



Chemical Distribution, Stability and Structures of Ternary Metal Chelates in Ethylene glycol–Water Media Involving Mercaptosuccinic acid and Some Non-Essential Amino acids

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<http://dx.doi.org/10.13005/ojc/380507>

(Received: July 06, 2022; Accepted: September 08, 2022)

ABSTRACT

There has been focus upon the study of stability constants of amino acids with metal ions since they processes antibacterial activities. The stability constants of ternary complexes were determined by pH metric analysis in aqueous solution at 25.0°C between ligands (Mercaprosuccinic acid (MSA), Glycine (GLY), L-Aspartic acid (ASP) and L-Asparagine (ASN)) and some transition metal ions (Co(II), Ni(II), Cu(II) and Zn(II)) in varying Ethylene glycol–water media. The "MINIQUAD75" software tool analysed the potentiometric data to create a complexation model for each system. Based on the calculated equilibrium and stability constants, the most likely binding mechanism for each ternary species was discussed. Their relative stabilities and plausible structures were also proposed.

Keywords: Glycine, L-Aspartic acid, BestFit, MINIQUAD75, Chemical speciation.

INTRODUCTION

In recent years, attention has been focused on transition metals amino acid complexes because they are effective antibacterial agents against *Staphylococcus aureus* and *Escherichia coli* and can be utilised to produce food for humans and animals¹. Many attempts have been made to evaluate different factors affecting the stability of the metal chelates along with their stability constants^{2,3}. There are 20 different amino acids that make up proteins out of which 9 are essential and 11 are non-essential

amino acids⁴. Through their amine and carboxylate groups, amino acids combine with different metal ions to form stable five-membered chelates⁵⁻⁷.

Glycine (Gly) is an important building block for all proteins in the body (Fig. 1(b)). It helps the body absorb calcium, build muscle protein, regulates protein turnover, heal after surgery or an injury, and make hormones, enzymes, and antibodies⁸. L-Aspartic acid (Asp) is an amino acid that is found in large amounts in plant proteins but is not essential to life (Fig. 1(c)). It is an important part of keeping



proteins soluble and ionic⁹. Asp is often used to treat chronic fatigue because it is a key part of how cells make energy. One of the 20 most common natural amino acids is L-Asparagine (Asn). Its side-chain group is carboxamide (Fig. 1(d)). It plays a role in how the brain and nervous system work. It is needed by the nervous system to keep things in balance, and the liver needs it to change amino acids from one form to another¹⁰. Mercaptosuccinic acid (MSA) is a potentially important tridentate ligand that can form strong complexes with many metal ions and has chemotherapeutic uses in a variety of solvents¹¹⁻¹⁴ (Fig. 1(a)). Metals like cobalt, nickel, copper, and zinc are important for living things.

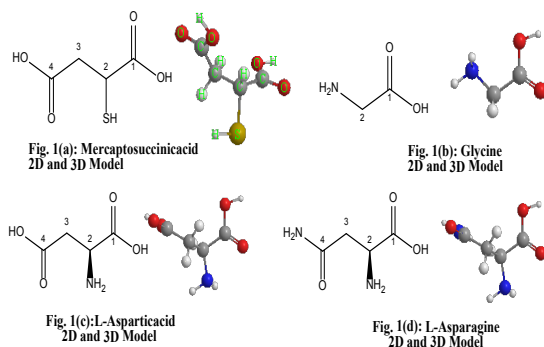


Fig. 1: 2D and 3D structures of a) Mercaptosuccinic acid; b) Glycine; c) L-Aspartic acid and d) L-Asparagine

The speciation study of ternary metal ion complexes of MSA with both essential and non-essential amino acids with transition metal ions have been studied in aqua-organic media have been studied separately by author. In the present study, complex formation between (Co(II), Ni(II), Cu(II), and Zn(II)) and MSA as primary ligand and some important non-essential amino acids namely Glycine, L-Aspartic acid and L-Asparagine as secondary ligands were carried out using a pH metric titration technique with an interpretation to determining the stability constants. The relative stabilities of complexes and plausible structures were proposed based on the results.

