

# A pH Metric Investigation on Mixed-Ligand Complexes of Transition Metal Ions with Selective Bio-Ligands in Surfactant - Aqua Mixture

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## Abstract

Chemical speciation of ternary complexes of L-Glutamine and L-Methionine with essential transition metal ions was studied pH metrically. The study was conducted by varying concentrations (0.0-2.5% v/v) of Triton X-100 (TX100), aqua mixture and at an ionic strength of  $0.16 \text{ mol dm}^{-3}$  using  $\text{NaNO}_3$  as an electrolyte at  $303.0 \pm 0.1 \text{ K}$ . The stability of various ternary species was established by modelling studies using the computer program MINQUAD75.

Titration curves were carried out in presence of relative concentrations of metal(M) to L-Glutamine(L) to L-Methionine(X) (M:L:X=1:2:2, 1:2:4, 1:4:2) with sodium hydroxide as titrant. The trend in the variation of stability constants with change in the mole fraction of the surfactant (neutral micellar) media is explained on the basis of electrostatic and non-electrostatic forces. Distribution diagrams in relation to pH and plausible structures were presented.

**Keywords:** Chemical speciation, Ternary complexes, TX100, MINQUAD75, L-Glutamine and L-Methionine.

## Introduction

Speciation study of essential metal ion complexes is becoming a vital factor to understand the role played by the active site cavities in biomolecules and the bonding behavior in peptide and protein residues with the metal ion. The species refined and their relative concentrations under experimental conditions represent the possible forms of amino acids in bio-fluids<sup>41</sup>.

Amino acids are the foundation stone of living organisms fostered by the vital role played by amino acids in our life studying their structural, chemical properties and stability constants of their corresponding complexes of metal ions Co(II), Ni(II) and Cu(II) in particular<sup>20</sup>. L-Glutamine (Gln) is a beneficial abundant, active free amino acid contributing more than 50% of the free amino acid pool in human muscle<sup>3, 4, 17</sup> and plasma which play an important role in Gut flora and immunity. Gln has a protective effect in maintenance of intestinal mucosal integrity, modulation of inflammatory response, nucleotide biosynthesis, energy metabolism and stimulation of immunity<sup>1,2,18,26,27</sup>. Low level of Gln causes infections, weight loss and muscle-wasting in

trauma treated by glutamine supplementation. Gln is involved in many metabolic and synthetic biochemical processes.

L-Methionine (Met) is a sulphur containing proteinogenic, essential amino acid and its dietary requirement is about 2 gm/day. In methionine metabolism, S-adenosyl methionine (SAM) is a key intermediate regarded as “the active methionine” acting as a coenzyme of remarkable versatility. SAM serves as a source of methyl donor in conversion of guandioacetate to creatine, methylene group donor for the synthesis of cyclopropyl fatty acids, amino groups donor in biotin synthesis, amino isopropyl group’s donor in synthesis of poly amines and in the synthesis of ethylene used to promote plant ripening<sup>7,8</sup>. Methionine supplementation is used to treat symptoms like depression, inflammation, liver diseases, muscle pains especially people suffering from estrogen dominance.

Cobalt plays a key role in the synthesis of many biomolecules like DNA and hemoglobin and also activates several metalloenzymes<sup>44</sup> like ribonucleotide reductase in DNA synthesis, glycylglycine dipeptidase in the hydrolysis of dipeptide and glutamate mutase in amino acid metabolism. Ni(II) is essential for some microbes. Urease, a Ni(II) dependent enzyme consists of 6% soluble cellular protein which catalyzes the urea (formed when proteins break down) to produce ammonia and carbamate<sup>9, 28</sup>. Copper is the third most abundant essential transition metal in body found mainly in heart, brain and liver where it is stored as copper thionein and released as ceruloplasmin or in the form of copper serum albumin complex. For the high metabolic rate, brain requires large number of Cu-metalloenzymes<sup>24, 29</sup>.

TX100 is a non-ionic surfactant having neutral charge on the micellar surface. In this medium the charged species are expected to be destabilized. Binary and ternary complexes of L-Glutamic acid and L-Methionine with Co(II), Ni(II) and Cu(II) in 1, 2-propanediol-water are reported earlier<sup>21-23</sup>. Hence, chemical speciation of Gln and Met with transition metal ions like Co(II), Ni(II) and Cu(II) in neutral surfactant-water mixture has been reported in this study.

## Material and Methods

AR Grade (E-Merck, India) samples of L-Glutamine, L-Methionine, Co(II), Ni(II) and Cu(II) nitrates, nitric acid, sodium nitrate and sodium hydroxide were prepared in triple

distilled water. The nitrates of all the metal solutions have been standardized by analytical methods<sup>45</sup>. Triton X-100 (Merck, India) was used as received. To increase the solubility of Gln and Met and to suppress the hydrolysis of metal Nitrates, the mineral acid concentration in the above solutions are maintained at 0.05 mol dm<sup>-3</sup>. To assess the errors in the concentration, the data was treated with the Analysis of Variance of one way classification (ANOVA). The strength of alkali was determined using Gran - Plot method<sup>11, 12, 31</sup>.

