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pH-metric Evaluation of Stability of Ternary Complexes of Aspartic acid and Ethylenediamine in Aqueous Dioxan medium

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Abstract

The stabilities of the ternary complexes of L-Aspartic acid (L) and Ethylenediamine (X) with Ca(II), Mg(II) and Zn(II) ions were determined pH-metrically at 303.0 K and an ionic strength of 0.16 mol L^{-1} (NaCl) in 0-60% v/v Dioxan-Water mixtures. The trend in the variation of stability and concentrations of the ternary species with the dielectric constant of the medium was explained on the basis of electrostatic and non-electrostatic forces. The formation and distribution of different species with relative concentrations of metal and ligands, with varying pH were represented in the form of distribution diagrams.

Key Words*: Aspartic acid, Dioxan, Ethylenediamine, Speciation, Ternary complexes.*

1. Introduction

Metal complexes have been extensively used in analytical and medicinal chemistry and pigments[1, 2]. Stability constants of metal complexes have been determined by Spectrophotometric [3] and pH metric methods. The formation of complexes containing two different ligands has been of interest to coordination chemists[4], The chemical speciation[5,6] and simultaneous determination[7] of metal ions have been increasingly needed in areas such as clinical chemistry, biochemistry, environmental pollution and industrial control. The ternary coordination plays an important role in biological processes. Ternary complex formation occurs commonly in biological fluids, with several potential ligands, including certain amino acids, peptides, peptide derivatives or their analogues[8].

L-aspartic acid (Asp) is a non-essential amino acid found in abundance in plants and animals. It plays an important role in maintaining the solubility and ionic character of proteins.[9] It assists the liver in removing excess ammonia and other toxins from the blood stream. It is also very important in the functioning of RNA and DNA, in immunoglobulin and antibody synthesis. Asp is popular as a drug for chronic fatigue as it plays crucial role in generating cellular energy, moves the coenzyme nicotinamide adenine dinucleotide molecules from the main body of the cell to its mitochondria, where it is used to generate adenosine triphosphate.[10] Ethylenediamine (en) is used as an important monodentate, bidentate or a bridging ligand.[11] It is also used in the manufacture of EDTA, carbamate fungicides, surfactants and dyes. It is involved in the synthesis of β-enaminoesters[12] and Schiff base compounds.[13]Calcium, Magnesium and Zinc are essential for the life of both flora and fauna. Hence the speciation of ternary complexes of Ca(II), Mg(II) and Zn(II) with L-Aspartic acid and Ethylenediamine has been studied in dioxan-water mixtures. The protonation constants[14] of Asp and En and their binary complexes[15,16] with Ca(II), Mg(II) and Zn(II) in Dioxan-water mixtures were reported earlier.