The types of complexes formed and their relative concentrations under the present experimental conditions represent the possible forms of metal ions in the biological fluids and speciation represents the biological activity of these metals in the presence of different complexing agents. Since the dielectric constant at the active site cavities is small compared to biofluids, the authors tried

to mimic low dielectric constant by using a water soluble organic solvent like ethylene glycol.

EXPERIMENTAL

MATERIALS AND METHODS

Standardized sodium hydroxide was used to test acid solutions^{15,16}. Merck p.a. reagent sodium chloride was used as a background electrolyte. By adding NaCl as a supporting electrolyte, the amount of ions in each solution was changed to 0.16 M. *Himedia India* was used to buy MSA, Glycine, L-Aspartic acid, and L-Asparagine. Using standard HCl, the Gran method was used to check the solutions' purity and figure out their exact concentrations. The metal(II) stock solutions were standardised by titrating them with EDTA and a suitable indicator¹⁷.

Measurements

The ternary stability constants of MSA as primary ligand, glycine, L-aspartic acid and L-asparagine as secondary ligands were determined using pH- titration with following composition.

- a – HCl +MSA+ glycine + metal(II);
 - b – HCl + MSA+L-aspartic acid + metal(II);
 - c – HCl + MSA+L-asparagine + metal(II).
- Metal(II) = Co(II) or Ni(II) or Cu(II) or Zn(II).

Approach

pH metric titrations were conducted in 0-60%v/v EG-Water media by using an auto-titrator Metrohm 877 titrino plus Switzerland in conjunction with pH sensitive electrode under the temperature of 298 K as described elsewhere¹⁸. The electrode was standardized with standard buffer solutions.

Metal-Ligand Ternary Systems

MINIQUAD75 computer programme was used to calculate stability constants^{19,20}. A set of typical species formed for the different 1: 1: 1 M(II): MSA: GLY, M(II): MSA: ASP and M(II): MSA: ASN systems is shown in Fig. 2.

RESULTS AND DISCUSSION

Modelling of chemical species

The metal-ligand stability constants of ternary complexes were evaluated assuming that the

formation of polynuclear complexes and hydrolyzed products were not formed. In this ternary (M-L-X) complexes it is observed that the metal forms the complex first with the primary ligand (L) at lower pH and then metal-primary ligand (M-L) curves comes in to contact with the secondary ligand (X) to form the mixed ligand complex at higher pH. Secondary ligand does not come into contact with metal ions at lower pH. In other words mixed ligand complex formation takes place when M-L complex formation is complete. The best fit models for ternary complexes with MSA and chosen amino acids (GLY, ASP & ASN) have been given in Tables 1-3. Species like MLX, MLX₂ and MLXH were detected for M(II)-MSA-X (X=GLY or ASN or ASP). Overall stability constants ($\log \beta$) with minimal standard deviation (SD) in specifies the exactness of these constraints.

Species Distribution Plots

The weak binary metal complexes render the essential metals accessible, while the

stable ternary complexes transport metal ions in biological systems. The plausible forms and their distribution of protonated and un-protonated complexes observed in 20% v/v EG–water mixtures that suit best for ternary complex hypothesis and their respective relative distribution plots of several species was plotted using computer program ORIGIN 8.5 and shown in Fig. 3. The present study found MLX, MLX₂ species for M(II)-MSA-Glycine and M(II)-MSA-L-Asparagine and MLX, MLXH species for M(II)-MSA-L-Aspartic acid in the pH range 3.0-9.5. At lower pH concentration of MLX species is high (Equilibria 1, 2, 3, 6, 7 and 8). As the concentration of MLX decreases with increasing pH and the concentration of MLX₂ (Equilibria 4, 5 and 9) increases gradually for M(II)-MSA-Glycine and and M(II)-MSA-L-Asparagine. As in the case of M(II)-MSA-L-Aspartic acid the pH increases the concentration of MLX decreased (Equilibria 1-3 and 6-8) and the concentration of MLXH are increased (Equilibria 10-12).

