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RESEARCH ARTICLE

CHEMICAL SPECIATION OF BINARY COMPLEXES OF L-PHENYLALANINE AND MALEIC ACID WITH SOME METAL IONS IN DMF- WATER MIXTURES.

Wondimu Tesfa Tefera, Wakgari Bungula Negassa, Tajedin Alansi, Tegene Desalegn Zeleke and Hadgu Hailekiros Belay.

Department of Applied Chemistry, Adama Science and Technology University, Adama, Oromia, Ethiopia.

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Abstract

Speciation of binary complexes of Co(II), Ni(II), and Cu(II) with L-phenylalanine and maleic acid in the presence of Dimethylformamide-water mixtures in the concentration range of 0–50% v/v DMF has been studied pH-metrically at a temperature of 303K and at an ionic strength of 0.16 mol L⁻¹. The selection of best fit chemical models is based on statistical parameters and residual analysis. The predominant species detected were ML, ML₂, and ML₂H₂ for Co(II), Ni(II), and Cu(II). The appropriateness of the experimental conditions were verified by introducing errors deliberately. The models containing different numbers of species were refined by using the computer program MINQUAD75. The chemical speciation was explained based on the distribution diagrams drawn using HYSS HYPERQUAD. The trend in variation of stability constants of the complexes with dielectric constant of the medium was attributed for the formation and possible structures of the complex species presented.

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Corresponding Author:- Hadgu Hailekiros Belay.

Address:- Department of Applied Chemistry, Adama Science and Technology University, Adama, Oromia, Ethiopia.

Introduction:-

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 [1]. For instance some elements can be highly toxic to various life forms; others are considered essential, but can become toxic at higher doses. Many of these effects depend strongly on the particular form in which the element is present in the system [2]. The bioavailability and toxicity of the complexes are critically dependent on their thermodynamic and kinetic stability. Identifying these species (i.e., speciation) and obtaining reasonable estimates of their thermodynamic and kinetic stabilities are the problems that continue to plague environmentalists and toxicologists [3]. Hence, extensive attention has been paid in recent years to the study of the chemical speciation of ligands with metal ions.

L-phenylalanine (LPA) is an essential α -amino acid. It is classified as non-polar because of the hydrophobic nature of the benzyl side chain. It is an electrically neutral amino acid, one of the twenty common amino acids used to biochemically form proteins, coded for by DNA. It is used in the manufacture of food and drink products and sold as a nutritional supplement for its reputed analgesic and antidepressant effects. A non-food source of phenylalanine is the artificial sweetener aspartame. LPA is the starting compound used in the flavonoid biosynthesis [4]. The genetic disorder phenylketonuria (PKU) is the inability to metabolize phenylalanine. Individuals with this disorder are known as "phenylketonurics" and must regulate their intake of phenylalanine. Phenylalanine uses the same active transport channel as tryptophan to cross the blood-brain barrier and, in large quantities, interferes with the production of serotonin. Individuals who cannot metabolize phenylalanine must monitor their intake of protein to control the buildup of phenylalanine as their bodies convert protein into its component amino acids [5]

Maleic acid (MA) is industrially derived by hydrolysis of maleic anhydride; maleic anhydride is produced by oxidation of benzene or butane. It is an industrial raw material for the production glyoxylic acid by ozonolysis. Maleic acid and fumaric acid do not spontaneously interconvert because rotation around a carbon-carbon double bond is not energetically favorable. However, conversion of the cis isomer into the trans-isomer is possible by photolysis in the presence of a small amount of bromine [6].

Cobalt is a central component of the vitamin, cobalamin (vitamin B₁₂). Cobalt in the form of cyanocobalamin is most efficiently stored in kidneys and liver. Cobalt is essential for the production of red blood cells. It acts as coenzyme in several biochemical processes. Nickel is associated with several enzymes,[7,8] and any variation in its concentration leads to metabolic disorders. Nickel is present in enzymes like urease, present in a wide range of plant species. The signs of nickel deprivation include depressed growth, reproductive performance, and plasma glucose. It also affects distribution and proper functioning of other nutrients including calcium, iron, zinc, and vitamin B₁₂. Copper is an essential element for life on earth. The biological functions include electron transfer, dioxygen transportation, oxygenation, oxidation, reduction, and disproportionation [9,10]. The melanin pigment of the skin is also a copper containing protein.

N,N'-Dimethylformamide (DMF) is a polar (hydrophilic) aprotic solvent with high boiling point. It facilitates reactions that follow polar mechanisms. It is able to form the H-bonded network structures with dipolar aprotic solvents [6]. Therefore, DMF-water mixture was selected as media to maintain the dielectric properties of the medium in comparable levels to those of the physiological fluids since the polarity at the active site cavities should generally be applicable when it is possible to compare ligand binding to the metal ion as in protein residues and solvent environment [11].