**Apparatus:** The pH metric titrations were carried out in the medium containing varying concentrations of TX100-water mixture (0.0-2.5% v/v) maintaining an ionic strength of 0.16 mol dm<sup>-3</sup> with sodium nitrate (inert electrolyte) at 303.0 ± 0.1 K. An ELICO pH meter (Model L1-120) of readability 0.01 in connection with glass electrode and calomel electrode was used to monitor the changes in hydrogen ion concentration. The glass electrode was calibrated in a well stirred TX100-water mixture containing sodium nitrate. The effects of variations in asymmetric potential, liquid junction potential, activity coefficient, sodium ion error and dissolved carbon dioxide on the response of glass electrode were accounted here in the form of correction factor<sup>34,37</sup> which was obtained from the experimental acid-base titration data and computed from the computer program SCPHD<sup>30,32</sup>.

Correction factor was applied on the pH meter readings to study the effect of solvent on pH. Titration of strong acid with strong alkali was carried out at regular intervals to check the complete equilibration of the glass electrode. The titrand consists of 1 mol dm<sup>-3</sup> mineral acid in a total volume

of 50 cm<sup>3</sup>. Titrations were carried out in presence of different relative concentrations (Table1) of metal(M) to Gln(L) and Met(X) with NaOH of concentration 0.4 mol dm<sup>-3</sup>.

## Results and Discussion

**Titration Curves:** For the present work a set of titrations were done separately with standard NaOH solution as analyte and the titration curves were presented in figure 1. The formation of ternary species in solution was best evidenced by these curves.

Figure 1 interpreted that:

- When the ligand (L/X) is added to the free acid solution, a decrease in pH indicates the release of protons due to deprotonation of protonated ligands maintained in acidic media of 0.05 mol L<sup>-1</sup> HNO<sub>3</sub>.
- Metal ligand curves (ML/MX) of binary species show lower pH, when compared to their corresponding protonation curves representing the formation of metal ligand complex species and liberation of protons.
- The mixed-ligand (MLX) curve of ternary species lie below the free ligand and those of the binary metal ligand curves indicating the formation of ternary complex species and release of more number of protons.
- Since the mixed-ligand curve did not merge with any one of the individual metal ligand titration curves, in the lower pH range confirm the formation of a ternary complex by simultaneous equilibria.
- The formation of mixed complex species in solution was supported by the absence of any solid phase during the titration of ternary mixture.

**Table 1**  
Total concentrations of reactants (in mol dm<sup>-3</sup>) for mixed-ligand titrations in TX100-Water mixtures.

% v/v TX100	TM0			TL0		TM0:TL0:TX0
	Co(II)	Ni(II)	Cu(II)	Gln	Met	
0.0	0.0999	0.0997	0.1001	0.1998	0.2003	1:2:2
				0.1998	0.4007	1:2:4
				0.3964	0.2003	1:4:2
0.5	0.0997	0.0999	0.0998	0.2002	0.2002	1:2:2
				0.2002	0.4004	1:2:4
				0.4003	0.2002	1:4:2
1.0	0.0999	0.0996	0.0997	0.1993	0.2000	1:2:2
				0.1993	0.4001	1:2:4
				0.3986	0.2000	1:4:2
1.5	0.0998	0.0996	0.0997	0.1998	0.1998	1:2:2
				0.1998	0.3996	1:2:4
				0.3996	0.1998	1:4:2
2.0	0.1001	0.0999	0.0998	0.2005	0.1998	1:2:2
				0.2005	0.3996	1:2:4
				0.4010	0.1998	1:4:2
2.5	0.0998	0.0997	0.0999	0.1999	0.1997	1:2:2
				0.1999	0.3994	1:2:4
				0.3999	0.1997	1:4:2

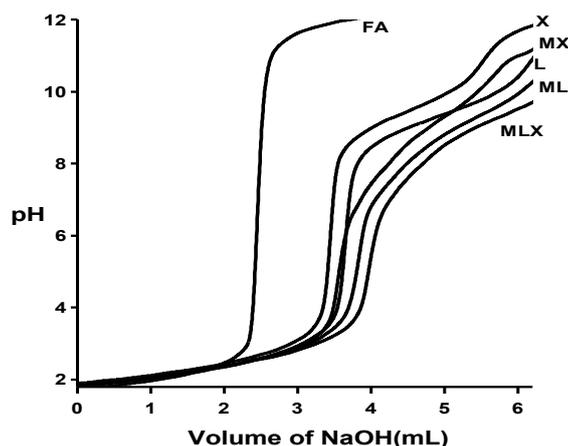


Figure 1: Titration curves (M= Co, L= Gln, X= Met and FA= HNO<sub>3</sub>)

Table 2  
Parameters of best fit chemical models of Gln-M(II)-Met complexes in TX100-water mixture.  
Temperature=303 K, Ionic strength=0.16 mol dm<sup>-3</sup>.