Table 1: Best fit model of M(II)-MSA- Glycine ternary complexes in 0-60% v/v EG-water mixtures

%V/V	$\log \beta_{MLXH}(SD)$ Co(II) pH Range: 2.5-9.5		$\log \beta_{MLXH}(SD)$ Ni(II) pH Range: 2.5-9.5	
	MLX	MLX ₂	MLX	MLX ₂
0.0	11.74(11)	20.17(14)	13.02(08)	23.98(09)
10	11.97(09)	20.57(22)	13.25(18)	23.40(17)
20	11.21(13)	20.98(15)	13.49(13)	24.83(15)
30	12.46(22)	21.40(11)	13.74(10)	25.27(11)
40	12.71(18)	20.83(18)	13.98(11)	25.71(14)
50	12.96(14)	22.27(21)	14.23(23)	26.17(18)
60	13.22(09)	22.71(09)	14.49(21)	26.63(20)
	Cu(II) pH Range: 2.5-9.5		Zn(II) pH Range: 2.5-9.5	
0.0	17.76(14)	31.55(21)	10.98(15)	20.04(11)
10	18.12(11)	32.02(14)	11.20(08)	20.28(14)
20	18.49(24)	32.50(19)	11.43(20)	20.52(09)
30	18.87(21)	32.98(07)	11.66(17)	20.77(18)
40	19.25(18)	33.47(11)	11.90(11)	21.02(24)
50	19.65(13)	33.97(14)	12.14(18)	21.27(15)
60	20.05(09)	34.48(21)	12.39(20)	21.53(13)

Table 2: Best fit model of M(II)-MSA- L-aspartic acid ternary complexes in 0-60% v/v EG-water mixtures

%V/V	$\log \beta_{MLXH}(SD)$ Co(II)pH Range: 2.5-9.5		$\log \beta_{MLXH}(SD)$ Ni(II) pH Range: 2.5-9.5	
	MLX	MLXH	MLX	MLXH
0.0	12.45(15)	15.09(14)	14.56(17)	16.66(11)
10	12.67(21)	15.51(21)	14.77(20)	17.17(21)
20	12.90(11)	15.13(15)	14.98(11)	17.68(24)
30	13.13(18)	16.36(10)	15.19(14)	18.21(09)
40	13.37(09)	16.80(18)	15.41(08)	18.74(15)
50	13.61(24)	16.24(26)	15.63(16)	19.29(12)
60	13.86(18)	16.70(09)	15.85(11)	19.85(14)
	Cu(II) pH Range: 2.5-9.5		Zn(II) pH Range: 2.5-9.5	
0.0	18.34(07)	22.21(17)	11.43(09)	14.84(11)
10	18.67(19)	22.53(25)	11.64(19)	14.23(14)
20	19.01(12)	22.86(24)	11.21(14)	14.63(21)
30	19.35(15)	23.19(19)	12.07(11)	15.04(15)
40	19.70(21)	23.52(22)	12.29(09)	15.46(08)
50	20.06(12)	23.85(14)	12.51(22)	15.88(19)
60	20.42(14)	24.19(09)	12.74(27)	16.31(20)

Table 3: Best fit model of M(II)-MSA- L-asparagine ternary complexes in 0-60% v/v EG-water mixtures

