

# CHEMICAL SPECIATION OF TERNARY COMPLEXES OF L-ASPARTIC ACID AND ETHYLENEDIAMINE WITH CO (II), NI(II) AND CU(II) IN LOW DIELECTRIC MEDIA

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## ABSTRACT

Chemical speciation of ternary complexes of Co(II), Ni(II) and Cu(II) ions with L-aspartic acid and ethylenediamine was studied pH metrically in 0.0–60.0% v/v Dioxan-water mixtures maintaining an ionic strength of 0.16 mol L<sup>-1</sup> at 303.0±0.1 K. Alkalimetric titrations were carried out in 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 aspartic acid (L) to ethylenediamine (X). Stability constants of ternary complexes were calculated and various models were refined with MINQUAD75. The best fit chemical models were selected based on statistical parameters and residual analysis. The species detected were MLX, ML<sub>2</sub>X and MLXH for Co(II), Ni(II) and Cu(II) in Dioxan-water mixtures. The stabilities of the complexes followed the Irving-Williams order, i.e., Co (II) < Ni (II) << Cu (II).

**Keywords:** Chemical Speciation, Ternary Complexes, Aspartic Acid, Ethylenediamine, Dioxan, MINQUAD75.

## I. INTRODUCTION

Chemical speciation of transition metals is important to study their mobility, distribution, bioavailability, toxicity and setting environmental standards. Bioavailability of a metal ion depends on whether it is in free state or complexed with various constituents during biological reactions. Controlling factors such as pH, temperature and ionic strength change the complexation behaviour of metals and binding sites. Thus complexation can signify the bioavailability of the metal ions in various biosystems. Most metabolic reactions are catalyzed by metal-containing enzymes, the activity of which is due to metal-enzyme-substrate complexes. The active site of the enzymes has a lower polarity than biofluids. The specificity and selectivity of enzyme-substrate reactions can be achieved by manipulating the equivalent solution dielectric constants (ESDC) at the active site [1]. Acidity and basicity of a molecule is governed by its structure and solvent effects [2, 3]. L-aspartic acid (Asp), a non-essential amino acid, plays an important role in maintaining the solubility and ionic character of proteins [4]. It acts as a tridentate ligand and also as a neurotransmitter [5].

Ethylenediamine (en) is used as monodentate, bidentate or a bridging ligand [6]. It is used in the manufacture of EDTA, carbamate fungicides, surfactants and dyes. It is also useful in manufacturing accelerator or curing agent in epoxy industry. It is involved in the synthesis of seven-membered ring components with β-ketoesters



resulting secondary amines and  $\beta$ -enaminoesters [7]. The en plays an important role in the synthesis of Schiff bases [8]. The protonation constants of ethylenediamine were reported earlier by theoretical calculations [9, 10]. Cobalt is essential for the production of the red blood cells, and its salts are widely used in industrial materials, paint products, fertilizers, feeds and disinfectants [11-13]. Special cobalt-chromium-molybdenum alloys are used for prosthetic parts such as hip and knee replacements [14]. Nickel is found in urease, which accounts for 6% of the soluble cellular proteins [15] and catalyses the hydrolysis of urea to yield ammonia and carbamate. Copper containing enzymes and proteins constitute an important class of biologically active compounds, whose biological functions include electron transfer, dioxygen transport, oxidation, reduction and disproportionation [16].

The aim of the present study is to understand the role of metal ions at active site cavities in bioactive molecules like enzymes and proteins and to know the effect of dielectric constant of the medium on the chemical speciation of the ternary complexes of Co(II), Ni(II) and Cu(II) with Asp and en. Since the dielectric constant at the active site cavities is very small compared to that in biofluids, low dielectric constant is mimicked by using a water soluble organic solvent like Dioxan (Dox) which is a non-polar solvent capable of acting as hydrogen bond acceptor with random structure. Protonation constants [17, 18] and binary stability constants of Asp [19] and en [20] with Co(II), Ni(II) and Cu(II) have been reported earlier. Hence, chemical speciation of their ternary complexes is reported in the present communication.

