Chemical Distribution, Stability and Structures of Ternary Metal Chelates in Ethyleneglycol – Water Media Involving Mercaptosuccinicacid and Some Essential Amino Acids

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Abstract

*There has been focus upon the study of stability constants of amino acids with metal ions since they processes antibacterial activities. Mixed-ligand complexes generated between Mercaprosuccinicacid (MSA), L-Histidine (His), and L-Valine (Val) with transition metals (Co(II), Ni(II), Cu(II), and Zn(II)) were examined pH metrically in aqueous solution at 25.0 ^0C and = 0.1M NaCl to determine stability constants. From potentiometric data, "**MINIQUAD75**" computer program created a complexation model for each system. Based on equilibrium and stability constants, the most likely binding mode for each ternary species was discussed. And also investigated solution concentration distributions of various species. Complex stability follows Irving-Williams' order of metal ions: Cu(II) > Ni(II) > Co(II) > Zn(II). The plausible structures for each species detected were proposed.*

Key Words: **L-Histidine, L- Valine, Best Fit, Ternary Complexes, Irving-Williams Series**

INTRODUCTION

Metal complexes have become importance in recent years especially at design from repository, release slow or long acting medicine in nutrition and by the study of metabolism [Akalpita et al,2012]. It is known that metal ions to accelerate the action of the drug [Sunil et al,2009]. Mixed ligand complexes are well known to play an important role in biological processes [Adkhis et al ,2000, Shivankar et al ,2003&Nomiya & Yokoyama ,2002]. Many researchers have extensively investigated metal complexes of biologically active ligands [Regupathy & Sivasankaran Nair ,2014 &Abdel-Rahman et al,2017]. The literature survey revealed that mixed ligand complexes of some transition metals with amino acids have been studied for their synthesis, characterization, biological significance and metabolic enzymatic [Choudhary et al,2011&Sharma et al,2018]. Amino acids containing $–NH_2$ and –COOH active groups are well known that, can form strong complexes with metals and have great significance at biological as well as pharmaceutical [Selvaganapathy & Raman,2016 &Liao et al,2013] domains. In last year's transition metals amino acids complexes were received lots of interest because they have proven to be beneficial antibacterial and antifungal agents used on Staphlococcus aureus, Escherichia Coli with humans and animals, etc[Liao et al,2007 &Liao et al,2010]

The study of the coordinated systems metal ion - amino acids has become increasingly important in recent years. The "Bio coordination Chemistry" was confirmed as one of seven priority research fieldsduring 1990's[14]. L-Valine (abbreviated as Val) is an α-amino acid with the chemical formula $HO_2CCH(NH_2)CH(CH_3)_2$(Fig. 1(a)).It is one of 20 proteinogenic amino acids [Yuichi Shimazaki et al1] widely distributed but rarely occurs in amount exceeding 10%. It is branched chain amino acid and can be derived from alanine by the introduction of two methyl group present on α-carbon atom [Aliyu and Isyaku ,2010,Deschamps et al,2005,Creighton et al,1999,Guo et al,1999, Vinnikova et al,1992,Sitton et al,1998, Hitomi-Ohmura et al,1992&Aoyama et al,1992] L-Histidine (His) is one of the most powerful metal coordinating ligands among amino acids, and it is essential for protein metal ion binding. Histidine is one of the amino acid residues found in the majority of biomolecule active sites.It has three potential metal-binding sites, namely, carboxylate oxygen ($O_{caboxyl}$), imidazole imidonitrogen (N_{im}) and aminonitrogen (N_{am}) [Cai et al,1995] (Figure 1(b)).

Mercaptosuccinic acid (MSA) is a potentially important tridentate ligand capable of forming strong complexes with a variety of metal ions and having chemotherapeutic applications in a variety of solvents [Muthusamy & Natarajan,2016,Pharr et al,2011, Wilson et al,1970&Lahsasni et al,2012,]Fig. 1(c)). Metals including cobalt, nickel, copper, and zinc are vital in biological activities.

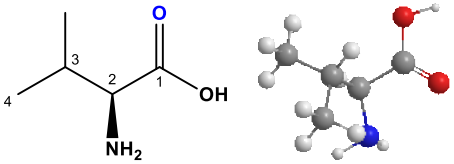

Fig. 1(a): L-Valine
2D and 3D model

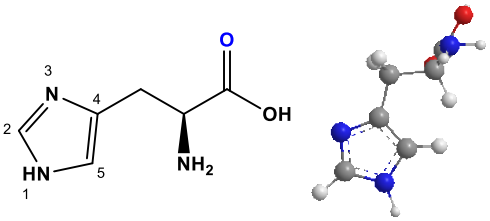

Fig. 1(b): L-Histidine
2D and 3D model

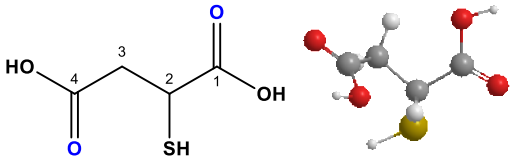

Fig. 1(c): Mercaptosuccinicacid
2D and 3D model

Fig. 1: 2D and 3D structures of a) Mercaptosuccinic Acid; b) L-Valine; c) L-Histidine.

