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Chemical Speciation of Ternary Complex of L-Aspartic Acid and Ethylenediamine with Essential Metal Mg (II), Ca (II) and Zn (II) Ions in Low Dielectric Media

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***Abstract**: Speciation studies of essential and toxic metal ion complexes are useful in order to understand the role played by the active site cavities in biological molecules and the bonding behavior of their residues with metal ions. Hence chemical speciation of ternary complexes of Mg(II), Ca(II) and Zn(II) with L-Aspartic acid and Ethylenediamine have been studied pH-metrically in various concentrations (0–60%, v/v) of Dimethylformamide-water mixtures maintaining an ionic strength of 0.16 mol L^{-1} using NaCl as an electrolyte at 310 K to mimic the different species formed in physiological condition. Alkalimetric titrations were carried out in different relative concentrations primary (Aspartic, L) and secondary (Ethylenediamine, X) ligands (M: L: X=1.0:2.5:2.5, 1.0:2.5:5.0, 1.0:5.0:2.5) where M = Ca (II), Mg (II) and Zn (II). Stability constants of ternary complexes were calculated and various models were refined with MINIQUAD75. The best-fit chemical models were selected based on statistical parameters and residual analysis. The species detected are MLX_2, MLX_2H and MLX_2H_2 for Ca (II), Mg (II) and Zn (II) in DMF- water mixtures. The chemical speciation, metal bioavailability and transportation are explained based on the stability constants*

***Keywords:** Chemical speciation, Ternary Complexes, L-Aspartic acid, Ethylenediamine and Low dielectric media*

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I. Introduction

L-Aspartic acid (D) is a non-essential amino acid. It is primarily used in the body's metabolic processes; it is also key for a variety of other biological processes such as production of immunoglobulin, cell functioning, and the movement of minerals across intestinal linings into blood. D has also been used in various clinical applications, showing promise in persons suffering from opiate addiction [1]. D is also significant in the removal of excess ammonia and other toxins from the blood stream. It also assists in the proper functioning of carriers for genetic information-RNA and DNA.

Ethylenediamine (en) is a colorless to yellowish hygroscopic liquid. It is involved in the synthesis of seven membered ring components with β-ketoesters resulting secondary amines and β-enaminoesters. Ethylenediamine plays an important role in the synthesis of Schiff base compounds [2].

N, N-Dimethylformamide (DMF) is an organic compound and a common solvent for chemical reactions that is miscible with water and the majority of organic liquids. It is a polar aprotic solvent with a high boiling point, which facilitates reactions that follow polar mechanisms.It can be hydrolyzed by strong acids and bases, especially at elevated temperatures[3].

The protonation constants of L-Aspartic acid and Ethylenediamine[4], their binary complexes with Mg (II), Ca (II) and Zn (II) DMF-water mixtures were reported earlier[1]. Based on the importance of D and en and their involvement in various physiological reactions, speciation studies of their ternary complexes with some essential metal ions like Mg (II), Ca (II) and Zn (II) in DMFwater mixtures have been undertaken.

II. Matrial And Methods

Chemicals

DMF was used as co-solvent. Aqueous solutions of L aspartic acid (E-Merck, Germany) and ethylenediamine (Qualigen, India) sodium chloride were prepared. Metal solutions of Ca (II), Mg (II) and Zn (II) chlorides (E- Merck, Germany) were prepared. To increase the solubility of ligand and to suppress the hydrolysis of metal salts, the mineral acid concentration in the above solutions was maintained at 0.05 M. To assess the errors that might have crept into the determination of the concentrations, the datawere subjected to analysis of variance of one-way classification (ANOVA)[5]. The strength (concentration) of alkali was determined using the Gran plot method.

Apparatus

The titrimetric data were obtained using ELICO (Model LI-120) pH meter (readability 0.01), which was calibrated with 0.05 mol L^{-1} potassium hydrogen phthalate in acidic region and 0.01 mol L^{-1} borax solution in basic region. The glass electrode was equilibrated in a well-stirred DMF-water mixture containing the inert electrolyte. All the titrations were carried out in the medium containing varying concentrations of DMF-water mixtures (0-60% v/v) by maintaining an ionic strength of 0.16 mol L^{-1} with sodium chloride at 310K. The effect of variation in asymmetry potential, liquid junction potential, activity coefficient, sodium ion error and dissolved carbon dioxide on the response of glass electrode was accounted for in the form of correction factor[6]

Modeling Strategy

The computer program SCPHD[7]was used to calculate the correction factor. By using pH metric titration data, the ternary stability constants were calculated with the computer program MINIQUAD75[7], which exploit the advantage of constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm. During the refinement of ternary systems, the correction factor and protonation constants of D and en and their binary complexes withCa (II), Mg (II) and Zn (II) DMF-water mixtures were fixed.