## **II. EXPERIMENTAL**

Aqueous solutions ( $0.1 \text{ mol L}^{-1}$ ) of Co(II), Ni(II) and Cu(II) chlorides (GR Grade, E-Merck, Germany) were prepared by dissolving them in triple distilled water.  $0.05 \text{ mol L}^{-1}$  solutions of Ethylenediamine (AR, Qualigen, India) and L-Aspartic acid (AR, Qualigen, India) were also prepared. To increase the solubility of the ligands and metal salts,  $0.05 \text{ mol L}^{-1}$  hydrochloric acid was maintained in the solutions. 1, 4- Dioxan (Finar, India) was used as received. The strength of alkali was determined using the Gran plot method [21, 22]. Errors in the concentrations of ligands, metal ions and alkali were subjected to analysis of variance (ANOVA) [23].

Titration were carried out in the medium containing varying concentrations of Dox maintaining an ionic strength of  $0.16 \text{ mol L}^{-1}$  with sodium chloride at  $303.0 \pm 0.1 \text{ K}$ . The measurements were recorded with an ELICO (Model LI-120) pH meter of 0.01 readability in conjunction with a glass and calomel electrode. The pH meter was calibrated with  $0.05 \text{ mol L}^{-1}$  potassium hydrogen phthalate in acidic region and  $0.01 \text{ mol L}^{-1}$  borax solutions in basic region. The glass electrode was equilibrated in a well stirred Dox-water mixtures containing inert electrolyte. The effect of variations in asymmetry potential, 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 ( $\log F$ ) [24] which was computed from the experimental and simulated acid-base titration data calculated by SCPHD program [25]. It also accounts for the solvent effect on pH. Titration of strong acid with alkali was carried out at regular intervals to check whether complete equilibration was achieved. The calomel electrode was refilled with Dioxan-water mixtures of equivalent composition as that of the titrand. In each of the titrations, the titrand consisted of 1 mmol of hydrochloric acid in a total volume of 50 mL. Titrations were carried out in the presence of different relative concentrations of the metal (M) to Asp (L) to en (X) (M:L:X =



1:2.5:2.5, 1:2.5:5.0, 1:5.0:2.5) with 0.4 mol L<sup>-1</sup> NaOH (Table 1). The best-fit chemical model for each system investigated was arrived at using a non-linear least squares analysis program MINQUAD75 [26].

### III. RESULTS AND DISCUSSION

#### 3.2 Modeling of chemical speciation

A preliminary investigation of alkalimetric titrations of mixtures containing different mole ratios of Asp and en in the presence of hydrochloric acid and inert electrolyte indicates that no condensed species were formed. The protonation constants and the stability constants of the binary metal complexes of these ligands were fixed in refining ternary complexes and in testing various chemical models using MINQUAD75. The best fit model was chosen based on the statistical parameters like  $\chi^2$ , R-factor, skewness and kurtosis given in Table 2. The ternary complex species detected are MLX, ML<sub>2</sub>X and MLXH for Co(II), Ni(II) and Cu(II).

A very low standard deviation (SD) in the overall stability constants (log  $\beta$ ) indicates the precision of these parameters. The small values of  $U_{\text{corr}}$  (sum of squares of deviations in the concentrations of the metal, the ligands and the hydrogen ion at all 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 the 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 most of the residuals are very nearer to leptokurtic and a few form mesokurtic patterns. The values of skewness recorded in Table 2 are between -2.34 and 3.07. These data evince 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 recorded.

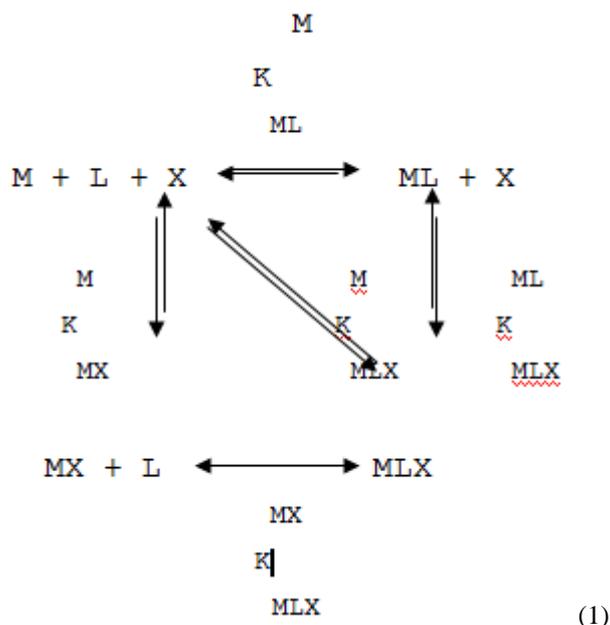
#### 3.3 Effect of dielectric constant on stability of ternary complexes