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

Experimental:-**Chemicals and Standard Solution:-**

All the chemicals used in this experiment were of analytical reagent grade purity. Triple distilled deionized water was used for the preparation of all the solutions. Solutions of 0.1 mol L⁻¹ of Co(II), Ni(II), Cu(II) chlorides (E-Merck, India) were prepared maintaining 0.05 mol L⁻¹ hydrochloric acid to suppress the hydrolysis of metal salts. A solution (0.05 mol L⁻¹) of L-phenylalanine and maleic acid (GR grade, E-Merck, Germany) were prepared by

maintaining a 0.05 mol L^{-1} hydrochloric acid concentration to increase its solubility. Dimethylformamide (Finar, India) was used as received. Solutions of 0.2 mol L^{-1} hydrochloric acid (GR grade, Merck, India) and 0.4 mol L^{-1} sodium hydroxide (E-Merck, India) were prepared. A solution of 2.0 mol L^{-1} sodium chloride (Merck, India) was prepared to maintain the ionic strength in the titrand. All the solutions were standardized by the usual oxalic acid and potassium hydrogen phthalate solutions, while the normality of hydrochloric acid was determined by using the standardized sodium hydroxide and the primary borax solutions [12]. The concentration of the metal ions were determined complex metrically by titrating against a standard solution of EDTA using xylenol orange indicator and hexamethylenetetramine powder as buffer to maintain the pH at 5.0-6.0 [13]. So as to assess the errors that might have crept into the determination of the concentrations, the data were subjected to one-way analysis of variance (ANOVA) by using the computer program, COST [14]. The strengths of alkali and mineral acid were determined using the Gran plot method [15,16].

Alkalimetric Titrations:-

The pH measurements of metal-ligand binary systems were carried out in aqueous media containing varying compositions of Dimethylformamide in the range of 0-50% (v/v) maintaining an ionic strength of 0.16 mol L^{-1} with sodium chloride at $303 \pm 0.1 \text{ K}$ by using a digital pH meter ELICO (Model Li 120, India) type readability of 0.01 (0-14). The electrode of the cell was calibrated with 0.05 mol L^{-1} potassium hydrogen phthalate solution in the acidic region and with 0.01 mol L^{-1} borax solution in the alkaline region to measure the response in the pH range 2.0-11.0. The effect of variations in asymmetry potential, liquid junction potential, activity coefficient, sodium ion error and dissolved carbon dioxide on the response of the glass electrode were accounted for in the form of correction factor [17, 18]. Mechanical stirring of the solution was carried out by means of a Teflon stirrer.

To verify whether the electrode was equilibrated, a strong acid was titrated with an alkali every day until no appreciable differences were observed between the pH values of two titrations at the corresponding volumes of titrant. A calomel electrode was refilled with DMF-water mixture of the equivalent composition to that of the titrand. Free acid titrations were performed before the metal-ligand titrations to calculate the correction factor. In each of the titrations, the titrand consisted of a mineral acid of approximately 1 mmol in a total volume of 50mL. Titrations with different metal-to-ligand ratios (1:2.5, 1:3.75, and 1:5) were carried out with 0.4 mol dm^{-3} sodium hydroxide.

Modeling Strategy:-

The computer program SCPHD [19] was used to calculate the correction factor. The binary stability constants were calculated from with the pH-metric titration data using the computer program MINIQuAD75 [20] which exploit the advantage of a constrained least-squares method in the initial refinement and reliable convergence of the Marquardt algorithm. During the refinement of the binary systems, the correction factor and the protonation constants of phenylalanine were fixed.

Results and Discussion:-

The results of the best fit models that contain the stoichiometry of the complex species and their overall formation constants along with some of the important statistical parameters are given in Table.1

Table. 1:-Parameters of best fit chemical models of M(II) – maleic acid complexes in DMF-water medium.

% v/v DMF	log β_{mlh} (SD)			NP	Ucorr	Skew- ness	χ^2	R- Factor	Kurtosis	pH- Range
	ML	ML ₂	ML ₂ H ₂							
Co(II)										
0.0	4.57(05)	6.89(05)	17.77(07)	50	1.06	1.34	14.57	0.0092	3.43	1.5-8.2
10.0	4.23(33)	6.09(08)	17.89(05)	67	1.48	0.13	12.09	0.0103	3.71	2.2-8.2
20.0	3.47(09)	6.30(06)	17.46(05)	69	1.11	0.12	19.54	0.0083	3.72	2.2-8.2
30.0	3.30(08)	5.90(05)	17.05(05)	72	1.86	0.33	13.70	0.0072	3.32	2.2-8.2
40.0	4.39(03)	7.09(06)	18.24(11)	75	1.09	-0.12	12.07	0.0182	3.49	2.0-8.2
50.0	3.12(03)	6.47(00)	17.35(09)	44	1.98	1.15	13.31	0.0111	6.51	2.0-8.2
Ni(II)										
0.0	5.46(08)	7.43(06)	16.39(02)	100	1.06	1.53	16.26	0.0131	4.92	1.8-6.6
10.0	3.77(02)	5.68(04)	17.31(02)	55	1.16	-0.14	9.59	0.0080	3.38	2.1-6.6

