Valine promotes muscle growth and tissue repair. It is a precursor in the penicillin biosynthetic pathway. It also lowers elevated blood sugar levels and increases growth hormone production. It is a bidentate ligand that has one dissociable and one associable proton. L-Valine is an essential proteinogenic amino acid [23].



L-valine

Figure 3: structure of L-valine

2.2. Surfactant

Surfactant (surface active agent) is an agent that decrease the surface tension between two media. The surface tension may develop between solid-liquid, liquid-liquid or liquid -gas media. Surfactants are usually organic compounds that are amphiphilic, meaning they contain both hydrophobic groups (their tails) and hydrophilic groups (their heads). A surfactant contains both a water-insoluble (or oil-soluble) component and a water-soluble component [24]. The addition of surfactants to protein solutions changes the physical properties of the protein i.e. unfolds proteins and causes aggregation and adsorption of proteins to surfaces. Both the structural stability of the protein and the molecular structure of the surfactant (charge, length, shape of the polar and apolar part of the surfactant) has an impact on their mutual binding affinity. The nature of the interactions of proteins with ionic surfactants is both electrostatic and hydrophobic [25].

2.2.1 Sodium Lauryl sulphate

Sodium lauryl sulphate (SLS) or sodium dodecyl sulphate is an anionic surfactant used in many cleaning and hygiene products, food, pharmaceuticals, and cosmetics. It is an anionic surfactant and profoundly influences the bulk properties of physiological systems. They can solubilise, concentrate and compartmentalize ions and molecules [26]. SLS has a negatively charged sulfonate group as its "hydrophilic" end and a saturated 12-carbon chain for its "lipophilic" end. SLS has a faint odor of fatty substances and at room temperature, occurs as white or cream-colored crystals, flakes, or powder or a clear to yellowish thick fluid. This compound works by disrupting non-covalent bonds in the proteins, denaturing them, and causing the molecules to lose their native shape (conformation)

SLS has the chemical formula $C_{12}H_{25}NaO_4S$ or CH_3-$(CH_2)11$-O-SO_3-Na^+ and its structure is presented in figure 4.

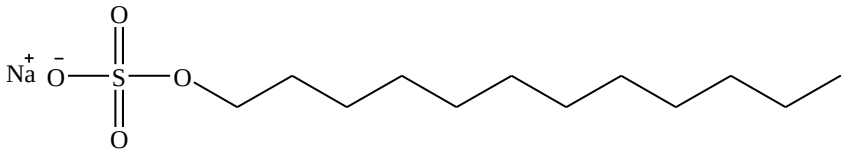


Figure 4: chemical structure of Sodium Lauryl Sulfate

2.3. Essential Elements

Chemical elements in both free state and a variety of chemical compounds are included in all cells and tissues of the human body. A chemical element is considered essential, if its absence or insufficient intake to the organism is accompanied by disturbance of normal life, delayed development, inability to reproduction [27]. The term trace elements refer to chemical elements present in a natural material at very small amounts. In biochemistry, a trace element is a dietary mineral that is needed in very minute quantities for the proper growth, development, and physiology of the organism [28]. Trace elements have several important roles in human bodies, some are essential for enzymes reactions where they attract and facilitate conversion of substrate molecules to specific end products. An element is considered essential to an organism when reduction of its exposure below certain limit results consistently in a reduction in a physiologically important function, or when the

element is integral part of an organic structure, preforming a vital function in the organism [29].

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 tissue is to be safeguared 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. The most well-known essential trace elements are: cobalt, copper, nickel, iron, zinc, manganese, chromium, molybdenum and iodine. Many of them act as catalysts in many enzymatic functions called as metalloenzyms [30].

2.3.1. Zinc

Zinc is an essential trace element for both animals and humans**.** Zinc plays vital roles in biological systems [31]. It can play either a predominantly catalytic role or a solely structural role to maintain the protein configuration. It is a versatile ion, as it can bind with different combinations of ligand types resulting in a broad range of stability, reactivity and functions. It is an essential trace element that functions as a cofactor for certain enzymes involved in metabolism and cell growth, it is found in nearly 300 specific enzymes [32]. The role of zinc in these metalloenzymes includes participation in catalytic functions, maintenance of structural stability and regulatory functions.

Zn is involved in the metabolism of proteins, carbohydrates, lipids, and energy. Zn is vital for the healthy working of many of the body's systems; it plays an essential role in numerous biochemical pathways. It is particularly important for healthy skin and is essential for a healthy immune system and resistance to infection. It plays a crucial role in growth and cell division where it is required for protein and DNA synthesis, in insulin activity, in the metabolism of the ovaries and testes, and in liver function [33]. In blood plasma, Zn is bound to and transported by albumin (60%, low-affinity) and transferrin (10%). Two examples of zinc-containing enzymes are carbonic anhydrase and

carboxypeptidase, which are vital to the processes of carbon dioxide (CO_2) regulation and digestion of proteins, respectively [34].

2.3.2. Manganese

Manganese is an essential trace element for human beings. It is essential for normal bone structure. The classes of enzymes that have manganese cofactors are very broad and include oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. It is a component of several enzymes, for example superoxide dismutase, pyruvate carboxylase and glutamine synthetase. [35]. Manganese exhibits a large scale of oxidation states from -III to +VII which makes this element an important cofactor of many enzymes, in particular for the catalysis of electron transfer reactions, e.g. Mn superoxide dismutase [36]. In biological systems the oxidation levels +II and +III are predominantly present. Mn helps the body to form connective tissue, bones, blood-clotting factors, and sex hormones. It also plays a role in fat and carbohydrate metabolism, calcium absorption, and blood sugar regulation. Mn is also necessary for normal brain and nerve function. In the human brain, the manganese is bound to manganese metalloproteins, most notably glutamine synthetase in astrocytes. Manganese deficiency in human being causes skeletal deformities due to impaired bone growth and reduced reproductive functions. It is mostly concentrated in the liver and kidneys [37]. In the human brain, manganese is bound to manganese metalloproteins, such as glutamine synthetase in astrocytes [38].

2.3.3. Calcium

Calcium is the most abundant metallic element in the human body, one of the most abundant in other organisms and the third most abundant element in vegetation. These facts alone suggest that this substance play a vital role in the maintenance, viability and regulation of certain biological system. Calcium is a structural component of bones, teeth and soft tissues and activates many metabolic processes It helps in blood clotting, nerve signaling and the release of certain hormones. It plays an important role in the cell membranes and as a structural component and also in muscle contraction. Ca^{2+} ions are essential components of plant cell walls and cell membranes and are used to balance organic anions in the plant vacuole [39]. Many different biological processes in eukaryotic cells are regulated by intracellular Ca^{2+} concentration levels. Examples of such processes

are muscle contraction, transport processes, cell division and growth, enzyme activities, and metabolic processes. The balance between the uptake of calcium from the blood for the formation of bone and the re-entry of calcium into the blood with resorption of bone is under hormonal control [40].

2.4. Chemical Speciation in Biological System

Among all chemical elements in the periodic table, biological systems utilize only a few [41]. In terms of trace element speciation, in addition to thermodynamic stability, kinetic factors strongly affect the occurrence of species in a biological system, organ, organ compartment, etc. The ligand exchange reactions of different metal ions are variable and their rate is influenced greatly by the chemical environment where the metal ion occurs. A high degree of chemical speciation can exist for any trace element in biological systems. Due to the predominantly aqueous nature of biological systems biologically active forms of all metallic elements tend to be ionic, these elements being complexed by molecules possessing electron-donating functional groups. Biological metals may exist in different forms, including as hydrated ions, tightly bound forms such as metal-bound cofactors and protein- or nucleic-acid bound species, or loosely bound forms in association with a diverse heterogeneous buffer, which can consist of low molecular weight species such as amino acids, glutathione, or citric acid, and labile species. The total metal content consists of the sum of all of these diverse forms [42].

2.5. Protonation constant

The data related to the protonation constants of biologically active ligands in various media will be valuable in further understanding of their chemistry in biological systems. Protonation constant of amino acids can be determined by analysis of acid –base titrations [10]. Knowledge of the equilibrium constants of some compounds is necessary for the calculation of the concentration of each ionized species at any pH, which is important for the complete understanding of the physicochemical behavior of such molecules. In addition, for the calculations of stability constants of the complex formation of biologically active ligands with metal ions, their protonation constants are used [43].

Protonation constant is an equilibrium constant that exist between ligand and proton. It can be calculated as:

$$L + H \rightleftharpoons LH; \qquad K_1^H = \frac{[LH]}{[L][H]} \quad \text{.....2.1}$$

$$LH + H \rightleftharpoons LH_2; \quad K_2^H = \frac{[LH_2]}{[LH][H]} \quad \text{.....2.2}$$

Natural and bio-fluids have a wide range of ionic strength and composition. Therefore, different equilibrium constant for a complex would have to be used for each individual system. The constant is used to calculate the speciation of the complex and its dissociation products in the fluid, which determine the mobility and toxicological effects of the complex. So, an understanding of the protonation-deprotonation equilibria of L- proline and L-valine is necessary before an attempt is made to investigate the metal-ligand equilibria associated with these ligands. Protonation equilibria of L-proline and L-valine in varying compositions (0.0-60.0% v/v) of 1, 2-Propanediol-water mixtures were investigated pH-metrically by Bhushanavathi *et al* [10] .

Table 1: Protonation constants of L-proline reported in literature

S.No.	Medium	Instrumental technique	Computer Program used	Ionic strength (mol dm^{-3})	LogK		Ref
					LogK1	LogK2	
1	Aqueous	pH metry	MINIQUAD75	0.15 KNO_3	10.46	12.41	[10]
2	"	Potentiometry	MINIQUAD75	0.10$NaNO_3$	10.52	12.03	[44]
3	"	"	MINIQUAD75	0.10$NaNO_3$	10.49	12.03	[45]
4	20%Dioxane	"	CHEMEQ	0.10KNO_3	10.30	12.52	[46]
5	40%methanol	"	CHEMEQ	0.10KNO_3	10.10	12.60	
6	aqaueous	pH metry	…….	0.10NaCl	9.25		[47]

Table 2: Protonation constants of L-valine reported in literature

S.No.	Medium	Instrumental technique	Computer Program used	Ionic strength (mol dm^{-3})	LogK		Ref
					LogK1	LogK2	
1	aqueous	pH metry	SCOGS	0.15 KNO_3	9.32	11.66	[48]
2	"	Potentiometry	CHEMEQ	0.10KNO_3	9.48	11.77	[49]
3	"	pH metry	SUPERQUAD	0.15KNO_3	9.31	11.55	[10]
4	0.3%Dioxane	Potentiometry	ESAB & PKPOT	0.10$NaNO_3$	9.67		[50]
5	20% Ethanol	"	PKAS	0.10NaCl	9.18	11.94	[51]
6	80%Ethanol	"	"	"	8.92	11.98	

2.6. Stability constant

The formation of various simple as well as ternary (mixed ligand) complex is always possible, depending on the affinity of the metal ions towards the various ligand present and the relative coordination sites [52]. Biochemical process in living system kept maintains their stability due to interaction of mixed metal ligand complex formation. Stability constant is well known tool for solution chemist, biochemist, and chemist to help for the determination of the properties of metal-ligand reaction in water and biological system. The formation of metal complexes can be represented as:

$$pM + qL + rH \rightleftharpoons M_p L_q H_r \quad \ldots\ldots\ldots\ldots 2.3$$

The equilibrium constant is also called as stability constant or formation constant is expressed as:

$$\beta_{pqr} = [M_p L_q H_r] / [M]^p [L]^q [H]^r \quad \ldots\ldots\ldots\ldots\ldots\ldots 2.4$$

In a system containing one metal ion (Mn^+) and two ligands, L and R with similar coordination tendencies lead to complexation simultaneously to form the mixed ligand complex, MLR

$$M + L + R \rightleftharpoons MLR \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 2.5$$

$$\beta_{111} = {}^{[M\ L\ R]}\Big/_{[M]\ [L]\ [R]} \quad \text{……2.6}$$

2.6.1. Binary and ternary stability constant of L-proline and L-valine complexes

Binary and ternary chelations occur commonly in biological fluids, as millions of potential ligands like amino acids, peptides or their derivatives or analogues, and heterocyclic N-bases are likely to compete for biologically important transition metal ions such as Cu(II), Ni(II) and Zn(II) found in vivo. Several literature reports have been devoted to the study of stability constants of metal-proline and valine complexes. Formation constants of mixed ligand complexes of Mn(II), Fe(II), Ni(II) with histidine as primary ligand and glutamic acid, proline and aspartic acid as secondary ligand were determined pH metrically by Durrani at 25℃ and 1.0 mol dm^{-3} ionic strength in aqueous and ethanol-water media [53]. Peketi Bhushanavathi & Gollapalli Nageswara carried out pH metric studies on the binary complexe formations of Co(II), Ni(II) and Cu(II) with L-valine [54].

Stability constants of ternary complexes of complexes of Ca(II), Zn(II) and Mn(II) with L-proline and L-valine was studied by Veeraswami *et al* in various concentrations (0–60% v/v) of acetonitrile–water mixtures at 303.0 K and ionic strength of 0.16 mol L^{-1} [55]. Potentiometric technique was used to study the binary and ternary complexes of Ni(II) with adenine as primary ligand and glycine, alanine, valine, proline, threonine, methionine, histidine, histamine, imidazole, lysine and ornithine as secondary ligands [45]. Binary and ternary complexes of Cu(II) with glycine, alanine, valine, cystene and penicillamine as primary ligands and nicotinamide as secondary ligand were studied potentiometrically in aqueous medium [56].

Table 3: Stability constants of complexes of proline with metal ions reported in literature

Metal ion	Medium	Instrumental technique	Computer Program used	$Log\beta_{mlh}$			Ref
				110	120	130	
Cu(II)	Aqueous	Potentiomety	CHEMEQ	8.73	16.08		[49]
Cu(II)	40%methanol	Potentiometry	CHEMEQ	9.11	16.74		[46]
Cu(II)	20% Meth	Potentiometry	CHEMEQ	8.91	16.42		
Ni(II)	Aqueous	Potentiometry	MINIQUAD 75	6.12	10.86		[45]
Co(II)	Aqueous	Potentiometry	ORIGIN 50			29.03	[57]
Fe(II)	"	"	"			28.78	

Table 4: Stability constants of complexes of L-valine with metal ions reported in literature

Metal ion	Medium	Instrumental technique	Computer Program used	$Log\beta_{mlh}$			Ref
				110	120	130	
Cu(II)	Aqueous	Potentiometry	CHEMEQ	8.10	14.9		[49]
Cu(II)	"	Potentiomety	MINIQUAD 75	8.02	14.98		[58]
Pd(II)	"	Potentiometry	MINIQUAD 75	10.33			[44]
Co(II)	30% acetonitrile	pH metry	MINIQOUD 75	4.4	8.07		[15]
Ni(II)	Aqueous	Potentiometry	SUPERQUAD	5.09	9.20	11.35	[59]
Zn(II)	Aqueous	pH metry	SCOGS	5.13	9.69	11.16	
Cu(II)	"	"	"	7.9	14.55		[48]

Table 5 : Stability constant of mixed ligand complexes reported in literature

System	$\frac{\text{Log}\beta\text{mlxh}}{1110}$	T°C	Ionic strength mol dm^{-3}	medium	ref
Mn(II)-his – proline	8.76	25	1.0 $NaClO_4$	aqueous	[60]
Fe(II)-his-proline	5.25	25	1.0 $NaClO_4$	aqueous	
Ni(II)-his-proline	7.92	25	1.0 $NaClO_4$	aqueous	

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

The materials used in this study were: Test tubes, pipette, pH meter, burette, glass electrode, funnel, 250 Erlenmeyer flasks, mass balance, measuring cylinder, dropper, volumetric flask, and stirrers, beaker

3.2. Chemicals

The chemicals used in this study were: L- proline (SRL, India), L- valine (SRL, India), Sodium lauryl sulfate, HCl, acetone, distilled water, NaOH pellet, Oxalic acid, potassium hydrogen phthalate, borax, EDTA, $ZnSO_4$, $CaCl_2$, $ZnCl_2$, $MnCl_2$, NaCl, and Eriochrome-black T indicator

3.3. Experimental procedure

3.3.1. Preparation of Solutions

3.3.1.1. L-proline and L-valine solution preparation

0.05 mol dm^{-3} Aqueous solutions of P and V AR grade (SRL, India) were prepared by dissolving samples in water. To increase the solubility of the ligands, 0.05 mol dm^{-3} hydrochloric acid concentration was maintained in their solutions. The probable errors that might have crept into the concentrations of the stock solutions of the ligands were determined by the computer program COST [61]. 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 approximately 0.1 mol dm^{-3} solution was prepared and standardized complexometrically with a 0.1 mol dm^{-3} standard zinc sulphate solution using Eriochrome Black-T as indicator.

3.3.1.3 Metal ion solutions

0.1 mol dm^{-3} Aqueous solutions of Ca(II), Zn(II) and Mn(II) chlorides were prepared by dissolving AR grade (Merck, India) salts in distilled water. The stock solutions were made slightly acidic to suppress hydrolysis of the metal ions. The concentrations of the metal

ions were determined complexometrically by titrating against a standard solution of EDTA using Eriochrome Black-T as indicator. The free hydrogen ion concentration in the stock solution was determined by Gran plot method [62].

3.3.1.4 Sodium hydroxide

A stock solution of 2.0 mol dm^{-3} sodium hydroxide was prepared by dissolving AR grade (Merck, India) sodium hydroxide pellets in distilled water. The solution was further diluted to the required concentration (0.4M). The strength of the alkali was determined by titrating it against a standard solution of oxalic acid and potassium hydrogen phthalate. 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) [63]. The strength of alkali was determined using the Gran plot method [62].

3.3.1.5 Hydrochloric acid

The concentration of stock hydrochloric acid (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 normality was determined with standardized sodium hydroxide.

3.3.2 Electrometric titration assembly

A Digital pH meter (Model Ad8000, India) of readability (0-14 pH) in conjunction with a glass combination pH electrode was used to monitor the changes in hydrogen ion concentration during either acido-basic or complex equilibria

3.3.3 Calibration of glass combination pH electrode

Potassium hydrogen phthalate (0.05 mol dm^{-3}) and borax (0.01 mol dm^{-3}) solutions were used to preliminarily check the response of the glass electrode in acidic (pH=4.01) and basic (pH=9.18) regions. The electrode system was calibrated in terms of hydrogen ion concentration instead of activities. It is assumed that the activity coefficient is constant, an assumption usually justified by working in a medium of a constant ionic strength (0.16 mol dm^{-3} sodium chloride). In electrometric studies, the glass electrode response is to be

established over a wide range of pH. The acid-base titrations were analysed by using the Gran plot method in which the functions ø and ø' were calculated by using the formulae

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

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

A typical Gran Plot is given in Fig. 5

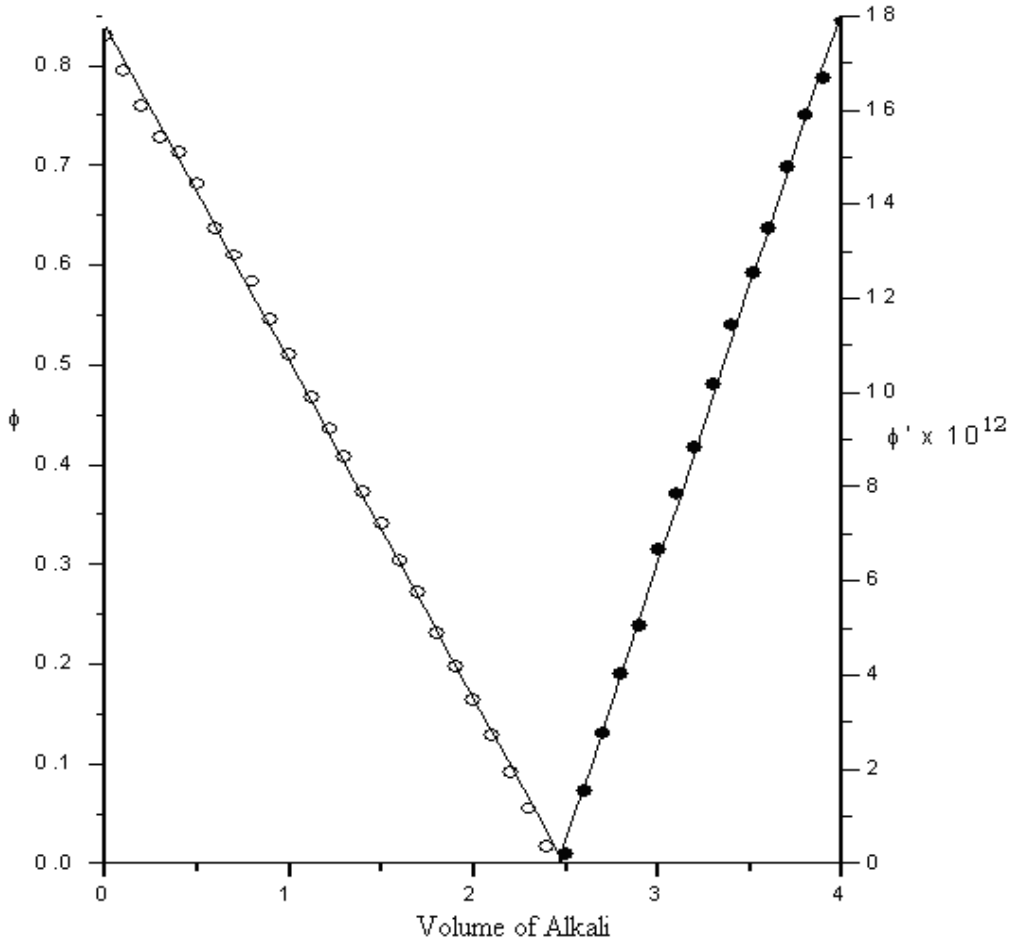


Figure 5: Gran plot for the titration of hydrochloric acid with alkali.

3.3.4 Equilibration of electrode in aqua- SLS mixtures

In order to obtain steady and reproducible results in aqua-surfactant media the electrode has to be pre-equilibrated with the respective media. In the present study the glass combination pH electrode was immersed in a well stirred mixture of required composition of surfactant-water mixture for 2 days before use. The electrode was tested intermittently by titrating a strong acid with sodium hydroxide in the medium of identical composition.

Before each titration in surfactant media the electrode was pre-conditioned for at least one hour in a solution of identical composition as that of the titrand. This practice resulted in a reasonably consistent and appropriate hydrogen ion activity in the outer layer of the glass membrane.

3.3.5 Determination of correction factor

The effect of variation in asymmetry potential, liquid junction potential, activity coefficient, sodium ion error and/or dissolved carbon dioxide on the response of glass electrode is to be considered for accurate determinations. The correction factor proposed by McBryde and others to account for the above parameters was used in the present electrometric titrations [64]. The simulated acid-base titrations data (pHC_i) calculated by SCPHD programs were used to compute correction factor (log F) for each of the solvent compositions. The log F value was used to convert pH meter dial reading into logarithm of reciprocal of hydrogen ion concentration (pHE_i) according to the Eq. 3.1.

$$\log F = pHC_i - pHE_i \quad \text{……..(3.1)}$$

Each point in the titration data past the equivalence point was corrected for sodium ion error (~<0.05 units) using the data available for aqueous system.

3.3.6 Titration procedure

The titrations were carried out in media containing varying amounts of surfactants maintaining an ionic strength of 0.16 mol dm^{-3} with NaCl at 310 K. In these titrations, the titrand consisted of mineral acid and ligands (P and V) in the presence and absence of metal ion, in a total volume of 50 cm^3. Titrations were performed by adding each time 0.1cm^3 portions of 0.4 mol dm^{-3} sodium hydroxide to the titrand. The details of experimental procedure and titration assembly have been detailed elsewhere [65]. The pH meter reading was recorded only after a constant value was displayed. Typical duplicate titrations showed that equilibration was fast and titration data did not differ by more than 0.02 pH units. A new glass combination pH electrode well tested in aqueous medium was used for each solvent system.

3.3.5.1 Proton - ligand equilibria

Requisite amounts of sodium chloride solution (to give overall concentration of 0.16 mol dm^{-3}), water and surfactant were added such that the overall percentages of SLS were in the range of 0.0-2.5% w/v for SLS. The amount of mineral acid maintained was 1 mmol, while the concentration of ligand was of the order of 0.25-0.50 mmol in different experiments.

Table 6: Total initial concentrations of ingredients (in mmol) in proton-ligand titrations in SLS- water mixtures. [NaOH] = 0.4 mol dm^{-3}; V_0 = 50 cm^3; Temperature = 310.0 K; Mineral acid = 1.0 mmol; μ = 0.16 mol dm^{-3}.