Dox is a protophilic dipolar protic solvent and acts as a structure former [27]. 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. 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. Born's classical treatment holds good in accounting for the electrostatic contribution to the free energy change [28]. According to this treatment, the energy of electrostatic interaction is related to dielectric constant. Hence, the log  $\beta$  values should vary linearly as a function of reciprocal of the dielectric constant (1/D) of the medium. The linear variation observed in the present study (Figure 1) indicates that electrostatic forces are dominating the equilibrium process under the present experimental conditions. This is due to protophilic dipolar protic and structure forming nature of the solvent. The stabilities of the complexes follow the Irving-Williams order, i.e., Co(II) < Ni(II) << Cu(II).

#### 3.4 Quantification of change in stability of ternary species

The change in the stability of the ternary complexes as compared to their binary analogues was quantified based on the disproportionation constant (log X) given by Equation 1 [29, 30] which corresponds to the equilibrium  $ML_2 + MX_2 \rightleftharpoons 2MLX$ . Under the equilibrium conditions one can expect the formation of 50% ternary

complexes and 25% each of the binary complexes statistically and the value of log X shall be 0.6. A value greater than this accounts for the extra stability of MLX.



Another approach to quantify the extra stability of ternary complexes was based on the difference in stability ( $\Delta \log K$ ) for the reactions ML with X and M (aq) with L and X [31-33], where L is the primary ligand (Asp) and X is the secondary ligand (en). It is compared with that calculated purely on statistical grounds as given in Equation 2. The equations for the calculation of  $\Delta \log K$  and log X are given in Table 3.

$$\Delta \log K = \log K_{MLX}^M - \log K_{ML}^M - \log K_M^M \quad \dots (2)$$

The electrostatic theory of binary complex formation and statistical arguments suggest the availability of additional coordination positions of the hydrated metal ion for the first ligand than for the second. Hence, the usual order of stability  $K_{ML}^M > K_{ML_2}^M$  applies. This suggests that  $\Delta \log K$  should be negative, although several exceptions have been found [34]. The statistical values of  $\Delta \log K$  for tridentate L and bidentate X are, -15.56 to 3.67 in Dox-water mixtures (Table 3). Negative values of  $\Delta \log K$  can be understood as the secondary ligand forms a more stable complex with hydrated metal ion than with L. Whenever the experimental values of  $\Delta \log K$  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.

The log X and  $\Delta \log K$  values could not be calculated for some systems due to the absence of relevant binary species. The log X values range from 1.39 to 10.50, some of which are found to be higher than those expected on statistical bases (0.6). These higher values account for the extra stability of the ternary complexes. The reason 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 and

stacking interactions [35, 36]. The extra stability of ternary complexes makes them more amenable for metal transport. The less stable binary complexes make the metals bioavailable.

#### IV. DISTRIBUTION DIAGRAMS

Based on the active forms of the ligands ( $LH_3^+$ ,  $LH_2$ ,  $LH$ ,  $L^-$  and  $XH_2^{2+}$ ,  $XH^+$  and  $X$ ) and the existence of the binary complex species ( $ML$ ,  $ML_2$ ,  $ML_2H_2$ ,  $ML_2H_3$  and  $ML_2H_4$  and  $MX$ ,  $MX_2$  and  $MX_3$ ), the plausible forms of species are assumed to form through the following equilibria.



The distribution of metal ions in various complexes species (chemical speciation) with pH is represented as distribution diagrams. Some typical distribution diagrams are given in Figure 2. Figure 2A represents the formation of Co-Asp-en complexes. In the pH range 4.0-10.0, the protonated ligands interact with metal ion (Equilibria 1 and 3) to form  $MLXH$  and  $MLX$ . And also  $MLX$  is formed by the dissociation of  $MLXH$  (Equilibrium 2).