% v/v TX100	log β <sub>m<sub>l</sub>x<sub>n</sub></sub> (SD)				pH range	NP	U <sub>corr</sub>	Skewness	Kurtosis	χ <sup>2</sup>	R-factor
	1110	1111	1112	1210							
<b>Co(II)</b>											
0.0	8.78(27)	16.20(24)	---	11.93(44)	2.00-10.5	44	3.2	-1.70	11.73	70.00	0.0227
0.5	8.79(25)	17.05(38)	---	10.55(50)	1.83-10.4	115	3.66	-0.87	4.90	119.90	0.0189
1.0	8.87(35)	16.76(43)	---	10.51(38)	1.96-10.5	105	10.06	-1.62	5.44	74.13	0.0332
1.5	8.99(23)	17.50(21)	---	11.10(32)	2.20-10.5	91	2.92	-0.66	5.04	33.80	0.0203
2.0	9.00(43)	17.85(18)	---	12.45(18)	1.72-9.3	165	2.76	0.35	2.72	22.50	0.0144
2.5	9.41(61)	17.46(41)	---	11.78(63)	1.74-10.5	137	9.55	0.84	2.33	158.63	0.0423
<b>Ni(II)</b>											
0.0	9.94(51)	17.60(29)	---	13.96(25)	2.6-10.5	70	4.10	-0.64	7.80	55.66	0.0279
0.5	11.59(36)	18.17(43)	---	14.72(51)	1.83-10.5	118	1.02	-1.66	5.08	279.93	0.0310
1.0	11.40(45)	17.98(57)	---	14.59(62)	1.76-10.5	127	1.56	-1.48	3.72	285.26	0.0349
1.5	11.65(19)	18.42(23)	---	14.41(24)	1.72-10.5	69	1.36	-1.23	3.34	83.42	0.0099
2.0	11.44(42)	19.27(23)	---	15.26(31)	1.72-10.5	138	4.48	-0.15	2.98	28.49	0.0183
2.5	13.74(37)	19.71(26)	---	15.12(19)	1.84-10.5	174	1.50	0.25	4.19	52.11	0.0413
<b>Cu(II)</b>											
0	16.32(51)	21.28(46)	24.89(51)	---	2.00-6.0	91	3.29	0.10	2.08	17.58	0.0181
0.5	16.44(16)	21.34(08)	24.45(09)	---	1.83-6.0	138	2.12	0.38	2.23	66.99	0.0124
1.0	17.71(04)	22.27(02)	25.33(04)	---	2.00-5.9	78	0.85	-1.06	7.08	28.97	0.0025
1.5	17.60(18)	22.56(09)	25.64(15)	---	2.00-6.0	109	2.17	0.42	2.23	56.66	0.0137
2.0	17.83(36)	23.14(24)	26.25(30)	---	2.00-6.0	103	3.50	0.28	1.98	71.11	0.0176
2.5	18.49(11)	23.46(04)	25.12(49)	---	2.00-6.0	43	3.17	0.42	2.79	5.40	0.0044

U<sub>corr</sub> = U / (NP-m).10<sup>8</sup>; NP = Number of points; m = number of formation constants; SD = Standard deviation

**Modeling of Chemical Speciation:** A preliminary investigation of alkali metric titrations of mixtures containing various mole ratios of Gln and Met in presence of mineral acid (HNO<sub>3</sub>) and inert electrolyte (NaNO<sub>3</sub>) inferred that no condensed species were formed. Assuming that the coordination number of metal ion is limited to six, the total number of primary and secondary ligands together to a

maximum of three are generating the possible ternary species for modeling studies.

The formation constants for acid-base equilibria of both primary and secondary ligands and those for binary metal complexes were fixed in testing various chemical models of ternary complexes using MINQUAD75<sup>5,10</sup>. The best fit models were chosen based on the statistical parameters like