Alkalimetric titration data

Initially strong acid was titrated against alkali at regular intervals to check the complete equilibration of the glass electrode. Then the calomel electrode was refilled with DMF-water mixture of equivalent composition as that of the titrand. In each of the titrations, the titrand consisted of approximately 1 mmol mineral acid in a total volume of 50 ml**.** Titrations were carried out in the presence of different relative concentrations of the metal (M) to primary (D, L) and secondary (en, X) ligands (M: L: X=1.0:2.5:2.5, 1.0:2.5:5.0, 1.0:5.0:2.5)with 0.4 mol dm^{-3} NaOH.Assuming that there is no expansion of the coordination sphere and three bidentate ligands are sufficient to satisfy the coordination number of the metal ion, the total number of primary and secondary ligands together was restricted to a maximum of three in generating the ternary species for modeling.

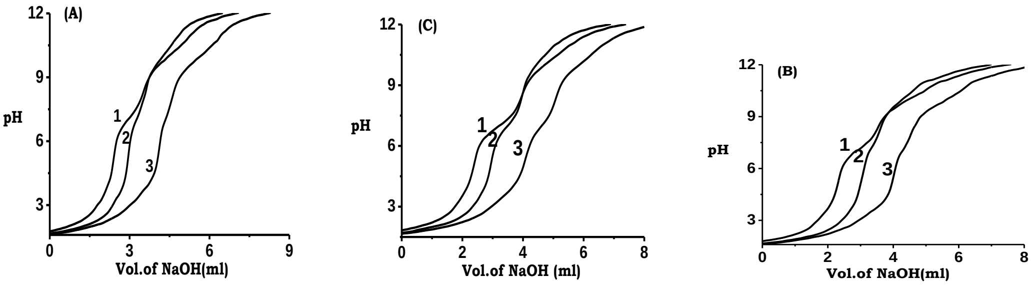


Figure 1: Alkali metric titration curves of D and en complexes in aqueous medium. (A) Ca (II), (B) Mg (II) and (C) Zn (II).Number of mmol of the ligands: (1)0.25,0.25 (2)0.25,0.50 (3) 0.50,0.25.

III. Modeling Of Chemical Speciation

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 that for any of the binary systems. The formation constants for acido-basic equilibria of both the primary and the secondary ligands and those for the binary metal complexes were fixed in testing various chemical models using MINIQUAD75. Exhaustive modeling was performed for all the systems and the final models are given in Table 1.for ternary complexes of D and en with Ca(II), Mg(II) and Zn(II) in DMF- water mixtures.The species detected are MLX_2, MLX_2H and MLX_2H_2 for Ca (II), Mg (II) and Zn (II) in DMF- water mixtures.

Table 1: Best fit chemical models of ternary complexes of Ca (II), Mg (II), and Zn (II) with D and en in DMF-water mixture