%V/V	log β _{MLXH} (SD)		log β _{MLXH} (SD)	
	MLX	MLX ₂	MLX	MLX ₂
	Co(II) pH Range: 2.5-9.5		Ni(II) pH Range: 2.5-9.5	
0.0	11.06(14)	20.25(10)	12.87(21)	23.61(11)
10	11.21(18)	20.53(14)	13.08(19)	23.89(18)
20	11.37(08)	20.82(19)	13.29(08)	24.18(06)
30	11.53(21)	21.11(21)	13.50(11)	24.47(24)
40	11.69(14)	21.41(09)	13.72(17)	24.76(19)
50	11.86(16)	21.71(21)	13.94(23)	25.06(22)
60	12.02(10)	22.01(15)	14.16(10)	25.36(15)
	Cu(II) pH Range: 2.5-9.5		Zn(II) pH Range: 2.5-9.5	
0.0	17.32(09)	31.04(18)	10.34(17)	18.69(10)
10	17.49(14)	31.32(24)	10.53(12)	19.06(15)
20	17.67(22)	31.60(21)	10.72(27)	19.45(11)
30	17.84(08)	31.89(18)	10.91(13)	19.83(18)
40	18.02(11)	32.17(17)	11.10(18)	20.23(22)
50	18.20(17)	32.46(11)	11.30(15)	20.64(10)
60	18.39(14)	32.75(22)	11.51(08)	21.05(14)

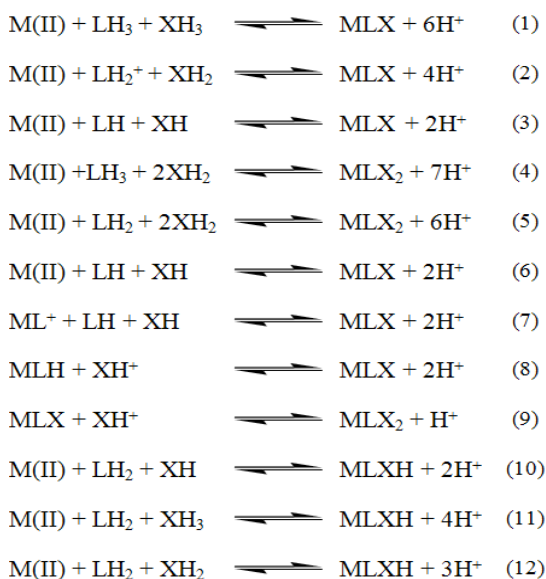


Fig. 2. The plausible forms of equilibria and their distribution of protonated and un-protonated complexes observed in the present study

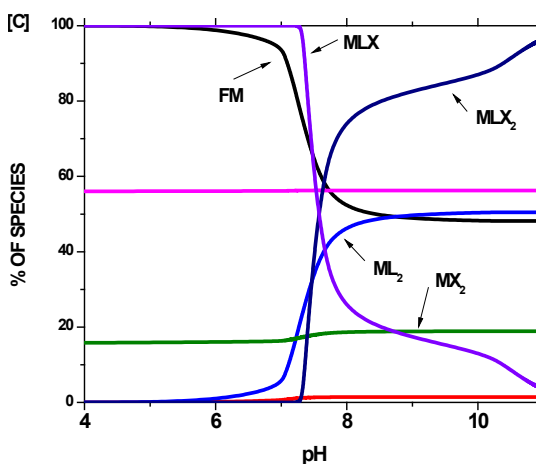
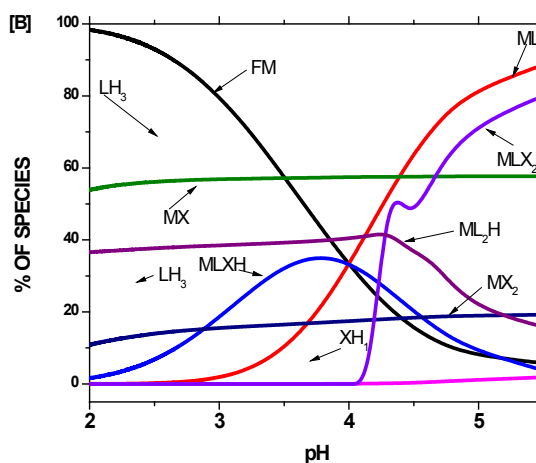
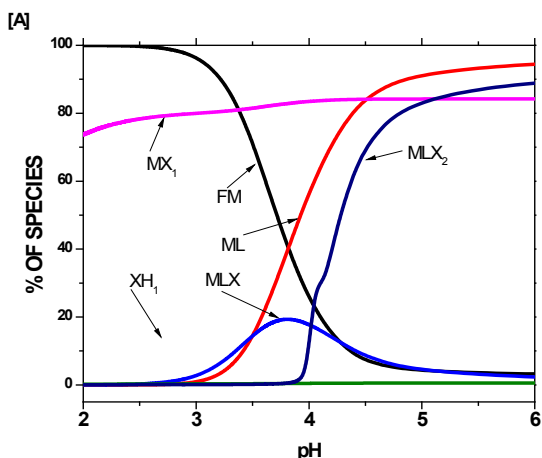