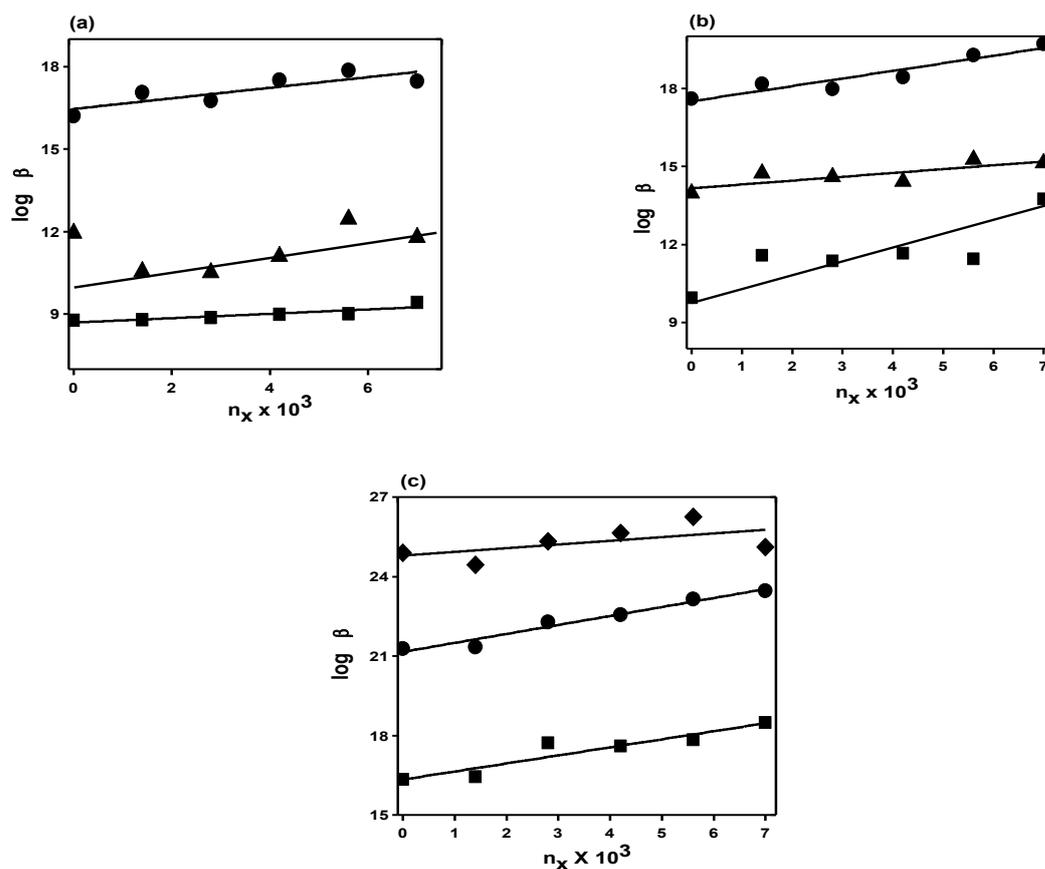
$\chi^2$ , R-factor, Skewness and Kurtosis given in the table 2. The ternary species detected were MLX, MLXH and ML<sub>2</sub>X for Co(II) and Ni(II) whereas for Cu(II) were MLX, MLXH and MLXH<sub>2</sub>. The small values of  $U_{\text{corr}}$  (The sum of the least squares of all experimental points, such as deviations in concentration of metal, ligand and hydrogen ion at all experimental points corrected for degrees of freedom) indicate that the model is consistent with 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 system, kurtosis value should be '3' and skewness must be a value of '0'. In the present study, mostly the residuals for Co(II) and Ni(II) systems follow platykurtic (flat peak, > 3) and for Cu(II) system, the residuals follow leptokurtic (sharp peak, < 3).

In the present data, skewness ranges from -1.70 to 0.84. This data is an evidence that the residuals follow a part of normal distribution, hence least squares method can be applied to the present data. The sufficiency of the models was proved by low crystallographic R-value, another statistical factor. Thus, these statistical parameters show the best fit models authenticating the mixed ligand equilibria in TX100-water mixture.

**Effect of Solvent on Stability of Ternary Complexes:** The effect of surfactant on complex equilibria and apparent shift in the magnitude of stability constants in micellar media compared to aqueous solution can be attributed to the creation of a concentration gradient of proton between the interface and the bulk solution<sup>43</sup>. The variation of overall stability constant values or change in free energy with co-solvent content depends upon two factors namely electrostatic and non-electrostatic. Born's classical treatment<sup>6</sup> holds good in accounting for the electrostatic contribution to the free energy change.

Hence, the  $\log \beta$  values should vary almost linearly as a function of mole fraction of the medium indicating that increase in electrostatic forces and decreased dielectric constant of the medium<sup>42</sup> are dominating the equilibrium process for Co(II), Ni(II) and Cu(II) of the medium under the present experimental conditions. The decrease or increase in the stability constants may arise primarily from the difference in the environment of the species as well as from the distribution of the micellar and bulk phase. TX100 is a non-ionic surfactant having neutral charge on the micellar surface, in this medium the charged species are expected to be destabilized<sup>33</sup>. This trend is observed in the present study as shown in figure 2.