Similarly  $ML_2X$  is formed either from interaction of  $ML$ ,  $LH$  and  $XH_2$  (Equilibrium 4) or from  $MLX$  (Equilibrium 5), because the concentrations of both  $MLX$  and  $LH$  are decreasing with increasing concentration of  $ML_2X$  in the pH range 8.0-10.0. Figure 2B shows formation of Ni-Asp-en complexes. The concentrations of  $MLX$  and  $MLXH$  increase in the pH range 4.0-10.0. Figure 2C shows the formation of Cu(II) complexes in the pH range 5.0-11.0.

#### 4.1 Structures of complexes

The literature [37, 38] suggests that Co(II), Ni(II) and Cu(II) complexes shall be octahedral. Amino nitrogen atom can associate with hydrogen ions in physiological pH ranges. Hence, there is often significant competition between hydrogen and metal ion for this donor site.

This situation results in the simultaneous existence of a number of equilibria producing an array of protonated complexes, which are detected in the present study. Asp acts as bidentate or tridentate ligand depending on the experimental conditions and en acts as bidentate ligand. Thus based on the above equilibria the plausible structures of the complexes are presented in Figure 3.



The following conclusions have been drawn from the modeling studies of the speciation of ternary complexes of Co (II), Ni (II) and Cu (II) with Asp and en in Dox-water mixtures.

1. The ternary metal complex species detected are  $MLX$ ,  $ML_2X$  and  $MLXH$ , for Co(II), Ni(II) and Cu(II), where  $L = Asp$  and  $X = en$ .
2. The values of  $\Delta \log K$  and  $\log X$  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 interactions between non-coordinated charge groups of the ligands.
3. The linear trend in the stabilities of ternary complexes with decreasing dielectric constants is due to the dominance of electrostatic forces over non-electrostatic forces.
4. The study also gives an insight into the metal availability/metal transport in biofluids. The ternary complexes are more amenable for "metal transport" because of their extra stability.

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### Table Captions

Table1: Total initial concentrations of reactants (in mmol) for mixed ligand titrations in Dox-water mixtures.

Table 2: Best fit chemical models of ternary complexes of Co (II), Ni (II) and Cu (II) with Asp and en in Dox-water mixtures.

Table 3:  $\Delta \log K$  and  $\log X$  values of ternary complexes of Asp and en with Co (II), Ni (II) and Cu (II) in Dox-water mixtures.



**Table1: Total initial concentrations of reactants (in mmol) for mixed ligand titrations in Dox-water mixture.**

[NaOH] = 0.4 mol L<sup>-1</sup>; V<sub>0</sub> = 50 mL; Temperature = 303.0±0.1 K; Mineral acid = 1.0 mmol; μ = 0.16 mol L<sup>-1</sup>.

PG % v/v	TM0			TL0	TX0	TM0:TL0:TX0
	Co(II)	Ni(II)	Cu(II)	Asp	en	
0.0	0.100	0.099	0.102	0.2500	0.2499	1:2.5:2.5
				0.2500	0.5000	1:2.5:5.0
				0.4999	0.2499	1:5.0:2.5
10.0	0.100	0.099	0.102	0.2498	0.2499	1:2.5:2.5
				0.2498	0.5000	1:2.5:5.0
				0.4998	0.2499	1:5.0:2.5
20.0	0.100	0.099	0.102	0.2492	0.2500	1:2.5:2.5
				0.2492	0.5000	1:2.5:5.0
				0.5001	0.2500	1:5.0:2.5
30.0	0.100	0.099	0.102	0.2510	0.2502	1:2.5:2.5
				0.2510	0.5000	1:2.5:5.0
				0.4998	0.2502	1:5.0:2.5
40.0	0.100	0.099	0.102	0.2550	0.2588	1:2.5:2.5
				0.2550	0.5000	1:2.5:5.0
				0.4999	0.2515	1:5.0:2.5
50.0	0.100	0.099	0.102	0.2500	0.2505	1:2.5:2.5
				0.2500	0.5000	1:2.5:5.0
				0.5001	0.2505	1:5.0:2.5
60.0	0.100	0.099	0.102	0.2500	0.2503	1:2.5:2.5
				0.2500	0.5000	1:2.5:5.0
				0.5001	0.2503	1:5.0:2.5