% v/v DMF	log β_{mlxh} (SD)			pH-Range	NP	U_{corr} x10^8	Skewness	Kurtosis	χ^2	R-factor
	MLX_2	MLX_2H	MLX_2H_2							
Ca(II)										
00.0	10.10(1)	19.56(4)	29.44(1)	3.0-9.8	66	7.75	-0.56	3.09	15.22	0.0014
10.0	11.27(0)	20.50(7)	30.22(4)	2.0-9.5	90	8.72	0.47	2.86	12.91	0.0015
20.0	11.33(4)	20.30(25)	29.61(5)	2.0-10.0	87	6.23	-0.88	3.28	16.36	0.0092
30.0	11.37(8)	20.57(27)	30.15(7)	2.0-9.7	65	3.20	-1.25	3.32	11.50	0.0072
40.0	11.35(1)	20.62(8)	30.11(17)	2.0-10.0	90	8.45	-0.96	2.43	12.61	0.0011
50.0	11.51(4)	21.09(11)	30.23(3)	2.0-9.4	45	8.07	-1.42	3.36	12.41	0.0040
60.0	11.76(6)	21.33(6)	30.36(0)	2.0-9.4	55	7.08	-2.22	3.03	12.41	0.0027
Mg(II)										
00.0	12.18(3)	21.14(0)	30.39(9)	3.0-9.4	64	8.41	-0.48	3.30	12.14	0.0086
10.0	12.36(0)	21.39(2)	30.64(8)	2.0-9.4	129	5.43	-1.01	3.31	17.17	0.0089
20.0	12.59(7)	21.17(4)	30.50(5)	2.0-9.4	35	2.53	-2.40	3.10	11.80	0.0080
30.0	12.63(8)	21.55(8)	30.73(6)	2.5.-9.4	24	4.65	-1.22	2.46	18.33	0.0032
40.0	12.79(6)	21.50(2)	30.72(5)	2.2-9.4	31	2.83	-1.50	3.60	12.54	0.0066
50.0	12.79(0)	21.90(8)	31.72(3)	2.0-10.0	87	6.63	-0.80	4.25	12.28	0.0079
60.0	13.47(7)	22.94(7)	31.80(8)	2.5-9.6	63	4.50	-1.44	3.24	13.08	0.0088
Zn(II)										
00.0	15.64(1)	24.54(7)	31.54(4)	3.0-10.0	82	1.78	0.47	3.21	16.61	0.0070
10.0	16.58(1)	23.76(2)	31.61(8)	4.0-10.4	76	2.50	3.21	4.52	15.00	0.0063
20.0	17.63(5)	25.42(7)	32.00(2)	3.0-8.0	48	3.35	1.08	2.85	15.67	0.0087
30.0	16.74(2)	24.77(8)	31.73(5)	3.0-11.4	99	7.00	2.88	3.35	12.71	0.0047
40.0	17.29(0)	25.29(4)	31.86(8)	3.0-9.0	99	5.93	-1.80	3.21	17.39	0.0052
50.0	17.28(2)	27.17(8)	34.39(2)	3.0-11.4	79	1.83	-2.53	3.50	12.46	0.0002
60.0	17.72(2)	26.97(6)	34.27(2)	2.0-10.0	84	6.60	-2.70	3.90	12.95	0.0019

U_{corr} = U/ (NP-m), 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 the parameters. The small values of U_{corr} indicate the consistency of the model with the experimental data[8]. The kurtosis values are around 3 for most of the systems. Hence, the residuals form mesokurtic pattern. Since the kurtosis values are more than 3 for most of the systems they form leptokurtic pattern. The skewness values -2.70 to 3.21 in DMF-water mixtures, respectively, show that the residuals form a part of normal distribution and hence a least squares method can be applied to the present data. The sufficiency of the model is further evident from the low crystallographic R factor values, which indicate the need for inclusion of additional species in the model. χ^2 is a special case of distribution, which measures the probability of residuals forming a part of standard normal distribution[9].

Effect of systematic errors

The computer programs refine the stability constants by minimizing the random errors in the data. However, in the presence of considerable systematic errors, not only the β's are in error, even some species may be rejected. This investigation is useful because the data acquisition is done under varied experimental conditions with different accuracies.

Errors were introduced into the concentrations of the ingredients intentionally to assess their effect on the perturbation of the stability constants. If the concentrations of ingredients determined and the experimental conditions maintained are appropriate, any variation in the concentrations of ingredients will affect the magnitudes of stability constants and worsen the statistical parameters. Even the species shall be rejected sometimes.The results of typical samples given in Table 2 emphasize that the errors in the concentrations of alkali and acid affect the stability constants more than those of the ligands and metal ions.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 2: Effect of errors in influential parameters on the stabilityconstants of D-M(II)-en ternary complexes in 10% v/v of DMF- water mixtures.