(% w/v) of SLS	No of titration	TL0	
		P	V
0.0	3	0.2490	0.2486
		0.3737	0.3735
		0.4981	0.4977
0.5	3	0.2504	0.2500
		0.3757	0.3764
		0.5000	0.5024
1.0	3	0.2473	0.2503
		0.3702	0.3750
		0.4955	0.5007
1.5	3	0.2486	0.2466
		0.3745	0.3745
		0.4973	0.4999
2.0	3	0.2480	0.2478
		0.3737	0.3747
		0.4971	0.4988
2.5	3	0.2513	0.2492
		0.3718	0.3741
		0.5036	0.4993

3.3.6.2 Metal-ligand complexes

In each of the titrations, the titrand consisted of 1 mmol hydrochloric acid in a total volume of 50 cm^3 and the ionic strength was adjusted to 0.16 mol dm^{-3} with sodium chloride. Solutions containing metal ions and ligands (metal to ligand ratios being in the range of 1:2.5 to 1:5) were titrated with 0.4 mol dm^{-3} sodium hydroxide. In these titrations metal ion was the last component added to the titrand. The actual concentrations of the constituents in different percentages of the SLS is given in Table 7. The stability constants of binary species of metal complexes with P and V were calculated from alkalimetric titrations obtained in the presence of different ratios of initial concentration of metal to ligand.

Table 7: Total initial concentrations of ingredients (in mmol) for metal-ligand titrations in SLS-water mixtures: [NaOH] = 0.4 mol dm^{-3}; V_0 = 50 cm^3; Temp = 310.0 K; Mineral acid = 1.0 mmol; μ = 0.16 mol dm^{-3}

(%w/v) of SLS	No. Of titration	TM0			TL0		TL0/TM0
		Ca(II)	Zn(II)	Mn(II)	P	V	
0	3	0.0892	0.0977	0.0844	0.254	0.252	2.50
					0.373	0.375	3.75
					0.500	0.501	5.00
0.5	3	0.0892	0.0977	0.0844	0.251	0.253	2.50
					0.373	0.377	3.75
					0.500	0.501	5.00
1	3	0.0892	0.0977	0.0844	0.253	0.248	2.50
					0.375	0.374	3.75
					0.504	0.497	5.00
1.5	3	0.0892	0.0977	0.0844	0.250	0.245	2.50
					0.375	0.376	3.75
					0.504	0.496	5.00
2	3	0.0892	0.0977	0.0844	0.256	0.245	2.50
					0.383	0.375	3.75
					0.500	0.490	5.00
2.5	3	0.0892	0.0977	0.0844	0.250	0.248	2.50
					0.376	0.373	3.75
					0.504	0.496	5.00

3.3.6.3 Mixed-ligand complexes

Titrations were carried out in the presence of different relative concentrations of metal (M) to primary (P, L) and secondary (V, X) ligands (M:L:X=1.0:2.5:2.5, 1.0:2.5:5.0 and 1.0:5.0:2.5) with sodium hydroxide. The other conditions were similar to those mentioned earlier. The actual concentrations of each of the ingredients are given in Table 8.

Table 8: Total initial concentrations of ingredients (in mmol) for mixed-ligand titrations in SLS-water mixtures: [NaOH] = 0.4 mol dm^{-3}; V_0 = 50 cm^3; Temp = 310.0 K; Mineral acid = 1.0 mmol; μ = 0.16 mol dm^{-3}

(%w/v) of SLS	No. Of titration	TM0			TL0		TM0: TL0:TX0
		Ca(II)	Zn(II)	Mn(II)	P	V	
0	3	0.0892	0.0977	0.0844	0.250	0.252	1.0:2.5:2.5
					0.251	0.502	1.0:2.5:5.0
					0.503	0.250	1.0:5.0:2.5
0.5	3	0.0892	0.0977	0.0844	0.250	0.251	1.0:2.5:2.5
					0.250	0.502	1.0:2.5:5.0
					0.502	0.250	1.0:5.0:2.5
1	3	0.0892	0.0977	0.0844	0.254	0.248	1.0:2.5:2.5
					0.254	0.498	1.0:2.5:5.0
					0.506	0.248	1.0:5.0:2.5
1.5	3	0.0892	0.0977	0.0844	0.250	0.247	1.0:2.5:2.5
					0.252	0.497	1.0:2.5:5.0
					0.501	0.247	1.0:5.0:2.5
2	3	0.0892	0.0977	0.0844	0.251	0.248	1.0:2.5:2.5
					0.251	0.498	1.0:2.5:5.0
					0.501	0.248	1.0:5.0:2.5
2.5	3	0.0892	0.0977	0.0844	0.251	0.246	1.0:2.5:2.5
					0.251	0.496	1.0:2.5:5.0
					0.502	0.248	1.0:5.0:2.5

3.4 Processing of data and modeling strategy

3.4.1 Speciation by computer modeling

Chemical speciation is often studied by using experimental methods however an alternative approach involves the application of theoretical chemical concepts to predict the distribution and transformations of chemical species. For equilibria in solution, usually the equilibrium constants are measured for the individual reactions and the species concentrations are calculated by solving the mass-balance equations [66]. The models use the law of mass action and mass balance and thermodynamic formation constants for all of the possible metal-ligand species, in conjunction with defined parameters such as pH, redox potentials and total metal and total ligand concentrations.

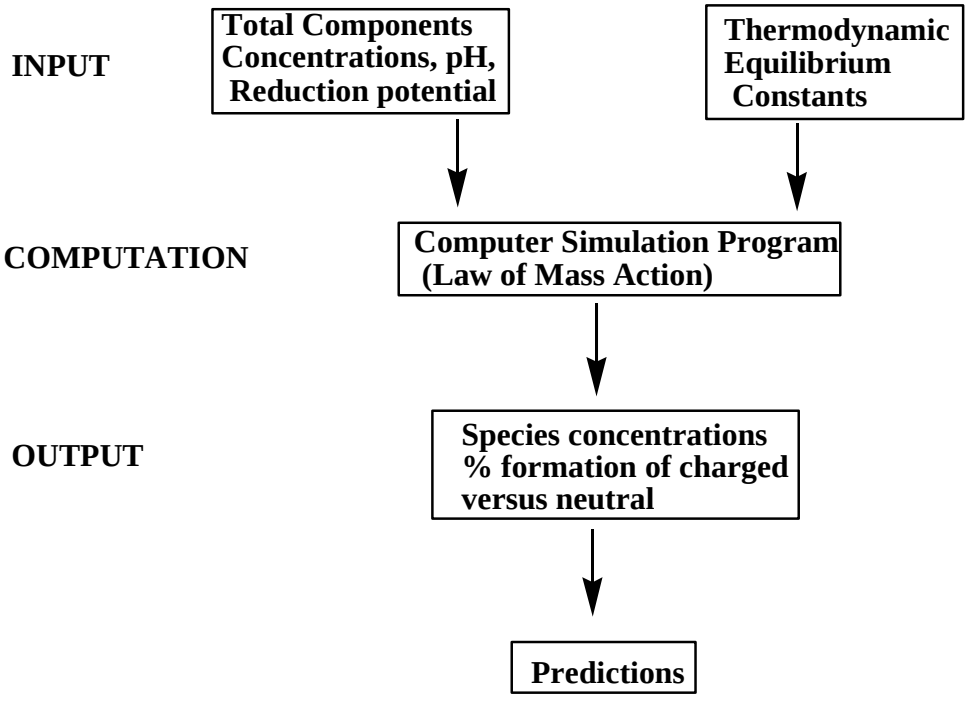


Figure 6: Basic components of a chemical speciation model

3.4.2. Software for modeling complex equilibria

Glass electrode measures protons associated with the solvent (including those in looser combinations). The appropriate protonation constants of P and V was calculated with the computer program SCPHD. The binary and ternary 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 [67]. The chemical speciation, metal bioavailability and transportation were explained based on the distribution diagrams drawn using HYSS HYPERQUAD [68].

3.4.3 Formulation of mathematical model

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

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

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

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

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. 3.2.

$$m(j)M + l(j)L + h(j)H \Leftrightarrow M_{m(j)}L_{1(j)}H_{h(j)} \qquad (3.2)$$

Then the formation constant for the j^{th} species can be written as Eq. 3.3.

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

$$= \frac{CS_{j,i}}{FM_i^{m(j)} * FL_i^{l(j)} * FH_i^{h(j)}} \qquad (3.3)$$

Where FM_i and FL_i are the free concentrations of metal ion and ligand and $CS_{j,,i}$ is the concentration of j^{th} complex at i^{th} experimental point: h(j) is negative for hydroxylated species. A titrand of total volume, V_0 containing an indifferent electrolyte, V_m cm^3 of metal ion (C_M mol dm^{-3}) and V_l cm^3 of ligand (C_L mol dm^{-3}) is titrated with alkali. 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 Eq. 3.4.

$$\left.\begin{aligned} TME_i &= V_m * C_M / (V0 + V_i) = TM0 * V0 / (V0 + V_i) \\ TLE_i &= V_l * C_L / (V0 + V_i) = TL0 * V0 / (V0 + V_i) \\ THE_i &= \frac{V_A * C_A}{V0 + V_i} - \frac{ALK * V_i}{V0 + V_i} + NDP * TLE_i + OH_i \end{aligned}\right\} \qquad (3.4)$$

where TM0 and TL0 are the analytical concentrations of metal and ligand in V_0 cm^3 of solution.

The calculated total metal ion concentration is given in Eq. 3.5.

$$TMC_i = FM_i + \sum_{j=1}^{NB} m(j) * \beta_{m(j)l(j)h(j)} * FM_i^{m(j)} * FL_i^{l(j)} * FH_i^{h(j)}$$
$$= FM_i + \sum_{j=1}^{NB} m(j) * CS_{j,i} \qquad (3.5)$$

Similarly, for ligand and hydrogen ion, the corresponding equations are given in Eq. 3.6.

$$\left.\begin{aligned} TLC_i &= FL_i + \sum_{j=1}^{NB} l(j) * CS_{j,i} \\ THC_i &= FH_i + \sum_{j=1}^{NB} h(j) * CS_{j,i} + OH_i \end{aligned}\right\} \qquad (3.6)$$

A large number of computer programs are available ranging in application from calibration of glass electrode to modeling strategies [69] applicable to quaternary complexes containing protonated, hydroxylated and polynuclear species. All the programs adopt this mathematical model for the above tasks.

3.4.4 Preprocessing of volume versus pH data

For the overlapping equilibria of the formation of two proton -ligand complexes shown in Eqs. 3.7 and 3.8, secondary formation function ($\bar{n}_H$) -average number of protons bound per mole of ligand- and the equilibrium constants are related by Eq. 3.9.

$$L + H \rightleftharpoons LH; \qquad K_1^H = \frac{[LH]}{[L][H]} \qquad (3.7)$$

$$LH + H \rightleftharpoons LH_2; \qquad K_2^H = \frac{[LH_2]}{[LH][H]} \qquad (3.8)$$

$$\bar{n}_H = \frac{\beta_{011} * FH_i + 2 * \beta_{012} * FH_i^2}{1 + \beta_{011} * FH_i + 2 * \beta_{012} * FH_i^2} \qquad (3.9)$$

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 several algebraic determination of transformations [10] useful for the equilibrium constants is

$$\frac{\bar{n}_{Hi}-1}{\left(2-\bar{n}_{Hi}\right)*FH_i}=\frac{\beta_{012}}{\beta_{011}}-\frac{\bar{n}_{Hi}}{\left(2-\bar{n}_{Hi}\right)*FH_i^{2}}*\frac{1}{\beta_{011}} \quad \text{……..(3.10)}$$

It may be represented as

$$Y_i = \beta_{012} * X_i + (-\beta_{011}) \text{ ……(3.11)}$$

The unweighted linear least squares treatment of Eq. 3.10 finds wide applicability in the estimation of β_{011} and β_{012}. For non-overlapping equilibria ($\left|\log K_{i+1}-\log K_i\right|>3$), stepwise stability constants at each point can also be calculated as

$$\log K_1 = pH_i + \log\left[\bar{n}_H/\left(1-\bar{n}_H\right)\right] \quad \text{and} \quad \log K_2 = pH_i + \log\left[\left(\bar{n}_H-1\right)/\left(2-\bar{n}_H\right)\right]$$

or the generalized equation can be written as

$$\log K_n = pH_i + \log\left[\left(\bar{n}_H-n+1\right)/\left(n-\bar{n}_H\right)\right] \qquad 3.12)$$

The computer program, SCPHD employs Eq. 3.13 for the calculation [70]

$$\bar{n}_{Hi} = \left(NDP_i + TLE_i + E_i + OH_i - ALK_i - FH_i\right)/TLE_i \qquad (3.13)$$

of paired data ($\bar{n}_{Hi}$, pH_i).where, NDP, TLE_i, E_i and OH_i are the number of dissociable protons of the ligand, total ligand concentration, total acid concentration and concentration of hydroxide ion due to autoionization of water, respectively. From this secondary data the stepwise stability constants are calculated from Eq. 3.12. 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. 3.14.

$$\bar{n}_i = \frac{\sum_{l=1}^{NML} l*\left(ML_l\right)_i}{FM_i+\sum_{l=1}^{NML}\left(ML_l\right)_i} = \frac{\sum_{j=1}^{NML} j*\beta_j*FL_i^{l(j)}}{1+\sum_{j=1}^{NML}\beta_j*FL_i^{l(j)}} \qquad (3.14)$$

Eq. 3.14 can be transformed into Eq. 3.15

$$\bar{n}_i = \frac{TLE_i*\left(\bar{n}_{Hi}-NDP\right)-\left(E_i+OH_i-ALK_i-FH_i\right)}{\bar{n}_{Hi}*TME_i} \qquad (3.15)$$

The value of $\bar{n}_{Hi}$ at FH_i is calculated using the Eq. 3.13, 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. 3.16.

$$FL_i = \frac{TLE_i - \bar{n}_i * TME_i}{1 + \sum_{j=1}^{NML} \beta_j * FL_i^{l(j)}} \qquad (3.16)$$

This algorithm was used in SCPHD to calculate the paired data ($\bar{n}_i$ versus PL_i). Some thumb rules [71] about formation function are given in Table 10.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 PROTONATION EQUILIBRIA OF L-PROLINE AND L-VALINE

Both L-proline(P) and L-Valine(V) act as a bidentate ligand and actively participates in acido-basic equilibria especially in physiological pH conditions. They exist as LH_2^+ at low pH and gets deprotonated with the formation of LH and L^- successively, with increase in pH.

4.1.1 Preprocessing of data

Amino acids present in biomolecules can be protonated or deprotonated depending on the availability of hydrogen ions in the solutions. This results in simultaneous existences of number of equilibria in solution. The alkalimetric titration curves for P and V in aqueous medium and SLS-water mixtures are shown in Figs.7-9. A perusal of the titration curves reveals that the acido-basic equilibria are active in the pH range 1.5-10.2 for P and 1.5-11.3 for V. The computer program SCPHD [70] was used to prune the data obtained in different experiments so that it contains more information than noise and the log β's obtained from the program are used as initial values for final refinement.

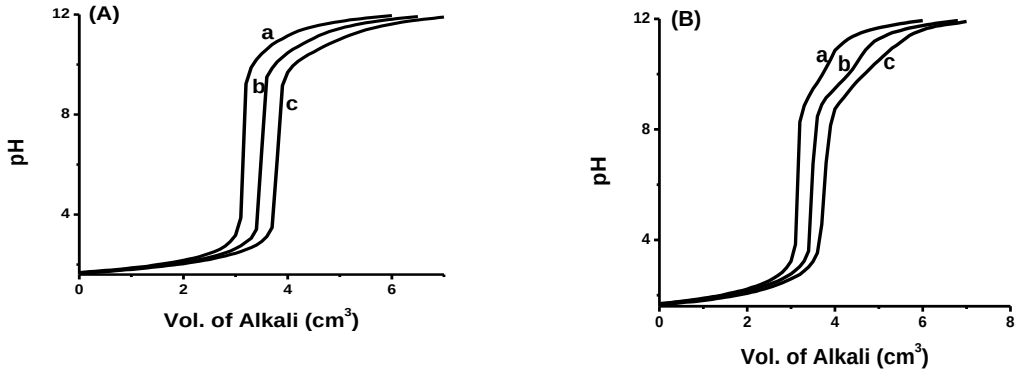


Figure 7: Alkalimetric titration curves for (A) P (B) V in aqueous medium; (a) 0.25, (b) 0.375 and (c) 0.50 mmols of P and V

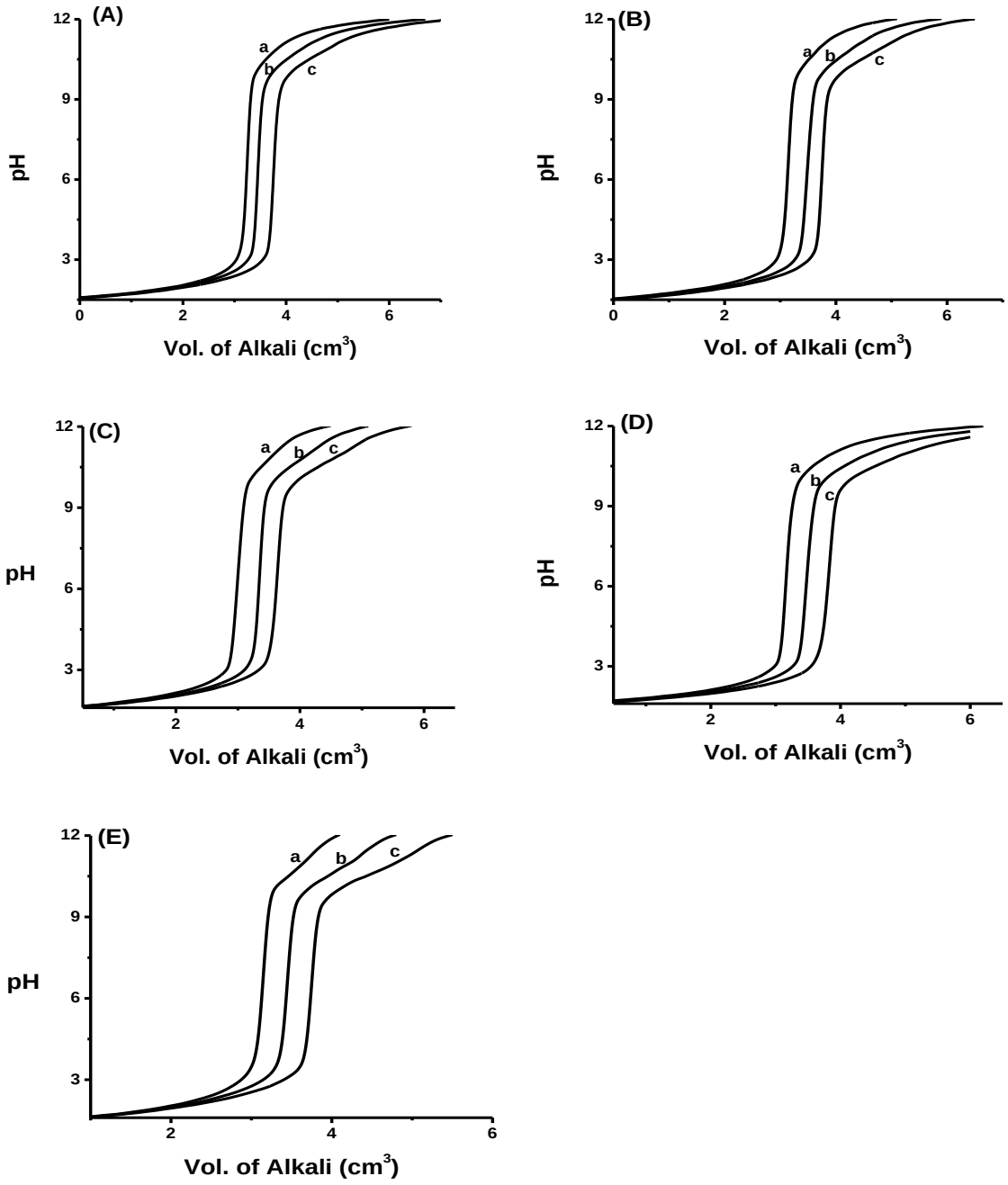


Figure 8 : Alkalimetric titration curves for P in SLS-water mixtures, (% w/v); (A) 0.5 (B) 1.0 (C) 1.5 (D) 2.0 (E) 2.5 ; (a) 0.25, (b) 0.375 and (c) 0.50 mmols of P

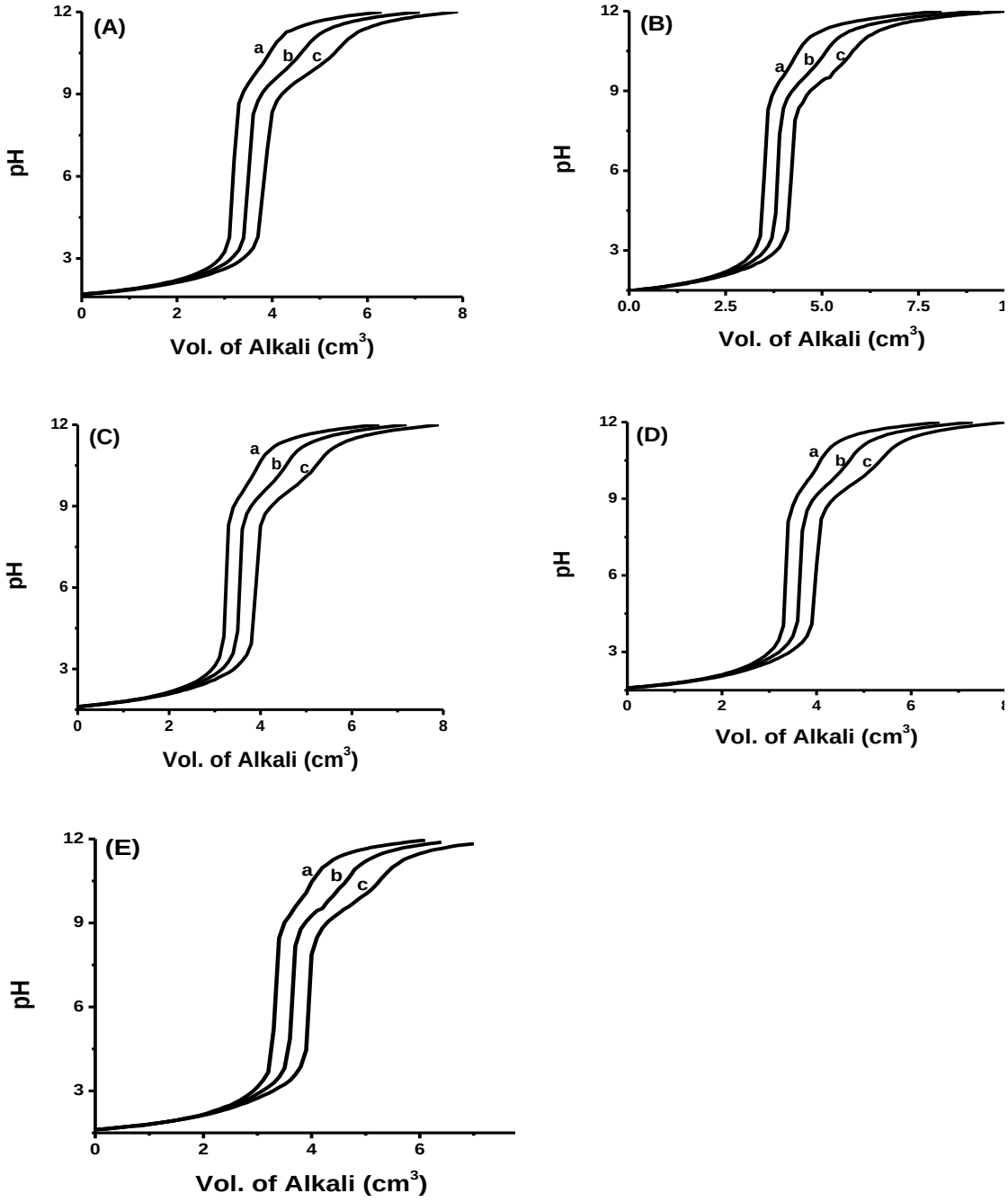


Figure 9: Alkalimetric titration curves for V in SLS-water mixtures, (% w/v); (A) 0.5 (B) 1.0 (C) 1.5 (D) 2.0 (E) 2.5 ; (a) 0.25, (b) 0.375 and (c) 0.50 mmols of V

4.1.1.1 Secondary formation function

Secondary formation functions like average number of protons bound per mole of ligand ($\bar{n}$H) and number of moles of alkali consumed per mole of ligand (a) are useful to detect the number of equilibria. Formation function is a useful parameter for the detection of polymeric species (L_2H_x). Plots of $\bar{n}$H versus pH for different concentrations of the ligand should overlap if there is no formation of polymeric species. Formation curves for different concentrations of ligands should overlap if there are no polymeric species [72]. Any deviation indicates the presence of polymeric species. Fig. 10 shows that there is no polymerization of P and V. In the present study two half integrals (0.5 and 1.5) in the case of P and V emphasize the existence of two protonation-deprotonation equilibria in the physiological pH range.