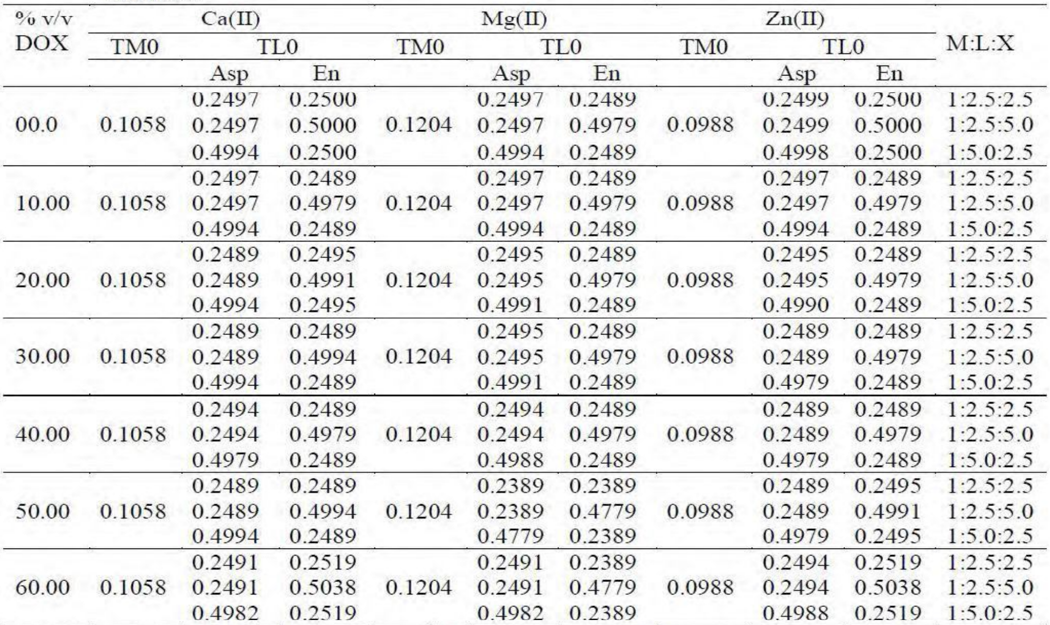
2. Experimental

0.05 mol L^{-1}solutions of Asp and En (Merck, India) were prepared in triple-distilled deionised water by maintaining 0.05 mol L^{-1} hydrochloric acid to increase the solubility. 1, 4-Dioxan (DOX) (Qualigens, India) was used as received. 0.2 mol L^{-1} Hydrochloric acid and 2.0 mol L^{-1} sodium chloride (Qualigens, India) solutions were prepared to maintain the acidity and ionic strength in the titrand, respectively. 0.1 mol L^{-1} Solutions of Ca(II), Mg(II) and Zn(II) chlorides (G.R. Grade, Merck, India) were prepared in triple distilled water maintaining 0.05 mol L^{-1} HCl to suppress the hydrolysis of metal ions. 0.4 mol L^{-1} Sodium hydroxide (Qualigens, India) solution was used as the titrant. Sodium hydroxide was standardized by standard solutions of oxalic acid and potassium hydrogen phthalate. Hydrochloric acid was standardized by standard borax and standardized sodium hydroxide solutions. The concentrations of Ca(II), Mg(II) and Zn(II) solutions were determined complexometrically by titrating against a standard solution of EDTA using Eriochrome Black T as indicator. The data were subjected to analysis of variance of one way classification[17] to assess the errors that might have crept into the determination of the concentrations. The strengths of alkali and mineral acid were determined using the Gran plot method.[18, 19]

2.1 Procedure

ELICO (Model LI-120) pH meter (readability 0.01) was used for obtaining titrimetric data. The effect of variations in asymmetry, liquid junction potential, activity coefficient, sodium ion error and dissolved carbon dioxide on the response of glass electrode were accounted for in the form of correction factor.[20] For the determination of stability constants of ternary species, 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 DOX-water mixture of equivalent composition as that of the titrand. All the titrations were performed pH metrically in medium containing varying concentrations (0.0-60.0% v/v) of DOX-water mixtures at 303.0 K. In each of the titrations, the titrand consisted of approximately 1.0 mol L^{-1} mineral acid in a total volume of 50.0 mL. Titrations were carried out in the presence of different relative concentrations of the metal (M), Asp (L) and en (X) (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 L^{-1} sodium hydroxide. The analytical concentrations of the ingredients are given in Table 1. The best-fit chemical model for each system investigated was arrived at using a non-linear least squares analysis program MINIQUAD75

Table 1. Total initial concentrations of ingredients (in mmol) for mixed-ligand titrations in DOX-water mixtures.



