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## Formation and Confirmation of Binary Complexes of Co (II), Ni (II ) and Cu (II) with L-Cysteine in SLS-water media.

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### ABSTRACT

Speciation of binary complexes of Co(II), Ni(II), and Cu(II) with L- Cysteine (Cys) in the presence of water-anionic surfactant mixtures in the concentration range of 0.0 –2.5% w/v SLS has been studied pH-metrically at a temperature of 303 K and at an ionic strength of 0.16 mol L<sup>-1</sup>. The selection of best fit chemical models is based on statistical parameters and residual analysis. The predominant species detected were ML<sub>2</sub>, ML<sub>2</sub>H and ML<sub>2</sub>H<sub>2</sub> for Co(II), Ni(II), and Cu(II). The trend in the variation of stability constants with the mole fraction of SLS was explained on the basis of electrostatic and non-electrostatic forces. Distribution of the species with pH at different compositions of SLS–water media was also presented.

**Keywords:** Chemical speciation, Stability constants, L- Cysteine, SLS, Bioavailability

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## INTRODUCTION

Chemical speciation of metals is important to understand their distribution, mobility, bioavailability, toxicity and for setting environmental quality standards [1]. A number of studies has been reported on binary stability constants of  $\alpha$ -amino acids in different media [2]. Investigations of acido-basic equilibria of amino and carboxylic acids and their interaction with metal ions in media of varying ionic strength, temperature and dielectric constant throw light on the mechanism of enzyme-catalyzed reactions. Bioavailability of a particular metal depends on its complex chemical reactions of dissolution, binding and complexation with the constituents of the environmental aquatic phase [3].

Cysteine is an important source of sulfur in human metabolism, and although it is classified as a non-essential amino acid, cysteine may be essential for infants, the elderly, and individuals with certain metabolic diseases or who suffer from malabsorption syndromes [4]. Essential elements are of important in many biological systems. Metal ions can be incorporated or naturally complexed in biological fluids [5]. Metals are involved in virtually all biological processes, including metabolism, energy transduction, gene expression, cell signaling, formation of endo- and exo-skeletons, and electron and information transfer[5-7]. The normal concentration range for each metal ion in biological systems is narrow, with both deficiencies and excesses causing pathological changes [8]. Hence, chemical speciation study of essential metal ion complexes is important to understanding of their distribution, mobility, bioavailability, toxicity and for setting environmental quality standards [9-10].

Cobalt is present as corrin coenzyme in glutamate mutase, diol dehydratase and arginase and in non-corrin form in dipeptidase [11]. Nickel is associated with several enzymes [12-14] and any variation in its concentration leads to metabolic disorders [15]. Copper is essential for the metabolism of many hormones and connective tissue [16-17]. Mixed solvents have been employed in different fields including pharmaceutical and analytical sciences [18].

Sodium lauryl sulphate (SLS) or sodium dodecyl sul-phate is an anionic surfactant used in many cleaning and hygiene products, food, pharmaceuticals, and cosmetics. Micellar–water mixtures are chosen in these studies to maintain the dielectric constants of the medium com-parable to those of the physiological fluids since the polarity of the active site cavities should generally be applicable, to compare ligand binding to the metal ion in protein and mixed solvent environments. The studies carried out on these systems under the present experimental conditions are useful to understand the role played by the active site cavities in biological molecules. Hence, speciation studies of L-Cysteine with some essential metal ions such as Co, Ni, and Cu in SLS–water mixtures are reported in this article. Extensive attention has been paid in recent years to the study of the chemical speciation of ligands with metal ions [19-25].

## EXPERIMENTAL

### **Materials**

0.05 mol dm<sup>-3</sup>aqueous solution of L-Cystein (GR grade, E-Merck, Germany) was prepared by dissolving sample in water. To increase the solubility of ligand, 0.05 mol dm<sup>-3</sup>hydrochloric acid concentration was maintained in the solution. GR samples of sodium lauryl sulphate (SLS, Qualigens, India) was used as such, and its purity was checked by determining critical micellar concentration (CMC) conductometrically. The CMC value of SLS was 0.0081 mol dm<sup>-3</sup>at 303 K. Solutions of Co(II), Ni(II), and Cu(II) chlorides (0.1 mol dm<sup>-3</sup>) were prepared by dissolving G.R. Grade (E-Merck, Germany) salts in tri-ple-distilled water maintaining 0.05 mol dm<sup>-3</sup>acid (HCl) to suppress the hydrolysis of metal salts. Sodium hydroxide (Qualigens, India) of 0.4 mol dm<sup>-3</sup> was prepared. All the solutions were standardized by standard methods. Cobalt, Nickel, and Copper [26] chlorides were standardized using EDTA. 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[27]. The strengths of alkali and mineral acid were determined using the Gran plot method[28, 29].