20.0	4.39(06)	7.30(03)	18.36(01)	56	1.77	1.51	10.53	0.0112	3.44	2.1-6.6
30.0	4.32(08)	7.47(03)	18.36(03)	77	1.73	-0.14	11.97	0.0151	2.64	2.1-6.6
40.0	4.23(01)	6.96(01)	18.04(10)	89	1.68	1.22	6.68	0.0202	3.26	1.9-6.6
50.0	3.40(07)	6.44(02)	17.63(06)	44	2.22	-0.22	10.44	0.0222	2.72	1.9-6.6
Cu(II)										
0.0	4.97(05)	7.80(09)	15.80(14)	63	1.59	0.01	8.84	0.0082	3.43	2.0-5.8
10.0	3.86(08)	6.24(08)	16.76(07)	69	1.57	-0.11	12.56	0.0042	3.34	2.0-5.8
20.0	4.77(08)	7.90(03)	18.44(12)	70	1.81	-0.13	9.36	0.0082	2.99	2.0-5.8
30.0	3.72(06)	6.95(09)	17.32(09)	88	1.68	0.62	6.69	0.0102	5.00	2.1-5.8
40.0	4.90(07)	7.85(01)	18.25(13)	448	1.58	-0.13	12.60	0.0203	3.90	2.1-5.8
50.0	3.30(06)	7.20(08)	17.38(34)	49	1.32	0.01	4.67	0.0185	3.33	2.5-5.8

$U_{\text{corr}} = U/(NP-m) \times 10^8$, where m = number of species; NP=Number of experimental points; SD=Standard deviation.

Table.2:-Parameters of best fit chemical models of M(II) – L-Phenylalanine complexes in DMF-water medium

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

$U_{\text{corr}} = U/(NP-m) \times 10^8$, where m = number of species; NP=Number of experimental points; SD=Standard deviation

A very low standard deviation in log β values indicates the precision of these parameters. The small values of U_{corr} (the sum of squares of deviations in concentrations of ingredients at all experimental points) corrected for degrees of freedom indicate that the model can represent the experimental data. Small values of mean, standard deviation, and mean deviation for the systems.

corroborate that the residuals are around a zero mean with little dispersion. Kurtosis 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) [21]. If the 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 as well as platykurtic patterns and very few form mesokurtic patterns. The values of skewness recorded in Table 2 are between -1.62 and 0.74. These data suggest that the residuals form a part of normal distribution. Hence, least-squares method can be applied to the present data. The sufficiency of the model is further evident from the low crystallographic R-values. These statistical parameters thus show that the best fit models portray the metal–ligand species in DMF-water media.

Effect of systematic errors on best fit model:-

In order to obtain the best chemical model for critical evaluation and application under varied experimental conditions with different accuracies of data acquisition, an investigation was under taken by introducing pessimistic errors in the influential parameters like concentrations of alkali, mineral acid, ligand, and metal (Table 3). The order of the ingredients that influence the magnitudes of stability constants due to incorporation of errors is alkali > acid > ligand > metal. Some species were even rejected when errors are introduced in the concentrations. The rejection of some species and increased standard deviations in the stability constants on introduction of errors confirm the suitability of the experimental conditions (concentrations of ingredients) and choice of the best fit models.

Table 3:-Effect of errors in influential parameters on the stability constants of Cu(II)-MA complexes in 30% v/v DMF-water medium.

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

Table 4:-Effect of errors in influential parameters on the stability constants of Co(II)-LPA complexes in 40% v/v DMF-water medium.

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

Effect of Solvent on Metal-Ligand Equilibria:-

Co-solvent influences the equilibria in solution due to change in the dielectric constant (D) of the medium that varies the relative contribution of electrostatic and non-electrostatic interactions which in turn vary the magnitude of stability constants [22].

DMF is a dipolar aprotic solvent. It is a structure former and enhances the water structure in DMF-water mixtures; hence, it removes water from the coordination sphere of metal ions, making them more reactive towards the ligands. As a result, the stability of the complexes is expected to increase. At the same time, it is a coordinating solvent, and it competes with the ligands for coordinating the metals [11]. This decreases the stability of the complexes. Hence, the stability of the complexes is expected to either increase or decrease. The variation of overall stability constants with co-solvent content depends upon electrostatic and non-electrostatic factors.

The co-solvent induced increased basicity of DMF-water mixtures increases the stabilization of protons. The trends of stability constant ($\log \beta$) values of complexes of LPA with $1/D$ of DMF-water mixture are given in Figs 1. The trend is almost linear which indicates that the dielectric constant or long range interactions are responsible for the stability trend. This linear increase indicates the dominance of the structure-forming nature of DMF over the complexing ability.