**Table 2: Best fit chemical models of ternary complexes of Co (II), Ni (II) and Cu (II) with Asp and en in Dox-water mixtures.**

%v/v	log $\beta_{mlxh}(SD)$			NP	U <sub>corr</sub>	$\chi^2$	Skewness	Kurtosis	R-factor
	1110	1210	1111						
Co(II) (pH=4.0-10.0)									
00.0	11.88(8)	15.51(23)	19.33(12)	23	0.82	12.35	-0.37	3.65	.0039
10.0	12.22(34)	16.01(25)	19.89(29)	52	6.81	51.08	1.74	9.72	.0177
20.0	12.91(78)	16.36(56)	19.98(36)	25	10.77	69.28	3.07	5.63	.0289
30.0	13.21(18)	16.85(17)	20.19(13)	33	8.73	59.03	2.49	8.97	.0169
40.0	13.85(28)	17.52(15)	20.27(11)	24	7.19	39.00	2.62	12.61	.0227
50.0	14.03(57)	18.67(61)	20.68(48)	25	11.66	42.72	2.44	10.44	.0346
60.0	15.17(8)	19.328(8)	21.01(8)	23	0.85	12.35	-0.37	3.65	.0039
Ni(II) (pH=4.0-10.0)									
00.0	15.01(32)	19.87(33)	21.15(57)	96	7.84	23.75	1.60	7.73	.0177
10.0	15.39(11)	20.44(13)	21.89 (12)	30	0.72	9.07	1.49	6.31	.0040
20.0	15.81(14)	20.78(14)	22.00(14)	77	3.00	14.55	-0.50	3.72	.0099
30.0	16.17(24)	20.91(18)	22.44(12)	24	0.75	6.50	0.01	3.56	.0044
40.0	16.64(18)	21.18(24)	22.85(15)	29	1.38	22.43	2.04	9.30	.0061
50.0	17.29(12)	22.23(20)	23.06(12)	22	0.85	5.82	2.14	3.53	.0066
60.0	18.91(13)	22.98(19)	23.89(16)	24	0.82	19.67	0.40	3.06	.0064
Cu(II) (pH=2.5-11.0)									
00.0	23.49(55)	30.39(53)	28.07(20)	33	2.66	7.15	0.64	4.62	.0115
10.0	22.38(24)	26.3(31)	27.25(12)	27	1.91	2.07	-0.53	4.06	.0059
20.0	22.25(17)	25.57(16)	27.81(13)	98	3.49	34.98	-1.22	4.45	.0221
30.0	21.64(55)	24.93(68)	26.95(24)	42	9.53	14.76	-0.88	3.65	.0126
40.0	21.50(41)	25.25(52)	26.22(43)	25	6.64	31.20	-2.34	10.45	.0168
50.0	21.25(14)	25.63(18)	26.08(20)	40	1.21	30.40	-1.45	3.86	.0173
60.0	21.12(19)	23.73(44)	25.55(62)	30	2.73	67.70	-2.27	8.87	.0101



**Table 3:  $\Delta \log K$  and  $\log X$  values of ternary complexes of Asp and en with Co(II), Ni(II) and Cu(II) in Dox-water mixtures.**

% v/v Dox	$\Delta \log K$		$\log X$	
	Co(II)			
	1110	1210	1110	1111
0.0	-0.23	-5.78	2.47	2.71
10.0	-1.02	-6.99	1.44	3.13
20.0	-0.36	-6.54	2.08	3.87
30.0	-0.35	-7.31	2.56	3.46
40.0	-0.24	-7.51	1.39	3.62
50.0	-0.09	-6.39	2.92	4.79
60.0	0.09	-7.59	3.13	3.83
	Ni(II)			
0.0	0.13	-6.47	3.68	4.69
10.0	0.16	-6.86	3.48	5.90
20.0	0.26	-6.49	4.35	5.89
30.0	0.07	-7.82	3.61	5.88
40.0	-0.06	-8.58	3.52	5.71
50.0	0.87	-7.61	4.74	5.04
60.0	1.69	-8.52	6.32	3.94
	Cu(II)			
0.0	3.67	-5.50	9.09	9.29
10.0	2.34	-10.0	8.46	9.57
20.0	1.63	-10.95	7.98	10.50
30.0	0.10	-12.39	5.96	6.92
40.0	-0.15	-13.17	4.58	4.90
50.0	0.06	-11.74	5.13	5.77
60.0	-1.35	-15.56	2.95	2.20