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. In this paper, we report the stability constants of all complexes which form in Ethylene glycol - Water media, including the most probable structures of all mixed species of Mercaptosuccinicacid (MSA) as primary ligand and L-Histidine and L-Valine as secondary ligands. Thus, in the present study, a systematic study of complex formation between some transition metal ions (Co(II), Ni(II), Cu(II), and Zn(II)) and ligands (MSA, His, Val) was carried out using a pH metric titration technique over a wide pH range at (25.0 ± 0.1)◦C and μ = 0.16M(NaCl), with a view to determining the stability constants.

Under the current experimental conditions, the types of complexes produced and their relative concentrations represent the potential forms of metal ions in biological fluids, and speciation symbolises the biological activity of these metals in the presence of diverse complexing agents. Because the dielectric constant at the active site cavities is low in comparison to biofluids, the authors attempted to simulate it by employing a water soluble organic solvent such as ethylene glycol.

MATERIALS AND METHODS

All compounds utilised in this study were analytical grade and unpurified. Alkali strength and lack of carbonate were frequently evaluated by pH-metry against oven-dried sodium hydrogen phthalate [Gran et al,1950 &Rossotti & Rossotti,1965,]. Merck's acid solutions were titrated against sodium hydroxide [Khan et al,1996]. NaCl was used to modify each solution's ionic strength to 0.16 M. Himedia india sold MSA, L-Histidine, and L-Valine. Analytical-grade chlorides were used to make 0.1 M stock solutions of Co(II), Ni(II), Cu(II), and Zn(II). Complexometric EDTA titration was used to standardise metal(II) stock solutions [Schwarzenbach et al,1969].

MEASUREMENTS

The ternary stability constants of MSA as primary ligand, L-Histidine and L-Valine as secondary ligands were determined by pH- titration and the total volume was (50.00 ± 0.01) cm^{-3} at the beginning of each pH metric titration. The following mixtures containing proton, metal(II) chloride and the two ligands (at different ratios 1:1:1, 1:1:1.5 and 1:1.5:1) were titrated by incremental additions of carbonate-free and standard NaOH.

a — HCl + metal(II) + MSA + L-Histidine;
b — HCl + metal(II)+ MSA + L-Valine;
Metal(II) = Co(II) or Ni(II) or Cu(II) or Zn(II).

APPROACH

pH metric titrations were performed in 0-60 percent v/v EG-Water using an auto-titrator Metrohm 877 titrino plus Switzerland and pH sensitive electrode at 298 K [Shoukry et al,1995].

Metal-Ligand Ternary Systems

The stability constants were calculated using the MINIQUAD75 computer programme [Gans et al,1976&Khan et al,1996]. A set of typical distribution curves for the different 1:1:1 M(II):MSA:His andM(II):MSA:Val systems at (25.0 ± 0.1)◦C and μ = 0.16M NaCl is shown in **Fig. 2**. Similar behaviour was observed for all metal ions (Co(II), Ni(II), Cu(II) and Zn(II)) studied.

$$M(II) + LH_3 + XH_3 \rightleftharpoons MLX + 6H^+ \quad (1)$$

$$M(II) + LH_2^+ + XH_2 \rightleftharpoons MLX + 4H^+ \quad (2)$$

$M(II) + LH + XH \rightleftharpoons MLX + 2H^+$ (3)

$M(II) + LH_3 + 2XH_2 \rightleftharpoons MLX_2 + 7H^+$ (4)

$M(II) + LH_2 + 2XH_2 \rightleftharpoons MLX_2 + 6H^+$ (5)

$M(II) + LH + XH \rightleftharpoons MLX + 2H^+$ (6)

$ML^+ + LH + XH \rightleftharpoons MLX + 2H^+$ (7)

$MLH + XH^+ \rightleftharpoons MLX + 2H^+$ (8)

$MLX + XH^+ \rightleftharpoons MLX_2 + H^+$ (9)

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

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) complex, the metal forms the complex first with the primary ligand (L) at lower pH and then with the secondary ligand (X) to form the mixed ligand complex at higher pH. At lower pH, the secondary ligand does not come into contact with metal ions. In other words, when M-L complex formation is complete, mixed ligand complex formation occurs.The best fit models for ternary complexes with MSA and chosen amino acids(His, Val) have been given in **Table 1 and 2**. Species like MLX and MLX_2 were detected for M(II)-MSA-X (X = His or Val). Overall stability constants (log β) with minimal standard deviation (SD) in specifies the exactness of these constraints.