% v/v DOX	Ca(II)			Mg(II)			Zn(II)			M:L:X
	TM0	TL0		TM0	TL0		TM0	TL0		
		Asp	En		Asp	En		Asp	En	
00.0	0.1058	0.2497	0.2500	0.1204	0.2497	0.2489	0.0988	0.2499	0.2500	1:2.5:2.5
		0.2497	0.5000		0.2497	0.4979		0.2499	0.5000	1:2.5:5.0
		0.4994	0.2500		0.4994	0.2489		0.4998	0.2500	1:5.0:2.5
10.00	0.1058	0.2497	0.2489	0.1204	0.2497	0.2489	0.0988	0.2497	0.2489	1:2.5:2.5
		0.2497	0.4979		0.2497	0.4979		0.2497	0.4979	1:2.5:5.0
		0.4994	0.2489		0.4994	0.2489		0.4994	0.2489	1:5.0:2.5
20.00	0.1058	0.2489	0.2495	0.1204	0.2495	0.2489	0.0988	0.2495	0.2489	1:2.5:2.5
		0.2489	0.4991		0.2495	0.4979		0.2495	0.4979	1:2.5:5.0
		0.4994	0.2495		0.4991	0.2489		0.4990	0.2489	1:5.0:2.5
30.00	0.1058	0.2489	0.2489	0.1204	0.2495	0.2489	0.0988	0.2489	0.2489	1:2.5:2.5
		0.2489	0.4994		0.2495	0.4979		0.2489	0.4979	1:2.5:5.0
		0.4994	0.2489		0.4991	0.2489		0.4979	0.2489	1:5.0:2.5
40.00	0.1058	0.2494	0.2489	0.1204	0.2494	0.2489	0.0988	0.2489	0.2489	1:2.5:2.5
		0.2494	0.4979		0.2494	0.4979		0.2489	0.4979	1:2.5:5.0
		0.4979	0.2489		0.4988	0.2489		0.4979	0.2489	1:5.0:2.5
50.00	0.1058	0.2489	0.2489	0.1204	0.2389	0.2389	0.0988	0.2489	0.2495	1:2.5:2.5
		0.2489	0.4994		0.2389	0.4779		0.2489	0.4991	1:2.5:5.0
		0.4994	0.2489		0.4779	0.2389		0.4979	0.2495	1:5.0:2.5
60.00	0.1058	0.2491	0.2519	0.1204	0.2491	0.2389	0.0988	0.2494	0.2519	1:2.5:2.5
		0.2491	0.5038		0.2491	0.4779		0.2494	0.5038	1:2.5:5.0
		0.4982	0.2519		0.4982	0.2389		0.4988	0.2519	1:5.0:2.5

[NaOH] = 0.4 mol L^{-1}; V_0 = 50.0 cm^3; temperature = 303.0 K; ionic strength = 0.16 mol L^{-1}; [HCl] = 1 mmol.

3. Results and discussion

3.1 Modeling of Chemical Speciation

A preliminary investigation of alkalimetric titrations of mixtures containing different mole ratios of Asp and en in the presence of mineral acid and inert electrolyte inferred that no condensed species were formed. The best fit models were chosen based on the statistical parameters like χ^2, R-factor, Skewness and Kurtosis given in Table 2. The ternary complex species detected are MLX_2, MLX_2H, MLX_2H_2 for Ca(II), Mg(II) and Zn(II). A very low standard deviation (SD) in log values of overall stability constants (log β) indicates the precision of these parameters. The small values of U_{corr} (sum of squares of the deviations in the concentrations of the metal, ligands and the hydrogen ion at all the experimental points corrected for degrees of freedom) indicate that the models represent the experimental data. Small values of mean, standard deviation and mean deviation for these systems corroborate that the residuals are around a zero mean with little dispersion. For an ideal normal distribution, the values of kurtosis and skewness should be three and zero, respectively. The kurtosis values in the present study indicate that some of the residuals are nearer to mesokurtic and others form leptokurtic patterns. The values of skewness recorded in Table 2 are between –2.56 and 3.21, which indicate that the residuals form a part of normal distribution. Hence the least–squares method can be applied to the present data. The sufficiency of the model is further evident from the low crystallographic R-values.

Table 2. Parameters of best fit chemical models of ternary complexes of Ca(II), Mg(II) and Zn(II) with Asp and En in DOX-water mixtures.