Equations for the calculation of  $\Delta \log K$  and  $\log X$

$$\Delta \log K_{1110} = \log \beta_{1110} - \log \beta_{1100} - \log \beta_{1010}$$

$$\Delta \log K_{1210} = \log \beta_{1210} - \log \beta_{1200} - \log \beta_{1020}$$

$$\Delta \log K_{1111} = \log \beta_{1111} - \log \beta_{1100} - \log \beta_{1010}$$

$$\log X_{1110} = 2\log \beta_{1110} - \log \beta_{1200} - \log \beta_{1020}$$

$$\log X_{1210} = 2\log \beta_{1210} - \log \beta_{1400} - \log \beta_{1020}$$

$$\log X_{1111} = 2\log \beta_{1111} - \log \beta_{1202} - \log \beta_{1020}$$

**Figure Captions**

Figure 1: Variation of stability constants of Asp-metal-en ternary complexes in Dox-water mixtures. (A) Co (II), (B) Ni (II) and (C) Cu (II); ( $\square$ )  $\log \beta_{MLX}$ , ( $\circ$ )  $\log \beta_{ML2X}$  and ( $\Delta$ )  $\log \beta_{MLXH}$ .

Figure 2: Species distribution diagrams of Asp and en ternary complexes in 40% v/v Dox-water mixtures. (A) Co (II), (B) Ni (II) and (C) Cu (II).

Figure 3: Structures of Asp-Metal-en complexes, where S is either solvent or water molecule.