Fig. 3. Species distribution plots of [A]: Co(II)-MSA-GLY; [B]: Ni(II)-MSA-ASP; [C]: Cu(II)-MSA-ASN in 20% v/v EG-water media

Inter Comparison of Stability Constants

By looking at Tables 1-3, we can see that due to the smaller size of Cu(II) makes its stability

constants higher than those of other studied metal complexes and shown in Fig. 4. This behaviour is in line with the Irving–Williams order²¹.

nature of EG, eliminates water from the metal ions coordination sphere and creating them highly reactive to ligands resulting the complexes more stable. Thus, $\log \beta$ values should be directly related to the reciprocal of the medium's dielectric constant ($1/D$), as shown in present study Fig. 5.

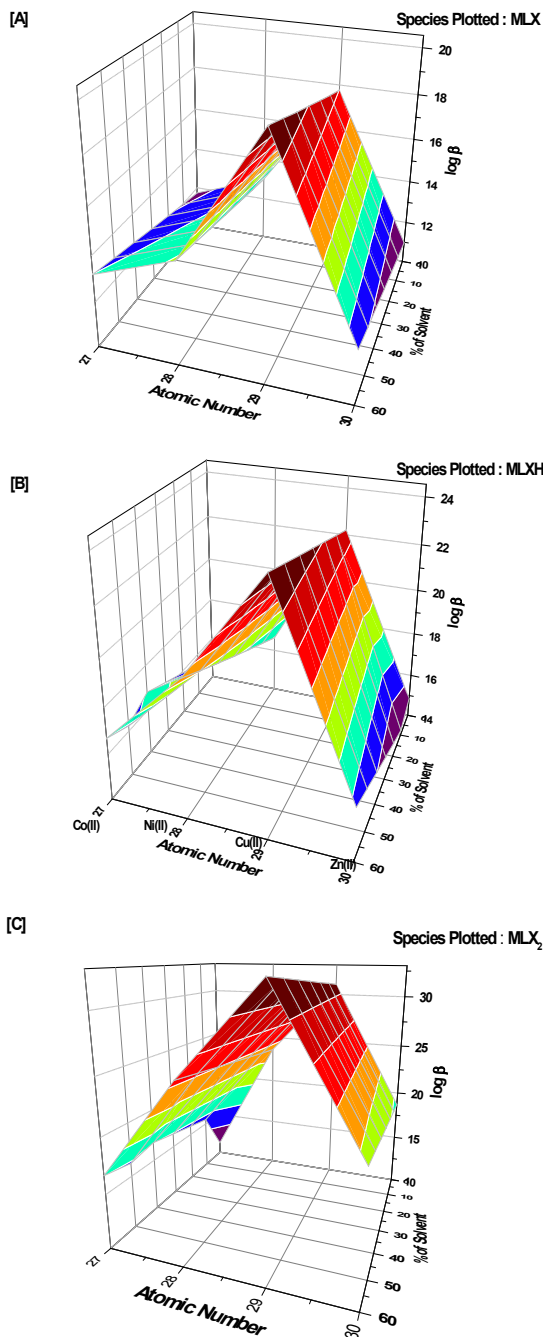


Fig. 4. Variation of $\log \beta$ with Atomic Number for [A]: M(II)–MSA–Gly; [B]: M(II)–MSA–ASP; [C]: M(II)–MSA–ASN in 0–60% v/v EG–water media