Table-1: Best fit model of M(II)-MSA- L-Histidine 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	13.03	23.75	15.76	28.51
10	13.29	24.23	16.04	29.01
20	13.56	24.71	16.33	29.52
30	13.83	25.20	16.63	30.04
40	14.10	25.71	16.93	30.57
50	14.39	26.22	17.23	31.11

60	14.67	26.75	17.54	31.66
Cu(II) pH Range: 2.5 – 9.5			**Zn(II) pH Range: 2.5 – 9.5**	
0.0	19.74	34.59	12.33	22.84
10	20.14	35.11	12.58	23.11
20	20.55	35.63	12.84	23.39
30	20.97	36.16	13.10	23.67
40	21.40	36.70	13.36	23.96
50	21.84	37.24	13.63	24.24
60	22.28	37.80	13.91	24.53

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

% V/V	**log β_{MLXH}(SD)**		**log β_{MLXH}(SD)**	
	MLX	**MLX2**	**MLX**	**MLX2**
Co(II) pH Range: 2.5 – 9.5			**Ni(II) pH Range: 2.5 – 9.5**	
0.0	10.75	19.26	12.55	23.34
10	10.94	19.61	12.73	23.78
20	11.14	19.96	12.91	24.24
30	11.34	20.32	13.10	24.70
40	11.55	20.68	13.28	25.17
50	11.75	21.06	13.47	25.64
60	11.96	21.44	13.67	26.13
Cu(II) pH Range: 2.5 – 9.5			**Zn(II) pH Range: 2.5 – 9.5**	
0.0	17.59	30.93	10.57	19.48
10	17.91	31.24	10.76	19.83
20	18.23	31.55	10.96	20.19
30	18.56	31.87	11.16	20.55
40	18.90	32.19	11.36	20.92
50	19.24	32.51	11.57	21.30
60	19.59	32.83	11.78	21.68

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, MLX_2 species were found for M(II)-MSA-L-His and M(II)-MSA-L-Val in the pH range 2.0-9.5. 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 MLX_2 (Equilibria 4, 5 and 9) increases gradually.

