



Scholars Research Library

Der Pharma Chemica, 2015, 7(5):147-156
(<http://derpharmachemica.com/archive.html>)



ISSN 0975-413X
CODEN (USA): PCHHAX

Chemical speciation of ternary complexes of L-Ornithine and Ethylenediamine with some metal ions in low dielectric media

Hadgu Hailekiros Belay, Budati Bala Venkata Sailaja and Gollapalli Nageswara Rao*

Department of Inorganic and Analytical Chemistry, Andhra University, Visakhapatnam, India

ABSTRACT

The stability constants of ternary complexes of Co(II), Ni(II) and Cu(II) with L-Ornithine as primary ligand and Ethylenediamine as a secondary ligand were determined pH-metrically. The study was conducted in varying concentrations (0-60% v/v) of DMSO-water mixtures at an ionic strength of 0.16 mol L⁻¹ using NaCl as an electrolyte at 303.0 K. The Value of $\Delta \log K$ which is responsible for the extra stability of ternary complexes was discussed on the basis of statistical parameters and the nature of the species formed. The formation of various ternary species was established by modeling studies using the computer program MINQUAD75. The formation and distribution of different species with relative concentrations of metal and ligands (M:L:X = 1.0:2.5:2.5, 1.0:2.5:5.0, 1.0:5.0:2.5) with varying pH were represented in the form of distribution diagrams drawn using HYSS HYPERQUAD. Influence of the solvent on the speciation was discussed based on the dielectric constant of the medium. The stability of the mixed ligand complexes are discussed in terms of the molecular structure of L-Ornithine and Ethylenediamine as well as the nature of the metal ion.

Keywords: Ternary complexes, Speciation, pH-metric study, L-Ornithine, Ethylenediamine.

INTRODUCTION

The specificity and selectivity of enzyme-substrate reactions can be achieved by manipulating the equivalent solution dielectric constants (ESDC) at the active site [1]. The ESDC at the active sites in bovine carbonic anhydrase and carboxy peptidase were estimated to be 35 and 70, respectively. In carbonic anhydrase the binding of water (substrate) to the zinc ion simply holds it in the correct stereochemical position suitable for attack by CO₂, where as in carboxy peptidase the binding of the substrate results in polarization, thus, facilitating hydrolysis. This variation is brought out by the changes in the interactions of side chains of the protein moiety among themselves and with those of the solvent molecules. Hence, modeling studies of ternary complexes have gained popularity in different aqua-organic mixtures with different dielectric constants [2-6].

L-Ornithine (Orn) is a tridentate amino acid containing nitrogen donor group in the side chain in addition to the nitrogen donor group found on the α -carbon. The nitrogen donor atoms can associate with hydrogen ions in the physiological pH range. Hence, there is often significant competition between hydrogen and metal ions for these donor sites which leads to the existence of a number of equilibria. This phenomenon may result in successively protonated complexes.

1, 2-Diaminoethane is more commonly known as ethylenediamine (en) which is a colorless to yellowish hygroscopic liquid with an ammonia-like odor. En is used as monodentate, bidentate or bridging ligand. It is also used in the manufacture of EDTA, carbamate fungicides, surfactants and dyes. It is involved in the synthesis of

seven membered ring components with β -ketoesters resulting secondary amines and β -enaminoesters [7]. En plays an important role in the synthesis of Schiff base compounds [8].

Dimethylsulfoxide (DMSO) is a dipolar aprotic solvent that dissolves both polar and non-polar compounds and is miscible in a wide range of organic solvents [9]. It is used as a cryoprotectant, added to cell media in order to prevent the cells from dying as they are being frozen [10]. In the present study, the protonation/deprotonation of Orn and en is reported in the presence of DMSO as a co-solvent.

Cobalt is an essential trace element for all multicellular organisms at the active center of coenzymes called cobalamins. These include vitamin B₁₂ which is essential for mammals [11]. Nickel is an essential nutrient. It is a component of the enzyme, urease, present in a wide range of plant species [12, 13]. Copper is an essential element for life and one of the transition elements frequently found at the active sites of proteins. Metallic copper has antibacterial properties [14]. Copper deficiency results anemia. Moreover, a congenital inability to excrete copper can result in toxic levels of copper accumulation, which leads to a metabolic disease known as Wilson's disease [15, 16].

Hence, speciation studies of the title systems have been undertaken based on their involvement in various physiological reactions.