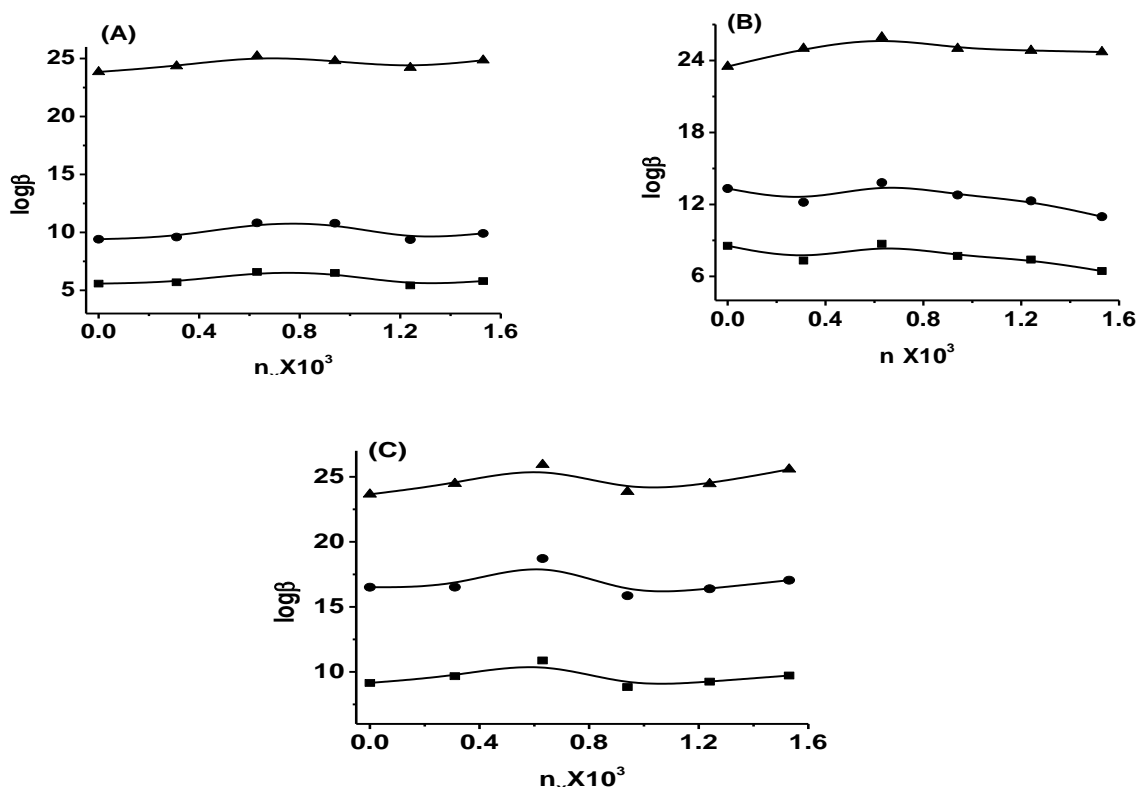


Fig. 1:-Variation of stability constant values of metal-phenylalanine complexes with mole fraction of DMF-water medium

(A) Co(II) (■) $\log \beta_{ML}$, (●) $\log \beta_{ML_2}$, (▲) $\log \beta_{ML_2H_2}$.

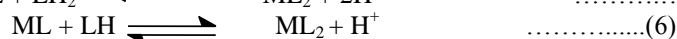
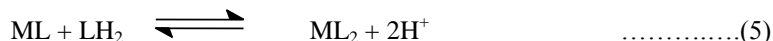
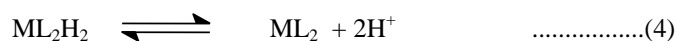
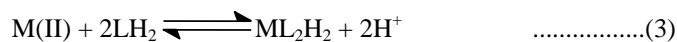
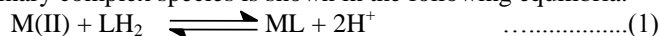
(B) Ni(II) (■) $\log \beta_{ML}$, (●) $\log \beta_{ML_2}$, (▲) $\log \beta_{ML_2H_2}$.

(C) Cu(II) (■) $\log \beta_{ML}$, (●) $\log \beta_{ML_2}$, (▲) $\log \beta_{ML_2H_2}$.

Distribution Diagrams

The present investigation reveals the existence of ML , ML_2 and ML_2H_2 for Co(II), Ni(II) and Cu(II). The ML_2 species is the predominant species (Figs.2. & 3.) at higher pH and ML_2H_2 is the predominant species at lower pH among all the binary complexes. Low concentration of free metal ion (FM) indicates the strong complexing nature of MA and LPA.

The formation of various binary complex species is shown in the following equilibria.



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

Some typical distribution diagrams of M(II)-LPA and M(II)-MA in DMF-water media are shown in the figs.2 to 5.