**Figure 2: Change in magnitude of stability of Metal-Gln-Met complexes in TX100-water mixture: (a) Co(II), (b) Ni(II) and (c) Cu(II); (■)  $\log \beta_{\text{MLX}}$ , (●)  $\log \beta_{\text{MLXH}}$ , (◆)  $\log \beta_{\text{MLXH}_2}$  and (▲)  $\log \beta_{\text{ML}_2\text{X}}$ .**

**Quantification of change in the Stability of Ternary Species:**

The change in the stability of the ternary complexes as compared to their binary analogues was quantified<sup>14-16,38</sup> based on the disproportionation constant ( $\log X$ ) given by the equation 1:

$$\log X = 2 \log K_{MLX}^M - \log K_{ML_2}^M - \log K_{MX_2}^M \quad (1)$$



Under these equilibrium conditions one can expect the formation of 50% of ternary complexes and 25% of each of the binary complexes statistically and the value of  $\log X$  shall be 0.6<sup>19</sup>. If  $\log X$  value is more than 0.6 accounts for the extra stability of  $MLX$ , another approach 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  $X$ <sup>25</sup> where  $L$  is the primary ligand (Gln) and  $X$  is the secondary ligand (Met). It is compared with that calculated value purely on statistical grounds as given by equation 3:

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

The electrostatic theory of binary complex formation and statistical arguments suggested the availability of additional coordination positions of the hydrated metal ion for the first ligand than for the second. This suggests that  $\Delta \log K$  should be negative, although several exceptions have been found<sup>40</sup>. The statistical values of  $\Delta \log K$  for bidentate  $L$  and  $X$  are -0.4, -0.6 and between -0.9 and -0.3 for octahedral, square planar and distorted octahedral complexes respectively. Negative values of  $\Delta \log K$  indicate the secondary ligand forming a more stable complex with hydrated metal ion than with  $ML$ .

Whenever the experimental values of  $\Delta \log K$  exceeds the statistical values, it can be inferred that ternary complex is formed as a result of interaction of  $ML$  with  $X$  or  $MX$  with  $L$ .  $\Delta \log K$  values of ternary complexes containing bipyridyl as the primary ligand are positive for O-donors (malonic acid, pyro catechol etc.), negative for N-donors (ethylene diamine) and intermediate or negative for amino acids with both 'N and O' coordination sites<sup>13,39</sup>. But a very high negative value (-2.3) for Cu(ethylene diamine) (iminodiacetic acid) and a positive value (0.82) for Cu(O-phen)-(6,7dihydroxynaphthaline-2-sulphonate) was also observed. The  $\log X$  and  $\Delta \log K$  values calculated from binary and ternary complexes are included in table 3.

**Table 3**

**$\Delta \log K$  and  $\log X$  values of ternary complexes of Co(II), Ni(II) and Cu(II) with Gln and Met in TX100-water mixture.**

% v/v TX100	$\Delta \log k_{MLX}$	$\Delta \log k_{MLXH}$	$\Delta \log k_{ML_2X}$	$\Delta \log k_{MLXH_2}$	$\log X_{MLX}$
<b>Co(II)</b>					
0.0	0.71	0.02	1.35	--	4.61
0.5	-0.22	0.31	-1.01	--	3.34
1.0	-0.22	-0.02	-1.52	--	2.49
1.5	-1.12	-0.06	-1.22	--	1.96
2.0	-1.08	0.35	-1.82	--	0.15
2.5	-2.57	-1.62	-3.24	--	5.58
<b>Ni(II)</b>					
0.0	-0.74	0.29	-0.3	--	2.23
0.5	0.41	1.19	-0.52	--	4.47
1.0	0.33	0.58	-0.52	--	3.60
1.5	-0.01	0.31	-1.03	--	4.08
2.0	-0.29	0.72	0.65	--	3.40
2.5	1.28	0.61	-1.03	--	5.88
<b>Cu(II)</b>					
0.0	1.19	2.76	--	2.38	4.76
0.5	0.05	1.35	--	0.03	3.79
1.0	0.6	1.23	--	-0.39	5.95
1.5	0.29	1.15	--	-0.30	4.97
2.0	1.28	1.61	--	0.45	5.76
2.5	0.92	1.88	--	-0.99	6.27
Calculations of $\log X$ and $\Delta \log K$ values from overall stability constants					
$\Delta \log K_{MLX} = \log \beta_{MLX} - \log \beta_{ML} - \log \beta_{MX}$ $\Delta \log K_{MLXH} = \log \beta_{MLXH} - \log \beta_{MLH} - \log \beta_{MX}$ $\Delta \log K_{MLXH_2} = \log \beta_{MLXH_2} - \log \beta_{MLH} - \log \beta_{MXH}$ $\Delta \log K_{ML_2X} = \log \beta_{ML_2X} - \log \beta_{ML_2} - \log \beta_{MX}$ $\log X_{MLX} = 2 \log \beta_{MLX} - \log \beta_{ML_2} - \log \beta_{MX_2}$					