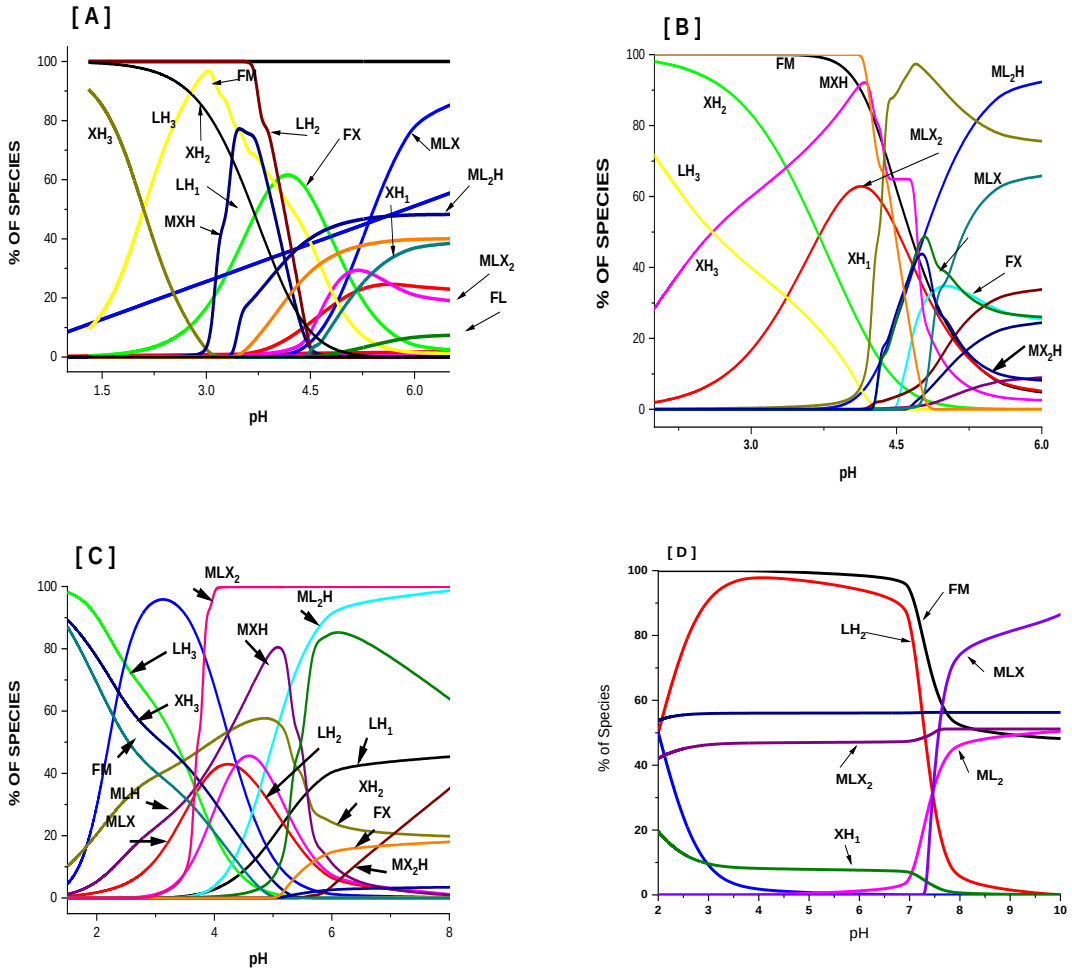

Fig. 3: Species distribution plots of [A]: Co(II) – MSA – His; [B] : Ni(II) – MSA – His; [C] : Cu(II) – MSA – Val; [D]: Zn(II) – MSA - Val in 20% v/v EG – water media.

INTER COMPARISON OF STABILITY CONSTANTS

Having inspected **Tables 1 and 2**, we can say that the stability constants of Cu(II) ternary complexes are higher than those of the other studied metal complexes due to the smaller size of Cu(II) and shown in **Fig. 4**. This behaviour is in line with Irving–Williams order [Irving & Williams ,1953s].

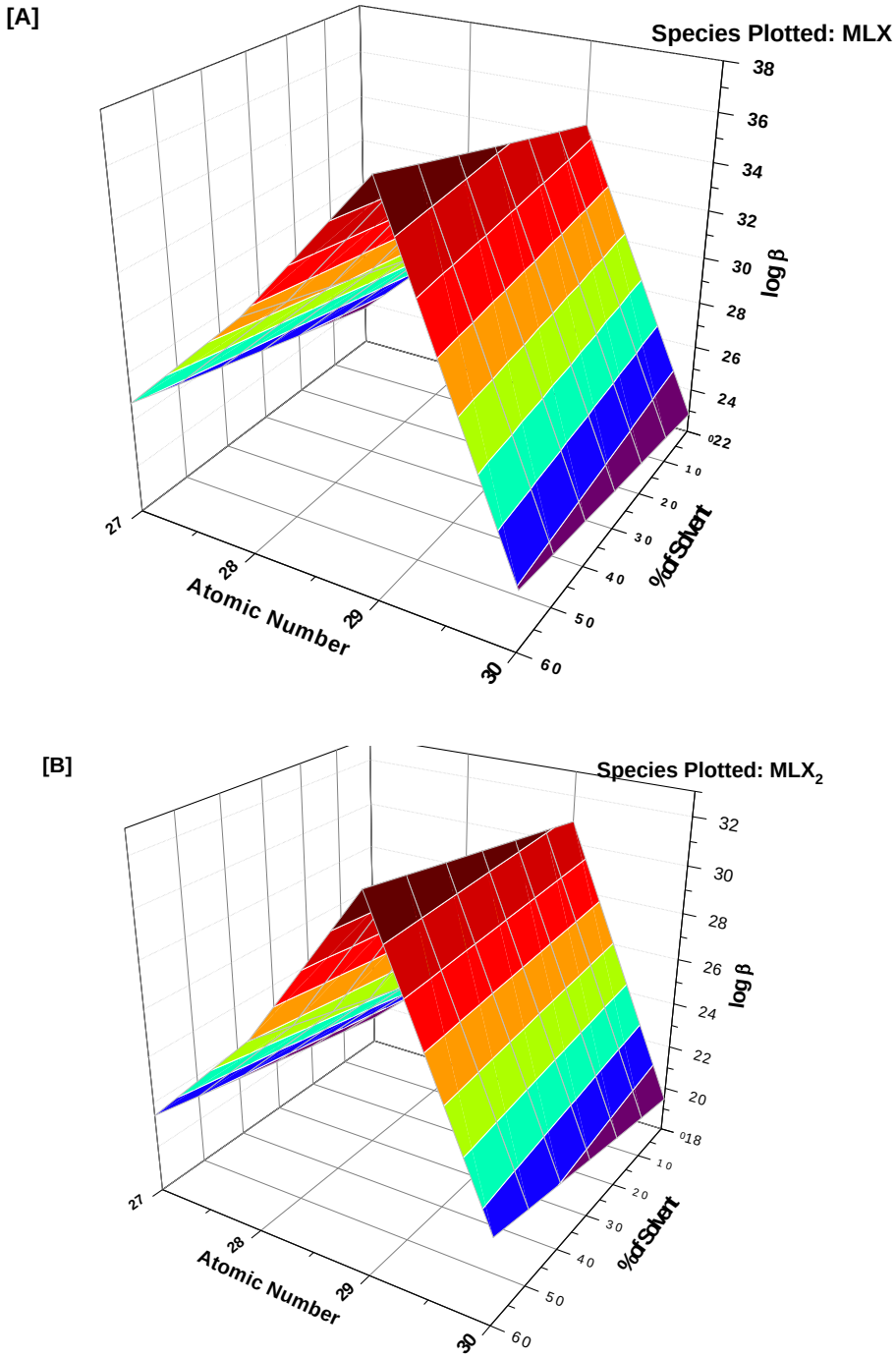

Fig. 4: Variation of log β with Atomic Number for [A]: M(II) – MSA – His; [B]: M(II) – MSA – Val in 0 – 60% v/v EG – water media.