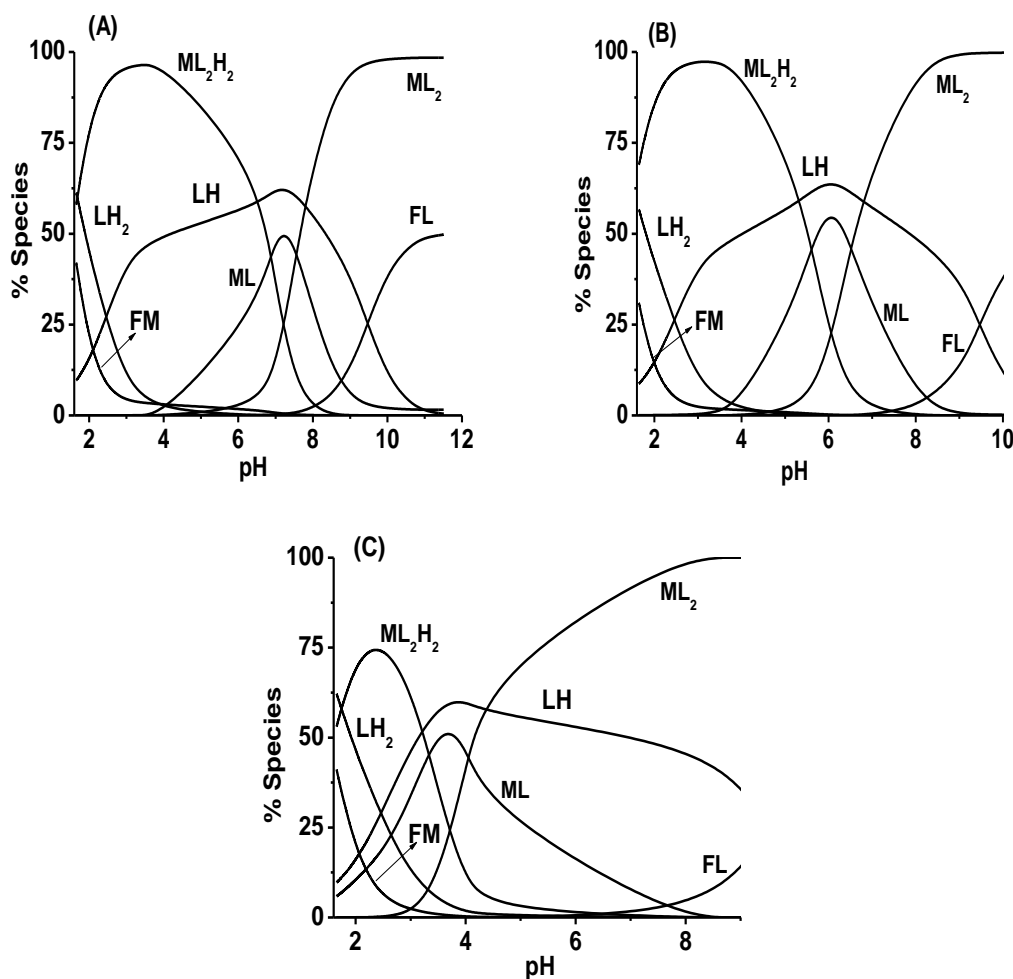


Figure.2:-Distribution diagrams of LPA complexes in 10 % v/v DMF – water medium.
Co(II) (B) Ni(II) and (C) Cu(II)