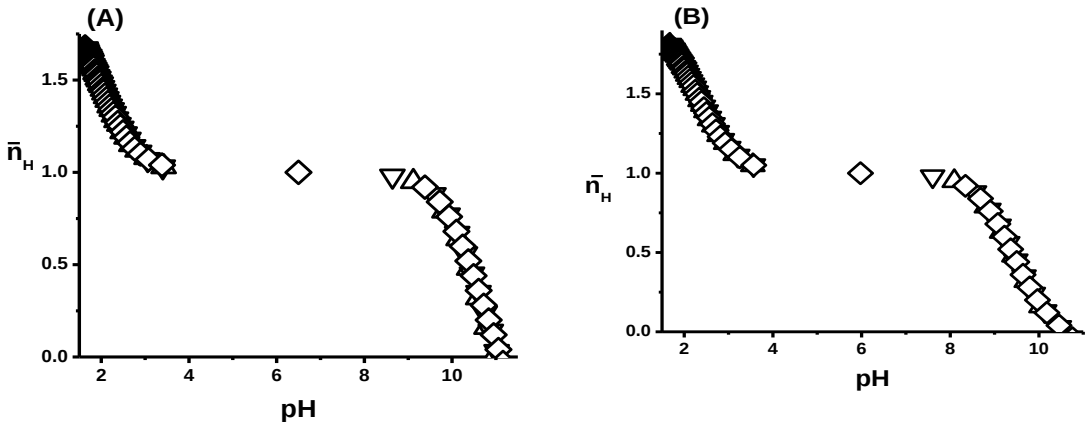


Figure 10: Formation curves of (A) P and (B) V in aqueous medium. Ligand concentrations are 0.25 (Δ), 0.375 (▽) and 0.50 (◊) in mmols.

The pH metric method is used for the determination of protonation constants or stability constants only when ligands are either weak organic acids or bases. Acids (eg. carboxylic acids and phenols) have dissociable protons (NDP) equal to the number of functional groups. If the ligand contains basic functional groups like amino group, they abstract protons corresponding to the number of such functional groups from the medium. Such number is called number of associable protons (NAP).

Another secondary function relevant to protonation equilibria is the number of moles of alkali consumed per mole of ligand, **a**. One can detect whether the given ligand solution contains acid or not by using **a**. The negative values of **a** correspond to excess of mineral acid present in the titrand and the number of associable protons of the ligand whereas the positive values correspond to the dissociable protons. In the present study (Fig. 11) the maximum value of **a** is +1, which indicates that both P and V have one dissociable proton.

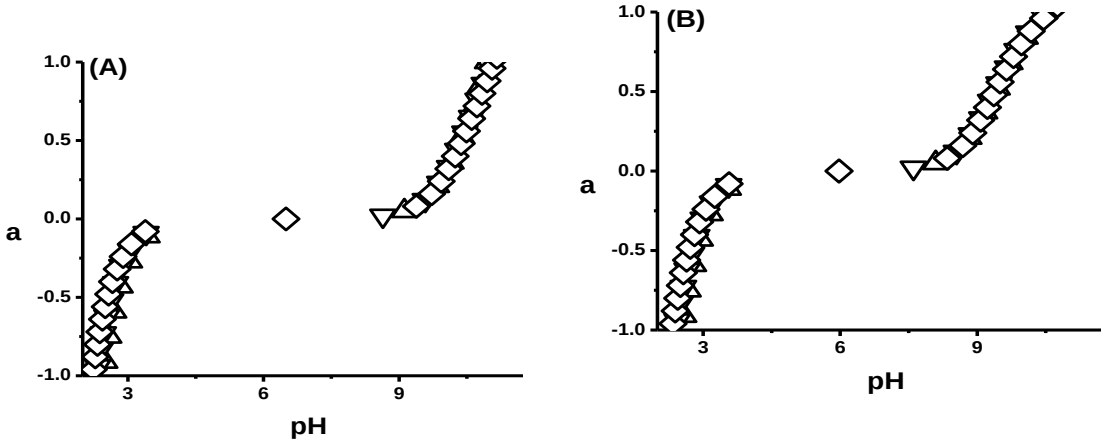


Figure 11 :The number of moles of alkali consumed versus pH curves of (A) P and (B) V in 0.5% w/v SLS-water mixture. Ligand concentrations are 0.25 (Δ), 0.375 (▽) and 0.50 (◊) in mmols.

4.1.2 Mathematical models for acido-basic equilibria

The computer program MINIQUAD75 [63] was used to refine the protonation constants of the proline and valine in SLS- water mixtures. The results of the best fit model along with some of the important statistical parameters of P and V are given in Tables 11. Very low standard deviations (SD) in log β values indicate their precision. Small values of U_{corr} (sum of squares of deviations in concentrations of the ligand and hydrogen ion at all experimental points corrected for degrees of freedom) indicate that the experimental data can be represented by the models.

Table 10: Best fit chemical models of acido-basic equilibria of L-Proline and L-Valine in SLS- water mixtures (Temp= 310 K, Ionic strength=0.16 mol dm^{-3})

P (pH range 1.5-11.2)								
SLS % w/v	log β_1(SD)	log β_2(SD)	NP	U_{corr} x10^8	Skewness	χ^2	Kurtosis	R-factor
0.0	10.44(1)	12.44(9)	100	0.33	0.05	12.16	2.22	0.004
0.5	10.47(0)	12.50(1)	99	0.45	0.03	11.47	1.44	0.009
1.0	10.55(3)	12.67(3)	78	0.21	0.08	12.07	2.04	0.005
1.5	10.62(2)	12.85(5)	102	0.55	0.09	11.56	3.60	0.004
2.0	10.46(9)	12.80(1)	68	0.12	-0.06	11.33	3.83	0.004
2.5	10.45(7)	12.95(8)	99	0.32	0.07	12.70	3.65	0.004
V (pH range 1.4-10.8)								
0.0	9.44(6)	11.73(8)	100	0.88	0.00	11.50	2.82	0.0041
0.5	9.39(7)	11.88(9)	67	0.92	0.08	12.00	1.40	0.0058
1.0	9.49(8)	12.19(2)	101	0.97	0.02	12.22	3.44	0.0044
1.5	9.55(5)	12.27(8)	100	0.66	-0.05	13.14	2.25	0.0033
2.0	9.47(6)	12.26(9)	99	0.90	1.03	13.30	1.32	0.0059
2.5	9.57(7)	12.40(0)	94	0.05	0.05	12.17	2.04	0.0019

Ucorr= U/(NP-m); NP = Number of points; m= number of protonation constants; SD = Standard deviation

4.1.2.1 Statistical parameters of the best-fit models

Residual Analysis [14, 73]

In data analysis with least squares methods, residuals (differences between the experimental data and the data simulated based on model parameters) are assumed to follow Gaussian or normal distribution [74]. 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 and following normal distribution in the least squares analysis, the residuals are tested for normal distribution. Such tests are χ^2, skewness, kurtosis and R-factor. These statistical parameters show that the best fit models portray the acido-basic equilibria of P and V in SLS- water mixtures, as discussed below.

χ^2 test

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

Crystallographic R-test

Hamilton's R factor ratio test [75] is applied in complex equilibria to decide whether inclusion of more species in the model is necessary or not. In pH-metric method the readability of pH meter is taken as the $R_{limit,}$ which represents the upper boundary of R beyond which the model bears no significance. When these are different numbers of species the models whose values are greater than R-table are rejected. The low crystallographic R-values given in Tables 11 indicate the sufficiency of the model.

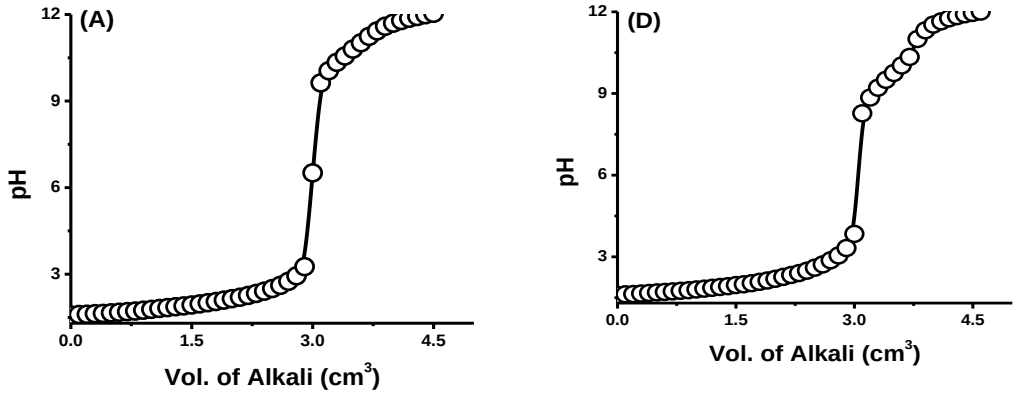
Skewness: It is a dimensionless quantity indicating the shape of the error distribution profile. A value of zero for skewness indicates that the underlying distribution is symmetrical.

If the skewness is greater than zero, the peak of the error distribution curve is to the left of the mean and the peak is to the right of the mean if skewness is less than zero. The values of skewness recorded in Tables 11 are between -0.06 and 1.03. These data evince that the residuals form a part of normal distribution; hence, least-squares method can be applied to the present data.

Kurtosis

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

The alkalimetric titration data are simulated using the model parameters given in Tables 11 and they are compared with the experimental data, to verify the sufficiency of the model. The overlap of typical experimental and simulated titration data indicates (Fig.12) that the proposed models represent the experimental data.



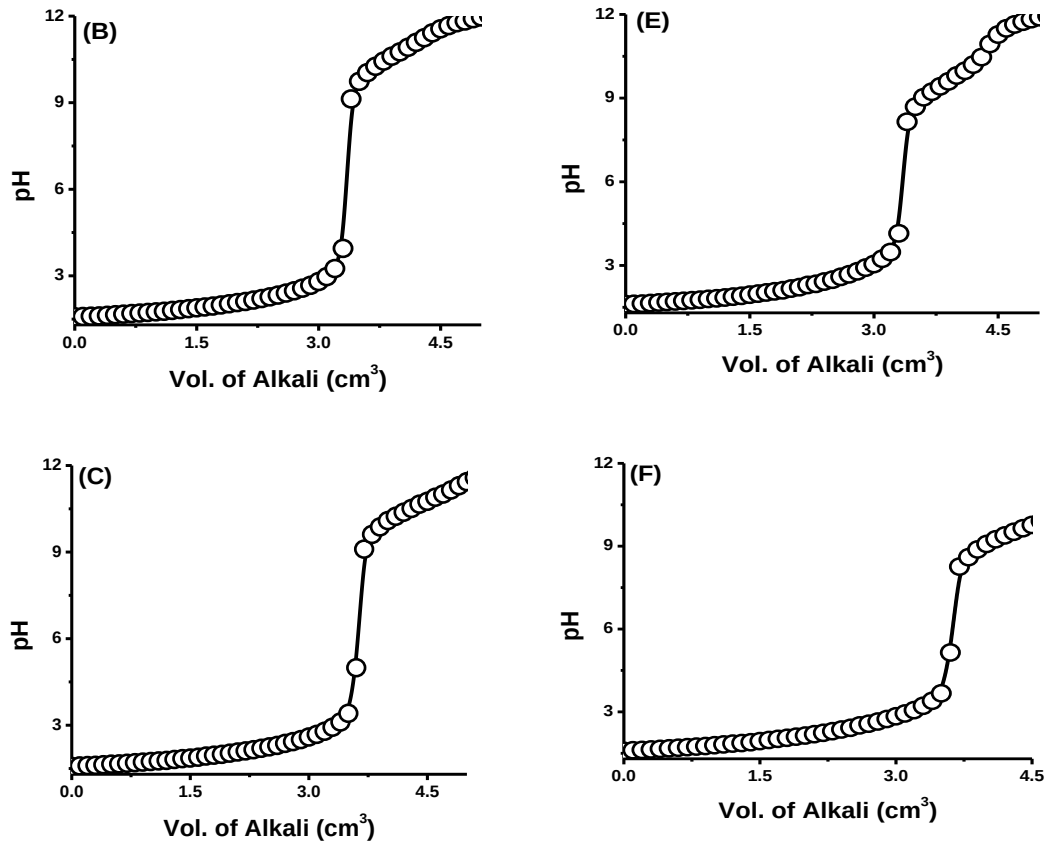


Figure 12 : Simulated (solid line) and experimental (o) alkalimetric titration curves in 1.0% w/v SLS-water mixtures; (A), (B) and (C) for 0.25, 0.375 and 0.50 mmols of P and (D), (E) and (F) for 0.25, 0.375 and 0.50 mmols of V.

4.1.3 Effect of Systematic Errors

In order to rely upon the best fit chemical model for critical evaluation and application, a brief investigation was made by introducing pessimistic errors in the concentrations of alkali, mineral acid, ligand, and volume [72]. Any variations in the parameters like concentrations of ingredients affect the magnitudes of equilibrium constants. Such parameters are called influential parameters.

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 12 emphasizes that protonation constants associated with carboxyl proton are more affected. This may be due to their lower magnitude than those for amino protons.

Similarly, errors in concentration of alkali and acid, affect more than volume. With the introduction of errors, the SD's are found to increase inferring the appropriateness of experimental conditions and correctness of analytical concentrations.

Table 11: Effect of errors in influential parameters on the protonation constants in 2.5% w/v SLS-water mixture.

Ingredient	% Error	log β_{mlh}(SD)			
		L-Proline		L-Valine	
		LH	LH_2^+	LH	LH_2^+
	0	10.45(7)	12.95(8)	9.57(7)	12.40(0)
Alkali	-5	Rejected	13.53(46)	Rejected	12.93(56)
	-2	10.76(55)	13.13(46)	9.72(77)	12.81(77)
	+2	10.73(45)	Rejected	9.40(99)	12.99(67)
	+5	10.18(77)	Rejected	9.17(42)	Rejected
Acid	-5	10.24(35)	12.88(37)	9.22(37)	11.54(32)
	-2	Rejected	12.60(36)	9.43(37)	11.96(29)
	+2	10.73(39)	13.16(33)	9.70(39)	12.54(37)
	+5	Rejected	13.60(35)	9.90(39)	Rejected
Ligand	-5	10.54(26)	12.88(28)	9.51(27)	12.26(25)
	-2	10.57(25)	12.88(27)	9.54(22)	12.25(38)
	+2	10.62(25)	12.88(27)	9.58(17)	12.25(19)
	+5	10.65(16)	12.88(28)	9.61(29)	12.24(17)
Volume	-5	10.59(17)	12.96(10)	9.54(18)	12.44(12)
	-2	10.59(15)	12.91(17)	9.54(16)	12.49(8)
	+2	10.40(06)	12.85(18)	9.56(15)	12.43(17)
	+5	10.41(18)	12.98(12)	9.54(18)	12.43(12)

4.1.4 Effect of Surfactants on Protonation Equilibria

The effect of surfactant on protonation equilibria was recognized long back. Amphiphilic molecules, containing both hydrophobic and hydrophilic moieties, associate in water above a certain concentration to form colloidal particles called micelles. Micellar systems can shift acid-base equilibria. This shift can be explained in terms of differences between the properties of the bulk solvent and of the interfacial region and perturbation of the acid-base equilibria by the electrostatic field effect of the charged interface. The apparent shift in the magnitude of protonation constants in micellar media compared to aqueous solution was attributed to the creation of a concentration gradient of protons between the interface and the bulk solution [76]. Further, the presence of micelles is known to alter the dielectric constant of the medium, which has a direct influence on the protonation – deprotonation equilibria [77]

The variation of step-wise protonation constant (log K^M) or change in free energy (ΔG) 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 (Eq. 4.1).

i.e

$$\Delta G^t = -RT (\ln K^M - \ln K^W) \qquad (4.1)$$

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

Born's classical treatment [78] 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

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

Eqs. 4.1 and 4.2 suggest that logarithm of protonation constant should vary linearly as a function of reciprocal of the dielectric constant (1/D) of the medium.

The primary factor in the surfactant effect on lower alkylamines is the electrostatic interaction of the amine cation and charged surface of the micelle while the hydrophobic interaction plays only a secondary role. Lower alkyl amines and carboxylic acids in their deprotonated state (RNH_2 and $RCOO^-$) stay in the bulk of the solution and protonated amines (RNH_3^+) will be located both in the bulk and on the surface of the anionic micelles [61].

In present study, the step-wise protonation constants of P and V exhibit non-linear trend with increasing mole fraction of SLS. This is because the interactions are not simple, the two ligands exist in zwitter ionic forms (Fig. 14) under these conditions. This trend observed in present study because of the existence of P and V different forms. This also indicates the dominance of both electrostatic and non-electrostatic interactions.

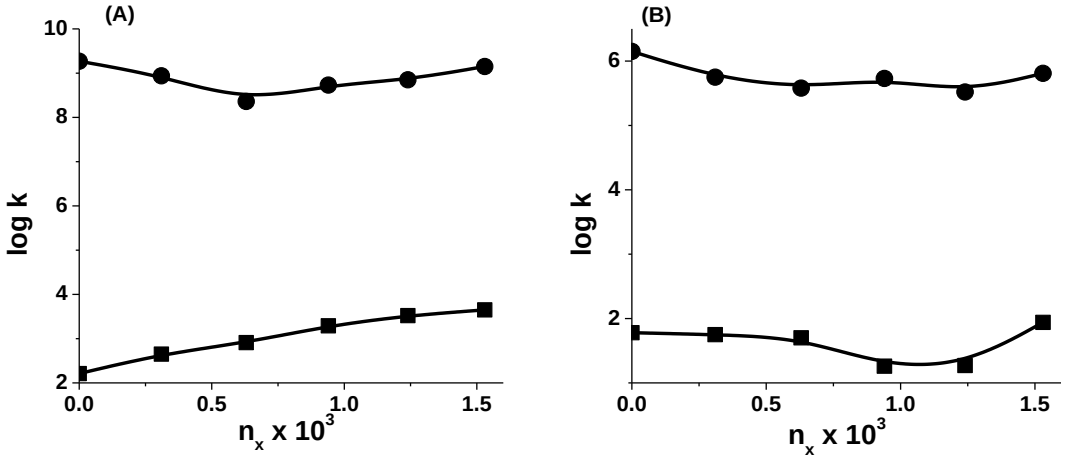


Figure 13: Variation of stepwise protonation constants (log K) with mole fraction of SLS, (A) P and (B) V in SLS-water mixtures; (■) log K_1, (●) log K_2.

The protonation-deprotonation equilibria of P and V are shown in Fig. 14. In these equilibria both the ligands can exist in anionic, zwitterionic and cationic forms. The protonation constants of P and V from the best fit models (Tables 11) show the existence of LH_2^+ and LH in different pH ranges. As the alkali is added to the titrand containing the ligands, the protonated forms of the ligands lose their protons.

$$LH_2^+ \rightleftharpoons LH + H^+ \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$LH \rightleftharpoons L^- + H^+ \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

In the pH range of present study P and V lose carboxylic protons first (Eq. 1) because this group is more acidic. P and V cations are successively deprotonated (Eq. 1 and 2), the charge of the species is decreased and low dielectric medium favours the

protonation reactions, since electrostatic interactions are dominating the non-electrostatic interactions.

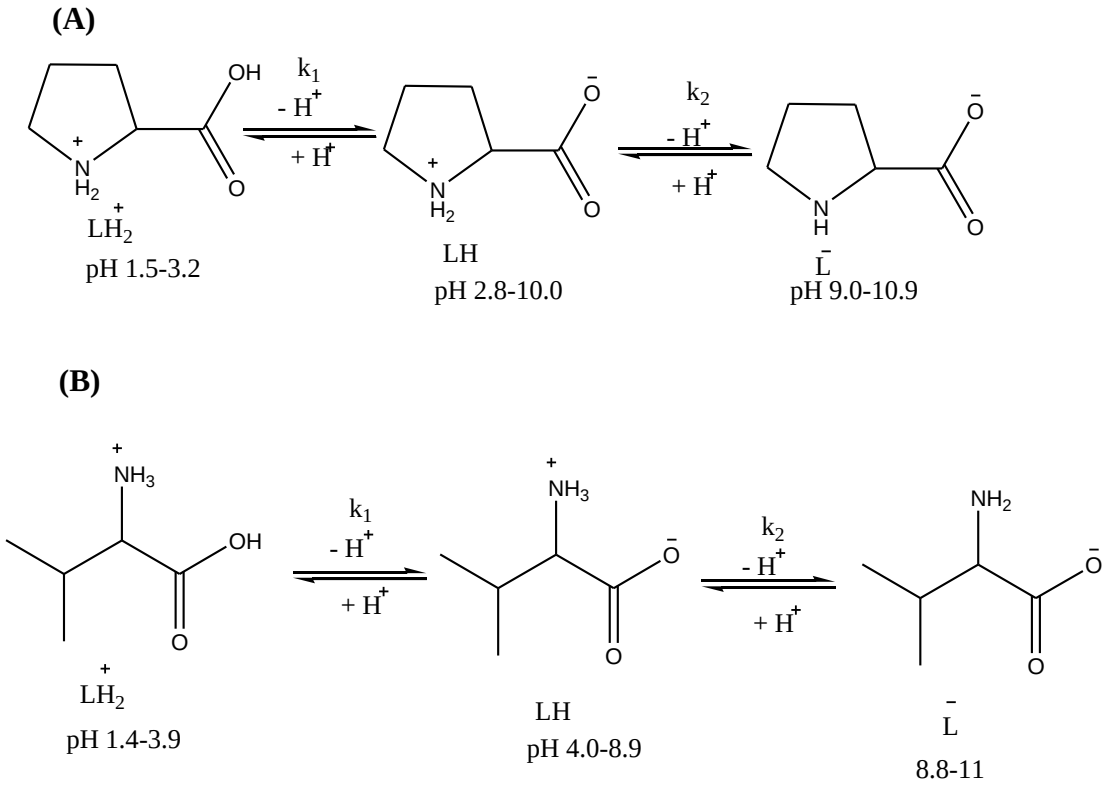


Figure 14: Protonation – deprotonation equilibria of P and V

4.1.5 Distribution diagrams

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

P has one dissociable carboxyl proton and one imino group that can associate with a proton. The imino proton is lost at pH greater than 10.3. Hence, under present experimental conditions the most deprotonated form of proline is L^-. V also has one associable proton and exists in LH_2^+ form below a pH of 2.9 and the amino proton is lost at pH greater than 9.3.

Typical distribution plots in SLS- water mixtures given in Figs.15-17 show the existence of LH_2^+, LH and L^- for P and V and the zwitterionic form is to an extent of 80-88% in the pH range 3-9.4 for P and 4.4-8.6 for V.

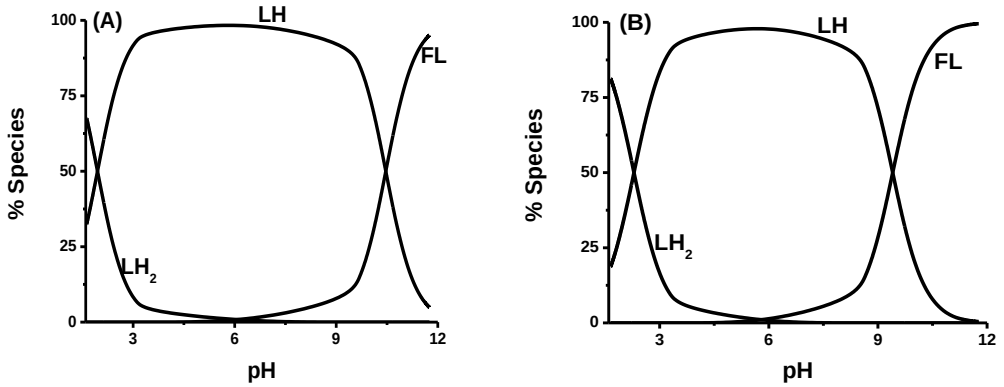
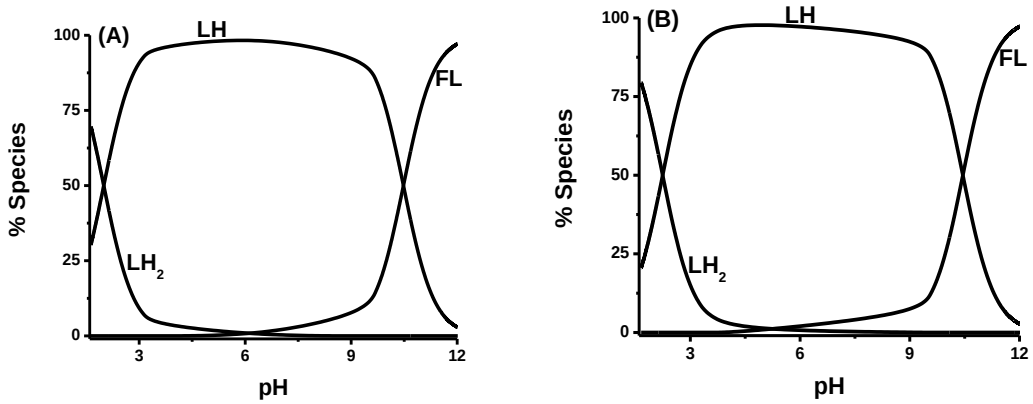


Figure 15: Distribution diagrams of (A) P and (B) V in aqueous medium.