ROLE OF DIELECTRIC CONSTANT,

EG's protophilic and structure forming nature removes water from metal ion coordination spheres, making complexes more stable. As indicated in the study, log values should be connected to the medium's dielectric constant (1/D). (**Fig. 5**).

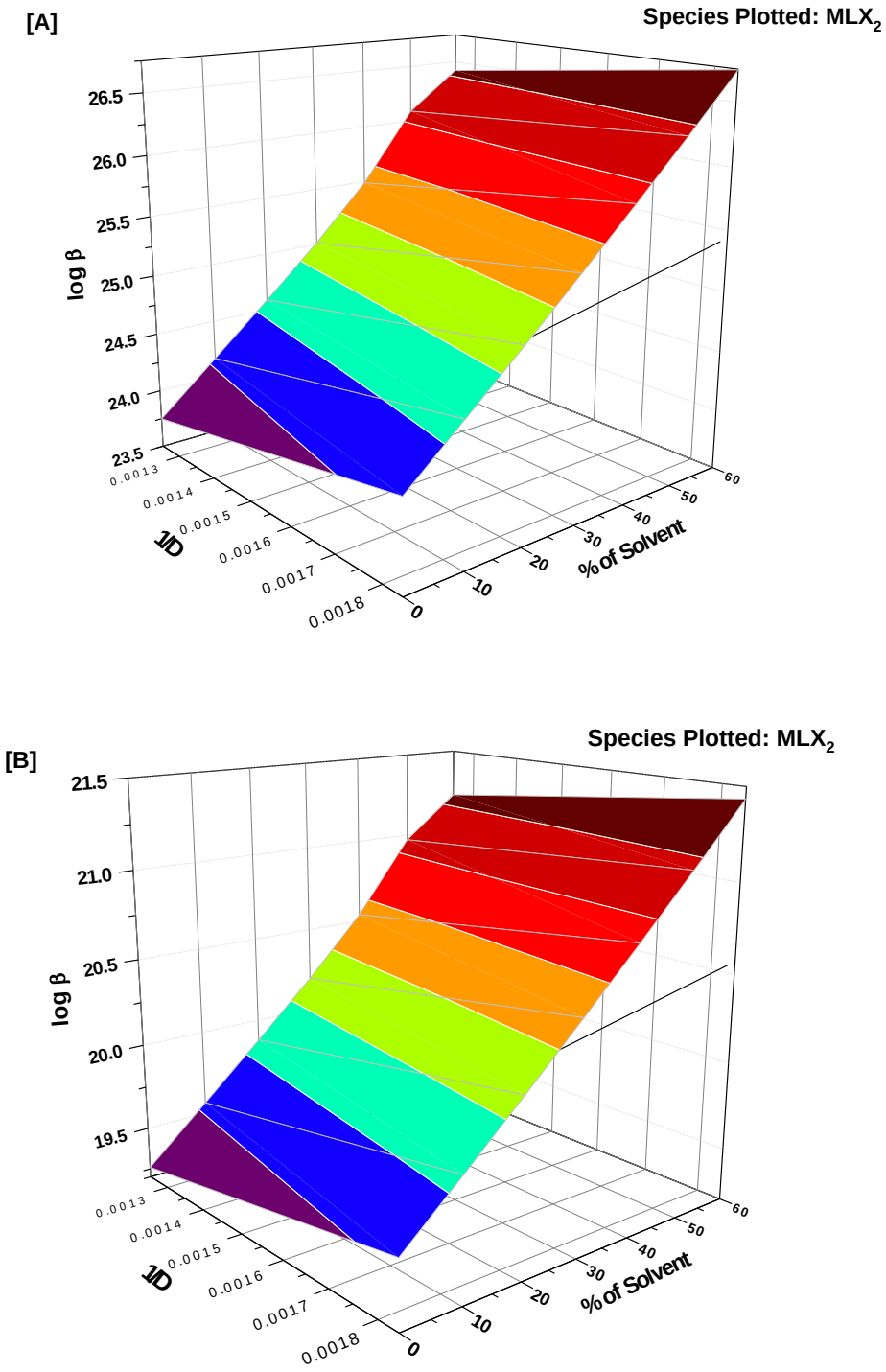

Fig. 5: 3D Surface plot for Variation of log β against reciprocal of Dielectric constant and percentage of solvent for [A]: M(II) – MSA – His; [B]: M(II) – MSA – Val; M(II): Co(II), Ni(II), Cu(II) and Zn(II).