% v/v DOX	log β_{mlxh} (SD)			pH-range	NP	U_{corr} x10^8	Skew-ness	Kurt-osis	χ^2	R-factor
	ML_2XH_2	$MLXH_2$	MLX_2							
				Ca(II)						
00.0	10.11(12)	19.51(28)	29.42(15)	3.0-10.0	72	4.79	-0.57	3.08	45.22	0.0113
10.0	11.25(23)	20.53(52)	30.12(14)	2.0-9.5	81	8.76	0.46	2.87	62.91	0.0114
20.0	11.30(14)	20.33(25)	29.60(15)	2.0-10.0	88	5.21	-0.90	3.18	76.36	0.0093
30.0	11.39(18)	20.56(27)	30.14(19)	2.0-9.8	86	3.70	-1.24	4.92	101.5	0.0074
40.0	11.34(11)	20.60(28)	30.10(13)	2.0-10.0	91	8.35	-0.93	2.73	80.66	0.0115
50.0	11.50(15)	21.08(41)	30.21(33)	2.0 9.5	35	27.65	1.47	3.26	84.46	0.0240
60.0	11.79(44)	21.31(68)	30.46(36)	2.0-9.5	33	26.33	-2.20	7.83	33.45	0.0257
				Mg(II)						
00.0	12.13(23)	21.12(70)	30.38(49)	3.0-9.5	64	85.41	-0.47	5.36	72.13	0.0486
10.0	12.37(10)	21.35(22)	30.64(8)	2.0-9.5	129	5.47	-1.05	5.51	67.19	0.0089
20.0	12.55(17)	21.14(84)	30.59(15)	2.0-9.5	35	12.55	-2.44	8.70	119.89	0.0180
30.0	12.60(18)	21.51(68)	30.74(16)	2.5.-9.5	24	14.67	-1.21	2.45	38.33	0.0232
40.0	12.78(36)	21.55(82)	30.70(35)	2.2-9.5	31	22.86	-1.59	3.68	70.84	0.0266
50.0	12.79(40)	21.94(85)	31.71(35)	2.0-10.0	87	46.67	-0.82	5.23	108.28	0.0279
60.0	14.48(87)	22.95(76)	31.87(81)	2.5-9.5	63	34.5	-1.44	3.66	73.08	0.0288
				Zn(II)						
00.0	15.62(11)	24.52(7)	31.51(14)	3.0-10.0	80	1.77	0.41	8.81	46.65	0.0070
10.0	16.55(31)	23.72(32)	31.65(28)	4.0-10.5	108	22.57	3.21	16.52	259.00	0.0263
20.0	17.60(25)	25.42(17)	32.04(52)	3.0-8.0	48	3.36	1.08	2.85	25.67	0.0087
30.0	16.78(22)	24.78(8)	31.71(15)	3.0-11.5	122	7.01	2.89	14.35	152.79	0.0147
40.0	17.20(40)	25.27(24)	31.87(68)	3.0-9.0	23	5.9	-1.80	5.21	57.39	0.0152
50.0	17.29(62)	27.15(58)	34.38(27)	3.0-11.5	79	31.84	-2.56	17.50	100.46	0.0302
60.0	17.70(62)	26.96(56)	34.29(28)	2.0-10.0	84	26.67	-1.70	6.90	91.95	0.0219

Ucorr = U/ (NP-m), m = number of species; NP =Number of experimental points; SD = standard deviation

3.2 Effect of Dielectric Constant on Stability of Ternary Complexes

DOX-water mixtures are a combination of aprotic and protic solvents with a wide range of dielectric constant.The increased basicity[22] of DOX-water mixtures which is induced by co-solvent increases the stabilization of the protons. At the same time the coordinating solvent (DOX) competes with the ligands for coordination with the metal ions which decreases the stability of the complexes. Hence, the stability of the complex is expected to either increase or decrease. According to Born's[23] equation the energy of electrostatic interaction is related to dielectric constant of the medium and log β versus 1/D (D is the dielectric constant of the medium) should be linear.The linear trends (Figure 1) indicate that either the dielectric constant or the long range interactions are responsible for the trend in stability. The linear increase in log β values indicates the dominance of the structure-forming nature of DOX over the complexing ability.