Role of dielectric constant
Being a protophilic and structural forming

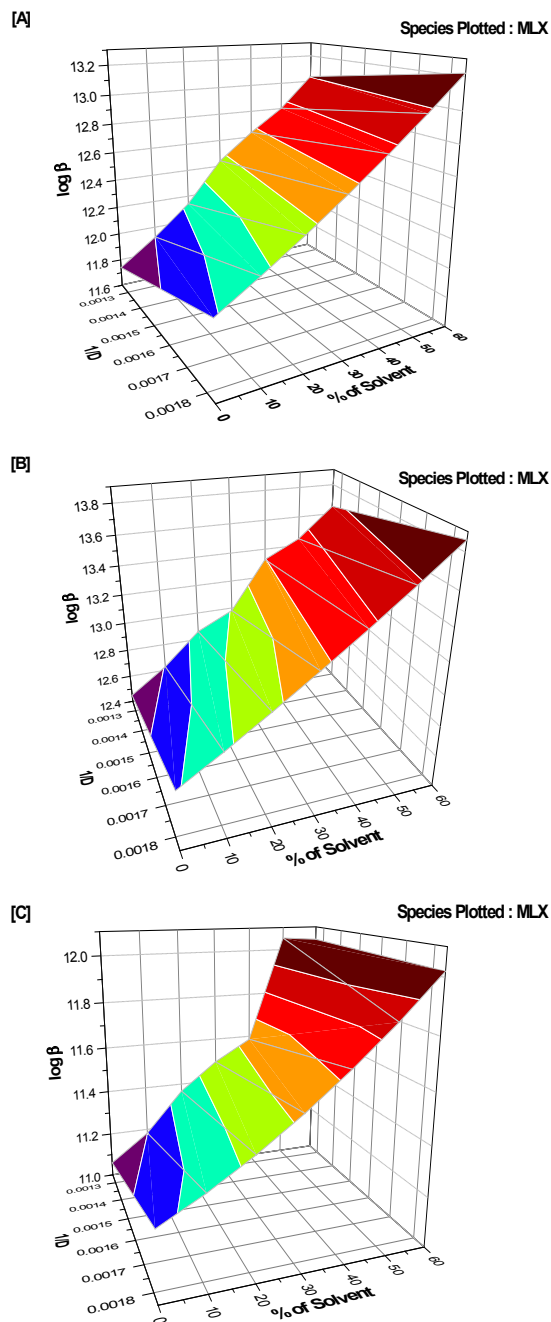


Fig. 5. 3D Surface plot for Variation of $\log \beta$ against reciprocal of Dielectric constant and percentage of solvent for [A]: M(II)–MSA–GLY; [B]: M(II)–MSA–ASP; [C]: M(II)–MSA–ASN

Proposed structure

Depending upon the nature of the ligands and the metal ions and based on the basic chemical knowledge the structures of the ternary complexes are proposed and sketched using Chem Draw 18.1

computer program as shown in Fig. 6. MSA form strong bidentate complexes with transition metals. MSA at higher pH favors the (O, S) coordination and at physiological pH amino acids bound only through the amino acid side chain²²⁻²⁸.