MATERIALS AND METHODS

2.1 Chemicals

DMSO (Qualigens, India) was used as received. Aqueous solutions of L-Ornithine and Ethylenediamine of 0.05 mol L⁻¹ (GR Grade Merck, India) and Hydrochloric acid of 0.2 mol L⁻¹ (AR, E-Merck) were prepared in triple-distilled deionized water. Sodium chloride (Qualigens, India) of 2 mol L⁻¹ was prepared to maintain the ionic strength in the titrand. 0.1 mol L⁻¹ Solutions of Co(II), Ni(II) and Cu(II) chlorides were prepared. To increase the solubility of ligands and to suppress the hydrolysis of metal salts, the mineral acid concentration in the above solutions was maintained at 0.05 mol L⁻¹. Sodium hydroxide (Qualigens, India) of 0.4 mol L⁻¹ was prepared. To assess the errors that might have crept into the determination of the concentrations, the data were subjected to analysis of variance of one way classification (ANOVA) [17]. The strengths of alkali and mineral acid were determined using the Gran plot method [18, 19].

2.2 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 DMSO-water mixture containing the inert electrolyte. All the titrations were carried out in the medium containing varying concentrations of DMSO-water mixtures (0-60% v/v) by maintaining an ionic strength of 0.16 mol L⁻¹ with sodium chloride at 303±0.1 K. 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 [5, 20].

2.3 Procedure

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 DMSO-water mixture of equivalent composition as that of the titrand. In each of the titrations, the titrand consisted of approximately 1 m mol mineral acid in a total volume of 50 ml. Titrations with different ratios (1:2.5, 1:3.75 and 1:5) of metal-to-ligand were carried with 0.4 mol L⁻¹ sodium hydroxide [21].

2.4 Modeling Strategy

The computer program SCPHD [22] was used to calculate the correction factor. By using pH metric titration data, the ternary stability constants were calculated with the computer program MINQUAD75 [23] 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 Orn and en and their binary complexes with Co(II), Ni(II) and Cu(II) in DMSO-water mixtures were fixed.

RESULTS AND DISCUSSION

The results of the best fit models that contain the stoichiometry of the complex species and their overall formation constants along with some of the important statistical parameters are given in Table 1. Very low standard deviation in overall stability constants ($\log \beta$) signifies the precision of these constants. The small values of U_{corr} (sum of

squares of deviations in concentrations of ligand and hydrogen ion at all experimental points corrected for degrees of freedom), small values of mean, standard deviation and mean deviation for the systems are validated by the residual analysis [24].

3.1 Residual Analysis

In data analysis with least squares methods, the residuals (the differences between the experimental data and the data simulated based on model parameters) are assumed to follow Gaussian or normal distribution. When the data are fit into the models, the residuals should ideally be equal to zero. If statistical measures of the residuals and the errors are assumed in the models are not significantly different from each other, the model is said to be adequate. Further, a model is considered adequate only if the residuals do not show any trend. Respecting the hypothesis that the errors are random following normal distribution in the least squares analysis, the residuals are tested for normal distribution. Such tests are χ^2 , skewness, kurtosis and R factor. These statistical parameters show that the best fit models portray the metal-ligand species in DMSO-water mixtures, as discussed below.

χ^2 Test

χ^2 is a special case of gamma distribution whose probability density function is an asymmetrical function. This distribution measures the probability of residuals forming a part of standard normal distribution with zero mean and unit standard deviation. If the calculated χ^2 is less than the table value, the model is accepted.

Crystallographic R-test

Hamilton's R factor ratio test is applied in complex equilibria to decide whether inclusion of more species in the model is necessary or not. In pH-metric method, the readability of pH meter is taken as the R_{limit} which represents the upper boundary of R beyond which the model bears no significance. When there are different numbers of species the models with values greater than R-table are rejected. The low crystallographic R-values given in Table 1 indicate the sufficiency of the model.

Skewness

It is a dimensionless quantity indicating the shape of the error distribution profile. A value of zero for skewness indicates that the underlying distribution is symmetrical. If the skewness is greater than zero, the peak of the error distribution curve is to the left of the mean and the peak is to the right of the mean if skewness is less than zero. The values of skewness recorded in Table 1 are between -1.12 and 1.11 for Co(II), -0.26 and 1.96 for Ni(II) and -0.28 and 1.13 for Cu(II). These data evince that the residuals form a part of normal distribution; hence, least-squares method can be applied to the present data.

Table 1 Best fit chemical models of ternary complexes of Co(II), Ni(II) and Cu(II) with Orn and en in DMSO-water mixtures. Temperature= 303.0 K, $\mu= 0.16 \text{ mol L}^{-1}$