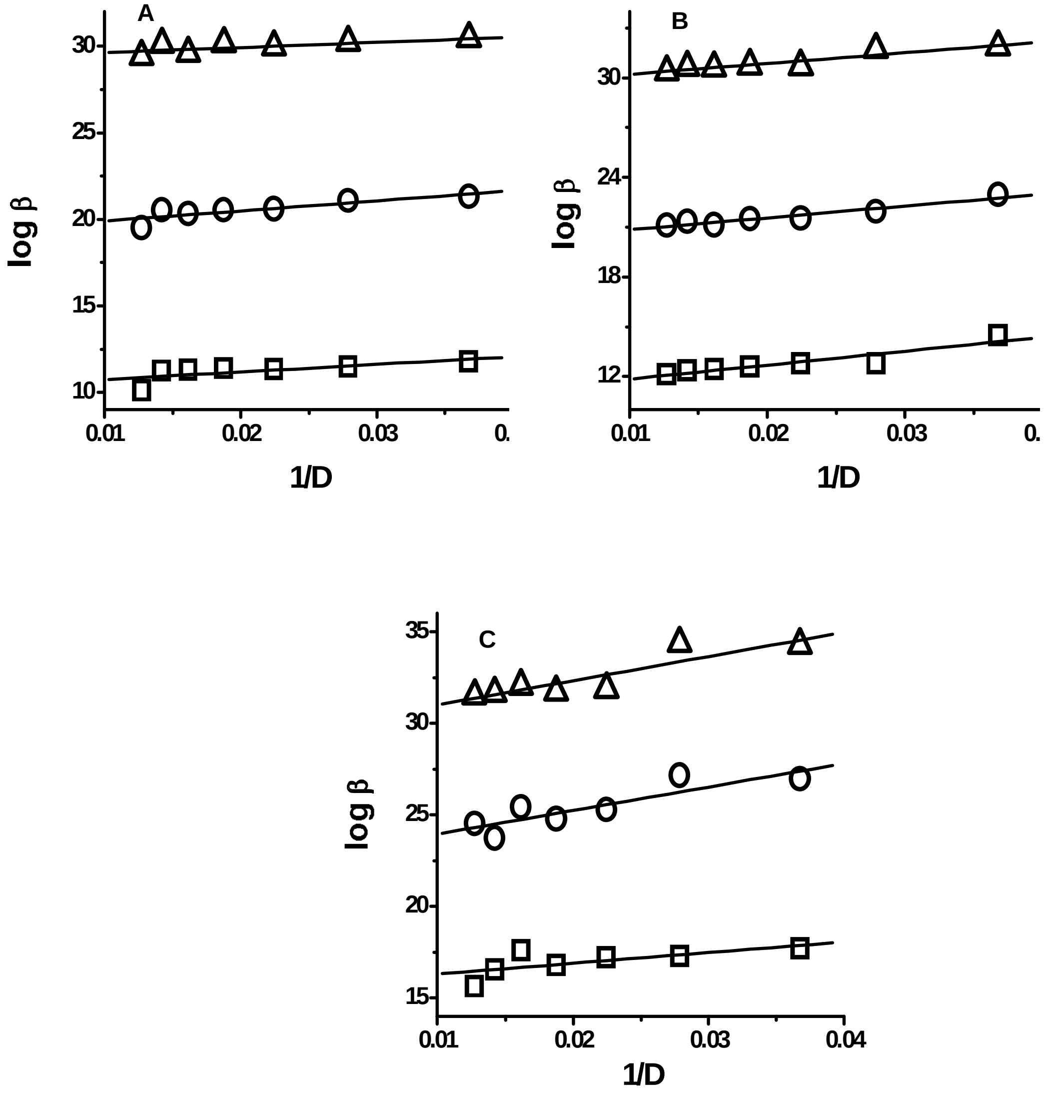


Figure 1: Variation of magnitude of stability constants (log β) of ternary complexes of (A) Ca(II), (B) Mg(II) and (C) Zn(II) with Asp and En with reciprocal of dielectric constant (1/D) of DOX-water mixtures: (□) log $\beta_{MLX2,}$ (O) log β_{MLX2H} and (**Δ**) log $\beta_{MLX2H2.}$

3.3 Stability of Ternary Complexes

The change in the stability of the ternary complexes as compared to their binary analogues was quantified[24-27] based on the difference in stability (Δ log K) for the reactions ML with X and $M_{(aq)}$ with L and X [24-28], where L is the primary ligand (Asp) and X is the secondary ligand (En). It is compared with that calculated purely on the statistical grounds as given in Equation (1).

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

----- (1)

The electrostatic theory of binary complex formation and statistical arguments suggest the availability of the additional coordination positions of the hydrated metal ion for the first ligand than for the second. Hence, the usual order of stability, $K^{M}_{ML} > K^{ML}_{MLX}$ applies. This suggests that Δ log K to be negative. The possible Δ log K values were calculated from the binary and ternary complexes (Table 3). The Δ log K values range from -0.09 to 3.48 for Ca(II), -0.56 to 4.38 for Mg(II) and -0.08 to 3.41 for Zn(II) and they are found to be higher than those expected on statistical bases. These higher values account for the extra stability of the ternary complexes. The extra stability of these ternary 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 and stacking interactions.