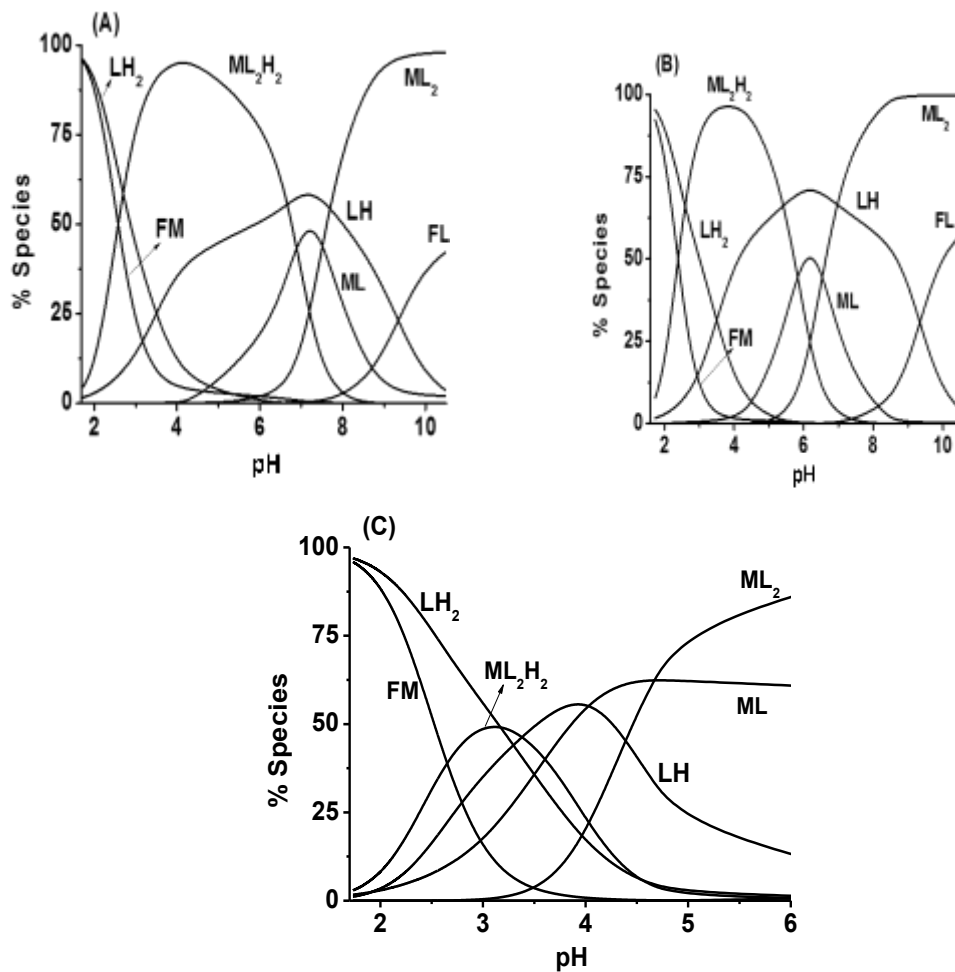
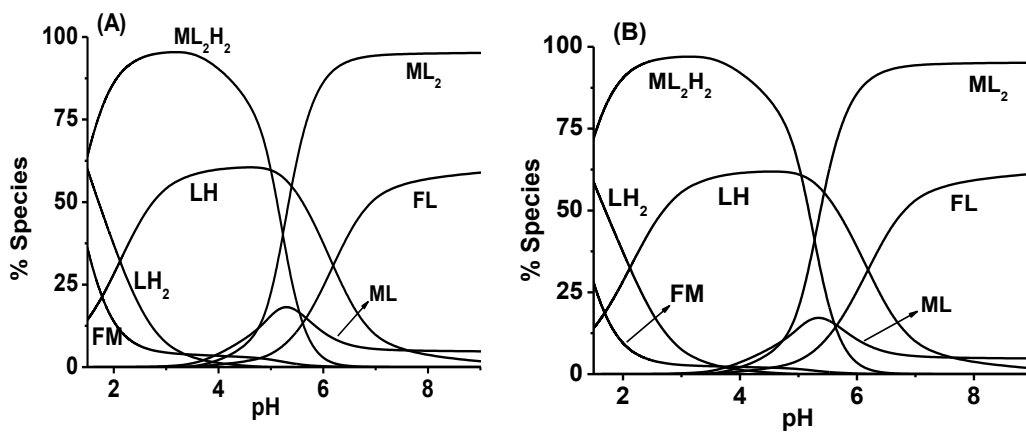


Figure.3:-Distribution diagrams of LPA complexes in 20 % v/v DMF-water medium. (A) Co(II) (B) Ni(II) and (C) Cu(II)



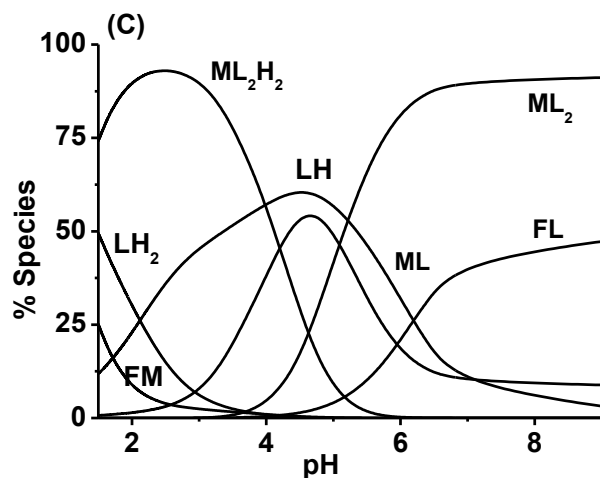


Figure.4:-Distribution diagrams of binary complexes of MA in 10% DMF – water mixtures: (A) Co(II), (B) Ni(II), and (C) Cu(II)