%v/v	log β_{mlsh} (SD)			NP	$U_{\text{corr}} \times 10^8$	χ^2	Skewness	Kurtosis	R- factor
	DMSO	1111	1110						
Co(II) (pH=5.0-10.0)									
0	19.30(02)	11.85(03)	14.60(15)	43	0.44	12.55	-0.3	3.05	0.0033
10	19.81(07)	12.14(14)	15.99(06)	33	1.13	11.51	0.36	4.02	0.0112
20	19.89(15)	12.82(19)	15.84(23)	40	1.76	16.41	0.45	3.17	0.0054
30	19.98(10)	13.00(10)	16.33(11)	42	1.45	12.08	-1.12	4.05	0.0173
40	20.23(11)	13.81(07)	17.00(17)	33	0.55	20.59	1.11	3.12	0.0195
50	20.48(09)	13.83(13)	18.15(10)	30	1.17	11.32	0.59	2.63	0.0022
60	20.93(08)	15.09(15)	18.80(12)	29	1.09	10.32	0.61	2.74	0.0036
Ni(II) (pH=4.0-10.0)									
0	21.10(08)	15.08(25)	19.62(09)	50	4.56	13.76	1.1	3.48	0.0055
10	21.99(23)	16.35(04)	20.98(31)	55	6.32	11.77	0.42	1.97	0.0162
20	22.45(29)	16.63(27)	21.11(23)	43	5.22	13.4	-0.14	3.01	0.0066
30	22.58(11)	16.80(20)	22.35(16)	30	4.33	9.75	1.37	3.41	0.0039
40	22.73(13)	17.13(15)	22.53(10)	29	1.34	8.63	-0.26	3.02	0.0046
50	22.97(02)	17.42(13)	22.84(14)	35	2.78	11.94	1.96	3.19	0.0026
60	23.42(21)	18.10(30)	23.00(31)	27	6.48	12.32	0.95	4.09	0.0047
Cu(II) (pH=2.0-8.0)									
0	28.14(10)	25.55(29)	30.69(11)	33	1.95	4.38	0.48	3.28	0.0033
10	30.65(13)	26.52(08)	33.94(10)	35	3.34	7.29	-0.28	3.12	0.0048
20	30.23(20)	26.89(13)	34.43(08)	30	2.13	13.4	0.67	3.33	0.0061
30	30.98(14)	27.34(19)	34.52(33)	40	5.39	15.96	0.71	3.49	0.0372
40	31.57(20)	27.55(12)	34.96(20)	28	3.9	20.52	2.13	4.06	0.0029
50	31.92(11)	27.93(17)	35.23(11)	37	0.52	15.95	-0.17	4.11	0.0036
60	32.43(07)	28.45(07)	36.01(14)	33	0.22	12.33	0.57	3.03	0.0044

Kurtosis

It is a measure of the peakedness of the error distribution near a model value. For an ideal normal distribution kurtosis value should be three (mesokurtic). If the calculated kurtosis is less than three, the peak of the error distribution curve is flat (platykurtic) and if the kurtosis is greater than three, the distribution shall have sharp peak (leptokurtic). The kurtosis values in the present study indicate that the residuals form leptokurtic pattern.

3.2 Effect of systematic errors on best fit model

In order to rely upon the best fit 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 influential parameters like concentrations of alkali, mineral acid, ligand and metal (Table 2). The order of the ingredients that influence the magnitudes of stability constants due to incorporation of errors is alkali > acid > primary ligand > secondary ligand > metal. Some species were even rejected and the standard deviation is high in few cases when errors are introduced in the concentrations. The rejection of some species and increased standard deviations in the stability constants on introduction of errors conform the appropriateness of the chosen best fit models. This study also indicates the relative sensitivities of model parameters.

Table 2 Effect of errors in influential parameters on the stability constants of Orn-Co(II)-en ternary complexes in 10% v/v of DMSO-water mixtures

Ingredient	% error	log β_{mixt} (SD)		
		1111	1110	1210
	0	20.48(09)	13.83(13)	18.15(10)
Alkali	-5	Rejected	12.99(89)	Rejected
	-2	19.52(27)	13.66(25)	18.27(14)
	+2	Rejected	14.61(27)	Rejected
	+5	Rejected	Rejected	20.93(30)
Acid	-5	24.44(63)	15.25(90)	20.83(40)
	-2	22.41(35)	Rejected	19.69(30)
	+2	21.19(15)	14.52(19)	14.03(17) Rejected
	+5	17.15(84)	Rejected	Rejected
Orn	-5	20.44(15)	13.33(18)	19.34(07)
	-2	20.09(09)	13.61(13)	19.80(25)
	+2	20.19(15)	14.27(18)	Rejected
	+5	Rejected	14.49(23)	19.55(17)
en	-5	21.21(10)	14.30(13)	19.75(15)
	-2	21.22(09)	14.19(15)	19.38(10)
	+2	Rejected	13.44(14)	Rejected
	+5	20.29(11)	13.80(14)	19.21(03)
Metal	-5	21.00(11)	14.09(11)	19.19(05)
	-2	20.41(11)	14.67(14)	19.10(15)
	+2	20.41(10)	13.80(14)	19.12(05)
	+5	20.25(09)	13.55(14)	19.10(03)

3.3 Effect of dielectric constant on stability of ternary complexes

DMSO is a dipolar aprotic solvent. It is a structure former and it enhances the water structures in DMSO-water mixtures; it removes water from 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 being a coordinating solvent, it competes with the ligands for coordinating the metals which decreases the stability of the complexes. Hence the stability of the complex is expected either to increase or decrease. The variation of overall stability constant values with co-solvent content depends upon two factors, viz., electrostatic and non-electrostatic. Born's [25] classical treatment holds good in accounting for the electrostatic contribution to the free energy change. According to this treatment, the energy of electrostatic interaction is related to dielectric constants.