Table 3. Δ log K values of ternary complexes of Ca(II), Mg(II) and Zn(II) with Asp and En in DOX-water mixtures calculated using equations given as foot note.

% v/v DOX	log K_{MLX2}	logK_{MLX2H}	logK_{MLX2H2}
	Ca(II)		
00.0	1.91	1.72	1.74
10.0	2.13	2.84	3.48
20.0	0.66	-0.27	2.1
30.0	1.34	0.41	0.91
40.0	0.53	-0.09	1.67
50.0	2.38	2.29	3.47
60.0	1.04	0.94	1.9
	Mg(II)		
00.0	4.38	3.35	3.47
10.0	3.8	2.92	3.71
20.0	0.97	0.18	2.29

30.0	3.69	2.73	3.84
40.0	2.4	1.33	2.75
50.0	1.91	1.54	3.43
60.0	0.23	-0.56	0.45
	Zn(II)		
00.0	-1.17	0.39	0.78
10.0	-0.34	-0.27	0.18
20.0	-0.88	0.3	-0.29
30.0	-1.01	0.01	-0.17
40.0	-0.22	0.65	0.72
50.0	0.58	3.18	3.41
60.0	-0.08	1.75	2.1

$\Delta \log K_{1120} = \log \beta_{1120} - \log \beta_{1100} - \log \beta_{1020}$

$\Delta \log K_{1121} = \log \beta_{1121} - \log \beta_{1100} - \log \beta_{1021}$

$\Delta \log K_{1122} = \log \beta_{1122} - \log \beta_{1100} - \log \beta_{1022}$

The study also gives an insight into the metal availability/metal transport in biofluids and toxicity of these metals. The ternary complexes are more amenable for ‘metal transport’ because of their extra stability and the binary complexes make the ‘metal available’ in biological systems due to their decreased stability.

3.4 Effect of influential parameters on stability constants

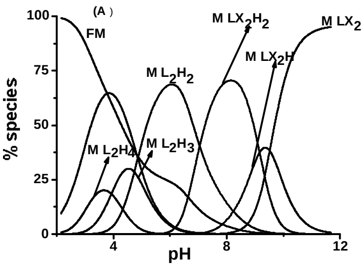
Any variation in the parameters like concentrations of ingredients affects the magnitudes of equilibrium constants. Such parameters are called influential or dangerous parameters. In order to rely upon the best chemical model for critical evaluation and application under varied experimental conditions with different accuracies of data acquisition, an investigation was made by introducing pessimistic errors in the concentrations of alkali, mineral acid, ligands, metal and log F. The results of typical samples given in Table 4 emphasize that the errors in acid and alkali affect stability constants more than those of the ligands, metal and log F.

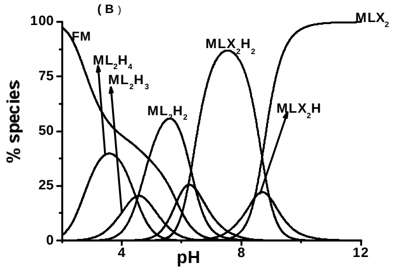
Table 4: Effect of errors in influential parameters on stability constants of ternary complexes of Asp-Mg(II)-En in 10% v/v DOX-water mixtures.