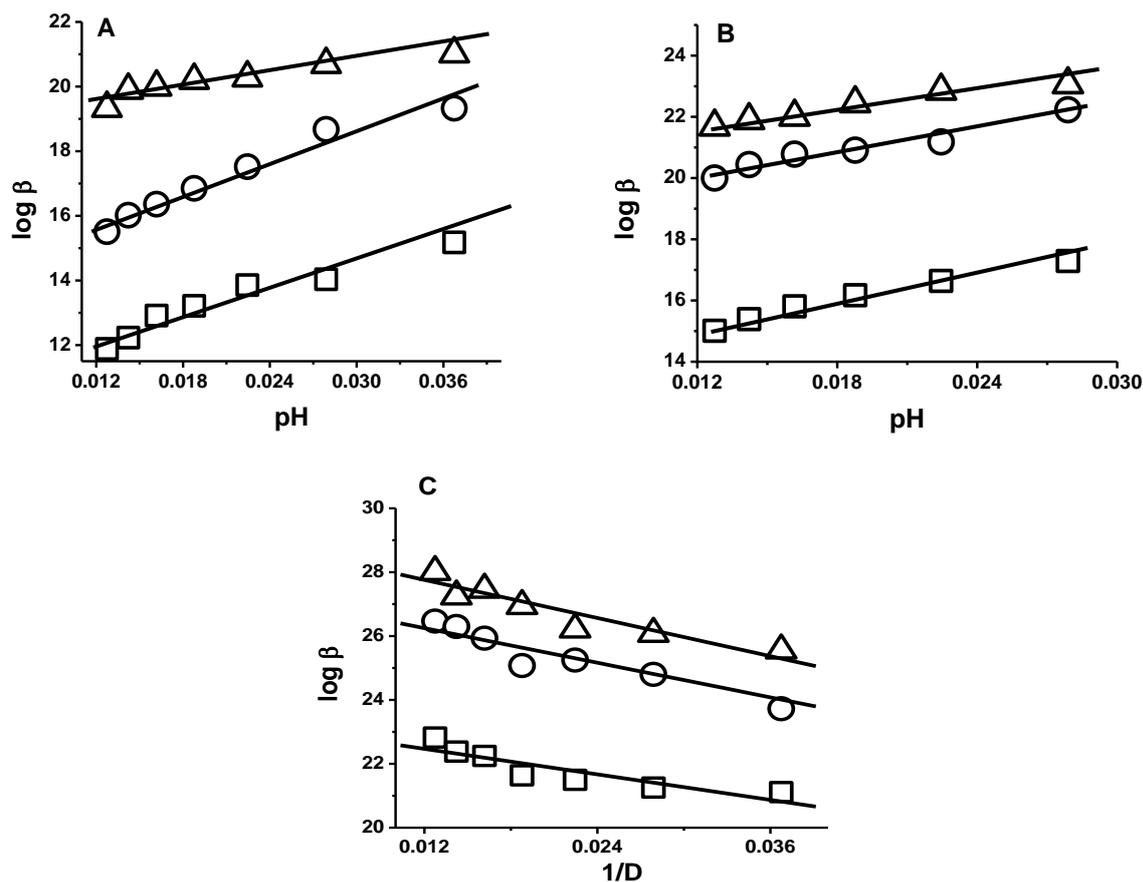




Figure 1: Variation of stability constants of Asp-metal-en ternary complexes in Dox-water mixtures. (A) Co(II), (B) Ni(II) and (C) Cu(II); ( $\square$ )  $\log \beta_{MLX}$ , ( $\circ$ )  $\log \beta_{ML_2X}$  and ( $\Delta$ )  $\log \beta_{MLXH}$ .