The trends of stability constants (log β) values of ternary complexes with 1/D (D is the dielectric constant of the medium) of DMSO-water mixtures are shown in Fig. 1. In all cases the trend is almost linear, implies that the dielectric constant or long range interactions are responsible for the stability trend. Linear increase in Fig. 1A, B, C indicates the dominance of the structure-forming nature of the solvent over its complexing ability. But the deviations from linearity are assumed due to some contribution from non- electrostatic forces.

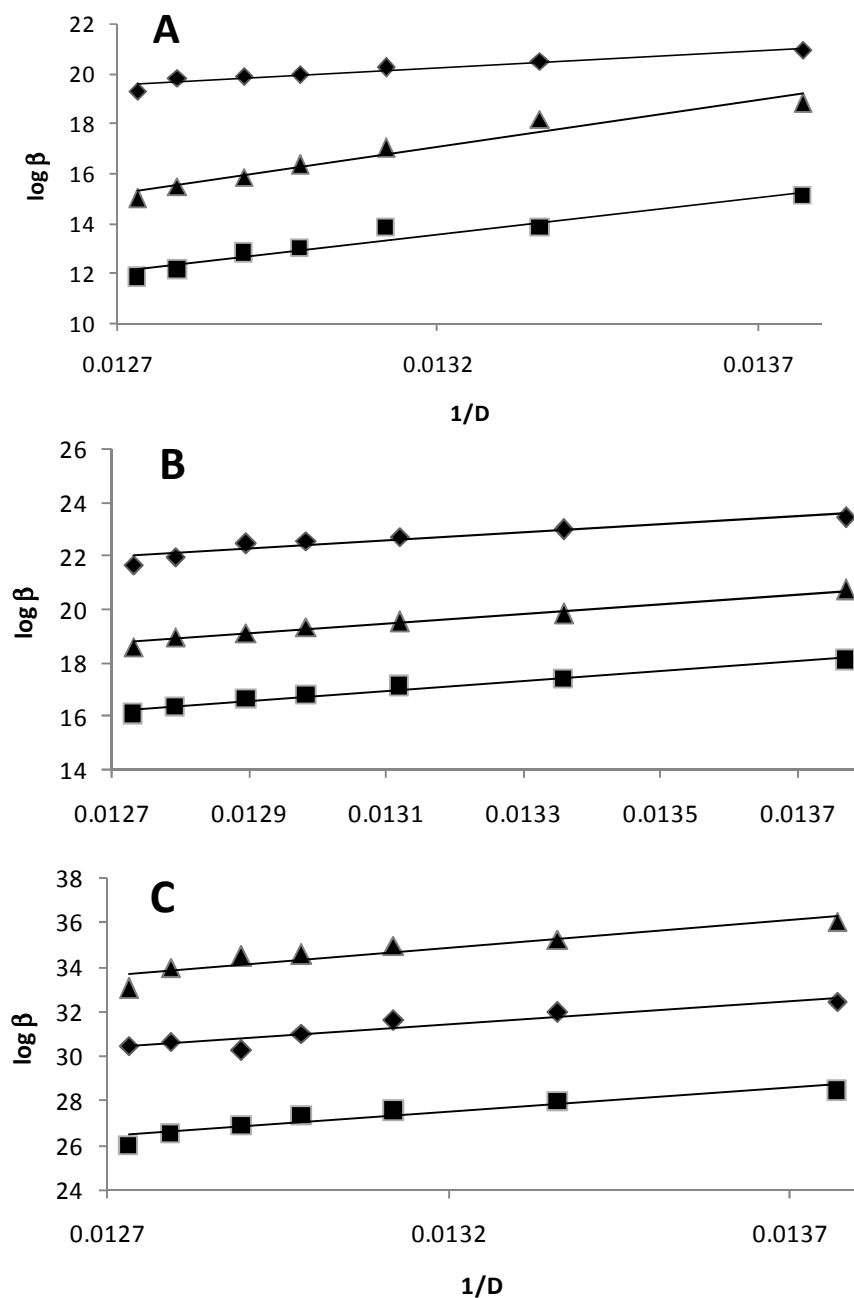


Fig. 1: Variation of stability constant values of metal-Orn-metal-en ternary complexes in DMSO-water mixtures. (A) Co(II); (B) Ni(II); (C) Cu(II); (■) log β MLX, (◆) log β ML₂X and (▲) log β MLXH.

3.4 Stability of Ternary Complexes

The change in the stability of the ternary complexes as compared to their binary analogues was quantified [26-29] based on the disproportion constant (log X) given by Equation 1 which corresponds to the equilibrium as shown Eq. 2.

$$\log X = 2 \log K_{MLX}^M - \log K_{ML}^M - \log K_{MX}^M \text{ -----(1)}$$



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 [19]. A value greater than this, accounts for the extra stability of MLX.