The equations for the calculation of  $\Delta \log K$  and  $\log X$  are given in the chart 1. These values could not be calculated for some systems due to the absence of relevant binary species. In the present study, the  $\log X$  values range from 0.15 to 6.27 and some values are found to be higher than those expected on statistical bases (0.6). This higher value infers the extra stability of ternary complexes.  $\Delta \log K$  values are in the range from -3.24 to 2.76, which indicates that the ternary complexes formed by the Cu(II) are more stable when compared to that of the other two metal ions namely Co(II) and Ni(II). The extra stability of these ternary complexes may be due to the interactions outside the coordination sphere such as charge neutralization, chelate effect of ligands, formation of hydrogen bonds between the coordinated ligands and stacking interactions<sup>35,36</sup>.

#### Effect of Influential Parameters on Stability Constants:

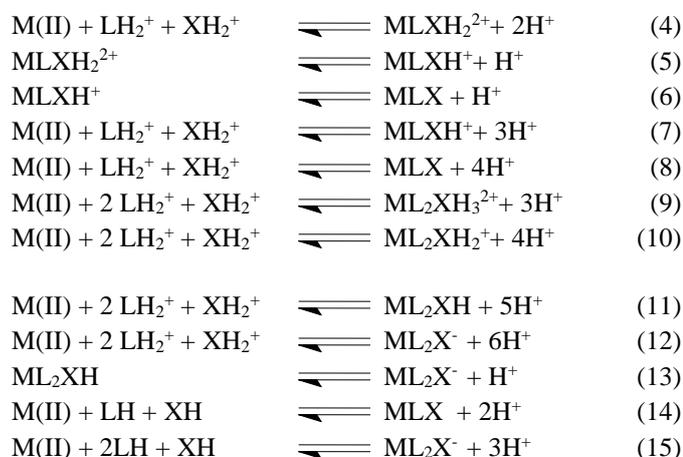
The variation in concentration of ingredients affects the magnitude of stability constants; such parameters are called influential parameters. In order to get the best chemical model for critical evaluation and application under different experimental conditions with different accuracies of data acquisition, an observation was made by applying pessimistic errors in the concentration of alkali, acid, ligands, metal and correction factor.

The results shows in the table 4 emphasize that the errors in the concentration of alkali and acid influence the equilibrium constants more than those of the ligands, metal,  $\log F$  and volume. Some species are rejected when errors are introduced in the concentration.

**Table 4**  
Effect of errors in influential parameters on the Cu(II)-Gln-Met complex stability constants in 1.0% v/v of TX100-water mixture.

Ingredient	% of error	$\log \beta_{mlxh}(SD)$		
		MLX	MLXH	MLXH <sub>2</sub>
	0	17.71(04)	22.27(02)	25.33(04)
Alkali	-5	Rejected	21.18(04)	24.81(06)
	-2	16.83(09)	21.85(04)	25.15(04)
	+2	18.13(05)	22.47(04)	25.40(04)
	+5	18.87(06)	24.79(09)	26.50(11)
Acid	-5	19.49(10)	23.50(08)	26.30(10)
	-2	18.04(05)	22.50(04)	25.55(05)
	+2	16.75(09)	21.72(04)	24.83(04)
	+5	Rejected	20.93(05)	Rejected
Ligand(L)	-5	18.71(09)	23.33(08)	26.48(10)
	-2	18.43(06)	23.05(07)	26.21(06)
	+2	18.20(05)	22.82(04)	25.99(05)
	+5	18.03(05)	22.66(04)	25.83(04)
Ligand(X)	-5	18.20(05)	22.82(04)	25.98(05)
	-2	18.28(06)	22.89(04)	26.06(09)
	+2	18.40(06)	23.01(07)	26.19(09)
	+5	18.50(05)	23.12(05)	26.31(05)
Metal	-5	17.80(04)	22.31(04)	25.43(04)
	-2	17.65(04)	22.24(03)	25.35(04)
	+2	17.45(04)	22.16(03)	25.26(05)
	+5	17.31(04)	22.10(03)	25.19(05)
$\log F$	-5	17.39(04)	22.03(04)	25.01(04)
	-2	17.48(04)	22.13(04)	25.19(04)
	+2	17.62(04)	22.27(04)	25.41(05)
	+5	17.80(05)	22.45(06)	25.65(04)
Volume	-5	17.40(04)	22.04(04)	25.04(04)
	-2	17.48(04)	22.13(04)	25.20(04)
	+2	17.62(04)	22.27(03)	25.40(04)
	+5	17.74(05)	22.39(04)	25.56(04)

**Distribution Diagrams:** A perusal of the distribution diagrams of figure 3 reveals that the concentrations of binary species are less compared to the ternary species which indicates the existence of more stable ternary complexes. The ternary species exist in the pH ranges 4.0-10.5, 3.8-10.5 and 2.0-6.0 for the three metal ions Co(II), Ni(II) and Cu(II) respectively. The formations of the complex species can be represented by the following equilibria.