Ingredient	% Error	log β_{mlxh}(SD)		
		1120	1121	1122
Mg(II)in10 % v/v DMF-water mixture				
Alkali	0	12.18(3)	21.14(0)	30.39(9)
	-5	Rejected	18.89(28)	Rejected
	-2	11.23(21)	Rejected	29.87(19)
	+2	13.59(23)	22.39(23)	31.37(20)
	+5	15.66(33)	23.89(49)	32.45(27)
Acid	-5	15.64(45)	Rejected	32.69(36)
	-2	13.56(27)	22.47(36)	31.46(23)
	+2	11.27(22)	20.25(44)	29.83(22)
	+5	Rejected	19.13(33)	27.45(32)
Asp	-5	13.05(23)	21.89(24)	31.07(19)
	-2	12.66(21)	21.57(23)	30.82(19)
	+2	12.18(28)	21.13(22)	30.44(18)
	+5	11.75(19)	20.78(32)	30.27(80)
En	-5	11.76(21)	20.74(26)	30.10(10)
	-2	12.14(19)	21.10(22)	30.41(8)
	+2	12.66(25)	21.62(25)	30.87(9)
	+5	13.09(27)	22.06(45)	31.25(21)
Metal	-5	12.54(20)	21.46(35)	30.74(18)
	-2	12.46(22)	21.38(39)	30.69(18)
	+2	12.33(25)	21.35(32)	30.64(18)
	+5	12.24(20)	21.27(31)	30.57(18)

Stability Of Ternary Complexes

The formation of mononuclear Unprotonated binary and ternary complexes from a mixture of metal ion (M) and primary (L) and secondary (X) ligands can be shown as the equilibria given below. The change in the stability of the ternary complexes as compared to their binary analogues as quantifiedbased on the disproportionation constant (log X) given by Eq. 1.1.

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

Which corresponds to theequilibrium

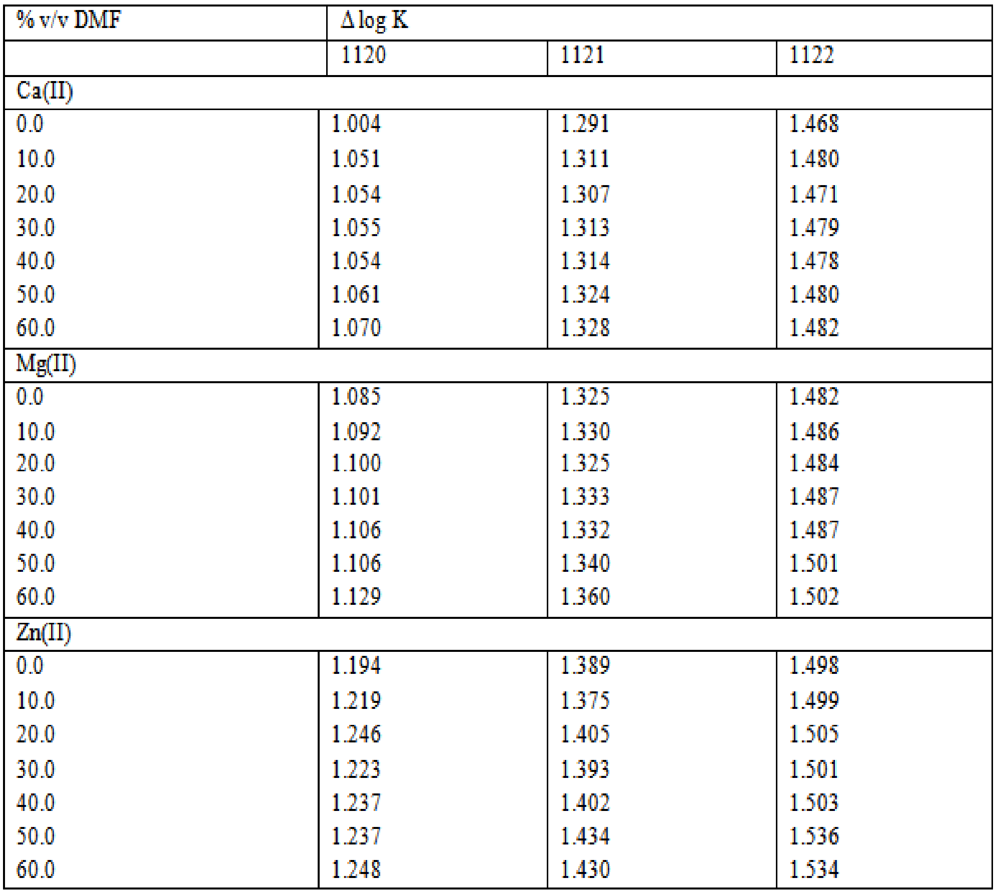
$ML_2 + MX_2$ 2 MLX $\rightleftharpoons$ ….1.2