Another approach [27-31] to quantify the 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, where L is the primary ligand (Orn) and X is the secondary ligand (en). It is compared with that calculated purely on the statistical grounds as given in Eq. 3.

$$\Delta \log K = \log K_{MLX}^M - \log K_{ML}^M - \log K_{MX}^M \text{ ----- (3)}$$

The $\Delta \log K$ values are calculated from binary and ternary complexes using the equations given in Chart 1. Various possible $\log K$ values obtained from these equations are given in Table 3. In the present study, the $\log K$ values range from -0.06 to 1.84 for Co(II), -0.74 to 1.34 for Ni(II) and 2.18 to 7.23 for Cu(II) and all values 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 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 [32, 33]. The $\log X$ values could not be calculated for the system due to the absence of relevant binary species.

Table 3 $\Delta \log K$ and $\log X$ values of ternary complexes of Co(II), Ni(II) and Cu(II)-Orn and en in DMSO-water mixtures

% v/v DOX	$\Delta \log K$		$\log X$	
	Co(II)		1110	1111
	1110	1210		
0.0	0.12	0.01	4.13	0.22
10.0	0.60	1.84	5.16	1.48
20.0	0.55	0.68	5.58	1.56
30.0	0.25	0.07	4.54	1.14
40.0	0.78	1.03	6.43	1.36
50.0	-0.06	1.39	5.83	1.94
60.0	1.05	1.18	7.46	2.63
	Ni(II)			
0.0	0.13	-0.74	3.79	-0.39
10.0	0.96	0.24	6.00	1.30
20.0	0.85	0.68	6.65	2.48
30.0	0.88	0.54	5.61	2.01
40.0	0.82	0.06	5.75	2.30
50.0	1.34	0.59	6.37	2.33
60.0	1.34	0.01	7.00	3.00
	Cu(II)			
0.0	2.18	4.24	15.62	2.17
10.0	3.02	6.15	16.35	6.99
20.0	3.30	7.23	18.56	7.44
30.0	3.04	6.64	17.58	6.51
40.0	2.60	6.42	17.30	8.29
50.0	3.13	6.75	18.16	9.40
60.0	3.37	7.22	18.68	9.22

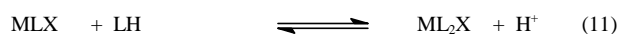
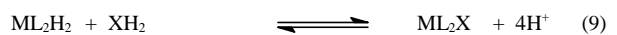
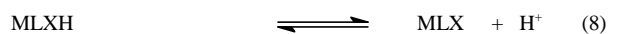
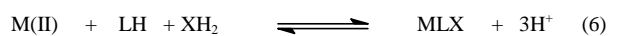
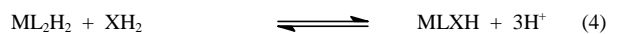
Chart 1 Equations for the calculation of $\Delta \log K$ and $\log X$

$\Delta \log K_{1110}$	$=\log \beta_{1110}$	$-\log \beta_{1100}$	$-\log \beta_{1010}$
$\Delta \log K_{1210}$	$=\log \beta_{1210}$	$-\log \beta_{1200}$	$-\log \beta_{1010}$
$\Delta \log K_{1111}$	$=\log \beta_{1111}$	$-\log \beta_{1101}$	$-\log \beta_{1010}$
$\log X_{1110}$	$=2\log \beta_{1110}$	$-\log \beta_{1200}$	$-\log \beta_{1020}$
$\log X_{1210}$	$=2\log \beta_{1210}$	$-\log \beta_{1400}$	$-\log \beta_{1020}$
$\log X_{1111}$	$=2\log \beta_{1111}$	$-\log \beta_{1202}$	$-\log \beta_{1020}$

3.5. Distribution diagrams

Some typical distribution diagrams in 10% DMSO-water mixture are drawn using the formation constants of the best fit model are shown in Fig. 2 which contain protonated and unprotonated species like $MLXH$, MLX and ML_2X for Co(II), Ni(II) and Cu(II). The active forms of these ligands are LH_3^{2+} , LH_2^+ , LH and L^- for Orn and XH_2^{2+} , XH^+ and X for en. The binary complex species of Orn are ML , ML_2 , MLH and ML_2H_2 and those of en are MX , MX_2 and MX_3 , for Co(II), Ni(II) and Cu(II). 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 binary 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.



The protonated ligands interact with metal ion (Equilibria 5 and 6) to form MLXH and MLX. MLX is also formed by the dissociation of MLXH (Equilibrium 7) which is formed by the interaction of ML_2H_2 with XH_2 (Equilibrium 4). Similarly ML_2X is formed by the interaction of ML_2H_2 with XH_2 (Equilibrium 9), ML and LH with XH_2 (Equilibrium 10) and MLX with LH (Equilibrium 11), because the concentrations of both MLX and LH are decreasing with increasing concentration of ML_2X .