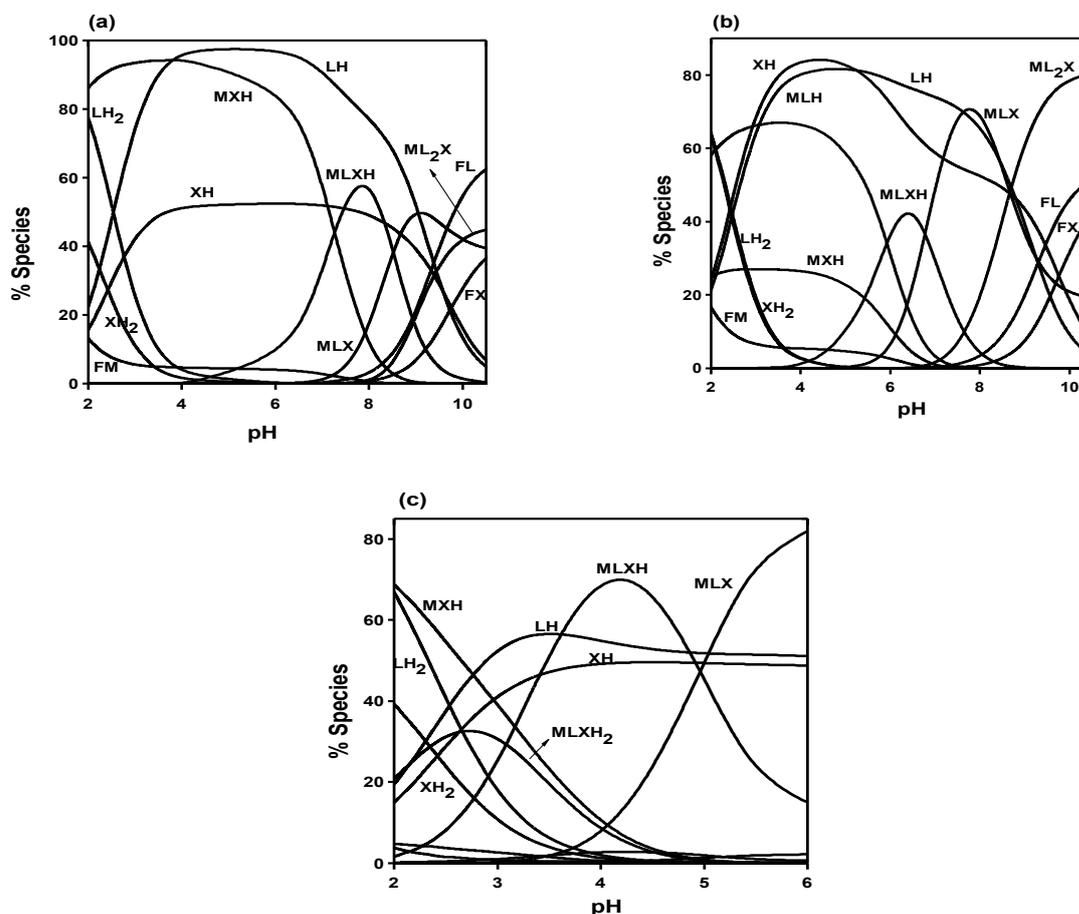


The distribution diagrams reveal that at very low pH, the concentrations of mixed ligand complexes are lower than