Another approach[10]to quantify the stability of ternary complexes was 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 the primary ligand and X is the secondary ligand. It is compared with that calculated purely on the statistical grounds.

$$\Delta \log K = \log K^{M}_{MLX} - \log K^{M}_{ML} - \log K^{M}_{MX} \quad ....1.3$$

The electrostatic theory of binary complex formation and statistical arguments suggest the additional coordination positions of give multivalent hydrated metal ion available for the first ligand than for the second.Δ log K values of ternary complexes containing bipyridyl as the primary ligand are positive[11] for O-donors (malonic acid, pyrocatechol etc.), negative[12] for N-donors (ethylene diamine) and intermediate or negative for amino acids with both N and O co-ordination sites. These values calculated from the binary and ternary complexes are shown in Table 3. In the present study, the values of Δ log K range from 1.004 to 1.536 for DMF- water mixtures.

Table 3: Δ log K values of ternary complexes of Ca(II), Mg(II) and Zn(II)-D and en in DMF- water mixtures



% v/v DMF	Δ log K		
	1120	1121	1122
Ca(II)			
0.0	1.004	1.291	1.468
10.0	1.051	1.311	1.480
20.0	1.054	1.307	1.471
30.0	1.055	1.313	1.479
40.0	1.054	1.314	1.478
50.0	1.061	1.324	1.480
60.0	1.070	1.328	1.482
Mg(II)			
0.0	1.085	1.325	1.482
10.0	1.092	1.330	1.486
20.0	1.100	1.325	1.484
30.0	1.101	1.333	1.487
40.0	1.106	1.332	1.487
50.0	1.106	1.340	1.501
60.0	1.129	1.360	1.502
Zn(II)			
0.0	1.194	1.389	1.498
10.0	1.219	1.375	1.499
20.0	1.246	1.405	1.505
30.0	1.223	1.393	1.501
40.0	1.237	1.402	1.503
50.0	1.237	1.434	1.536
60.0	1.248	1.430	1.534

The higher values account for the extra stability of the ternary complexes compared to the corresponding binary complexes. The reason[13]for this extra stability might 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 electrostatic interactions between non-coordinated charge groups of the ligands.

Effect Of Dielectric Constant Of Medium

DMF-water medium is the combination of aprotic and protic solvents with a wide range of dielectric constant and with high solubility for polar as well as non-polar solutes. The increased basicity[14]of DMF-water mixtures that is induced by co-solvent increases the stabilization of the protons. The variation of overall stability constant values with co-solvent content depends upon two factors, viz., electrostatic and non-electrostatic. Born's[13] classical treatment holds good in accounting for the electrostatic contribution to the free energy change. The trends of the stability constant (log β) values of the mixed ligand complexes with 1/D (D is the dielectric constant of the medium) of DMF-water media are given in Figure 2.The trend is almost linear which indicates that either the dielectric constant or the long-range interactions are responsible for the trend in stability. This linear increase in log β values indicates the dominance of the structure-forming nature of DMF over the complexing ability. The cation stabilizing nature of co-solvents, specific solvent-water interactions, charge dispersion and specific interactions of co-solvent with solute (indicated by the changes in the solubility of different species in the aqua-organic mixtures) account for the little deviation of linear relationship.