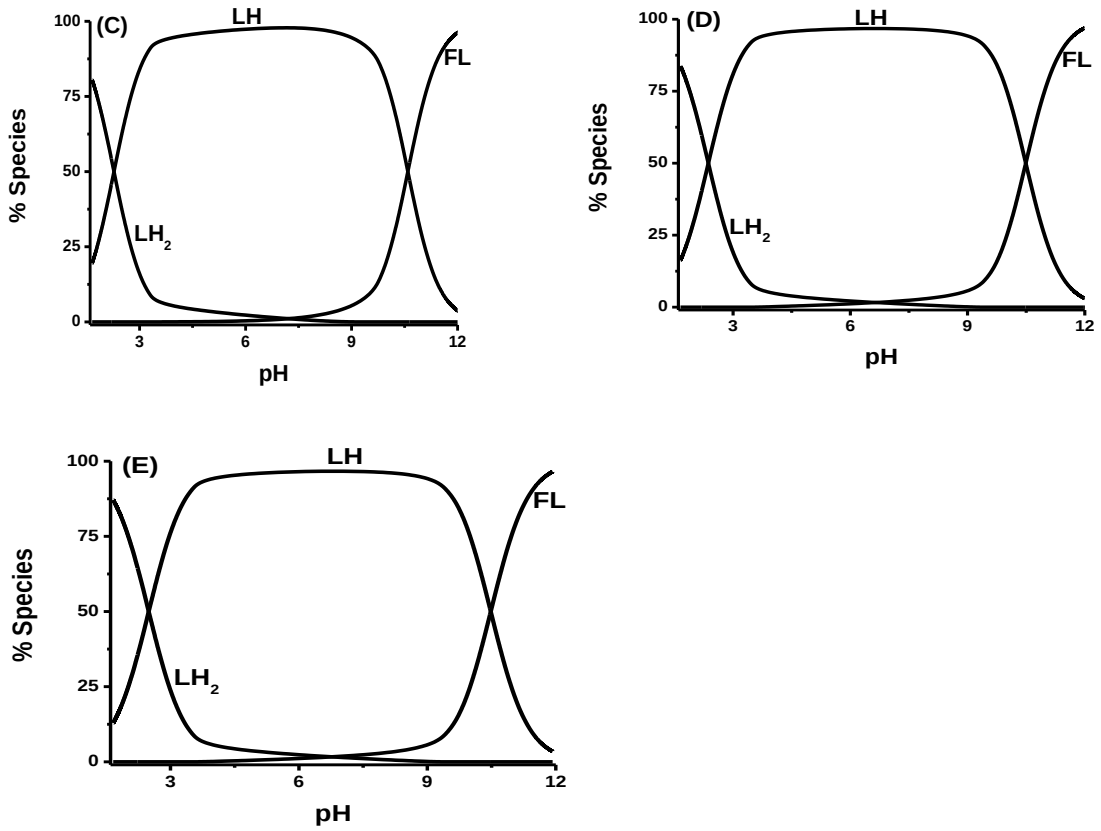
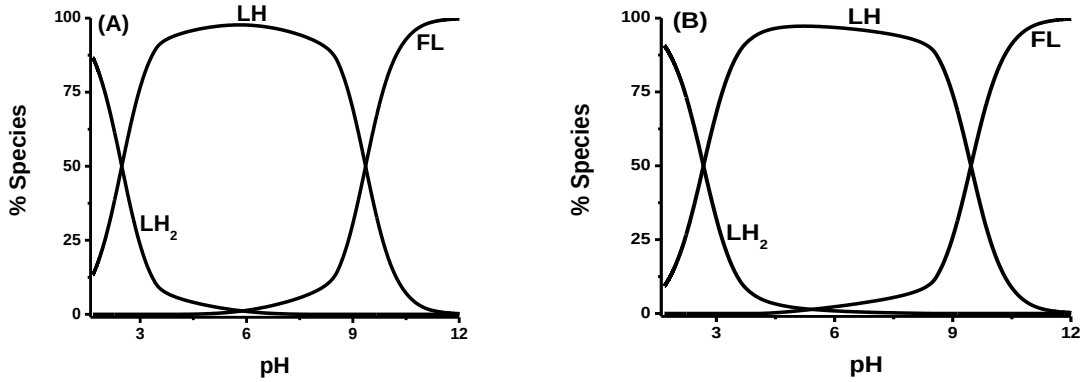


Figure 16: Distribution diagrams of P in SLS-water mixtures, (% w/v); (A) 0.5, (B) 1.0, (C) 1.5, (D) 2.0 and (E) 2.5.



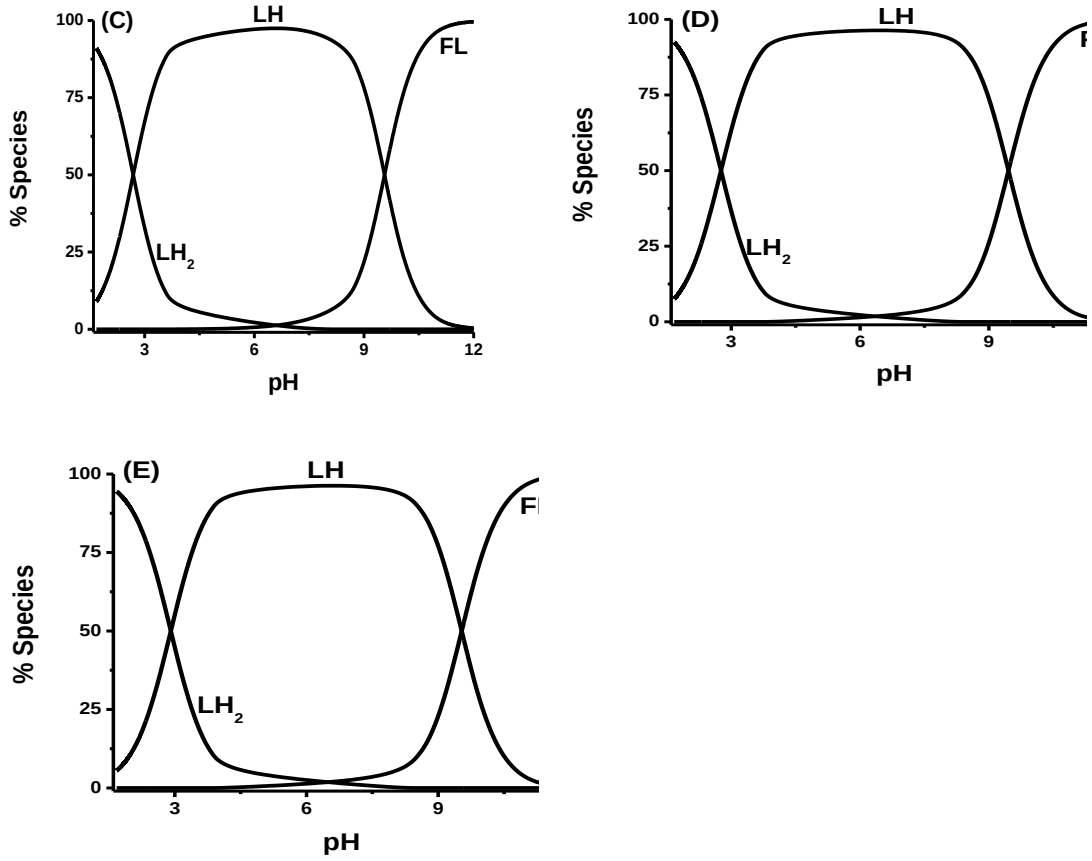


Figure 17: Distribution diagrams of V in SLS-water mixtures, (% w/v); (A) 0.5, (B) 1.0, (C) 1.5, (D) 2.0, (E) and 2.5.

4.2 DETERMINATION OF BINARY STABILITY CONSTANTS

In this section, the complex equilibria of P and V with Ca(II), Zn(II) and Mn(II) in SLS-water mixtures had been investigated. This type of study throws light on 1) the effect of electrostatic and non-electrostatic interactions on complex formation in vitro, 2) the nature of active site cavity of enzymes, 3) detection of specific solute-solvent interactions, 4) understanding the selectivity and sensitivity of various protonated complexes and relative abundance of free components, 5) the type of complex formed by the metal ion, and 6) the bonding behavior of the protein residues with the metal ion. The species refined and their relative concentrations under the present experimental conditions represent the possible forms of metal ions in biological fluids.

4.2.1 Formation of Species

If the ligand contains one or more donor atoms than those participating in complexation, there is possibility for its participation in acido-basic equilibria, thus resulting in the formation of protonated species, ML_lH_h in addition to ML_l type of complexes. 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 $ML_l(OH)_k$.

Typical alkalimetric titration curves are given in Fig.18 from which the stability constants of binary metal-ligand complexes were determined. Since the ratio of TL0 to TM0 was greater than unity in all the experiments, as clear from the data in Table 7 of Chapter 3, formation of polynuclear complexes was not considered. The formation of polymeric species is possible if TL0: TM0 $\leq$ 2 [79]. The data from the table (table 7) reveals that, TL0: TM0 are 2.5, 3.75 and 5.0 in all the experiments. This indicates that formation of polymeric species are impossible in the present study.

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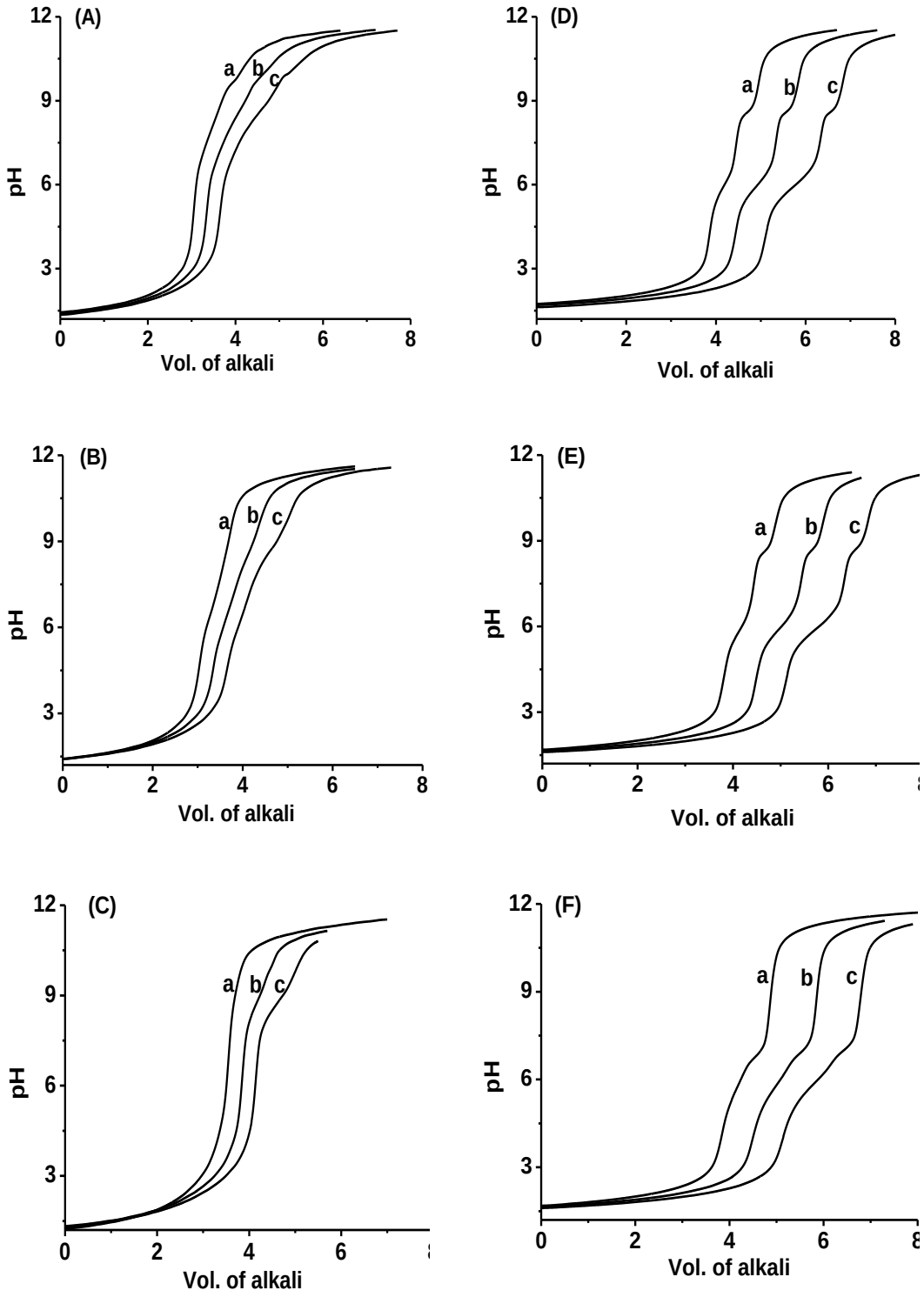
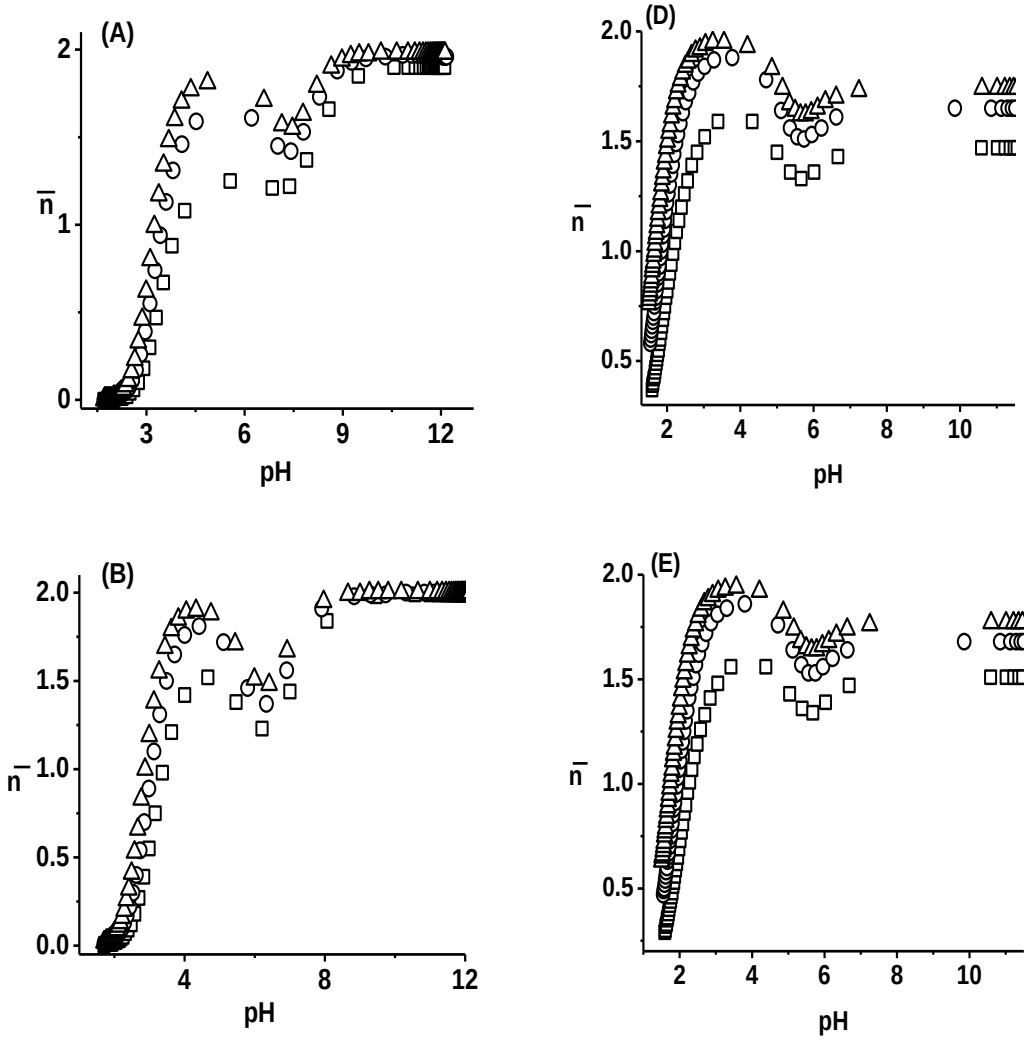


Figure 18: Alkalimetric titration curves for P complexes of (A) Ca(II), (B) Zn(II) and (C) Mn(II) and V complexes of (D) Ca(II), (E) Zn(II) and (F) Mn(II) in 2.0% w/v SLS-water mixture; (a) 0.25, (b) 0.375 and (c) 0.50 mmols of ligand.`

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 pH at all the ligand concentrations, with fixed metal ion concentration should overlap and any deviation indicates the presence of the protonated species. Some typical plots given in Fig. 19 shows spread in some pH regions, indicating the presence of protonated metal complexes



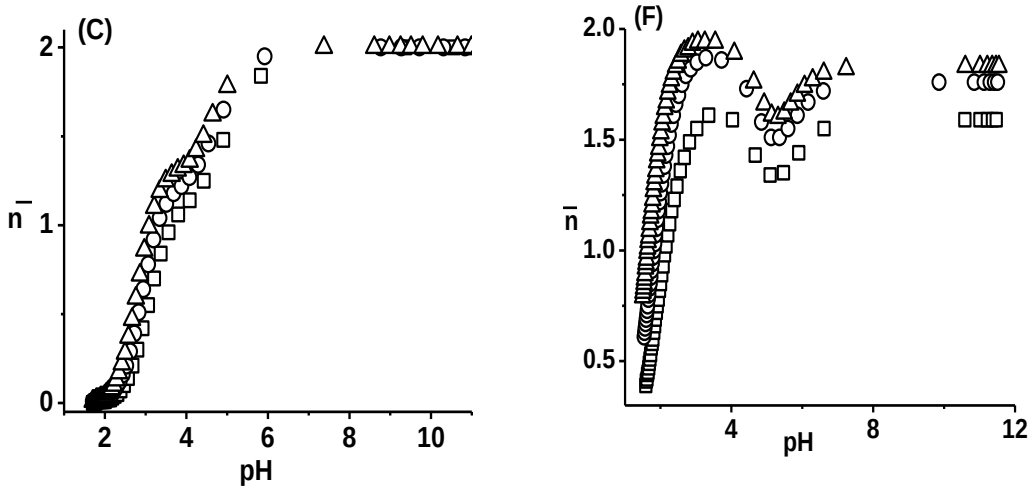
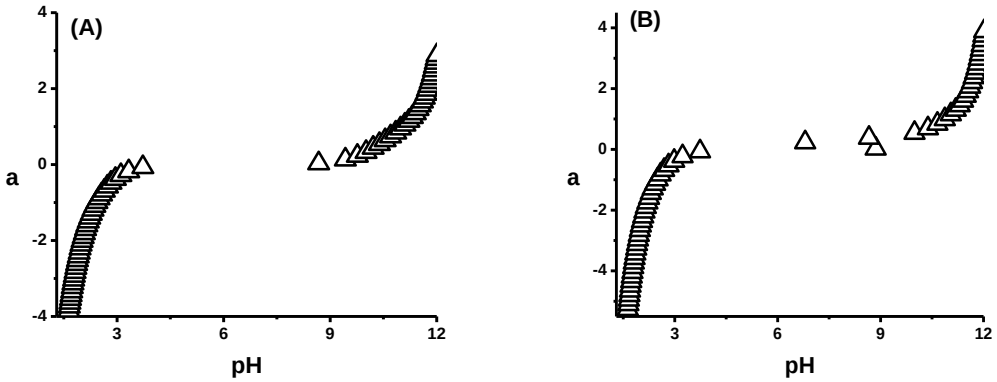


Figure 19: Formation curves of P complexes of (A) Ca(II), (B) Zn(II), (C) Mn(II) and V complexes of (D) Ca(II), (E) Zn(II), (F) Mn(II) in 1.5% w/v SLS-water mixture; (□) 0.25, (○)0.375 and (Δ) 0.50 mmol mmol of ligand.

Fig. 20 gives the relation between the number of moles of alkali consumed per mole of metal ion (**a**) and the pH. The number of protons released in the equilibrium can be guessed depending on the moles of alkali consumed.



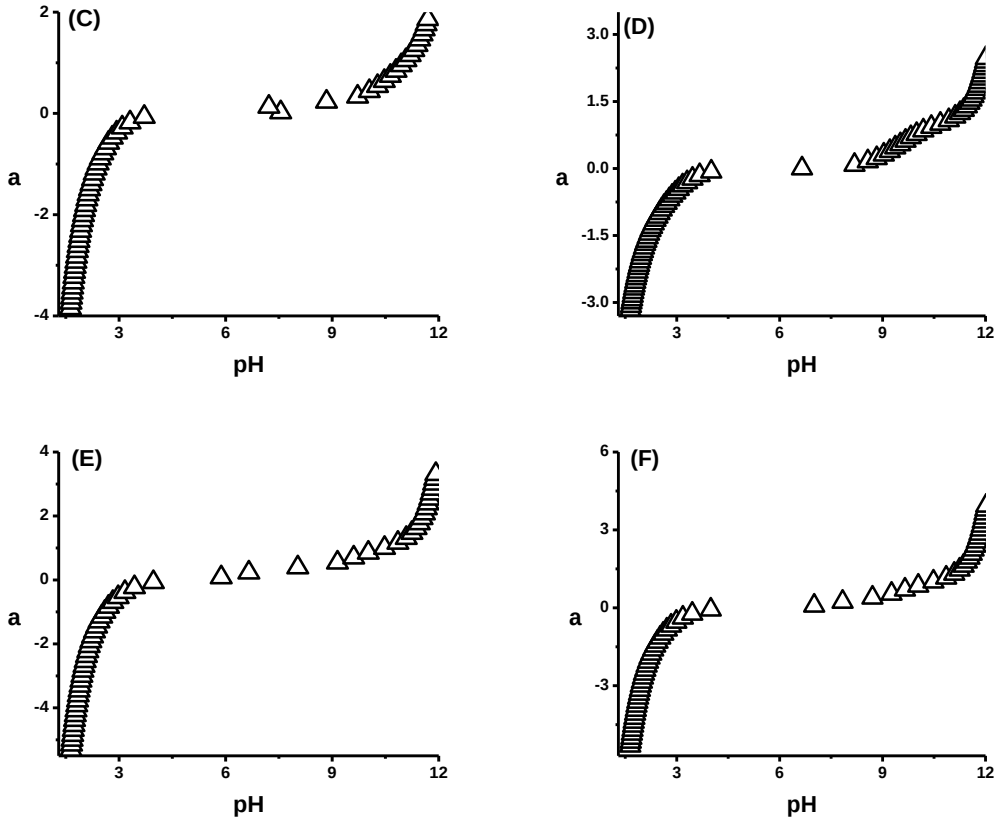


Figure 20: Number of moles of alkali (**a**) versus pH curve of P complexes of (A) Ca(II), (B) Zn(II), (C) Mn(II) and V complexes of (D) Ca(II), (E) Zn(II), (F) Mn(II) in 0.5% w/v SLS-water mixture; (□) 0.25, (○) 0.375 and (Δ) 0.50 mmol of ligand

4.2.2 Selection of Species

The earlier reports reveal that 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 [71]. The $\bar{n}$ values greater than three or ascending slope indicates the presence of a complex species with higher ligand number

In view of the large number, it is not pragmatic to consider all models for refinement process. Hence, several thumb rules based on chemical principles and well-established practices in multiple linear regression analysis were resorted to, for this purpose. Hence the species considered to develop different models were MLH^{2+}, ML^{+}, $ML_2H_2^{2+}$, ML_2H^{+}, ML_2, ML_3H and ML_3^{-}. The preliminary screening of a number of species based on several thumb rules [80] resulted in a short list of MLH^{2+}, ML^{+}, $ML_2H_2^{2+}$, ML_2H^{+} and ML_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 [63]. 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 was less than 10.