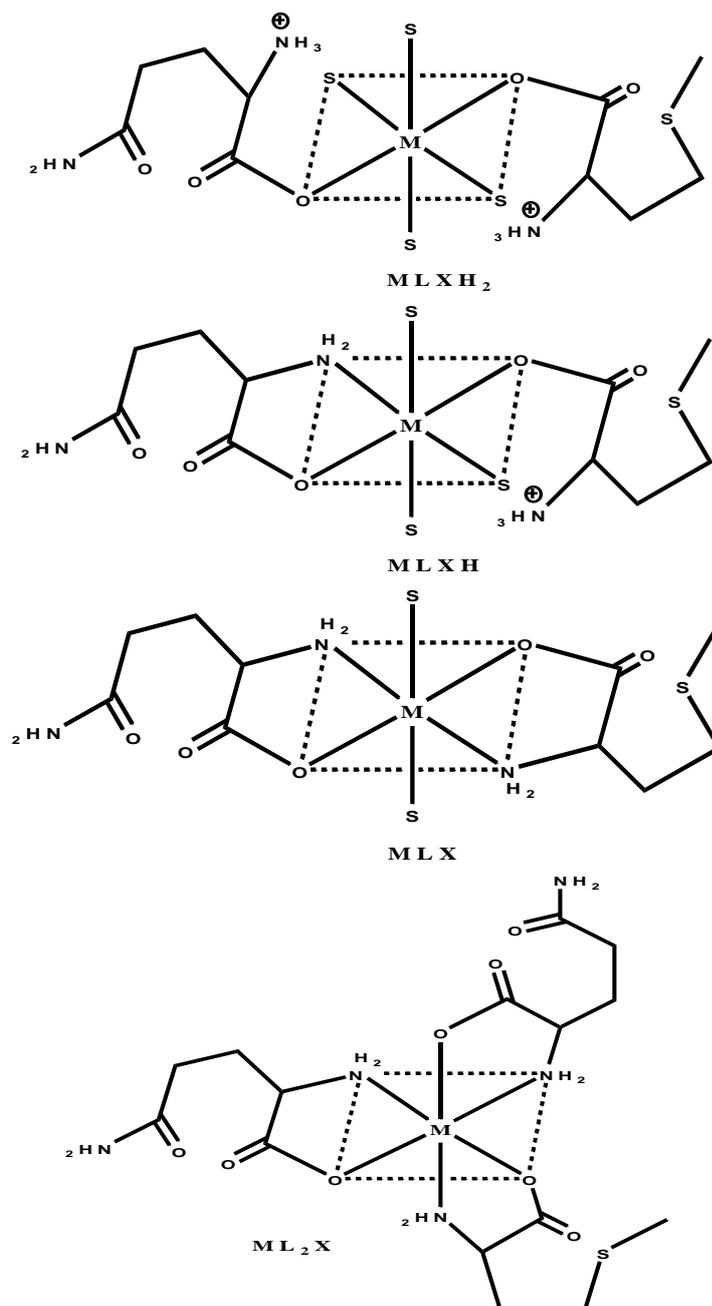
those of the protonated ligands. The concentration of the ternary species increases with increasing pH. The protonated ternary species (MLXH, MLXH<sub>2</sub>) are distributed at lower pH and the deprotonated ternary species (MLX, ML<sub>2</sub>X) are distributed at higher pH values. The dominative species for Co(II), Ni(II) and Cu(II) systems were MLXH, ML<sub>2</sub>X and MLX respectively. MLXH species are found in the pH region 4.5-10.0 (maximum at pH 7.8) for Co(II), 4.0-8.0 (maximum at pH 6.1) for Ni(II) and 2.0-6.0 (maximum at pH 4.0) for Cu(II) (Equilibria 5 and 7).

MLX species are found in the pH region > 7.0 (maximum at pH 9.0) for Co(II), 5.8-10.5 (maximum at pH 8.0) for Ni(II) and > 3.5 (maximum at pH 6.0) for Cu(II) (Equilibria 6, 8 and 14), ML<sub>2</sub>X species formation starts at a pH region of > 8.0 for Co(II) and > 7.0 for Ni(II), as pH increases, the % of species also increases (Equilibria 12, 13 and 15) and MLXH<sub>2</sub> species in Cu(II) system are detected in the pH 2.0-5.0 range (maximum at pH 2.8) (equilibria 4).

**Proposed Structures for Ternary Complexes:** The possible structures of these ternary complexes are proposed in figure 4. The existence of ML<sub>2</sub>X and the absence of MLX<sub>2</sub> are due to the higher affinity of LH than XH towards the metal ion.



**Figure 3: Distribution diagrams of ternary complexes of Gln and Met complexes in 1.5% v/v TX100-water mixture. (a) Co(II), (b) Ni(II) and (c) Cu(II).**



**Figure 4: Structure of ternary complexes (S is either solvent or water molecules).**

## Conclusion

The following conclusions have been inferred from the modeling studies.

1. The ternary metal complex species detected are MLXH, MLX and ML<sub>2</sub>X for Co(II), Ni(II) and MLXH<sub>2</sub>, MLXH and MLX and for Cu(II) systems. Only these species are refined due to the restricted pH ranges and the possible active forms of ligands like LH<sub>2</sub><sup>+</sup>, LH and XH<sub>2</sub><sup>+</sup>, XH for Gln and Met respectively
2. The order of stability of these complexes was formed to be Co(II) < Ni(II) << Cu(II).
3. The values of  $\Delta \log K$  indicate that the ternary species have extra stability compared to their binary species which 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 between non-coordinated charge groups of the ligands.

4. The linear increase in the stabilities of ternary complexes with decreasing dielectric constant is due to the dominance of electrostatic forces than non-electrostatic forces.
5. The order of the ingredients that influence the magnitudes of stability constants due to the incorporation of errors is alkali > acids > Gln > Met > metal > log F > Volume.

The study also gives an insight into the metal availability / metal transport in bio fluids. The ternary complexes are more amenable for "metal transport" because of their stability.

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