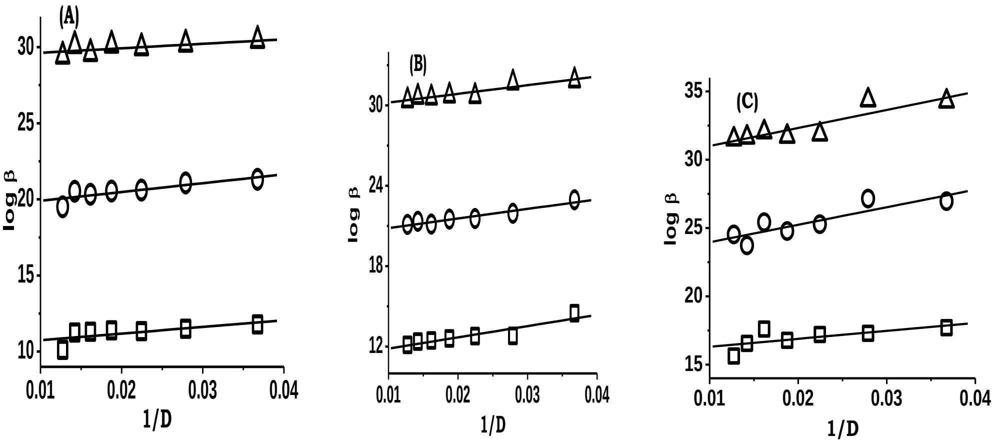


Figure 2: Variation of stability constants of D-metal-en ternary complexes in DMF- water mixtures. (A) Ca (II), (B) Mg (II) and (C) Zn (II) (□) $\log\beta MLX_2$, (○) $\log\beta MLX_2H$ and (Δ) $\log\beta MLX_2H_2$

DISTRIBUTION DIAGRAMS

The distribution diagrams indicate the relative abundance of various forms of metal ions (chemical speciation) at different pH's and dielectric conditions. 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.

The distribution diagrams were drawn using the formation constants of the best-fit model (Figure 3). They reveal that the concentrations of binary complexes are less than those of the ternary species, due to the extra stability of the ternary complexes. The ternary complex species of D (L) and en(X) in DMF- water mixtures are MLX_2, MLX_2H and MLX_2H_2. The protonated ternary species, MLX_2H and MLX_2H_2 exist at lower pH than the unprotonatedternary species, MLX_2. The formation of these complex species can be represented by the equilibria given in Equilibriums 1.1-1.9.

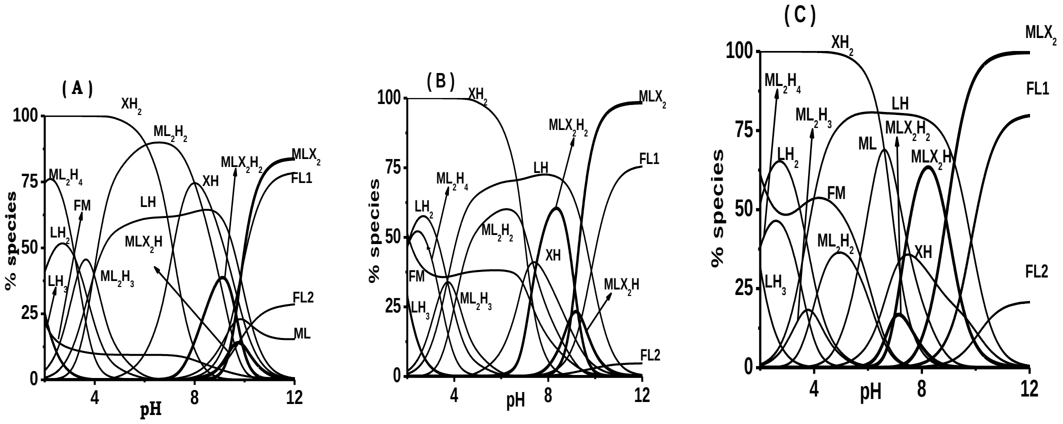


Figure 3: Distribution diagrams of D-en complexes of (A) Ca (II), (B) Mg (II) and (C) Zn (II) in aqueous medium. Ratios of mmols of metal, primary ligand secondary ligand are Ca(II) and Mg (II) 1.0:5.0:2.5 and Zn (II) 1.0:10.0:5.0.

The active forms of the ligands are LH_3^+, LH_2, LH^-, $XH_2^{2+}$and XH^+. The binary complexes of D for Ca (II), Mg (II) and Zn (II) are ML, ML_2H_2 ML_2H_3 and ML_2H_4. All the metal ions with en form protonated complexes MLX_2, MLX_2H and MLX_2H_2 in both the media.$MLX_2$is formed by the interaction of the protonated ligands with the free metal ion and on deprotonation of MLX_2H_2 and MLX_2H (Equilibria 1.5, 1.6 -1.8 and 1.9). MLX_2H species is formed by the interaction of free metal ion with LH_2 and XH_2 or by the deprotonation of MLX_2H_2 (Equilibria 1.3 and 1.4). MLX_2H_2 species is formed by the reaction of free metal ion with LH_2 with XH_2 and ML withXH_2 (Equilibria 1.1 and 1.2).