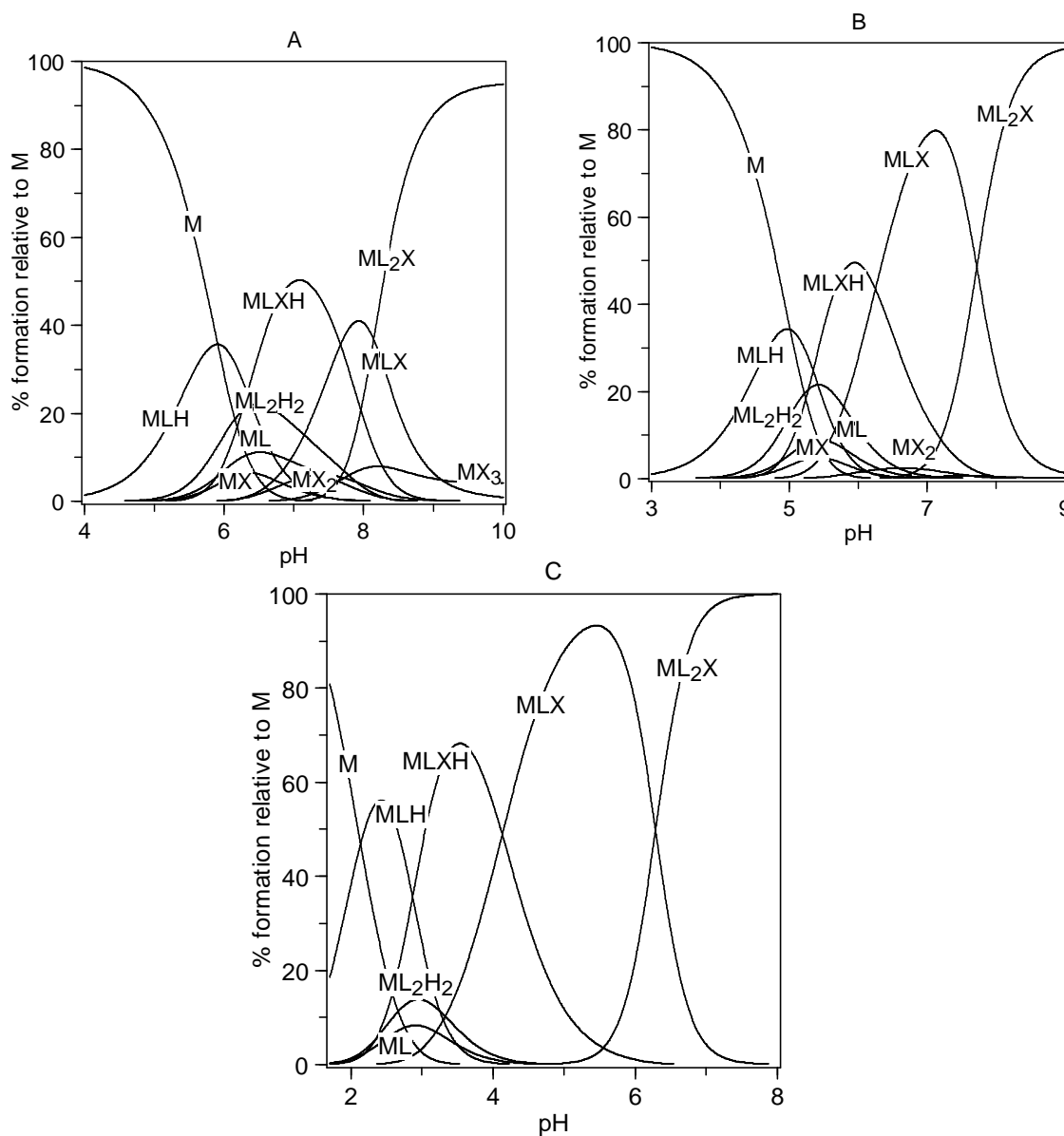


Fig. 2: Species distribution diagrams of ternary complexes of Orn and en in 10% v/v Dox-water mixture. (A) Co(II), (B) Ni(II), and (C) Cu(II)

3.6 Structures of complexes

Based on the protonation and deprotonation equilibrium of Orn 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 Fig. 3. Octahedral structures are proposed for all the metals based on literature reports, [34-37] with Cu(II) normally being Jahn-Teller distorted (38,39). Orn form strong tridentate and en bidentate complexes with transition metals. Orn at higher pH favors the (N, N) coordination and at physiological pH it is bound through ω -amino group to form (O, N) coordination.