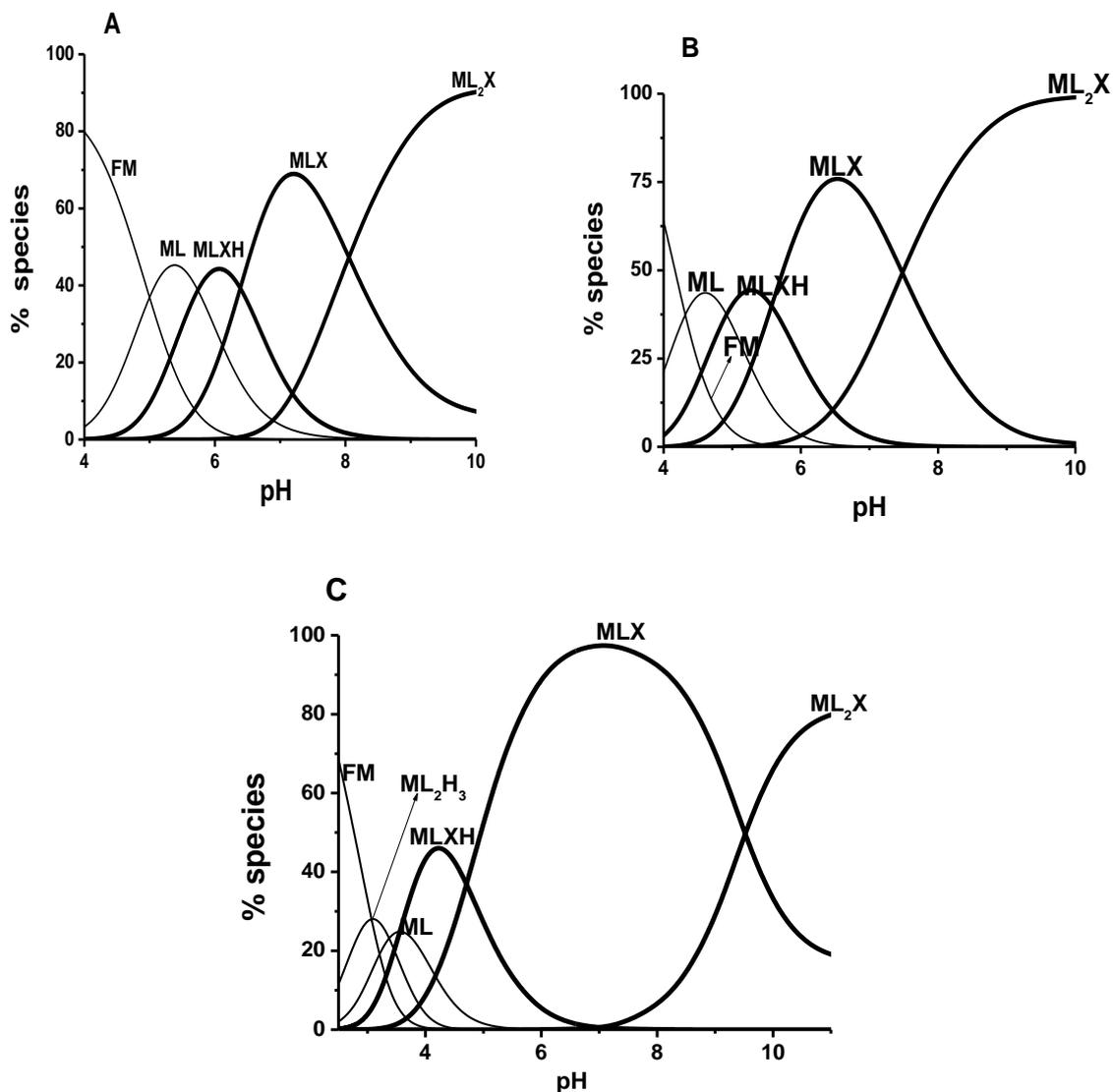


Figure 2: Species distribution diagrams of Asp and en ternary complexes in 40% v/v Dox-water mixtures. (A) Co (II), (B) Ni (II) and (C) Cu(II). The amounts of ingredients are Asp: 5.0 mmol, en: 2.5 mmol and M (II): 1.0 mmol.

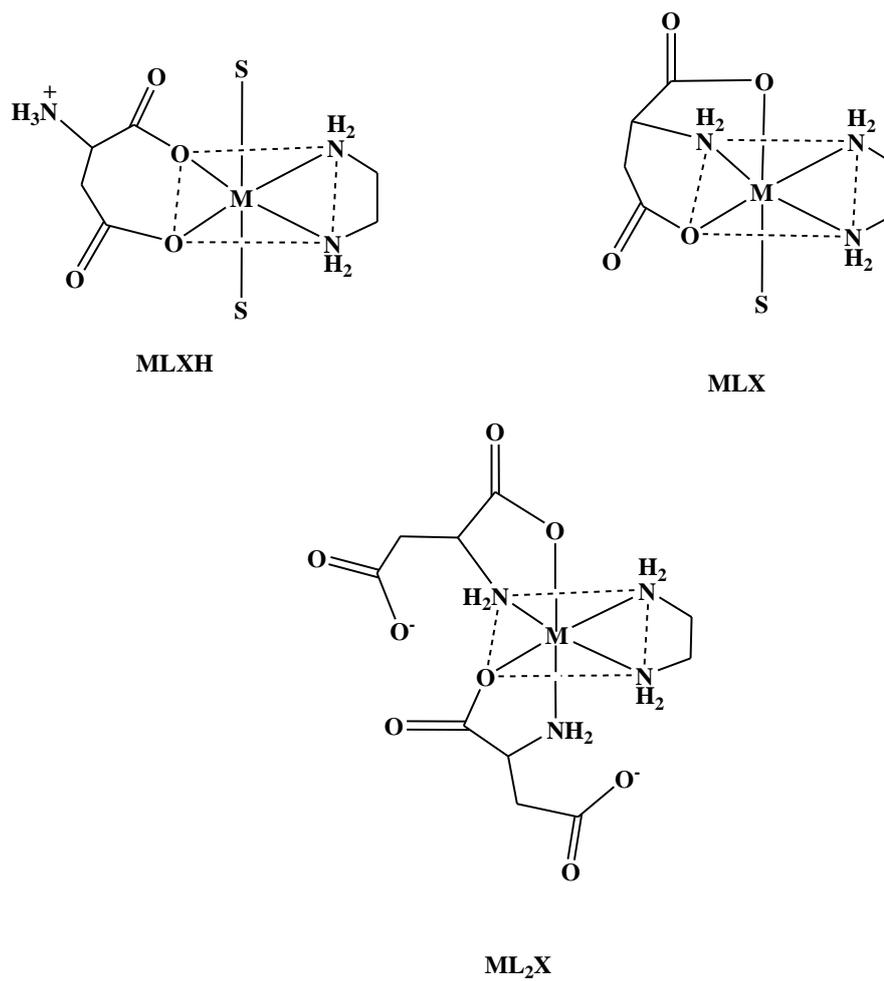


Figure 3: Structures of Asp-M(II)-en complexes, where S is either solvent or water molecule.