Existence of species was determined by performing exhaustive modeling. The models were evaluated assuming the simultaneous existence of different combinations of species. Models containing various number and combinations of species were refined using MINIQUAD75. As the number of species was increased, the models gave better statistics denoting better fit. This indicates that the final model appropriately fits the experimental data. Such exhaustive modeling study was performed for all the systems and the final models are given in Tables 13 and 14 for Ca(II), Zn(II) and Mn(II) complexes of P and V in SLS-water mixtures. The tables contain the stoichiometric coefficients and stability constants of the complex species, standard deviations in the stability constants and residual statistics of the best fit models

Table 12: Parameters of best fit chemical models of Ca(II), Zn(II) and Mn(II)-P complexes in SLS-water mixtures.

%w/v	log β_{mlh}(SD)			NP	U_{corr} $*10^8$	Skew-ness	χ^2	Kurto-sis	R-factor	pH-Range
	ML^+	MLH^{2+}	ML_2H^+							
Ca(II)										
0.0	2.15(2)	12.71(4)	15.10(1)	100	0.1	0.6	12.0	2.5	0.005	2.6-11.4
0.5	2.30(0)	12.96(1)	15.50(2)	120	1.4	0.1	11.8	3.0	0.002	2.4-11.0
1.0	3.55(1)	12.98(9)	15.89(0)	99	0.6	0.5	12.5	2.5	0.006	1.5-11.0
1.5	3.49(6)	13.12(0)	16.55(2)	90	0.5	0.9	11.2	1.6	0.001	1.5-11.0
2.0	2.31(8)	13.20(2)	15.40(1)	103	3.7	0.3	11.0	3.1	0.005	1.7-11.0
2.5	3.09(3)	12.87(3)	15.75(0)	107	3.1	0.0	12.1	2.8	0.004	1.7-11.0
Zn(II)										
0.0	6.70(0)	13.30(3)	20.35(1)	99	1.9	0.0	12.09	3.5	0.002	1.5-8.7
0.5	6.55(9)	12.56(9)	19.40(0)	100	2.5	0.3	12.08	3.9	0.005	1.5-9.2
1.0	6.61(1)	13.77(0)	19.75(9)	130	2.0	0.9	11.09	3.1	0.009	1.5-9.2
1.5	6.60(0)	13.30(3)	20.29(9)	88	3.8	0.4	11.08	2.8	0.005	1.5-9.2
2.0	6.92(1)	13.99(6)	20.38(8)	90	3.0	0.1	12.01	2.5	0.010	1.5-9.2
2.5	6.45(2)	14.19(2)	20.00(1)	104	3.0	0.2	10.09	2.3	0.013	1.5-9.2
Mn(II)										
0.0	4.00(1)	12.39(9)	16.71(0)	99	1.1	0.6	12.03	3.0	0.001	1.6-9.7
0.5	4.80(0)	12.50(0)	17.67(7)	100	3.6	0.2	11.09	3.0	0.004	1.6-9.7
1.0	5.24(9)	13.57(9)	17.90(2)	103	3.3	0.1	12.06	2.9	0.005	1.6-9.7
1.5	5.38(5)	13.79(8)	17.68(1)	105	2.9	0.6	12.09	3.8	0.002	1.6-9.7
2.0	5.90(1)	13.10(6)	18.20(0)	90	3.0	0.4	10.09	3.1	0.001	1.6-9.7
2.5	6.67(3)	13.70(6)	18.99(9)	80	2.6	0.8	11,99	2.9	0.003	1.5-9.7

Table 13: Parameters of best fit chemical models of Ca(II), Zn(II) and Mn(II)-V complexes in SLS-water mixtures.

%w/v	log β_{mlh}(SD)			NP	U_{corr} $_{*10^8}$	Skew-ness	χ^2	Kurto-sis	R-factor	pH-Range
	ML^+	MLH^{2+}	ML_2H^+							
Ca(II)										
0.0	2.40(7)	11.4(0)	14.00(0)	88	0.7	-0.6	12.9	3.1	0.007	1.7-10.6
0.5	1.80(9)	11.60(9)	13.89(1)	50	0.7	0.6	11.2	3.8	0.010	1.7-10.6
1.0	2.79(0)	11.49(8)	14.40(2)	56	1.0	0.1	11.0	2.9	0.007	1.7-10.6
1.5	2.71(9)	12.15(6)	14.37(3)	49	0.6	-0.2	11.0	2.0	0.004	1.7-10.6
2.0	2.89(1)	12.59(6)	14.80(1)	100	1.6	1.0	12.8	3.6	0.020	1.7-10.6
2.5	3.37(5)	11.90(3)	14.63(4)	76	1.4	-0.3	12.8	3.3	0.001	1.7-10.6
Zn(II)										
0.0	5.00(5)	11.61(1)	16.56(0)	140	0.0	0.2	11.7	3.5	0.005	1.5-8.5
0.5	5.60(9)	11.37(0)	17.00(0)	77	1.9	0.8	10.9	3.9	0.013	1.5-8.5
1.0	5.67(2)	12.50(7)	17.49(1)	110	0.6	0.4	12.0	4.0	0.007	1.5-8.5
1.5	6.00(0)	12.60(8)	17.51(3)	40	0.9	0.7	12.7	2.9	0.009	1.5-8.5
2.0	6.59(3)	13.19(2)	18.09(1)	89	1.0	0.9	11.0	3.1	0.014	1.5-8.5
2.5	6.01(1)	13.09(1)	18.17(0)	89	1.5	0.1	12.7	2.8	0.020	1.5-8.5
Mn(II)										
0.0	3.0(6)	11.58(5)	14.70(0)	99	0.9	-0.5	13.9	3.4	0.001	1.5-9.0
0.5	3.65(1)	11.80(3)	15.34(2)	90	0.7	0.4	13.4	3.7	0.009	1.5-9.0
1.0	3.60(0)	12.20(0)	15.51(2)	85	1.9	-1.0	11.0	2.1	0.003	1.5-9.0
1.5	4.79(7)	12.15(6)	16.30(9)	100	0.3	-0.5	11.4	3.1	0.007	1.5-9.0
2.0	4.67(5)	12.81(2)	15.80(8)	106	7.0	0.4	12.4	2.8	0.007	1.5-9.0
2.5	5.67(1)	13.14(0)	17.00(3)	80	9.0	0.1	13.9	2.7	0.003	1.5-9.0

4.2.4 Analysis of Residuals

The residuals-difference 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.

Very low-standard deviation in overall stability constants (log β) signifies the precision of these data. 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. The SD and confidence intervals in β are meaningful only when unweighted residuals follow χ^2 distribution, which measures the possibility of residuals forming a part of standard normal distribution with zero mean and unit standard deviation. Higher χ^2 values than expected are due to 1) inadequacy of the model although the experimental data are of high quality, 2) use of poor data even though the model is appropriate and 3) invoking optimistic estimates of errors in primary data.

A perusal of Tables 13-14 indicates that the χ^2 values range between 10.09 – 13.9 for SLS-water mixtures. The values of kurtosis and skewness range from 1.6 – 4.0 and -1.0 – 0.9 for SLS-water mixtures, respectively. Deviation of the values of kurtosis and skewness from three and zero, respectively, show the tendency of these residuals to concentrate more to the left or right of the mean and broadening of the peak. However, the values of U_{corr} in all the three mass-balance equations are very low confirming the adequacy of the chemical model to represent the experimental data.

4.2.5 Effect of Influential Parameters

The computer programs refine the stability constants by minimizing the random errors in the data. But in the presence of considerable systemic errors, not only the β's are in errors; even some species may be rejected. MINIQUAD75 has no provision to vary the influential parameters. In order to rely upon the best-fit chemical model for critical evaluation and application under varied experimental conditions with different accuracies of data acquisition, an investigation was undertaken by introducing pessimistic errors in the influential parameters like concentrations of alkali, mineral

acid, ligand, metal and total volume [81]. Hence, some representative systems were studied in order to have a cognizance of the effects of errors in concentrations of ingredients on the stability constants of binary metal complexes. These results are given in Table 15**.**

The data show that the order of affecting the magnitudes of stability constant is alkali > acid > ligand > metal. The increased standard deviation in stability constants and even rejection of some species on the introduction of errors confirms the correctness of the proposed models. This type of investigation is significant as the data acquisition was done under varied experimental conditions with different accuracies.

Table 14: Effect of errors in influential parameters on the stability constants of Ca(II)-P complexes in 1.5% w/v of SLS-water mixture.

		log β_{mlh}(SD)		
Ingredient	% Error	ML^{+}	MLH^{2+}	ML_2H^{+}
	0	3.49(6)	13.12(0)	16.55(2)
Alkali	-5	Rejected	16.34(55)	Rejected
	-2	Rejected	16.09(34)	Rejected
	+2	Rejected	16.99(22)	Rejected
	+5	6.99(34)	16.10(56)	Rejected
Acid	-5	6.90(**)	15.45(**)	Rejected
	-2	6.45(60)	15.34(45)	20.09(30)
	+2	Rejected	15.90(33)	Rejected
	+5	Rejected	Rejected	Rejected
Ligand	-5	4.65(50)	Rejected	17.45(23)
	-2	4.45(30)	14.43(40)	17.45(29)
	+2	4.08(30)	14.26(45)	17.34(20)
	+5	4.34(66)	14.62(34)	17.00(40)
Metal	-5	3.40(12)	13.14(14)	16.50(12)
	-2	3.41(19)	13.11(14)	16.46(19)
	+2	3.55(18)	12.13(14)	15.49(11)
	+5	3.46(20)	13.15(14)	16.40(10)

**High standard deviation

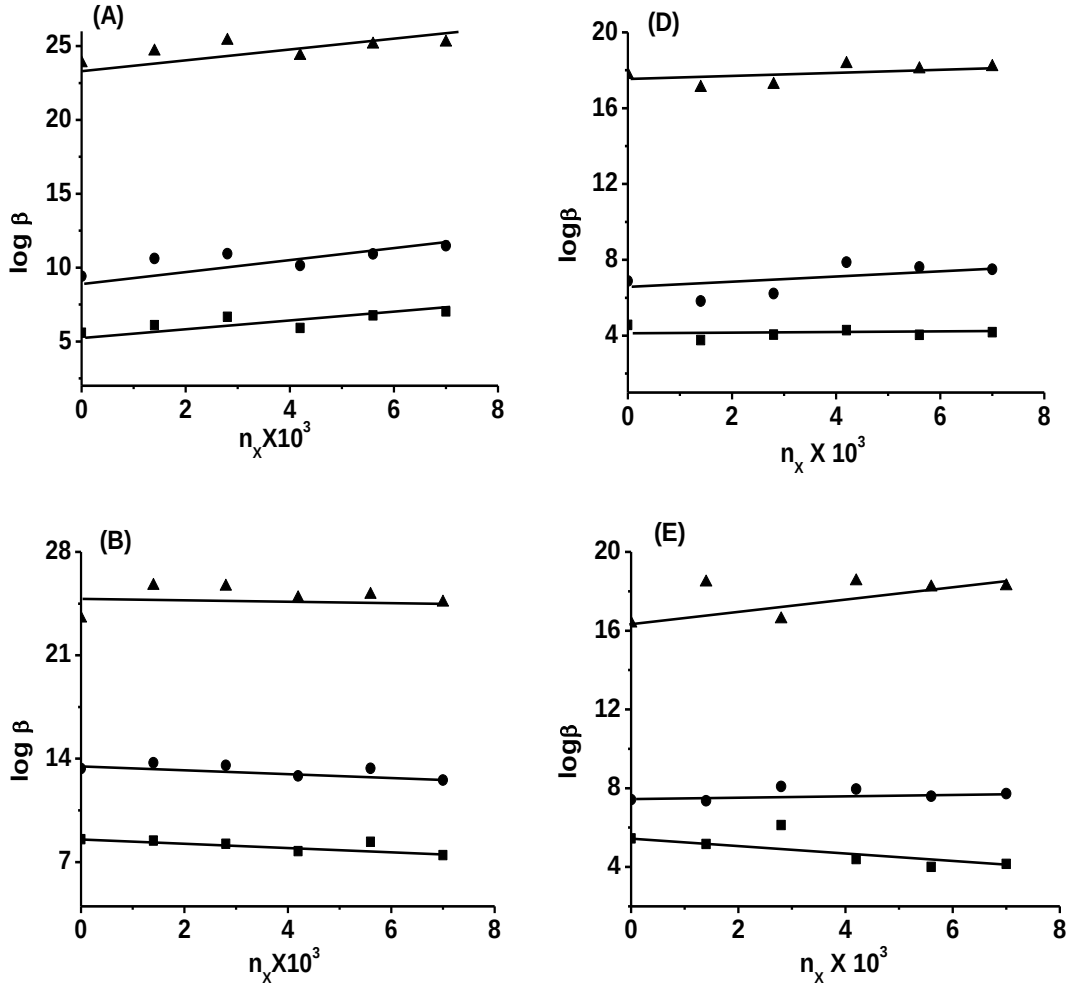
4.2.6. Effect of Surfactant on Metal-ligand Equilibria

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. The surfactant in aqueous solution considerably decreases the dielectric constant and these solutions are expected to mimic the physiological conditions. The formation of micelles creates low dielectric environment at the core of the micelles and the reactions taking place at the core [82]. The surfactant molecules in water aggregate to form micelles above its CMC. The role of micelles is simple, but vital, resulting in compartmentalization of the energy transfer partners. It separates non-polar molecules from each other by encapsulating them in hydrophobic cavity. By bonding the metal ions electrostatically to the negatively charged micellar surface (in SLS), their effective concentration is substantially increased.

The equilibria in solution is influenced by the surfactants 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. Micelles alter the reaction rates and shift equilibria primarily by concentrating reactants within the small volume of micellar pseudo phase. The extent of change is a product of the micellar effect on the reaction within the micellar pseudo phase and the distribution of reactants between the two phases. Micellar effects on reactivity are generally independent of changes in micellar size and shape. The effect of micelles on overall reaction rates and equilibria depends upon the incorporation of solutes into the micellar pseudo phase.

The stabilities of the complexes decrease with increase in surfactant concentration, which is due to the destabilization of these species with increasing surfactant content [83]. The variations of stability constants (log β) with mole fraction of different SLS-water media are shown in Fig. 21. The linear variation of the stability constants of the P and V complexes of Ca(II), Zn(II) and Mn(II) in SLS–water mixtures with mole fraction indicates that electrostatic forces dominate the equilibrium process under the employed experimental conditions. The stability of a complex depends on the polarity of the medium and the electrostatic attraction or repulsive forces operating between the complex species and the micellar surface. The species should be stabilized in the micellar medium with opposite charges due to electrostatic interactions but these

charged species should be destabilized due to the decreased dielectric constant of the medium. This trend was observed in SLS-water media (Figs. 21)



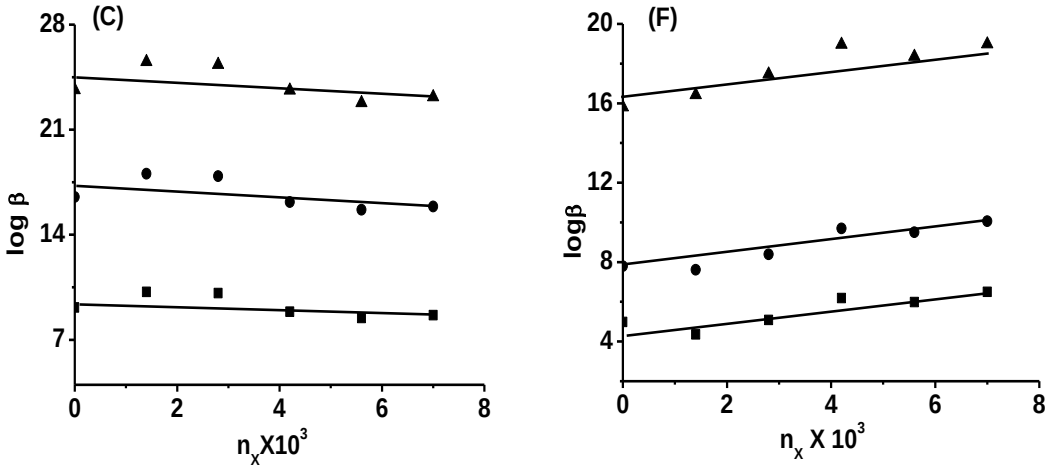


Figure 21: Variation of stability constants of P complexes of (A) Ca(II), (B) Zn(II) and (C) Mn(II) and V complexes of (D) Ca(II), (E) Zn(II) and (F) Mn(II) with reciprocal of dielectric constant (1/D) in SLS-water mixtures; (■) $\log \beta_{ML}$, (●) $\log \beta_{MLH}$ and (▲) $\log \beta_{ML2H}$.

4.2.7 Distribution Diagrams

The distribution diagrams indicate the relative abundance of various forms of metal (chemical speciation) at different pH and dielectric conditions. The percentage of metal ion in the form of various complex species is given by Eq. 4.3

$$PS = \frac{m(j) * \beta_{m(j)l(j)h(j)} * FL_i^{l(j)} * FH_i^{h(j)}}{\sum_{j=0}^{N} \beta_{m(j)l(j)h(j)} * FL_i^{l(j)} * FH_i^{h(j)}} * 100 \qquad (4.3)$$

Using the formation constant in the best fit model, distribution diagrams were obtained with the computer programs HYSS HYPERQUAD [84] . 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 SLS- water mixtures. The variation of species concentration with pH is shown in Figs. 22-28 for metal-P and metal-V complexes in

SLS-water mixtures. The patterns of the distribution of species with pH show that the concentrations of species are affected by solvent.

4.2.7.1. L-Proline and L-Valine Complexes

The protonation equilibria of P and V were discussed in the section 1. Hence, the plausible binary metal-ligand complexes can be predicted from these data. P exists as LH_2^+, LH and L^- in the pH ranges 1.5-3.2, 2.8-10.0 and 9.0-10.9, and V in the pH ranges 1.4-3.9, 4.0-8.9 and 8.8-11.0, respectively. Under the present experimental conditions, the predominant forms of the ligands are LH_2^+ and LH. The species that are refined are ML^+, MLH^{2+} and ML_2H^+ for Ca(II), Zn(II) and Mn(II) with P and V in SLS-water mixtures. The stability constants of these species are found to follow the trend Ca(II)< Mn(II) < Zn(II).

In the present study the binary species of Ca(II), Zn(II) and Mn(II) ions with P and V in SLS-water mixtures refined are ML^+, MLH^{2+} and ML_2H^+ in the pH range 1.3-10.9. The formation of various binary complex species is represented as Equilibria 1-5 for both the ligands.

$$M(II) + LH_2^+ \rightleftharpoons MLH^{2+} + H^+ \quad (1)$$

$$MLH^{2+} \rightleftharpoons ML^+ + H^+ \quad (2)$$

$$M(II) + LH \rightleftharpoons ML^+ + H^+ \quad (3)$$

$$MLH^{2+} + LH \rightleftharpoons ML_2H^+ + H^+ \quad (4)$$

$$ML^+ + LH_2^+ \rightleftharpoons ML_2H^+ + H^+ \quad (5)$$

For all metal-ligand systems, MLH^{2+} species is formed by the interaction of metal ion with LH_2^+ (Equilibrium 1), where the percentages of FM and LH_2^+ are decreasing with increasing percentage of MLH^{2+}. The ML^+ species is formed by deprotonation of MLH^{2+} (Equilibrium 2) or by the interaction of FM with LH species (Equilibrium 3). But Equilibrium 2 is more predominant than Equilibrium 3, because the concentration of FM decreases while concentration of LH increases with pH. ML_2H^+ species is formed through Equilibria 4 and 5. But Equilibrium 4 is predominant than Equilibrium 5, because the concentration of MLH^{2+} and LH are decreasing with increasing concentration of ML_2H^+.

An observation made from the distribution diagrams (Figs.22-28) is that the free metal ion concentration is more in case of V than P for Ca(II), Zn(II) and Mn(II) in SLS-water mixtures. This infers the stronger complexing ability of P than V, even though the distinctive cyclic structure of proline's side chain gives an exceptional conformational rigidity to P compared to other amino acids.

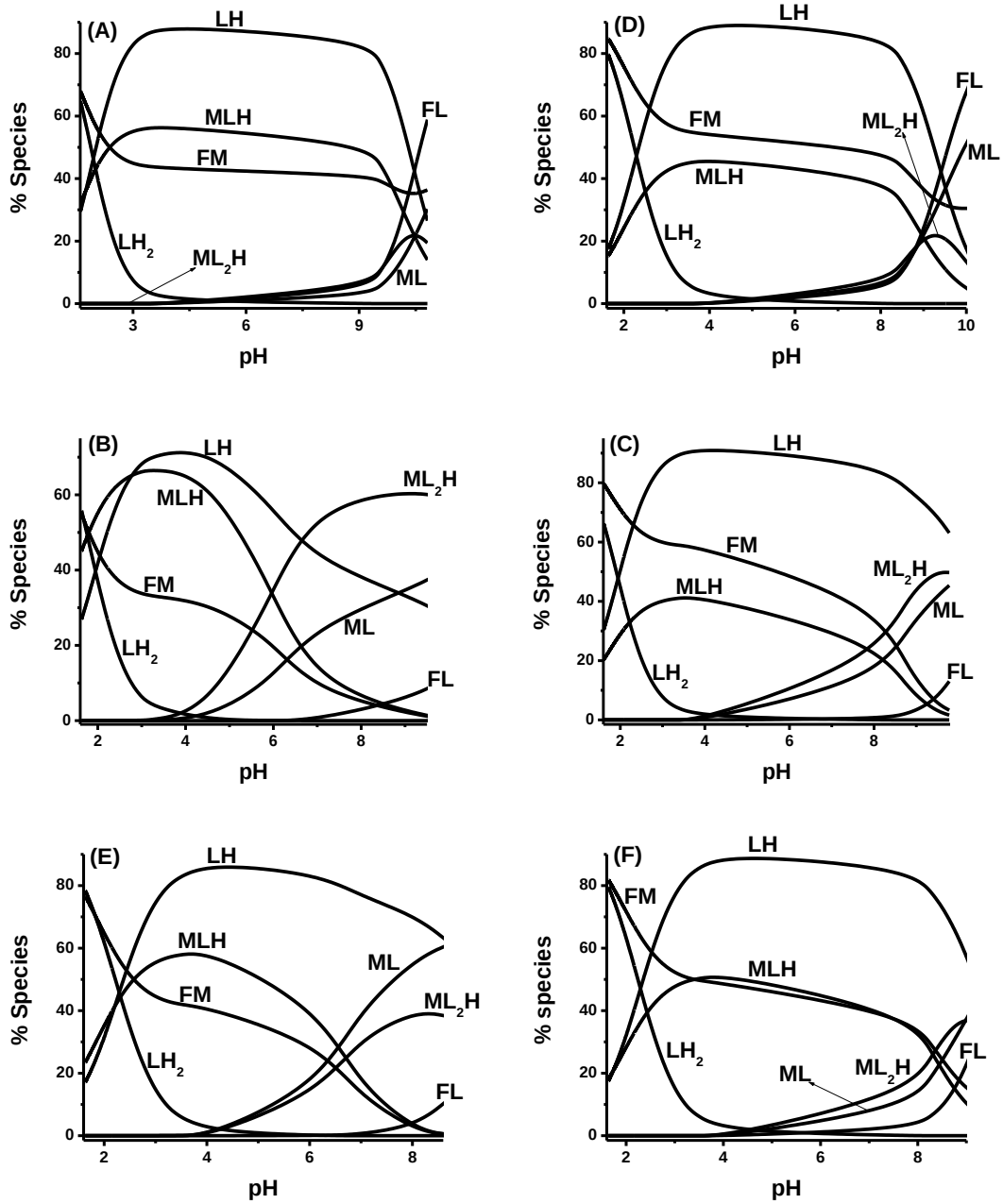


Figure 22: Distribution diagram of M(II)-P and V complexes in aqueous medium (A) Ca(II)-P, (B) Zn(II)-P, (C) Mn(II)-P, (D) Ca(II)-V, (E) Zn(II)-V ,(F) Mn(II)-V.