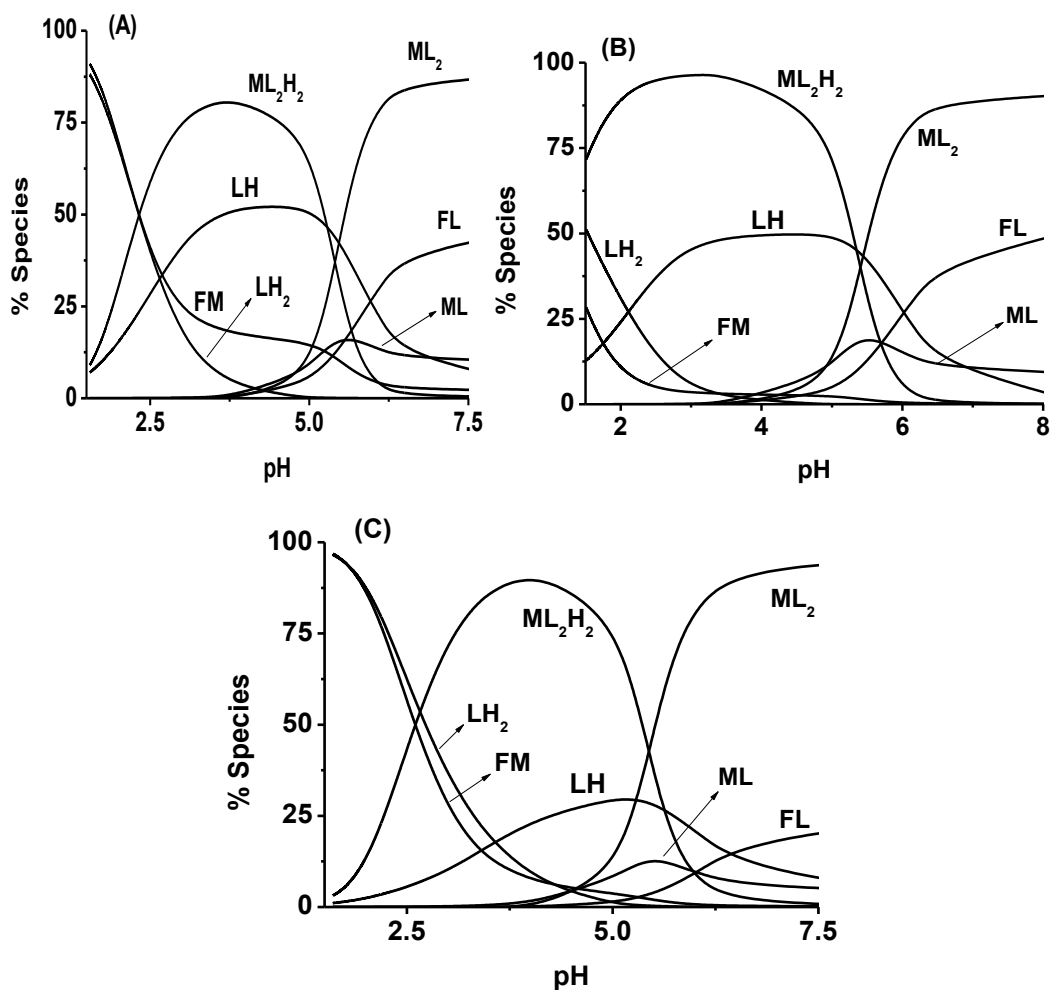


Figure.5:-Distribution diagrams of MA complexes in 20 % v/v DMF – water medium. (A) Co(II) (B) Ni(II) and (C) Cu(II).

Structures of Complexes:-

Although it is not possible to elucidate or confirm the structures of complex species pH metrically, they can be proposed based on the literature reports and chemical knowledge. In aqueous solutions, metal ions are coordinated by six water molecules. Amino acids replace water molecules and form metal-amino acid complexes [23]. Depending upon the nature of the ligands and metal ions and based on the basic chemical knowledge tentative structures of the complexes are proposed as shown in Figs.6 and 7 for LPA and MA complexes.