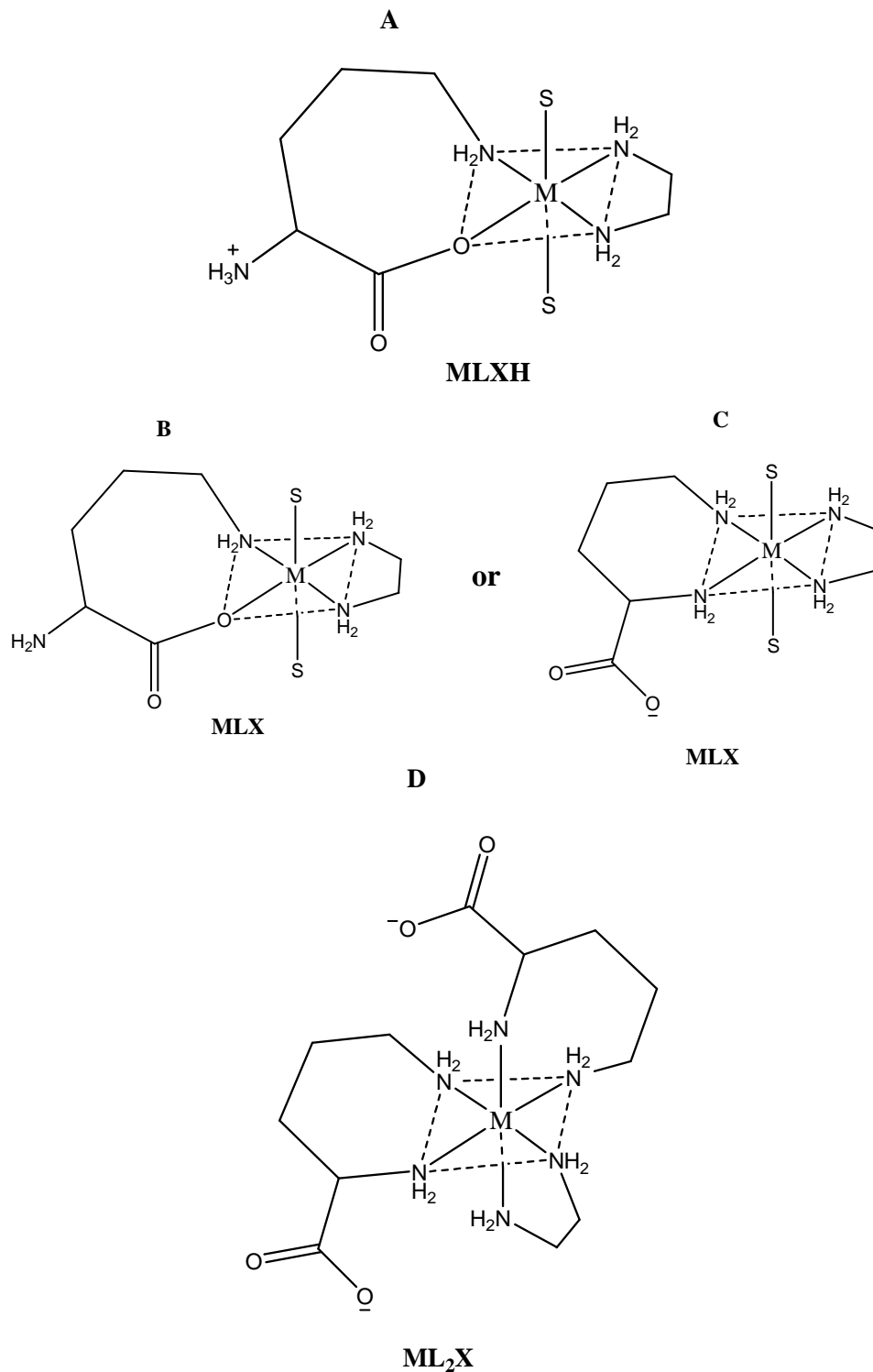


Fig. 3: Speculative structures of Orn-M(II)-en complexes, where S is either solvent or water molecule and M is Co(II), Ni(II) or Cu(II)

CONCLUSION

1. The predominant species detected are MLXH, MLX, and ML₂X for Co(II), Ni(II), and Cu(II) where L = Orn and X = en. The active forms of these ligands are LH₃²⁺, LH₂⁺, LH and L⁻ for Orn and XH₂²⁺, XH⁺ and X⁻ for en.
2. The Δ log K values 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.
5. 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.

Acknowledgements

One of the authors (HHB) gratefully acknowledges the financial support provided to him by the Ministry of Education of the Federal Democratic Republic of Ethiopia.