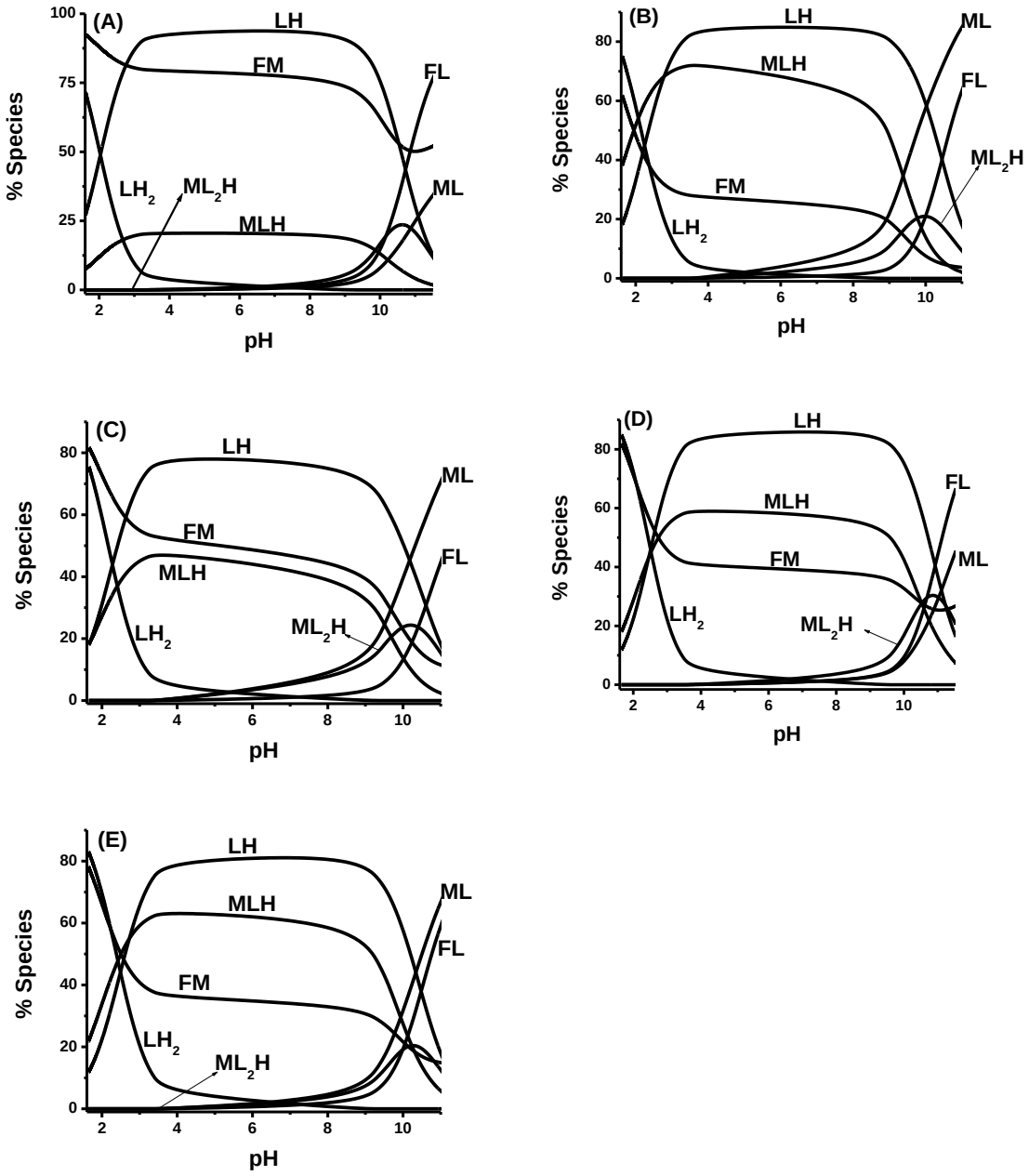


Figure 23: Distribution diagrams of Ca(II)-P complexes in SLS-water mixtures, (% w/v); (A) 0.5, (B) 1.0, (C) 1.5, (D) 2.0 and (E) 2.5.

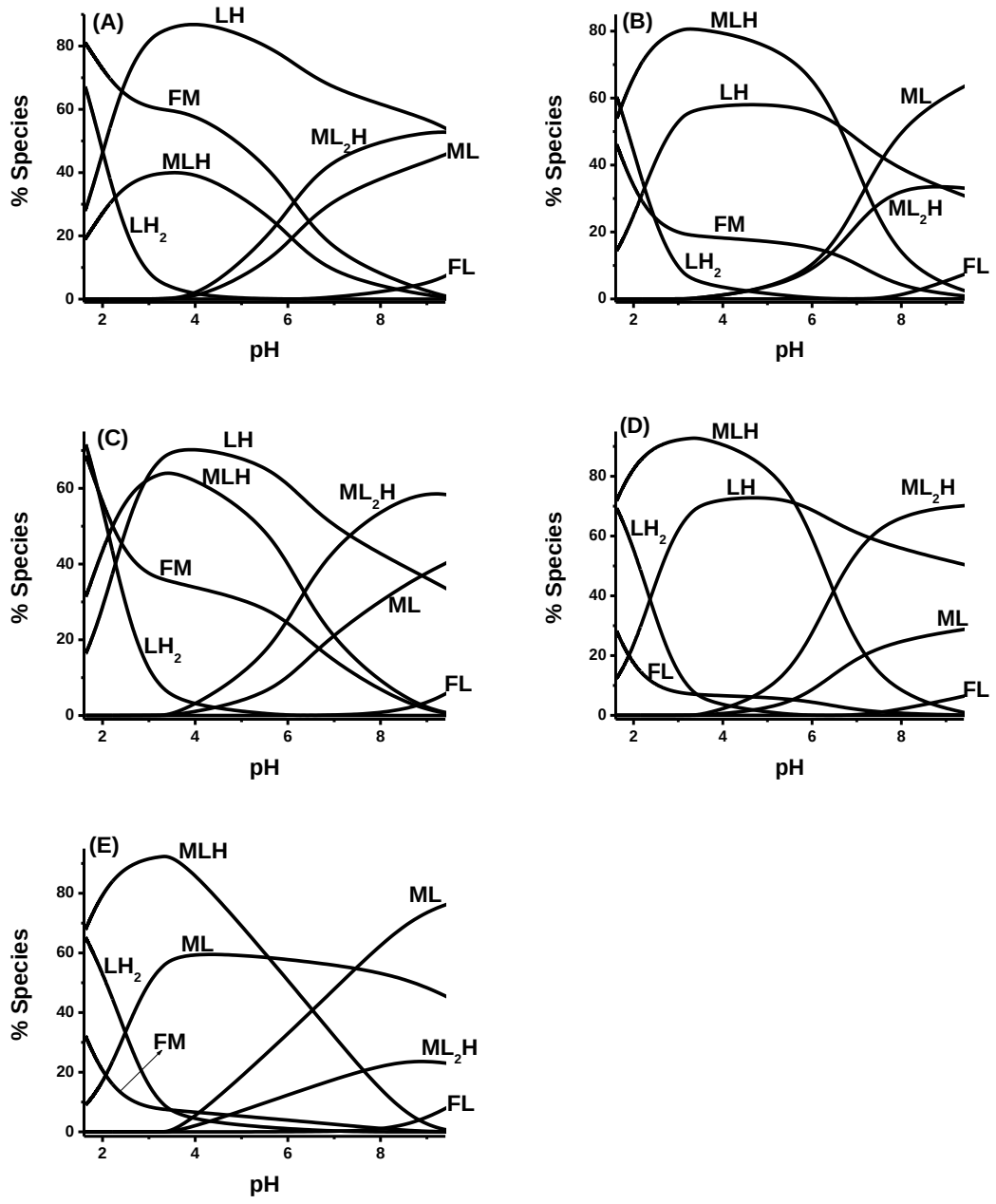


Figure 24: Distribution diagrams of Zn(II)-P complexes in SLS-water mixtures, (% w/v); (A) 0.5, (B) 1.0, (C) 1.5, (D) 2.0 and (E) 2.5.

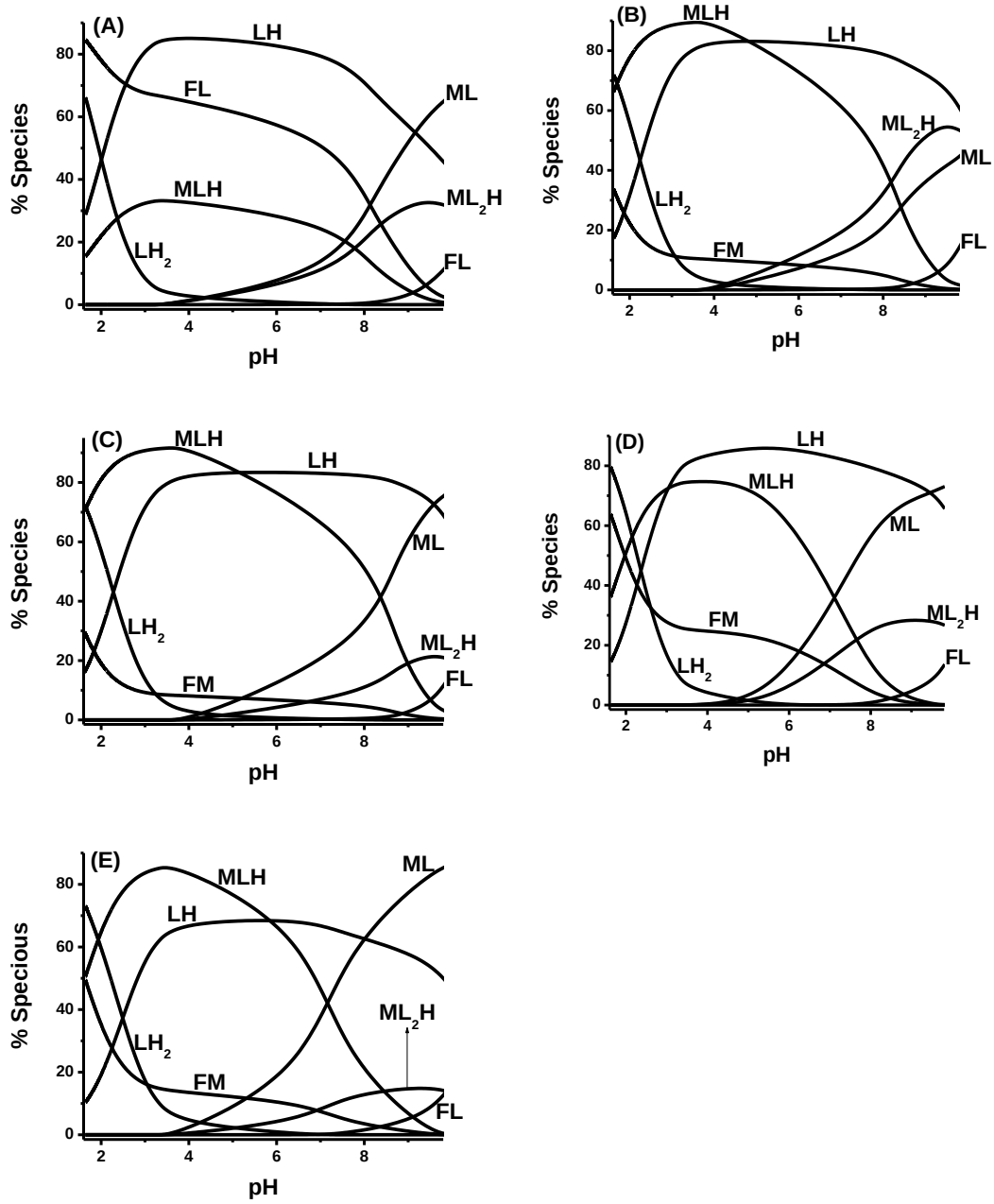


Figure 25: Distribution diagrams of Mn(II)-P complexes in SLS-water mixtures, (% w/v); (A) 0.5, (B) 1.0, (C) 1.5, (D) 2.0 and (E) 2.5.

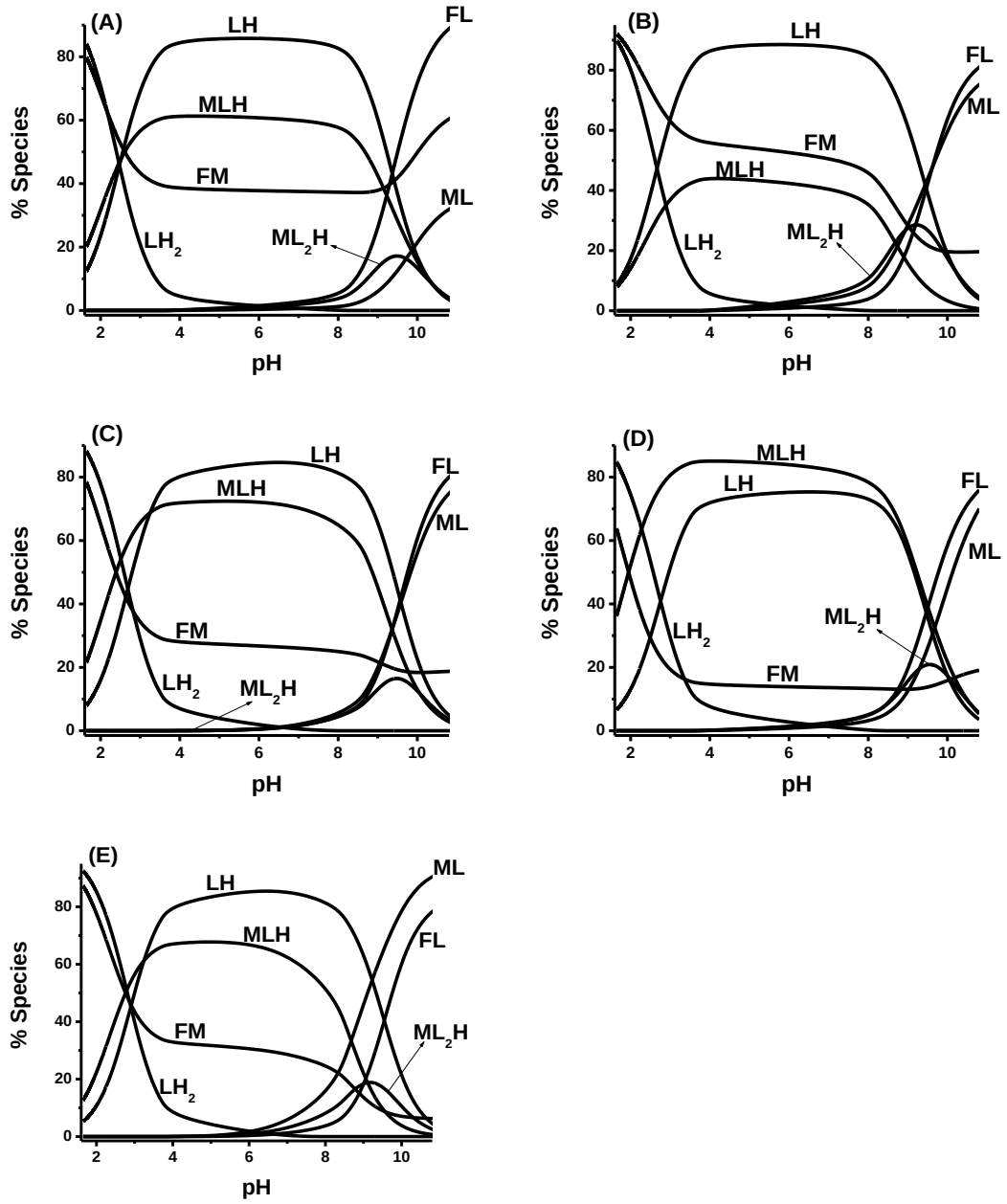


Figure 26: Distribution diagrams of Ca(II)-V complexes in SLS-water mixtures, (% w/v); (A) 0.5, (B) 1.0, (C) 1.5, (D) 2.0 and (E) 2.5.

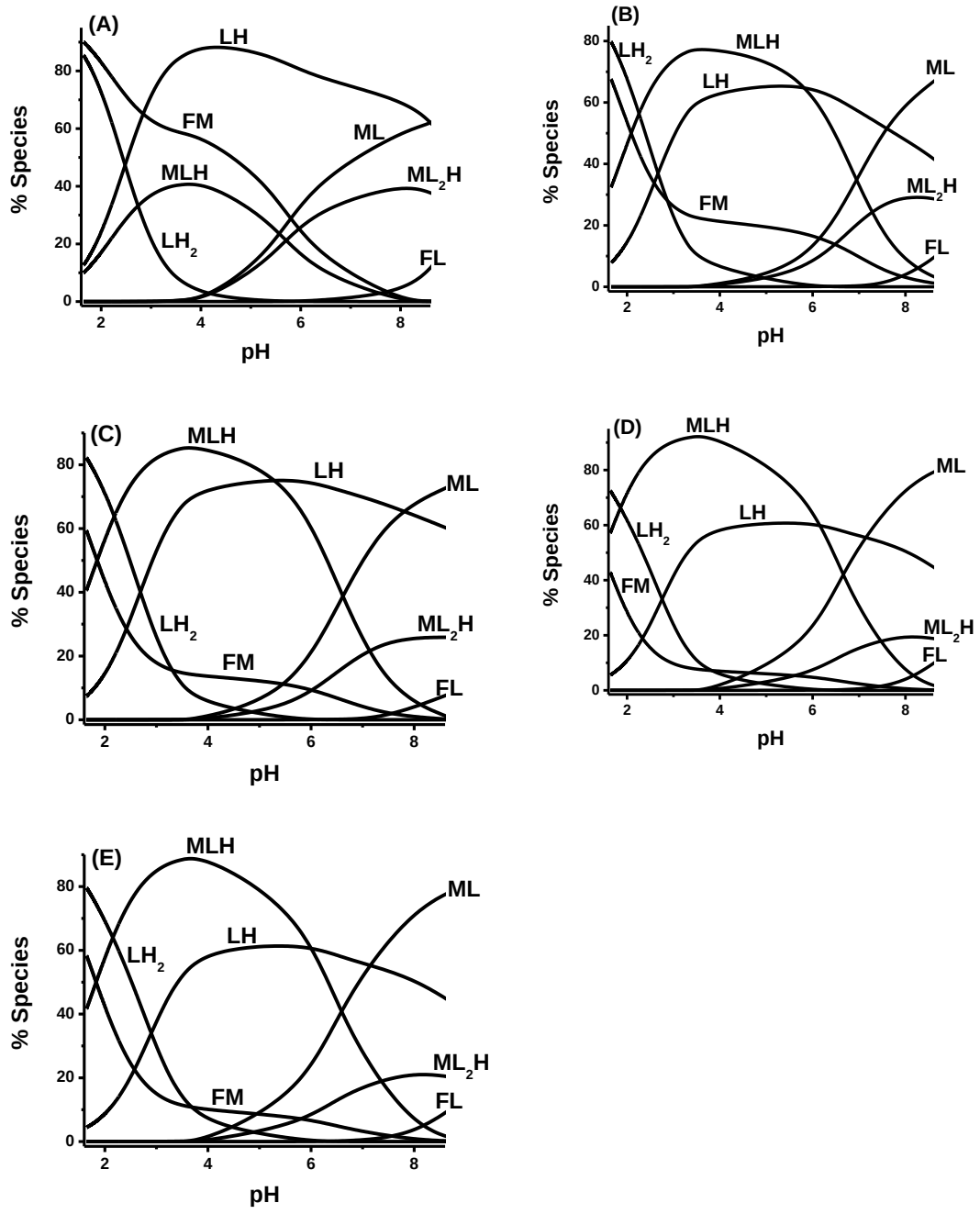


Figure 27: Distribution diagrams of Zn(II)-V complexes in SLS-water mixtures, (% w/v); (A) 0.5, (B) 1.0, (C) 1.5, (D) 2.0, and (E) 2.5.

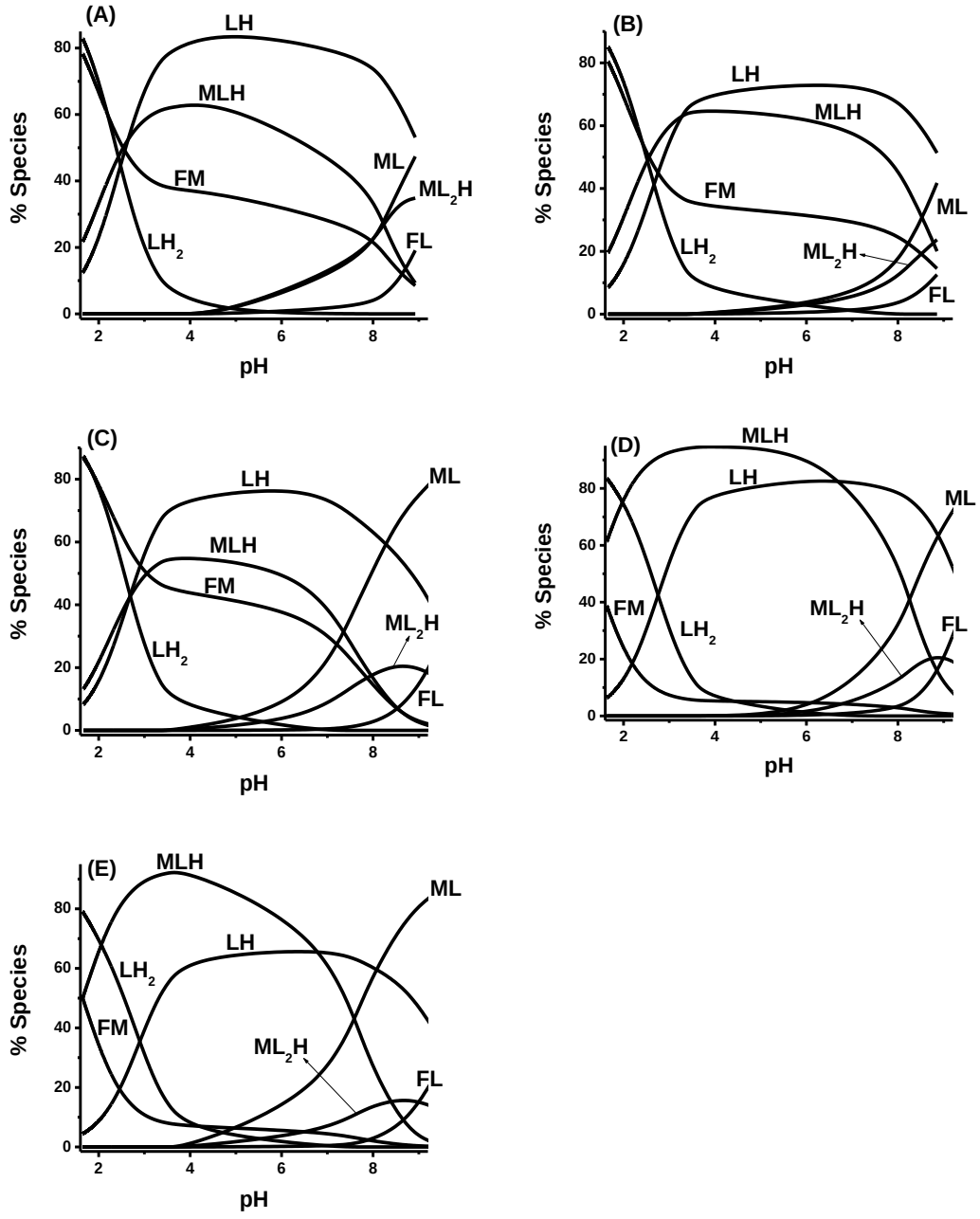


Figure 28: Distribution diagrams of Mn(II)-V complexes in SLS-water mixtures, (% w/v); (A) 0.5, (B) 1.0, (C) 1.5, (D) 2.0, and (E) 2.5.

4.2.7.2 Structures of Binary Complexes

Although it is not possible to elucidate or confirm the structures of the complex's pH metrically, they can be proposed based on literature reports and chemical knowledge. Amino nitrogen and carboxyl oxygen of P and V participate in bonding with metal ions. Amino nitrogen atoms can also associate with hydrogen ions in physiological pH ranges. Hence, there is often significant competition between hydrogen and metal ions for this donor site. This situation results in the simultaneous existence of a number of equilibria producing an array of protonated complexes as found in this study. Literature shows that, in aqueous solution, Ca(II), Zn(II) and Mn(II) ions typically form octahedral complexes. Thus, octahedral structures have been proposed tentatively as given in Figs.29 and 30.