Ingredient	% Error	log β_{mlxh} (SD)		
		MLX_2	MLX_2H	MLX_2H_2
	0	12.37(10)	21.35(22)	30.64(8)
Alkali	-5	Rejected	18.85(18)	27.57(**)
	-2	11.22(11)	20.18(35)	29.86(9)
	+2	13.57(13)	22.39(23)	31.35(10)
	+5	15.62(23)	23.89(37)	32.43(16)
Acid	-5	15.63(31)	24.19(**)	32.66(22)
	-2	13.54(14)	22.44(25)	31.41(11)
	+2	11.26(12)	20.22(36)	29.81(10)
	+5	Rejected	19.10(22)	27.45(**)
Asp(L)	-5	13.03(11)	21.89(24)	31.05(9)
	-2	12.63(10)	21.57(23)	30.80(9)
	+2	12.13(9)	21.13(22)	30.471(8)
	+5	11.77(9)	20.79(23)	30.23(8)
En(X)	-5	11.75(11)	20.74(26)	30.10(10)
	-2	12.12(9)	21.10(22)	30.41(8)
	+2	12.65(11)	21.62(25)	30.87(9)
	+5	13.08(15)	22.04(31)	31.23(11)
Metal	-5	12.55(10)	21.45(24)	30.73(8)
	-2	12.44(11)	21.39(23)	30.67(8)
	+2	12.31(12)	21.32(22)	30.60(8)
	+5	12.22(10)	21.26(21)	30.55(8)
log F	-5	12.33(11)	21.35(22)	30.61(8)
	-2	12.35(11)	21.36(22)	30.62(8)
	+2	12.39(11)	21.36(22)	30.65(8)
	+5	12.42(11)	21.38(23)	30.66(8)

** = Standard Deviation is very high

3.5 Distribution diagrams





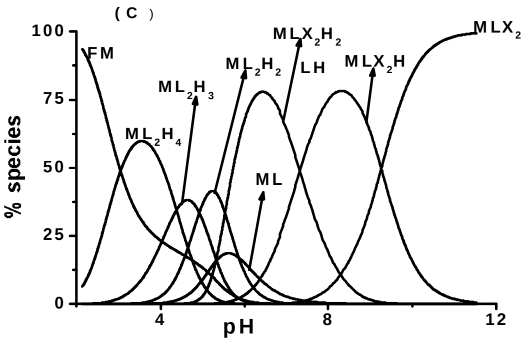


Figure 2: Species distribution diagrams of ternary complexes of Asp and En in 60% DOX-water mixture: (A) Ca(II), (B) Mg(II) and (C) Zn(II).

Some typical distribution diagrams in 60% DOX-water mixture drawn using the formation constants of the best fit model are shown in Figure 2, which contain protonated and unprotonated species like MLX_2H_2, MLX_2H and MLX_2 for Ca(II), Mg(II) and Zn(II). The active forms of these ligands are LH_3^+, LH_2, LH^- and L^{2-} for Asp and XH_2^{2+}, XH^+ and X for En. The binary complexes[15,16] of Asp are ML, ML_2H_2, ML_2H_3 and ML_2H_4 and those of En are MX_2, MX_2H, MX_2H_2. The distribution diagrams indicate the relative abundance of various forms of metal (chemical speciation) at different pH and dielectric conditions. A stable ternary complex shall be responsible for metal ion transportation in biological systems and the weak binary metal complexes make the essential metals bioavailable. The increased concentrations of complexing agents make the essential metal ions unavailable due to the formation of stable metal complexes. The formation of the ternary complex species can be represented by the following equilibria. The charges of the species are omitted for clarity.

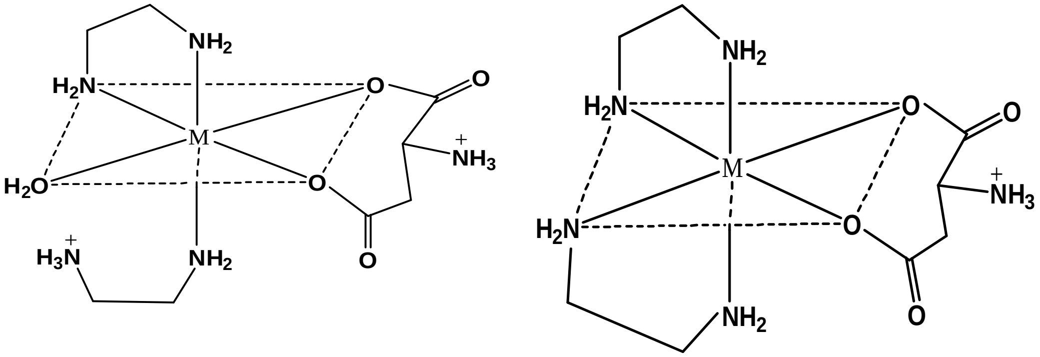
$$M(II) + LH_2 + 2XH_2 \rightleftharpoons MLX_2H_2 + 4H^+ \qquad (2)$$