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 and 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 chain [Wang et al,Peter et al,2014,Zambelli et al,2013,Padmaja et al,2011,Vashi, & Shelat,2011,Raman et al,2004&Kumar & Kiremire,2007].

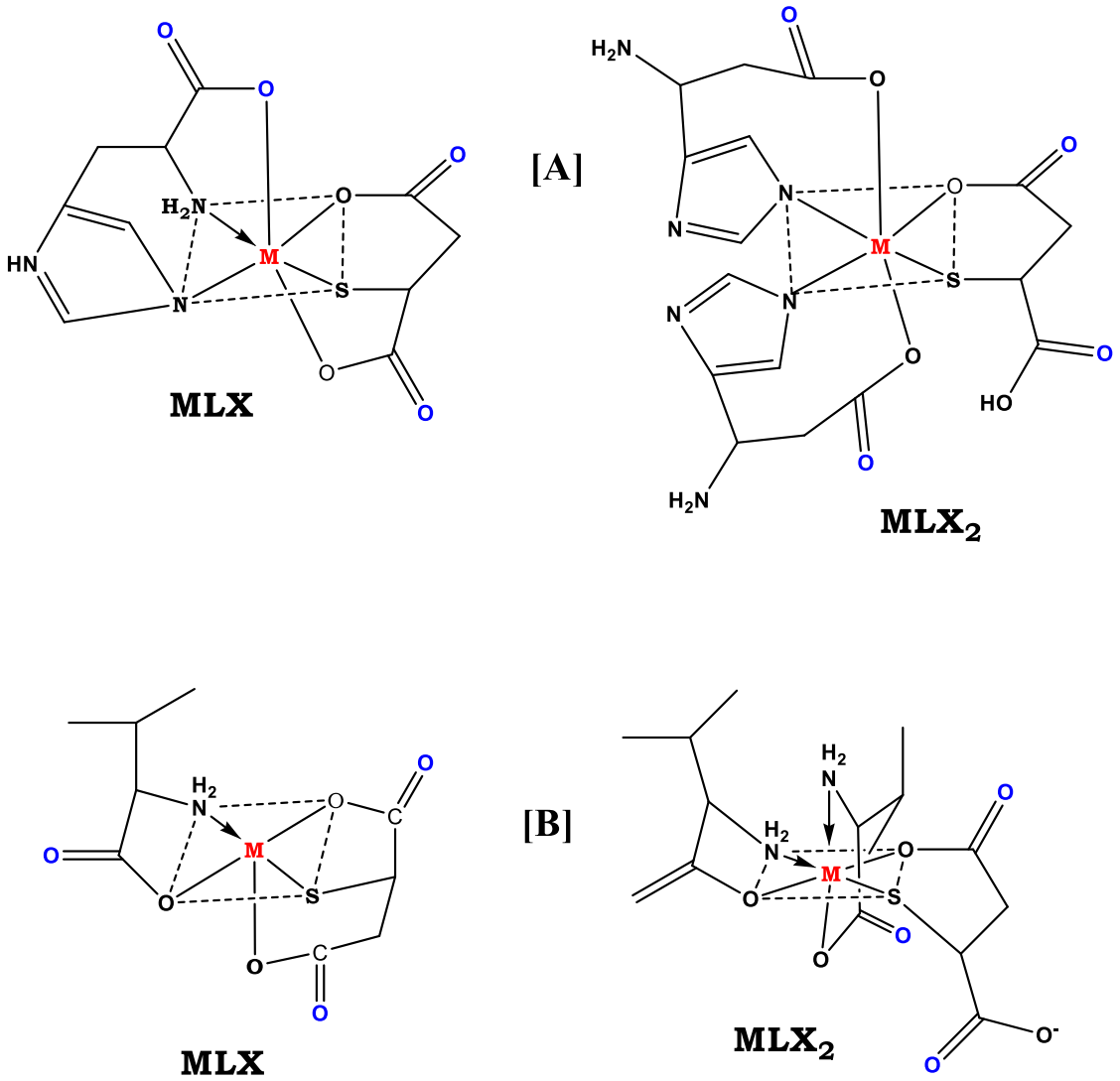

Fig. 6: Proposed Structures of Ternary Complexes of [A]: M(II) - MSA - His; [B]: M(II) - MSA - Val. M(II) : Co(II). Ni(II), Cu(II) or Zn(II); S = either H_2O or Solvent.

CONCLUSION

In this study, the results of a pH-metric study of ternary systems of MSA and three essential amino acids (Val and His) 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, L-Valine and L-Histidine 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

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CONFLICTS OF INTEREST

There are no conflicts to declare.