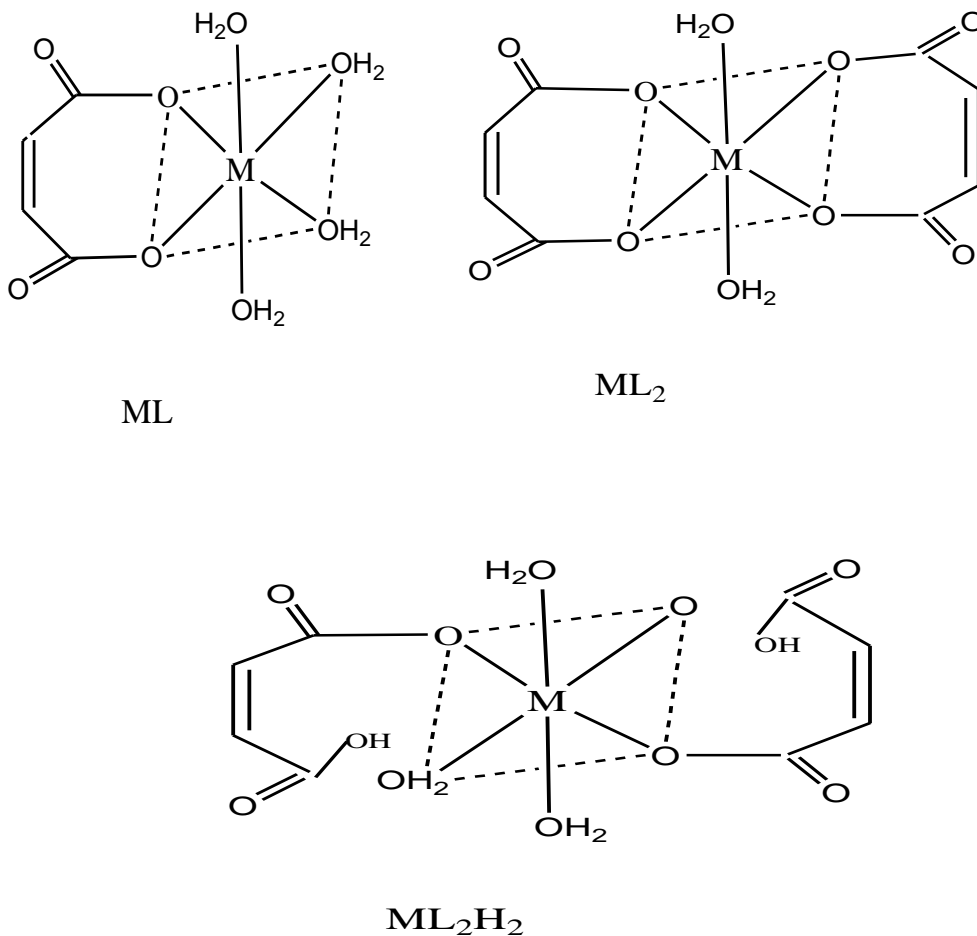
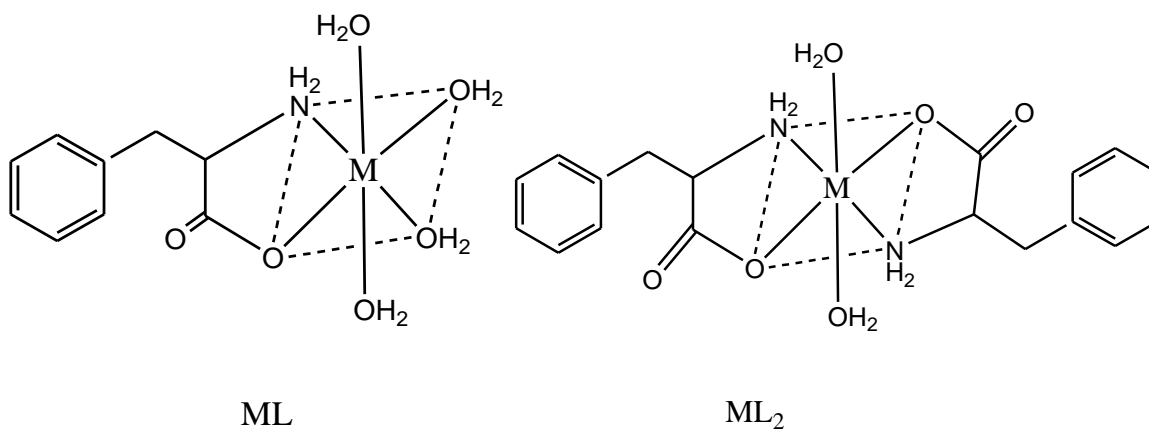


Fig.6:-Speculative Structures of binary complexes of MA with M(II).



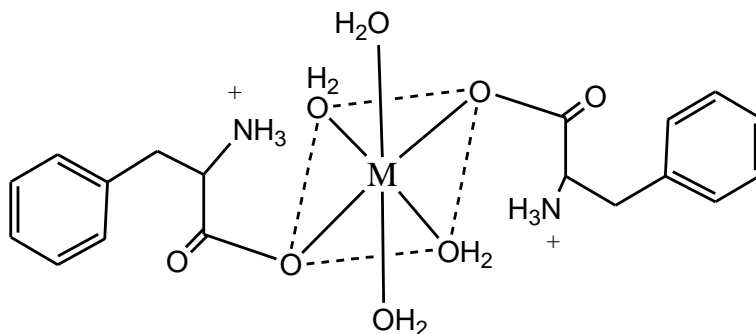


Fig.7:-Speculative Structures of binary complexes of LPA with M(II).

Conclusions:-

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

1. The binary species refined due to the interaction of LPA and MA with metals are ML, ML_2 and ML_2H_2 for Co(II), Ni(II) and Cu(II). These models are validated by statistical treatment of the data.
2. The ingredients that affect the magnitudes of stability constants of metal complexes were found to be: alkali > acid > ligand > metal.
3. The stabilities of the complexes followed the Irving-Williams order, i.e., Co(II) < Ni(II) < Cu(II).
4. The study also gave an insight into the metal ion availability/metal ion transport in biofluids which may help in assessing the toxicity of these metal ions if found to be in excess. The binary complexes with higher stability were more amenable for "metal transport" because of their extra stability and the binary complexes with less stability constants make the 'metal ion to be available' in biological systems due to their decreased stability.

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