$M(II) + LH_2 + 2XH_2 \rightleftharpoons MLX_2H_2 + 4H^+$ ----- 1.1

$ML + 2XH_2 \rightleftharpoons MLX_2H_2 + 2H^+$ ----- 1.2

$M(II) + LH_2 + 2XH_2 \rightleftharpoons MLX_2H + 5H^+$ ----- 1.3

$MLX_2H_2 \rightleftharpoons MLX_2H + H^+$ ----- 1.4

$M(II) + LH_2 + 2XH_2 \rightleftharpoons MLX_2 + 6H^+$ ----- 1.5

$M(II) + LH + 2XH \rightleftharpoons MLX_2 + 3H^+$ ----- 1.6

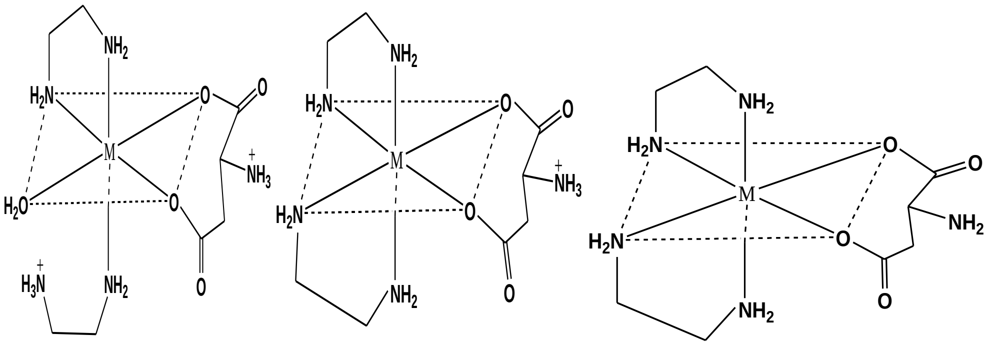
$MLX + XH \rightleftharpoons MLX_2 + H^+$ ----- 1.7

$MLX_2H_2 \rightleftharpoons MLX_2 + 2H^+$ -----1.8

$MLX_2H \rightleftharpoons MLX_2 + H^+$ -----1.9

Structures Of Ternary Complexes

Based on the nature of the metal ions, the protonation and deprotonation equilibria of (1.1-1.9) D and en, and based on basic coordination chemistry principles, the probable structures of the ternary complexes are givenin Figure 4. Octahedral structures are proposed for all the metals based on literature reports[15]. D and en form strong bidentate complexes with transition metals. D at higher pH favors the (O, O) coordination and at physiological pH it is bound through the amino acid side chain[16].



MLX_2H_2 MLX_2H MLX_2

Figure 4: Speculated structures of ternary complexes of Ca(II), Mg(II) and Zn(II).

IV. Conclusion

A study of the speciation of ternary complexes of Ca(II), Mg(II) and Zn(II) with D and en in DMF-water mediareveals the compartmentalization of metabolic reactions. The following conclusions have been drawn from the modeling studies**:**The ternary metal complex species detected are MLX_2, MLX_2H and MLX_2H_2 for Ca(II), Mg(II) and Zn(II) in DMF-water media where L = D and X = en. Only these species are refined due to the restricted pH ranges and the possible active forms of ligands taken as LH^{3+}, LH_2, LH^-, L^{2-} and $XH_2^{2+,}$ XH^+, X.The study also gives an insight into the metal availability/metal transport in bio fluids. The ternary complexes are more amenable for “metal transport” because of their extra stability.

The linear increase in the stabilities of the ternary complexes with decrease in the dielectric constants is due to the dominance of the electrostatic forces.The order of the ingredients that influence the magnitude of stability constants due to incorporation of errors is alkali > acid >Ligand > metal ion >.The ternary complexes are more amenable for ‘metal ion transport’ because of their extra stability and the binary complexes make the ‘metal ion to be available’ in biological systems due to their decreased stability.

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