REFERENCES

[1] Akalpita S. B., Sunil S. P. and Manzoor M. S. Synthesis, characterization and antibacterial studies on mixed ligand copper complexes with polydentate ligands, *Acta Poloniae Pharmaceutica – Drug Research*, **69(5)**, 871-877.(2012)

[2] Sunil S. P., Ganesh A. T. and Vishwanath R. P. Synihesis, spectral and biological studies on some mixed ligand Ni(II) complexes, *Acta Poloniae Pharmaceutica - Drug Research*, **66 (3)**, 271-277.(2009)

[3] Adkhis A., Benali-Baïtich O., Khan M. A., Bouet G. Synthesis, characterization and thermal behaviour of mixed-ligand complexes of cobalt(III) with dimethylglyoxime and some amino acids. *Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry*, **30(10),** 1849–1858. (2000). https://doi.org/10.1080/00945710009351873

[4] Shivankar V. S., Vaidya R. B., Dharwadkar S. R., Thakkar N. V. Synthesis, characterization, and biological activity of mixed ligand Co(II) complexes of 8-hydroxyquinoline and some amino acids. *Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry*. **33(9),** 1597–1622.(2003). https://doi.org/10.1081/SIM-120025443

[5] Nomiya K., Yokoyama H. Syntheses, crystal structures and antimicrobial activities of polymeric silver(I) complexes with three amino-acids [aspartic acid (H2asp), glycine (Hgly) and asparagine (Hasn)] *Journal of the Chemical Society, Dalton Transactions*. **(12),** 2483–2490.(2002). **DOIhttps://doi.org/10.1039/B200684G**

[6] Regupathy S. and Sivasankaran Nair M. Studies on the mixed ligand complexes of copper(II) involving a sulfa drug and some potentially bi or tridentate ligands under physiological conditions, *Arabian Journal of Chemistry*, **7(6),** 1003-1012.(2014). https://doi.org/10.1016/j.arabjc.2010.12.027

[7] Abdel-Rahman L.H., Abu-Dief A.M., Ismail N.M. and Ismael M.M. Synthesis, characterization, and biological activityof new mixed ligand transition metal complexesof glutamine, glutaric, and glutamic acid withnitrogen based ligands, *Inorganic and Nano-Metal Chemistry*, **28(3),** 467-480. (2017). https://doi.org/10.1080/15533174.2015.1137057

[8] Choudhary A., Sharmaa R. and ,Nagara M. Synthesis, characterization and antimicrobial activity of mixed ligand complexes of Co (II) and Cu (II) with N, O/S donor ligands and amino acids, *International Research Journal of Pharmacy and Pharmacology*, **1(6)**, 172-187. (2011).

[9] Sharma A.K., Sharma R. and Gangwal A. Antifungal activities and characterization of some new environmentally safe Cu (II) surfactants substituted 2-amino-6- methyl benzothiazole, *Open Pharmaceutical Sciences Journal*,**5**, 1-11.(2018). **DOI:** 10.2174/1874844901805010001

[10] Selvaganapathy M and Raman N. Pharmacological activity of a few transition metal complexes: a short review, *Journal of Chemical Biology & Therapeutics*, **1(2)**, 1-17.(2016).DOI:10.4172/2572-0406.1000108

[11] Liao C., Wang T., Koehler AV., Fan Y., Hu M. and Gasser RB. Cellular and computational studies of proteasome inhibition and apoptosis induction in human cancer cells by amino acid Schiff base-copper complexes, *J Inorg Biochem.*, 118:83-93.(2013). DOI: 10.1016/j.jinorgbio.2012.10.006

[12] Paláčková H.,Vinklárek J.,Holubová J., Císařová I. and Erben M. The interaction of antitumor active vanadocene dichloride with sulfur-containing amino acids, *Journal of Organometallic Chemistry*, 792(17), 3758-3764.(2007).

[13] Sobel S., Haigney A., Kim M., Kim D., Theophall G.,Nuñez J.,Williams D.,Hickling B. and Sinacori J. The complexation of aqueous metal ions relevant to biological applications. 2. Reactions of copper(II) citrate and copper(II) succinate with selected amino acids, *Chemical Speciation and Bioavailability*, 22(2), 109-114. (2010).https://doi.org/10.3184/095422910X12692705325385

[14] Yuichi Shimazaki, Masako Takanib and Osamu Yamauchi , Metal complexes of amino acids and amino acid side chain groups. Structures and properties , *Dalton Trans*.,7854–7869(2009).**DOIhttps://doi.org/10.1039/B905871K**

[15] AliyuH. N. and IsyakuS.,(Spectroscopic and Potentiometric Studies of N-(2-Hydroxybenzyl)-L-α-Valine Cobalt (II) Complex)., *J.Biokemistri*., Volume 22, No. 2 pp91-97 (2010).

[16] DeschampsP., KulkarniP. P., Gautam-BasakM., and SarkarB., "The saga of copper(II)-L-histidine," *Coordination Chemistry Reviews*, vol. 249, no. 9-10, pp. 895–909, (2005). DOI:10.1016/j.ccr.2004.09.013

[17] CreightonT. E., Encyclopedia of Molecular Biology, vol. 2, Wiley, New York, NY, USA, (1999).