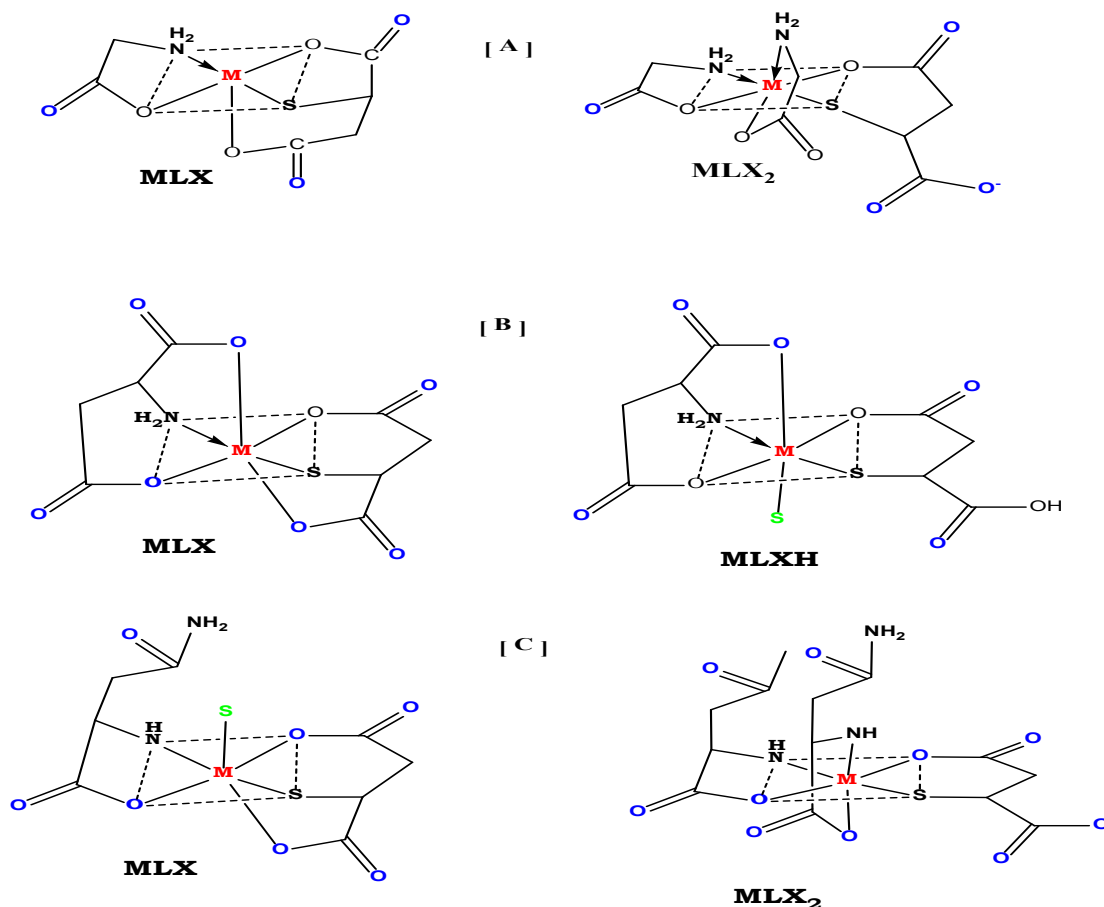


Fig. 6. Proposed Structures of Ternary Complexes of [A]: M(II)-MSA-GLY; [B]: M(II)-MSA-ASP; [C]: M(II)-MSA-ASN. M(II): Co(II), Ni(II), Cu(II) or Zn(II) and S=Either H₂O or Solvent

CONCLUSION

In this study, the results of a pH-metric study of ternary systems of MSA and three non-essential amino acids (Glycine, L-Aspartic acid, and Asparagine) with transition metal ions were used to figure out the constants. It was clear that MSA could form stable ternary complexes with studied metal ions, with glycine, L-aspartic acid, and L-asparagine

as the secondary ligands. Due to the effect of the N- and O- donors, the formation of mixed-ligand complexes probability is more than binary.

ACKNOWLEDGEMENT

The author is grateful to Department of Basic Sciences, Vishnu Institute of Technology for their support.

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