REFERENCES

- [1] H. Sigel, R.B. Martin, R. Tribolet, U.K. Haring, R.M. Balakrishnan, *Eur. J. Biochem*, **985**,152, 187, 1.
- [2] S.B. Ronald, G.N. Rao, *Proc. Nat. Acad. Sci. India*, **2002**, 72, 301.
- [3] S.B. Ronald, G.N. Rao, *J. Indian Chem. So*, **2002**, 79, 799.
- [4] M.S. Babu, G.N. Rao, K.V. Ramana, M.S.P. Rao, *Proc. Nat. Acad. Sci. India*, **2003**,73, 173.
- [5] B.B.V. Sailaja, T. Kebede, G.N. Rao, M.S.P. Rao, *Proc. Nat. Acad. Sci*, **2004**, 74, 399.
- [6] G.N. Rao, K.G. Sudarsan, *Chem. Speciaiton. Bioavail*, **2006**, 18, 71.
- [7] F. Hiromichi, M. Kenichi, Y. Kita, K. Ozora, O. Yuruke, *Org. Lett*, **2007**, 9, 1687.
- [8] L. Arash, M. H. Habibi, R. W. Harrington, M. Morteza, C. William, *J. Fluorine Chem*, **2006**, 127, 769.
- [9] S. Raju, B. Ananda Kumar, K.B.K. Naik, G.N. Rao, *Asian J. Research Chem*, **2011**. 4, 1908.
- [10] D.E. Pegg, Principles of cryopreservation methods. *Mol. Biol*, **2007**, 368, 39.
- [11] C. Sennett, L. E. G. Rosenberg, I. S. Millman, *Annu. Rev. Biochem*, **1981**, 50, 1053.
- [12] R.A. Poellot, T.R. Shuler, E. O. Uthus, F. H. Nielsen, *Proc. Natl. Acad. Sci., USA*, **1990**, 44, 80.
- [13] H. Kim, R. J. Maier, *J. Biol. Chem*, **1990**, 265, 18729.
- [14] W. B. Schaap, D. L. McMasters, *J. Am. Chem. Soc*, **1961**, 83, 4699.
- [15] K. D. Karlin, Z. Tyeklar, (Eds) Bioinorganic Chemistry of Copper, Chapman and Hall, New York, **1993**, 506.
- [16] W. Kaim, B. Schwederski, (Eds) Inorganic Elements in the Chemistry of Life, Wiley, Chichester, **1994**, 187.
- [17] G. N. Rao, A. Ramakrishna, *Proc. Nat. Acad. Sci., India*, **2005**, 75,245.
- [18] G. Gran, *Anal. Chim. Acta*, **1988**, 206, 111.
- [19] G. Gran, *Analyst*, **1952**, 77, 661.
- [20] M. P. Latha, V. M. Rao, T. S. Rao, G. N. Rao, *Bull. Chem. Soc. Ethio*, **2007**, 21, 363.
- [21] K. V. Lavanya, G. N. Rao, M. Rajesh, M. S. Babu, *J. Indian Chem. Soc*, **2004**, 81, 384.
- [22] G. N. Rao, Ph.D. Thesis, Andhra University, Visakhapatnam, India, **1989**.
- [23] P. Gans, A. Sabatini. A. Vacca, *Inorg. Chim. Acta*, **1976**, 18, 237.
- [24] G. N. Rao. A. Ramakrishna, *Proc. Nat. Acad. Sci., India*, **2005**, 75, 245.
- [25] M. Z. Born, *Phy*, **1920**, 1, 45.
- [26] R. Griesser H. Sigel, *Inorg. Chem*, **1970**, 9, 1238.
- [27] R. Griesser H. Sigel, *Inorg. Chem*, **1971**, 10, 2229.
- [28] R. Griesser H. Sigel, *Inorg. Chem*, **1974**, 13, 462.
- [29] H. Sigel, P.R. Huber, R. Griesser, B. Prijs, *Inorg. Chem*, **1974**, 12, 1198.
- [30] S. Kida, *Bull. Chem. Soc. Jpn*, **1956**, 29, 805.
- [31] R. B. Martin, R. Prados, *J. Inorg. Nucl. Chem*, **1974**, 36, 1665.
- [32] T. Sakurai, O. Yamauchi, A. Nakahara. *Bull. Chem. Soc. Jpn*, **1976**, 49, 169.
- [33] T. Sakurai, O. Yamauchi, A. Nakahara, *Bull. Chem. Soc. Jpn*, **1977**, 50, 1776.
- [34] M. Padmaja, J. Pragathi, C. Gyana Kumari, *J. Chem. Pharm. Res*, **2011**, 3, 602.
- [35] R. T. Vashi, C. D. Shelat, *Int. J. of Chem. Tech. Res*, **2011**, 3, 911.
- [36] N. Raman, S. Ravichandran, C. Thangaraja, *J. Chem. Sci*, **2004**, 116, 215.
- [37] G. Kumar, E. M. R. Kiremire, *Chemistry*, **2007**, 16, 386.
- [38] J. Sheals, P. Persson, B. Hedman, *Inorg. Chem*, **2001**, 40, 4302.

[39] J. K. Cherutoil, L. L. Cheruiyot, C. P. Kiprono, *Bull. Chem. Soc. Ethiop*, **2005**, 19, 295.