$$MLX_2H_2 \rightleftharpoons MLX_2H + H^+ \qquad (3)$$

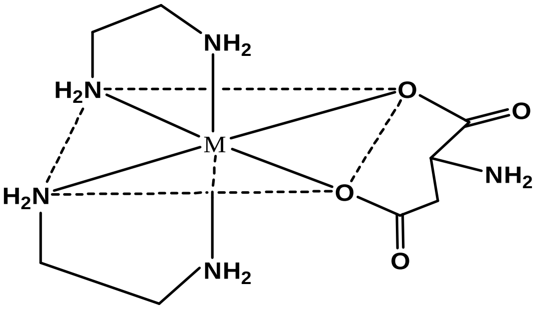
$$M(II) + LH + 2XH \rightleftharpoons MLX_2 + 3H^+ \qquad (4)$$

$$MLX_2H \rightleftharpoons MLX_2 + H^+ \qquad (5)$$

MLX_2H_2 species is formed by the reaction of free metal ion with the protonated ligands (Equilibrium 2). MLX_2H species is formed by the deprotonation of MLX_2H_2 (Equilibrium3).MLX_2 is formed by the interaction of free metal ion with LH and XH or on deprotonation of MLX_2H (Equilibria 4, 5). Based on the protonation and deprotonation equilibria of Asp and En, depending on the coordinating sites in the ligands and the nature of the metal ions, basic coordination chemistry principles, the possible structures of the ternary complexes are proposed as given in Figure 3.



MLX_2H_2 MLX_2H



MLX_2

Figure 3: Structures of Asp-M(II)-En ternary complexes, where S is either solvent or water molecule.

4. Conclusions

An electrometric study of the speciation of ternary complexes of Ca(II), Mg(II) and Zn(II) with Asp and En in DOX–water media reveals the compartmentalization of metabolic reactions. The following conclusions have been drawn from the modeling studies:

1. The predominant species detected are MLX_2, MLX_2H and MLX_2H_2 for Ca (II), Mg (II) and Zn (II). Where L = Asp and X = En.
2. The values of $\Delta \log K$ indicate that the ternary species have 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.

3. The linear increase in the stabilities of ternary complexes with decreasing dielectric constants is due to the dominance of electrostatic forces.

4. The order of the ingredients that influence the magnitudes of stability constants due to incorporation of errors is alkali > acid > Asp > En > metal > log F.

5. The ternary complexes are more amenable for 'metal transport' because of their extra stability and the binary complexes make the 'metal available' in biological systems due to their decreased stability.

References

1 G. N. Schrauser and J. Kohnie, Chem. Ber., 1964, 97, 3056-3059

2 S. Kuse, S. Motomizu and K. Toei, Anal. Chim. Acta, 1974, 70, 65-76

3 K. Takacs-Novak and K. Y. Tam, Anal. Chim. Acta, 2001, 434, 157-167.

4 W. B. Schaap and D. L. McMasters, J. Am. Chem. Soc., 1961, 83, 4699.

5 T. M.Florence, Talanta, 1982, 29, 345.

6 E. Nakayama, Y. Suzuki, K. Fujiwara and Y. Kitano, Anal. Sci., 1989, 5, 129.

7 M. D. Luque de Castro and M. V. Cases, Analyst, 1984, 109, 413.

8 H. Sigel and A. Sigel, Metal Ions in Biological Systems, Vols. 12 and 13, Marcel Dekker, New York, 2005.

9 H. Tapiero, G. Mathe, P. Couvreur and K.D. Tew, Biomed Pharmacother. 2002; 56: 446-67.

10 A.V. Fitsanakis and A. Michael, Toxicol. Appl. Pharm. 2005; 204: 343-354.

11 P. Paoletti. Pure Appl. Chem. 1984; 56: 491-497.

12 F.Hiromichi, M.Kenichi, Y.Kita, K. Ozora and O. Yuruke. Org. Lett. 2007; 9: 1687-1690.

13 L. Arash, M.H. Habibi, R.W. Harrington, M. Morteza and C. William. J. Fluorine Chem.2006; 127: 769-775.

14 A. C. Leela, R. S. Rani and G. Nageswara Rao Proc. Nat. Acad. Sci., India. 2012; 82, 197-204.