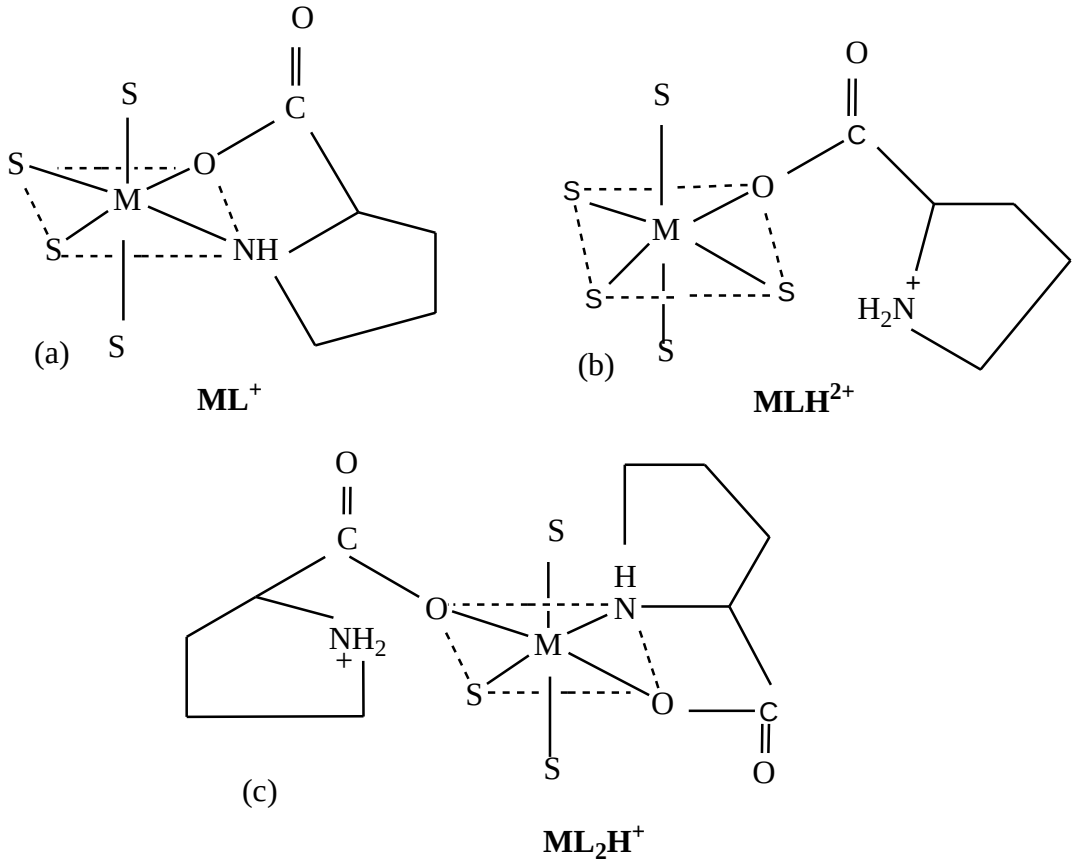


Figure 29: Speculated structures of P complexes with Ca(II), Zn(II) and Mn(II), where S is either solvent or water molecules

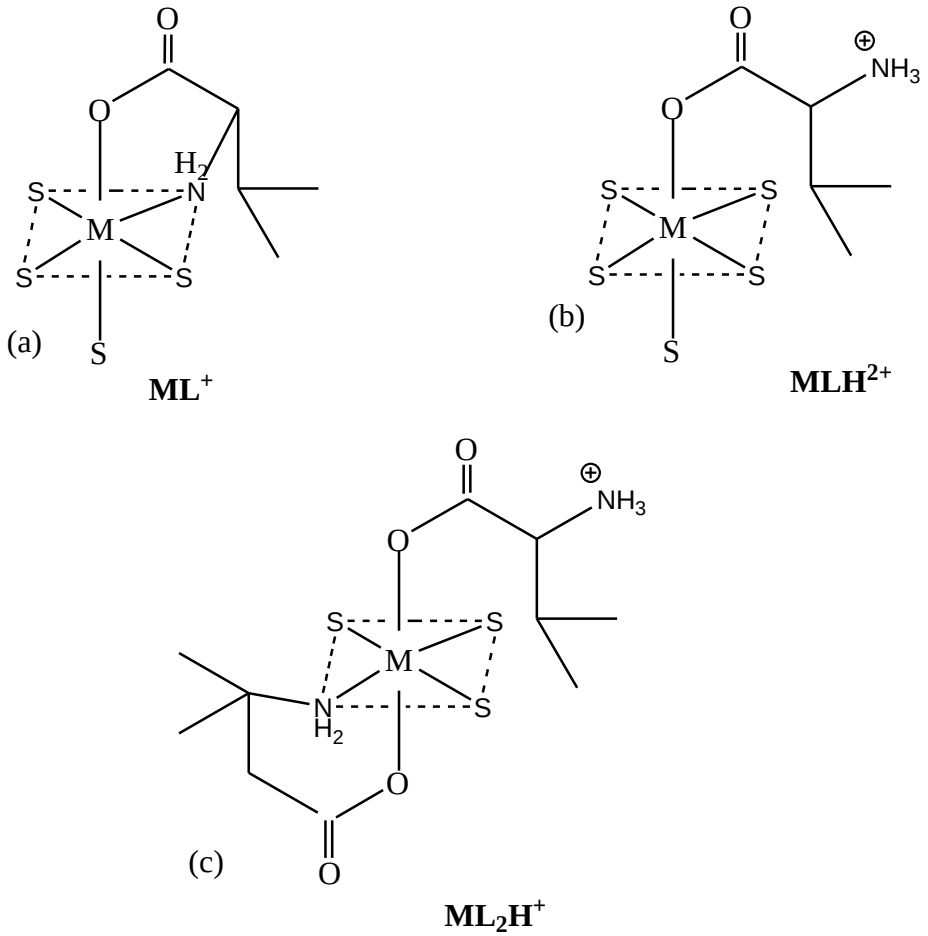
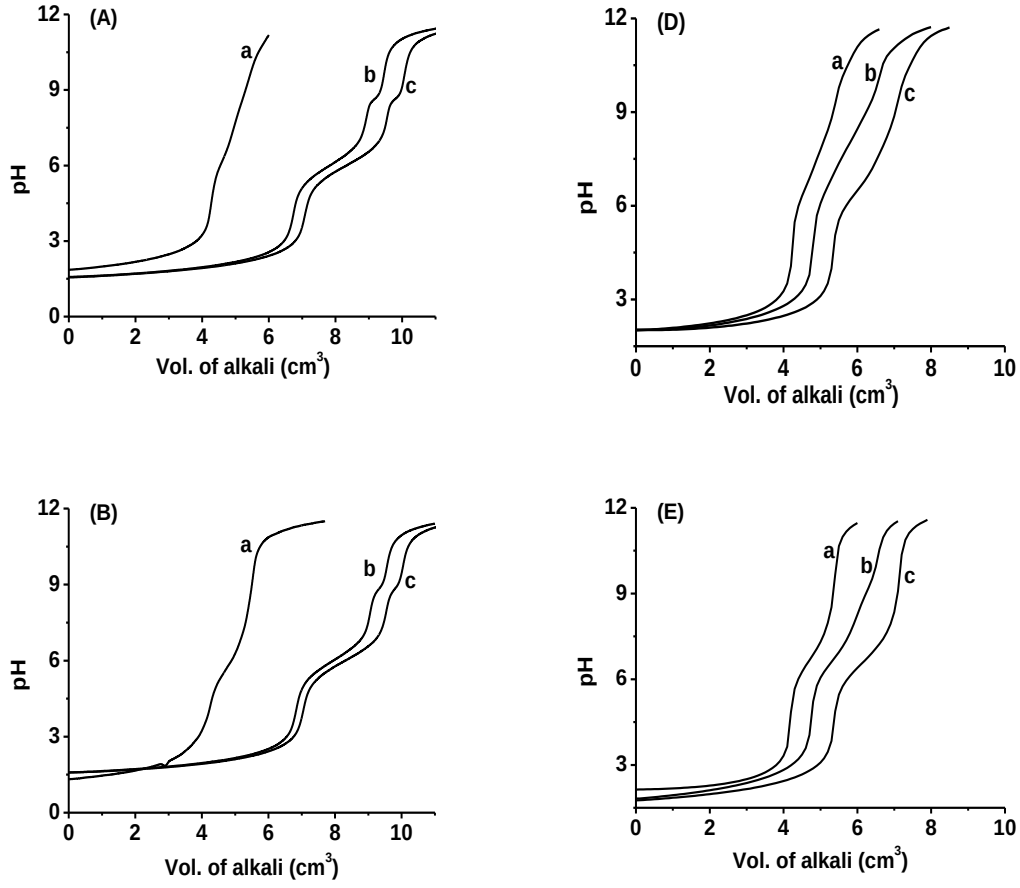


Figure 30: Speculated structures of V complexes with Ca(II), Zn(II) and Mn(II), where S is either solvent or water molecules

4.3 DETERMINATION OF TERNARY STABILITY CONSTANTS

The models containing different number of species were tested from the primary alkalimetric data. Only a few species were refined while other species were rejected by MINIQUAD75. Existence of species was determined by performing exhaustive modeling [85]. The alkalimetric titration curves of mixtures containing different mole ratios of P and V in the presence of mineral acid and inert electrolyte are given in Fig. 31. A preliminary investigation of the alkalimetric titration data inferred that these two ligands do not form any condensed species. In modeling studies, the total number of primary and secondary ligands was restricted to maximum of three in generating the ternary species.



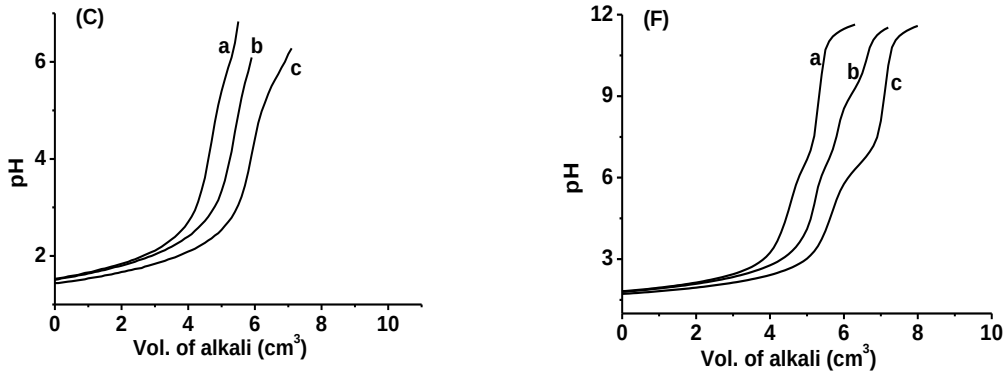


Figure 31: Alkalimetric titration curves for P-V complexes of (A) Ca(II), (B) Zn(II) and (C) Mn(II) in 0.5% w/v SLS-water mixture and (D) Ca(II), (E) Zn(II) and (F) Mn(II) in 1.5% w/v SLS-water mixture. M(II):P:V is (a) 1.0:2.5:2.5 (b) 1.0:2.5:5.0 and (c) 1.0:5.0:2.5.

4.3.1 Selection of Best fit Models

The qualitative evidence for the formation of mixed ligand complexes was obtained from the shift of the precipitation point of mixed ligand systems compared to those of the corresponding binary systems. In all these systems, the pH for precipitation of the mixed ligand systems was found to be more than any of the binary systems. Models containing various number and combinations of species were generated and refined using MINIQUAD75. The best fit models were chosen based on the statistical parameters [86] like $\chi 2$, R-factor, skewness and kurtosis. Such exhaustive modeling study was performed for all the systems and the final models are given in Table 16 for ternary complexes of Ca(II), Zn(II) and Mn(II) with P and V in SLS-water mixtures. Various possible equilibria for the formation of the ternary complexes refined in the present study have been proposed based on the existence of free metal, Various forms of the ligands and the binary complexes as found in the distribution diagrams (Figs. 33-35). They contain protonated and unprotonated ternary species like MLX, ML_2XH and $MLXH_2^{2+}$ for Ca(II), MLX, ML_2X^{-}, $MLXH_2^{2+}$ for Zn(II) and MLX, ML_2XH and $MLXH_2^{2+}$ for Mn(II) in SLS- water mixtures.

.

Table 15: Parameters of best fit chemical models of proline-M(II)-valine complexes in SLS-water mixtures. Temperature=310 K, Ionic strength=0.16 mol dm^{-3}.

SLS % w/v	log β_{mlxh}(SD) MLX	ML_2X^-	$MLXH^+$	$MLXH_2^{2+}$	MLX_2H_2	NP	U_{corr} *10^8	Skew-ness	χ^2	Kurt-osis	R-factor
Ca(II) (pH= 2.2-11.3)											
0.0	6.39(9)	---	---	26.22(4)	28.40(7)	100	0.1	0.08	12.04	3.08	0.001
0.5	5.72(8)	---	---	25.53(0)	28.51(6)	89	0.5	0.05	11.48	2.90	0.003
1.0	5.90(3)	---	---	26.21(6)	28.79(0)	99	3.0	1.05	11.09	3.08	0.005
1.5	6.51(1)	---	---	26.15(0)	29.23(9)	79	0.5	1.02	12.90	1.99	0.007
2.0	6.59(2)	---	---	26.39(7)	29.50(1)	106	0.6	1.01	13.07	2.78	0.013
2.5	7.63(0)	---	---	25.80(5)	29.72(0)	76	9.0	0.06	12.06	3.05	0.002
Zn(II) (pH= 3.0-10.0)											
0.0	11.63(8)	14.70(2)	---	26.00(6)	---	99	0.9	0.07	11.09	2.00	0.003
0.5	11.12(6)	14.60(0)	---	26.13(0)	---	87	1.7	0.01	11.34	3.09	0.014
1.0	11.59(1)	15.12(6)	---	25.20(3)	---	91	1.0	1.00	12.90	2.90	0.007
1.5	11.66(4)	15.39(5)	---	26.49(9)	---	76	0.7	0.44	13.00	2.12	0.021
2.0	12.50(0)	15.80(2)	---	25.60(2)	---	65	1.0	0.22	12.09	3.10	0.008
2.5	12.49(1)	16.51(3)	---	25.71(0)	---	100	0.6	1.00	11.90	3.09	0.002
Mn(II) (pH= 2.0-9.)											
0.0	7.54(1)	---	16.72(1)	24.65(7)	---	78	0.1	-0.13	12.90	3.11	0.002
0.5	9.49(1)	---	18.64(4)	26.57(3)	---	98	0.5	0.03	12.09	3.01	0.003
1.0	9.69(0)	---	18.78(2)	27.65(2)	---	90	0.7	-0.22	13.09	2.90	0.008
1.5	10.30(9)	---	19.19(1)	27.90(4)	---	76	0.2	0.89	12.05	2.05	0.001
2.0	10.42(2)	---	19.58(9)	27.81(9)	---	55	0.2	0.04	11.98	3.01	0.006
2.5	11.54(1)	---	20.00(3)	28.22(0)	---	90	1.0	0.11	12,09	3.00	0.009

4.3.2 Residual Analysis

A very low standard deviation in log β values indicates the precision of the parameters. The small values of U_{corr} indicate the consistency of the model with the experimental data [87]. For most of the systems, the kurtosis values are more than 3 and hence the residuals form leptokurtic pattern. Very few systems kurtosis values are less than 3 and hence they form a platykurtic pattern. The values of skewness between -0.22 and 1.05 shows that the residuals form a part of normal distribution and hence least squares method can be applied to the present data

4.3.3 Effect of Systematic Errors

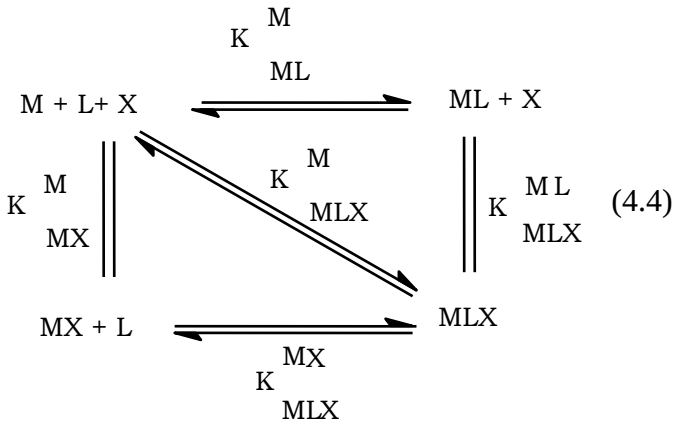
The results of typical systems are given in Table 17 emphasize that the errors in the concentrations of alkali and acid affect the stability constants more than those of the ligands and metal ions in SLS-water mixtures. The increased SD's and rejection of some species on the introduction of errors in the concentrations clearly infer the appropriateness of the experimental conditions.

Table 16: Effect of errors in influential parameters on the stability constants of ternary complexes of P-Ca(II)-V in 0.5% w/v SLS-water mixture.

Ingredient	Error %	log β_{mlxh}(SD)		
		MLX	$MLXH_2^{2+}$	$MLXH_2$
	0	6.39(9)	26.22(4)	28.40(7)
Alkali	-5	Rejected	Rejected	31.44(44)
	-2	Rejected	Rejected	31.22(32)
	2	10.59(32)	30.30(34)	Rejected
	5	Rejected	Rejected	31.44(50)
Acid	-5	9.45(44)	29.56(40)	30.06(45)
	-2	9.21(55)	29.88(36)	30.10(43)
	2	Rejected	Rejected	30.20(22)
	5	Rejected	Rejected	30.02(31)
Proline (L)	-5	7.03(32)	27.23(40)	29.44(9)
	-2	7.22(37)	27.15(24)	29.13(15)
	2	7.26(22)	27.17(34)	29.62(33)
	5	7.26(27)	27.36(32)	29.00(32)
Valine (X)	-5	7.33(17)	27.31(30)	29.91(12)
	-2	7.70(23)	27.84(28)	29.29(20)
	2	7.39(**)	27.63(45)	29.01(34)
	5	7.74(**)	27.04(33)	29.40(23)
Metal	-5	6.47(16)	26.37(18)	28.55(19)
	-2	6.44(19)	26.30(20)	28.56(20)
	2	6.41(19)	26.29(13)	28.57(27)
	5	6.46(11)	26.32(15)	28.53(16)

4.3.3 Stability of ternary complexes

The formation of mononuclear unprotonated binary and ternary complexes from a mixture of metal ion (M), primary (L) and secondary (X) ligands can be shown as the equilibria given in Eq. 4.4.



(4.4)

The change in the stability of the ternary complexes as compared to their binary analogues was quantified [88]based on the difference in stability (Δ log K) for the reactions ML with X and $M_{(aq)}$ with L and X, where L is primary ligand and X is the secondary ligand. It is compared with that calculated purely on statistical grounds. Eq. 4.5 can be formulated based on the properties of the cyclic systems reported earlier from which it is clear that both the ligands in the ternary complex influence mutually to the same extent.

$$\Delta \log K = \log K^{M}_{MLX} - \log K^{M}_{ML} - \log K^{M}_{MX} \qquad (4.5)$$

The electrostatic theory of binary complex formation and statistical arguments suggest the additional coordination positions of given multivalent hydrated metal ion available for the first ligand than for the second ligand. Hence, the usual order of stability $K^{M}_{ML} > K^{ML}_{MLX}$ applies. This suggests that Δ log K should be negative, although several exceptions [89] have been found. The statistical values of Δ log K for bidentate L and X are -0.4, -0.6 and between -0.9 and -0.3 for octahedral, square planar and distorted octahedral complexes respectively. Negative values of Δ log K can be understood as

the secondary ligand forms a more stable complex with hydrated metal ion than with ML. Whenever the experimental values of Δ log K exceed the statistical values, it can be inferred that the ternary complex is formed as a result of interaction of ML with X or MX with L.

Another approach to quantify [90] the stability of ternary complexes was based on the on the disproportionation constant (log X) given by Eq. 4.6.

$$\log X = 2\log K^{M}_{MLX} - \log K^{M}_{ML_2} - \log K^{M}_{MX_2} \quad \text{.....(4.6)}$$

This corresponds to the equilibrium

$$ML_2 + MX_2 \rightleftharpoons 2MLX$$

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

The Δ log K and log X values calculated from binary and ternary complexes are included in Tables 20. These values could not be calculated for some systems due to the absence of relevant binary species. In the present study, the Δ log K values range from -1.58 to 2.67 for SLS-water mixtures and some values are found to be higher than those expected on statistical basis (-0.4). These higher values account for the extra stability of the ternary complexes. The log X values are found to be higher than 0.6 which accounts for the extra stability of the ternary complexes. The reason [91] for the extra stability of these complexes may be due to interactions outside the coordination sphere such as the formation of hydrogen bonds between the coordinated ligands, charge neutralization, chelate effect, stacking interactions and electrostatic interaction between non-coordinated charge groups of the ligands.

Table 17: Δ log K and log X values of P-M(II)-V ternary complexes in SLS-water mixtures.

SLS % w/v	Δ log K_{MLX}	Δ log K_{MLXH2}	Δ log K_{MLXH}	Δ log K_{MLX2H2}	log X_{MLXH}
Ca(II)					
0.0	1.84	2.11	---	1.67	---
0.5	1.62	0.97	---	1.66	---
1.0	-0.44	1.74	---	1.41	---
1.5	0.31	0.88	---	1.74	---
2.0	1.39	0.6	---	1.5	---
2.5	1.17	1.03	---	1.22	---
Zn(II)					
0.0	-0.07	1.09	---	---	---
0.5	-1.03	2.2	---	---	---
1.0	-0.69	-1.07	---	---	---
1.5	-0.94	0.59	---	---	---
2.0	-1.01	-1.58	---	---	---
2.5	0.03	-1.57	---	---	---
Mn(II)					
0.0	0.54	2.67	1.33	---	1.14
0.5	1.04	2.27	2.49	---	2.04
1.0	0.85	1.88	1.61	---	1.34
1.5	0.13	1.96	0.61	---	1.66
2.0	-0.15	1.9	1.81	---	0.87
2.5	-0.8	1.38	0.63	---	0.19

3.3.4 Effect of Surfactant on Mixed ligand equilibria

The stability of the complex depends on the polarity of the medium and the electrostatic attraction or repulsive forces operating between the complex species and the micellar surface [82]. The variations of stability constants as a function of mole fraction of the different surfactants are shown in Fig. 32. The variation of overall stabilities constant values or change in free energy with co-solvent content depends upon two factors, viz., electrostatic and non-electrostatic contribution to the free energy change. Hence, the linear or almost linear variations of stability constants (logβ) with the mole fraction of SLS (Figure 32) indicate the dominance of electrostatic forces over non-electrostatic forces [92] are dominating under present experimental condition.

The decrease or increase in the stability constants may arise primarily from the difference in the environment of the species as well as from the distribution of the

micellar. When the complex species have negative charge, they shall be destabilized by anionic micelles due to the same environment on the micellar surface. This trend is observed in the present study (Fig. 32b).

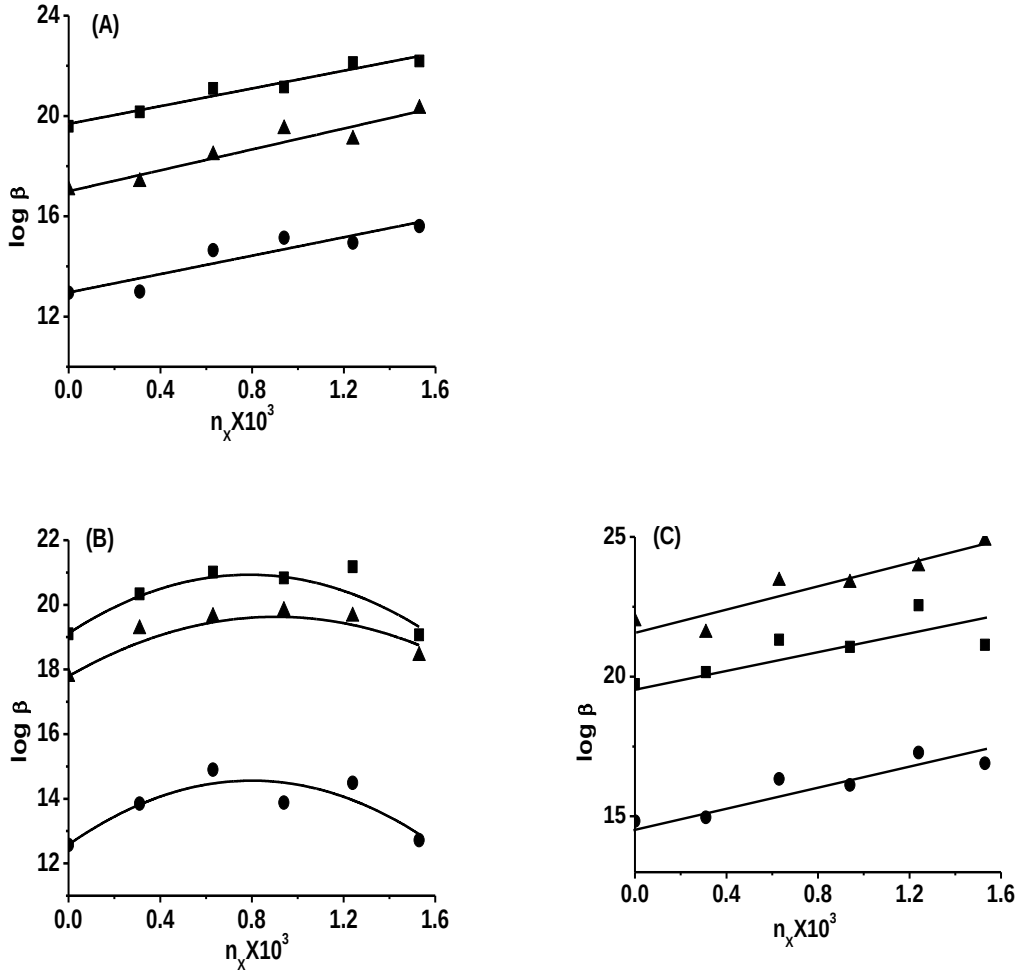


Figure 32 : Variation of magnitude of stability constants (log β) of ternary complexes of P and V with mole fraction of SLS-water mixtures: (A) Ca(II), (B) Zn(II) and (C) Mn(II).