[18] GuoC. Nan, W. Xiao Ping, D. Jian Ping, and C. Hong Qing, "A study on electrochemistry of histidine and its metabolites based on the diazo coupling reaction," *Talanta*, vol. 49, no. 2, pp. 319–330, (1999).

[19] VinnikovaA. K., KukrejaR. C., and HessM. L., "Singlet oxygen-induced inhibition of cardiac sarcolemmal Na+K+-ATPase," *Journal of Molecular and Cellular Cardiology*, vol. 24, no. 5, pp. 465–470, (1992). DOI: 10.1016/0022-2828(92)91835-s

[20] SittonN. G., DixonJ. S., AstburyC., FrancisR. J., BirdH. A., and WrightV., "Kinetic investigations into the possible cause of low serum histidine in rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 47, no. 1, pp. 48–52, (1988). doi: 10.1136/ard.47.1.48

[21] Hitomi-OhmuraE., AmanoN., AoyamaY., and YoshidaA., "The effect of a histidine-excess diet on cholesterol synthesis and degradation in rats," *Lipids*, vol. 27, no. 10, pp. 755–760, (1992). https://doi.org/10.3177/jnsv.45.773

[22] AoyamaY., TsudaT., Hitomi-OhmuraE., and YoshidaA., "Effect of dietary excess-histidine on fructose 1,6-bisphosphatase and 6-phosphofructokinase activities, and activation of fructose 1,6-bisphosphatase by basic amino acids in rat liver," *International Journal of Biochemistry*, vol. 24, no. 6, pp. 981–985, (1992). DOI: 10.1016/0020-711x(92)90107-c

[23] CaiQ., TakemuraG., and AshrafM., "Antioxidative properties of histidine and its effect on myocardial injury during ischemia/reperfusion in isolated rat heart," *Journal of Cardiovascular Pharmacology*, vol. 25, no. 1, pp. 147–155, (1995).

[24] MuthusamyS. and NatarajanR., *Journal of Chemical Biology and Therapeutics*, 1, 2 (2016). DOI: 10.4172/2572-0406.1000e102

[25] PharrD.Y., *Analytical Letters*, 44, 2287 (2011); https://doi.org/10.1080/00032719.2010.551689

[26] WilsonE.W., KasperianM.H. and MartinR.B., *Journal of the American Chemical Society*, 92, 5365 (1970); https://doi.org/10.1021/ja00721a013.

[27] LahsasniS.A., AmmarR.A., AminM.F. and ShoukryM.E., *International Journal of Electrochemical Science*, 7, 7699 (2012).

[28] GranG., *Acta Chem. Scand*. 4, 559 (1950).**DOI number:** 10.3891/acta.chem.scand.04-0559

[29] RossottiF. J. C. and RossottiH., *J. Chem. Edu*. 42, 375 (1965).https://doi.org/10.1021/ed042p375

[30] KhanM. A., BouetG., VierlingF., MeullemeestreJ., and Schwing M. J., *Trans. Met. Chem*. 21, 231 (1996).

[31] SchwarzenbachG., Komplexometrishe Titration, Titration Procedures and Working Instructions; Second Edition, pp. 145–265 (London, Methuen, 1969).

[32] ShoukryM.M., HosnyW.M. and KhalilM.M., *Transition Metal Chemistry*, 20, 252 (1995); https://doi.org/10.1007/BF00143487.

[33] GansP., SabatiniA. and VaccaA., *Inorganica Chimica Acta*, 18, 237 (1976); https://doi.org/10.1016/S0020-1693(00)95610-X..

[34] KhanM.A., BouetG., VierlingF., MeullemeestreJ. and SchwingM.J., *Transition Metal Chemistry*, 21, 231 (1996). https://doi.org/10.1023/A:1018577127619

[35] IrvingH. M. and WilliamsR. J. P., *J. Chem. Soc.* 3192 (1953).**https://doi.org/10.1039/JR9530003192**

[36] Wang, V.C. C.; Ragsdale S.W.; Armstrong, F.A. *Metal Ions in Life Sciences 14. (Eds.)*

[37] Peter, M.H.; Kroneck, M. E.; Sosa, T.;*Springer*, U.S.A., **2014**, 71–97.

[38] Zambelli, B.; Ciurli, S. Nickel and Human Health" In *Metal Ions in Life Sciences* 13 "Interrelations between Essential Metal Ions and Human Diseases" (Eds.), **2013.**

[39] Padmaja, M.; Pragathi, J.; Gyana, K. C.*J. Chem. Pharm. Res.***2011,***3*, 602.

[40] Vashi, R. T. and Shelat, C. D., *Int.J. of Chem. Tech. Res.***2011,***3*, 911.

[41] Raman, N.; Ravichandran, S.; Thangaraja, C., *J. Chem. Sci*. **2004,***116*. 215.

[42] Kumar, G.; Kiremire, E. M. R., *Chemistry.* **2007,***16*, 386.