4.3.4 Distribution diagrams

The distribution diagrams indicate the relative abundance of various forms of metal ions (chemical speciation) at different pH and dielectric conditions. Various possible equilibria for the formation of the ternary complexes refined in the present study have been proposed based on the existence of free metal, Various forms of the ligands and

the binary complexes as found in the distribution diagrams (Figs. 33-35). The formation of the ternary complex species can be represented by the following equilibria.

They contain protonated and unprotonated ternary species like MLX, $MLXH_2^{2+}$ and

$M(II) + LH_2^+ + XH_2^+ \rightleftharpoons MLXH_2^{2+} + 2H^+$ ……(1)

$MLH^{2+} + XH_2^+ \rightleftharpoons MLXH_2^{2+} + H^+$ ……(2)

$MXH^{2+} + LH_2^+ \rightleftharpoons MLXH_2^{2+} + H^+$ ……(3)

$M(II) + LH_2^+ + XH_2^+ \rightleftharpoons MLXH^+ + 3H^+$ ……(4)

$MLH^{2+} + XH_2^+ \rightleftharpoons MLXH^+ + 2H^+$ …… (5)

$MXH^{2+} + LH_2^+ \rightleftharpoons MLXH^+ + 2H^+$ (6)

$MLXH_2^{2+} \rightleftharpoons MLXH^+ + H^+$ (7)

$MLXH_2^{2+} \rightleftharpoons MLX + 2H^+$ ..8)

$MLXH^+ \rightleftharpoons MLX + H^+$.(9)

$M(II) + 2LH + XH \rightleftharpoons ML_2X^- + 3H^+$ (10)

$MLXH^+ + LH \rightleftharpoons ML_2X^- + 2H^+$.(11)

$MLX + LH \rightleftharpoons ML_2X^- + H^+$.(12)

$M(II) + 2LH + XH \rightleftharpoons ML_2XH + 2H^+$ (13)

$MLXH_2^{2+} + XH \rightleftharpoons MLX_2H_2^+ + H^+$.(14)

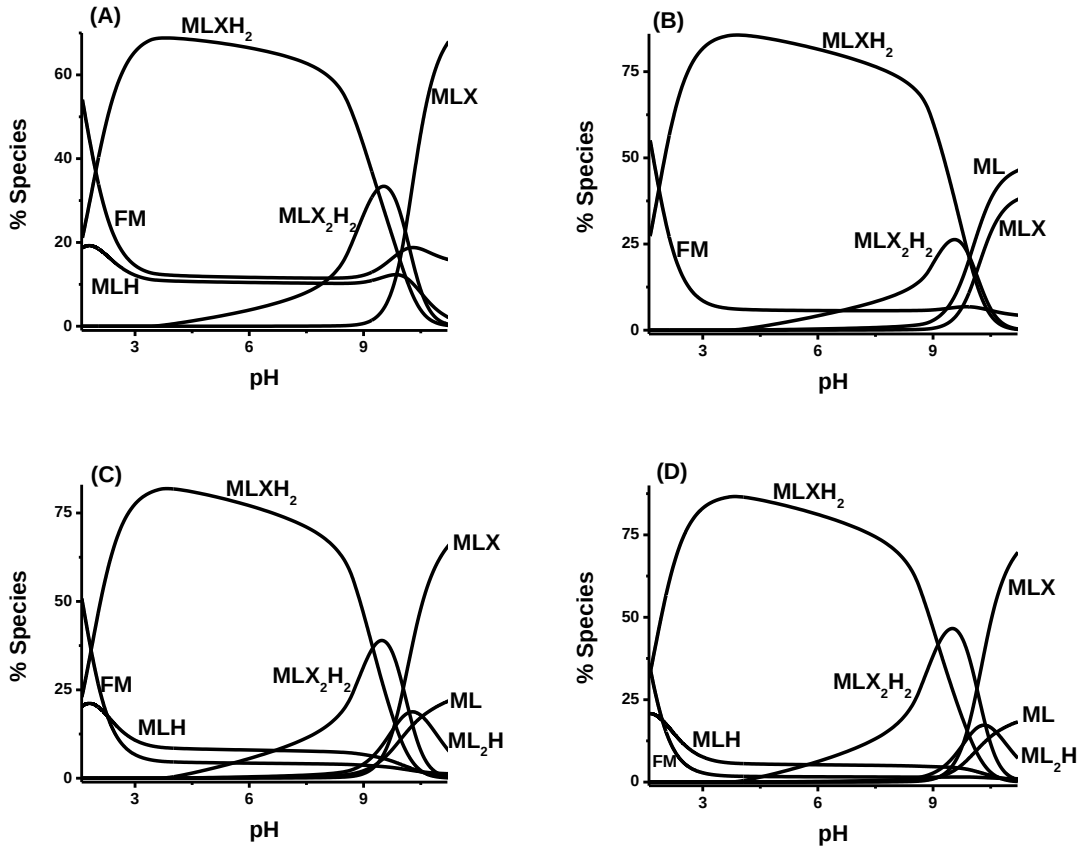
$MLXH^+ + XH \rightleftharpoons MLX_2H_2^+$ …(15)

$MLXH^+ + XH_2^+ \rightleftharpoons MLX_2H_2^+ + H^+$ ..(16)

MLX_2H_2 for Ca(II) in the pH range 2.2-9.5, MLX, MLX^-, $MLXH_2^{2+}$ for Zn(II) in the pH range 1.7-10.5 and MLX, $MLXH^+$ and $MLXH_2^{2+}$ for Mn(II) in the pH range 1.4-9.6 in SLS-water mixtures.

For all the metal-ligand systems $MLXH_2^{2+}$ species is formed by the interaction of metal ion with LH_2^+ and XH_2^+, because the percentage of metal ion (M(II)), LH_2^+ and XH_2^+ are decreasing with increasing percentage of $MLXH_2^{2+}$ (Equilibrium 1). It may also be formed by the interaction of MXH^{2+} with LH_2^+ (Equilibrium 3) because the percentages of LH_2^+ and MXH^{2+} (which are formed at lower pH) are decreasing with increasing percentage of $MLXH_2^{2+}$. MLX species is formed by the deprotonation of $MLXH_2^{2+}$ (Equilibrium 8) and $MLXH^+$ (Equilibrium 9).

Equilibrium 8 is more predominant for Ca(II), Zn(II) and Equilibrium 9 is more predominant for Mn(II) because the percentage of $MLXH^{+}$ is decreasing where the percentage of MLX increasing. For Ca(II), ML_2XH species can be formed by the interaction of metal ion (M) with LH and XH (Equilibrium 13). For Zn(II), ML_2X^{-} species can be formed by the Equilibria 10-12, but the equilibrium 12 is more predominant. For Mn(II), $MLXH^{+}$ species is formed by deprotonation of $MLXH_2^{2+}$ (Equilibrium 7). A stable ternary complex shall be responsible for metal ion transportation in bio-systems and the weak binary metal complexes make the essential metal ions bioavailable. The increased concentrations of complexing agents make the essential metal ions unavailable due to the formation of stable metal complexes. Distribution diagrams were drawn using the formation constants of the best fit model and are given in Figs. 33-35 for SLS-water mixtures.



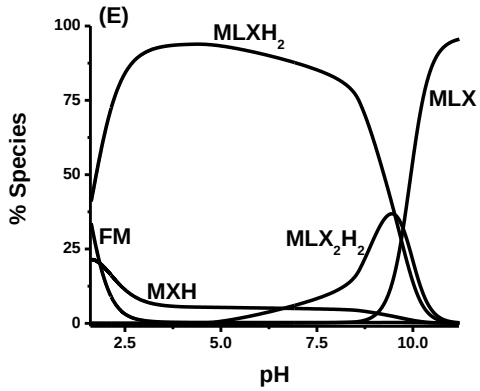
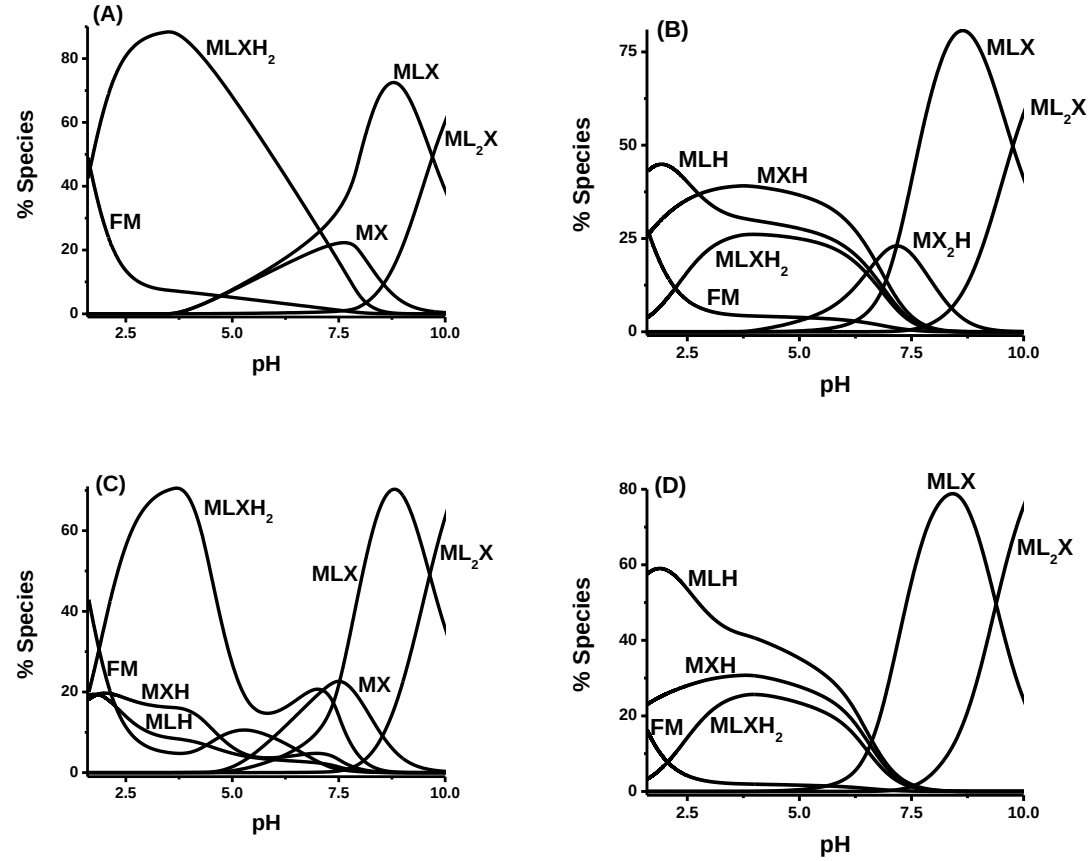


Figure 33: Distribution diagrams of P-Ca(II)-V complexes in SLS-water mixtures, (% w/v); (A) 0.5, (B) 1.0, (C) 1.5, (D) 2.0 and (E) 2.5. Ratio of Ca(II) :P:V is 1.0:5.0:2.50.



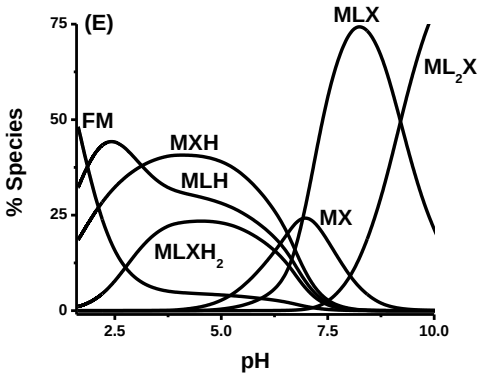


Figure 34: Distribution diagrams of P-Zn(II)-V complexes in SLS-water mixtures, (% w/v); (A) 0.5, (B) 1.0, (C) 1.5, (D) 2.0 and (E) 2.5. Ratio of Zn(II):P:V is 1.0:5.0:2.50.

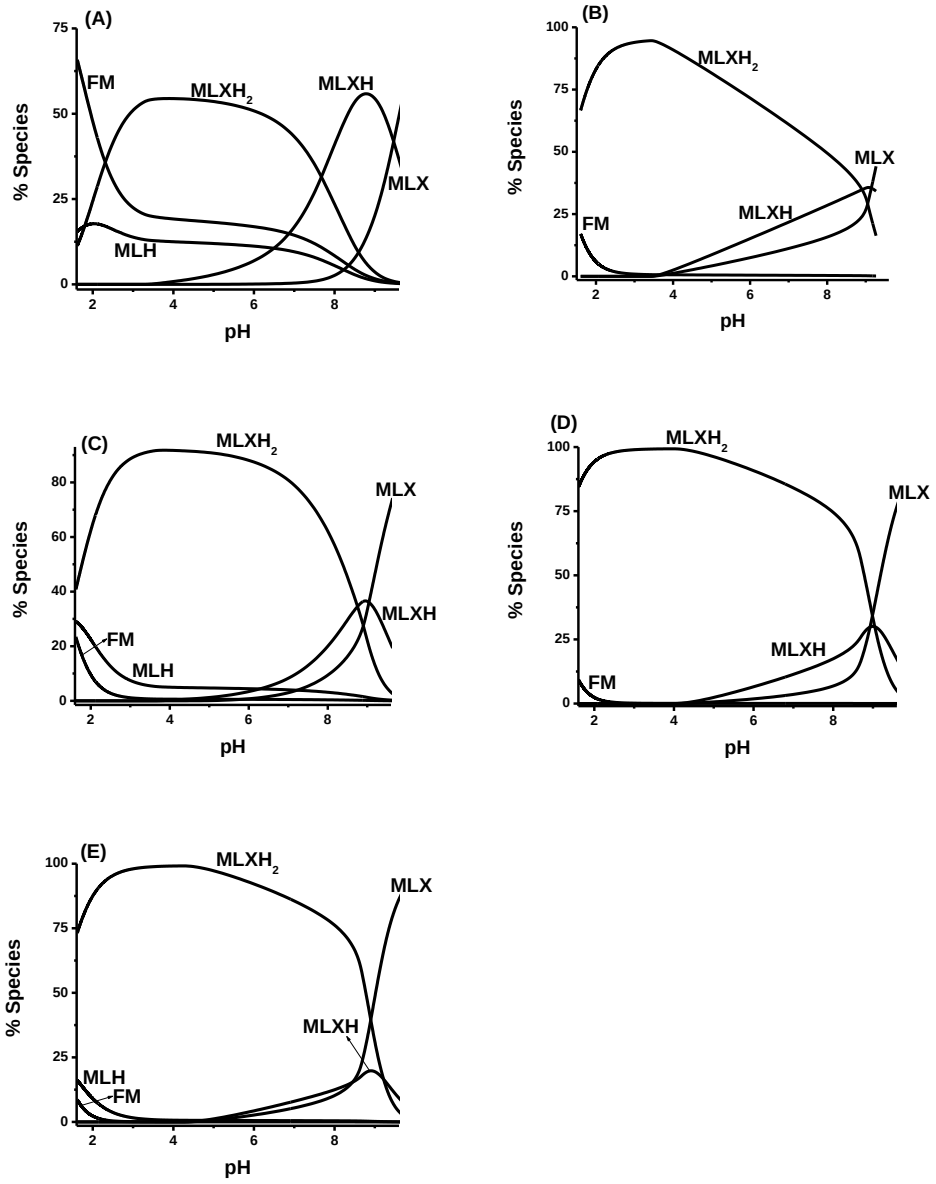


Figure 35 : Distribution diagrams of P-Mn(II)-V complexes in SLS-water mixtures, (% w/v); (A) 0.5, (B) 1.0, (C) 1.5, (D) 2.0 and (E) 2.5. Ratio of Mn(II):P:V is 1.0:5.0:2.50.

4.3. 5 Structures of ternary complexes

Although it is not possible to elucidate or confirm the structures of complex species pH metrically, they can be proposed based on the basic chemical knowledge, the nature of the ligand and metal ion, protonation and deprotonation equilibria of P and V. In aqueous solutions Ca(II), Zn(II) and Mn(II) are coordinated by six water molecules $[M(H_2O)_6]^{2+}$. Amino acids replace the coordinated water molecules to form metal-amino acid complexes [93] through carboxyl oxygen and amino nitrogen.

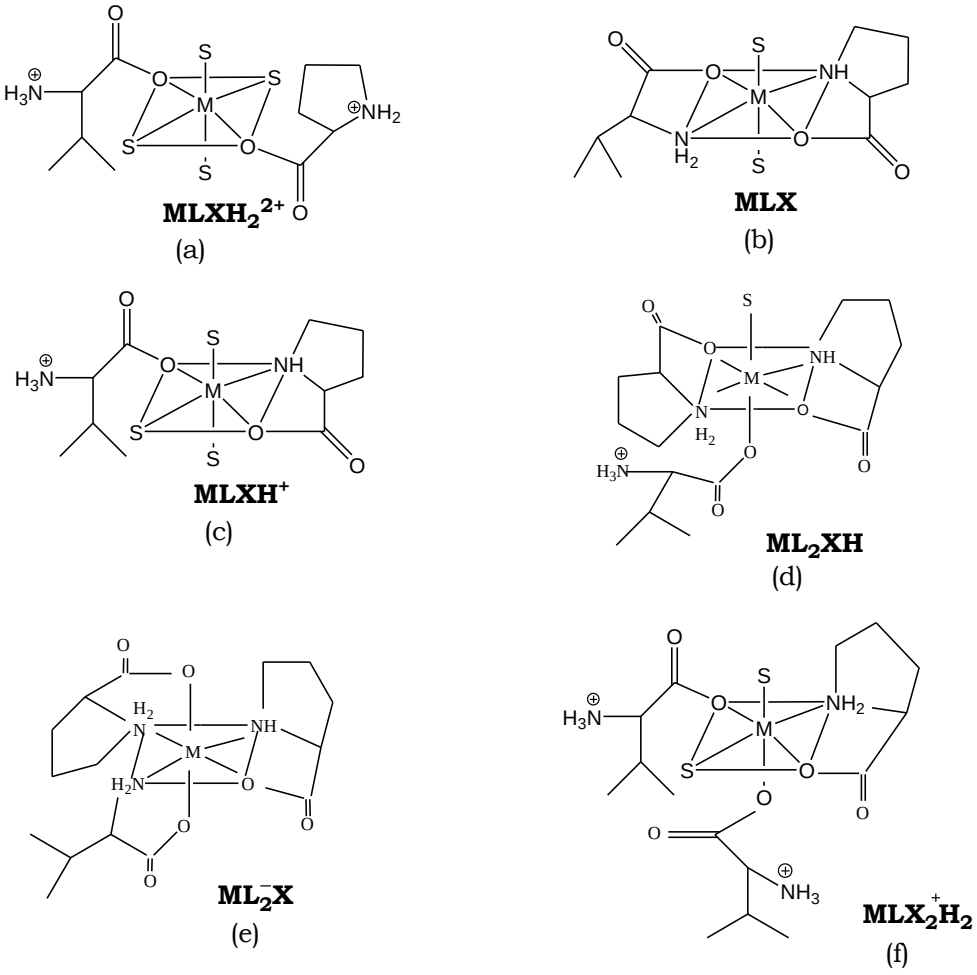


Figure 36: Speculative structures of ternary complexes of P and V with Ca(II), Zn(II) and Mn(II), where S is either solvent or water molecule

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The speciation studies of essential metal ion complexes are useful to identify those metal species that are likely to have adverse effect on biota. Knowledge of chemical speciation of metal ions is needed in human biology, nutrition, toxicology, in biomedical applications, and in clinical practice. Speciation includes oxidation state, concentration and composition of each of the species present in a chemical sample. The studies carried out on these systems under the present experimental conditions are useful to understand the role played by the active site cavities in biological molecules. Hence, chemical speciation of Ca(II), Mn(II), and Zn(II) complexes with L-Proline and L-Valine in varying compositions of SLS-water mixture has been carried out. The following conclusions are drawn based on the experimental results.

Protonation Equilibria

The investigation by the biomimetic studies indicates the pH ranges of protonation equilibria of P and V in SLS-water mixture as 1.5-11.2 and, 1.4-10.8 respectively. Both P and V exists as LH_2^+ at low pH and gets deprotonated with the formation of LH and L^- successively with increase in pH. Overlapping of formation function verses pH curves for different concentrations of the ligand indicates non-polymerization of this ligand under the pH condition employed in the presented study. Non-linear trend of step-wise protonation constants of P and V as a function of mole fraction of the medium indicates the dominance of non-electrostatic forces over electrostatic forces. Effect of errors in the ingredients indicated that the protonation constants associated with carboxyl proton were more affected than the amino proton. This is probably due to lower magnitude of carboxyl protons than amino proton. The overlap of experimental and simulated titration curves indicates the adequacy of the experimental data.

Metal-Ligand Equilibria

The biomimetic studies of metal ion complexes with P and V indicate the formation of protonated complexes at lower pH and unprotonated complexes at higher pH. The binary species refined for both P and V complexes of Ca(II), Zn(II) and Mn(II) were ML^{+}, MLH^{2+} and ML_2H^{+} . None overlapping of the formation curves evinced the formation of protonated complexes. The possibility of the formation of poly-nuclear complexes was excluded as the ratio of TLO to TMO was greater than unity. The observed more free metal ion concentration in V complexes than P complexes indicates that stronger complexing ability of P than V. The linear variation of the stability constants of the P and V complexes of Ca(II), Zn(II) and Mn(II) with mole fraction indicates the dominance of electrostatic forces. Errors introduced in the concentrations of ingredients affected the magnitudes of stability constants of metal complexes which were in the order: alkali > acid > ligand > metal.

Mixed-Ligand Equilibria

The species detected for mixed ligand complexes are: MLX, ML_2XH and $MLXH_2^{2+}$ for Ca(II), MLX, ML_2X^{-}, $MLXH_2^{2+}$ for Zn(II) and MLX, ML_2XH and $MLXH_2^{2+}$ for Mn(II). Complex species having negative charges are destabilized by anionic micelles due to the same environment on the micellar surface. The values of Δ log K indicated that the ternary species had extra stability compared to their binary species, may be due to the interactions outside the coordination sphere, such as the formation of hydrogen bonds between the coordinated ligands, charge neutralization, chelate effect, stacking interactions and the electrostatic interaction between non-coordinated charge groups of the ligands. This study also gives an insight into the bioavailability/bioaccumulation of these metals. The ternary complexes are more amenable for metal transport because of their extra stability while the binary complexes make the metal bioavailable due to their decreased stability.

5.2 RECOMMENDATION

- Using the present finding data as baseline, further investigation can be conducted to synthesize the maximum yield of solid complexes at fixed pH value.
- In our country Ethiopia there were no more investigation was carried out regrading chemical speciation of elements to ensure the environmental quality and bioavailability of the chemical species, hence the author recommend that researchers focus more on this study.
- The structure of the complexes in the present study were proposed potentiometrically. Therefore, NMR and FTIR Spectroscopy should further confirm it.

Research Fund Acknowledgment

This research project is funded by Adama Science and Technology University under the grant number ASTU/SM-R/007/19 , Adama,Ethiopia

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