### Procedure

An ELICO (Model Li 120, India) pH meter of 0.01 readability (0–14 pH) in conjunction with a glass combination pH electrode was used to monitor changes in hydrogen ion concentration. The glass electrode was equilibrated in a well-stirred micellar solution containing an inert elec-trolyte. All the titrations were performed at  $303.0 \pm 0.1$  K in a medium containing varying concentrations of SLS (0.5–2.5% w/w) maintaining an ionic strength of  $0.16 \text{ mol dm}^{-3}$  with sodium chloride. In each of the titra-tions, the titrand consisted of approximately  $1 \text{ m mol}$  mineral acid in a total volume of  $50 \text{ cm}^3$ . Titrations were carried out in presence of different relative concentrations of metal (M) to Cys (L) (M:L = 1.0:2.5, 1.0:3.75 and 1.0:5.0) were carried out with  $0.4 \text{ mol dm}^{-3}$  sodiumhydroxide.

### Modeling Strategy

The computer program SCPHD [30] was used to calculate the correction factor. By using pH metric titration data, the binary stability constants were calculated with the computer program MINIQUAD75 [31] which exploits the advantage of constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm. During the refinement of binary systems, the correction factor and protonation constants of Cys were fixed. The variation of stability constants with the dielectric constant of the medium was analyzed on the basis of electrostatic/non-electrostatic, solute-solute and solute-solvent interactions.

## 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.

**Table 1: Parameters of best fit chemical models of Co(II), Ni(II) and Cu(II) with Cys complexes in SLS-water- mixtures at pH : 2.0 – 10.5.**

SLS % w/v	log $\beta_{mlh}$ (SD)			NP	$U_{corr}$ * $10^8$	$\chi^2$	Skew- ness	Kurt- osis	R-factor
	ML <sub>2</sub>	ML <sub>2</sub> H	ML <sub>2</sub> H <sub>2</sub>						
<b>Co(II)</b>									
0.0	4.38(12)	12.14(17)	20.15(12)	65	10.12	12.16	0.58	4.19	0.0114
0.5	4.58(18)	12.58(26)	20.33(16)	56	11.63	13.84	0.69	2.91	0.0158
1.0	5.45(06)	13.06(7)	21.13(06)	63	12.23	16.84	0.39	6.21	0.0110
1.5	5.78(20)	13.33(13)	21.29(17)	68	10.83	71.18	0.35	5.39	0.0224
2.0	5.86(13)	13.55(18)	21.50(20)	65	14.04	86.51	-0.37	6.20	0.0135
2.5	5.99(09)	13.84(41)	21.72(22)	58	16.80	13.25	0.25	5.10	0.0421
<b>Ni(II)</b>									
0.0	4.49(10)	12.26(10)	20.50(09)	78	8.76	15.68	0.98	6.13	0.0125
0.5	4.67(15)	12.60(24)	20.62(12)	69	7.43	27.92	-0.20	5.86	0.0215
1.0	5.74(18)	12.87(15)	20.97(12)	59	9.98	16.50	-0.10	5.18	0.0165
1.5	5.32(14)	13.23(11)	21.27(18)	74	9.31	25.00	-1.20	5.80	0.0110
2.0	5.57(03)	13.45(22)	21.50(13)	79	8.29	382.81	-0.18	3.13	0.0167
2.5	5.77(28)	13.71(11)	21.67(12)	83	8.29	23.25	0.33	4.26	0.0140
<b>Cu(II)</b>									
0.0	5.07(11)	13.16(16)	20.86(18)	53	10.26	11.78	0.82	6.01	0.0186
0.5	5.01(13)	13.46(16)	20.94(20)	49	16.53	12.49	1.06	4.37	0.0208

1.0	6.36(17)	14.25(20)	21.86(09)	65	15.77	20.93	-0.12	7.72	0.0168
1.5	6.43(20)	14.34(18)	22.15(12)	71	12.34	11.25	0.77	6.46	0.0316
2.0	6.48(19)	14.82(18)	22.31(21)	53	14.20	40.98	-0.25	8.77	0.0256
2.5	6.72(16)	15.02(11)	22.62(25)	56	13.21	22.00	-0.18	3.87	0.0272

$U_{corr} = U / (NP - m) \times 10^8$ ;  $m$  = number of species;  $NP$  = number of experimental points;  $SD$  = standard deviation

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 ingredients 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.

### **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 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 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  $\chi^2$ , skewness, kurtosis and R-factor. These statistical parameters show that the best fit models portray the metal-ligand species in SLS-water mixtures, as discussed below.

### **$\chi^2$ Test**

$\chi^2$  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  $\chi^2$  calculated is less than the table value, the model is accepted.

### **Crystallographic R-test**

Hamilton's R-factor ratio test [32] 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 these are different numbers of species the models whose values are 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 -0.37 and 0.69 for Co(II), -1.20 and 0.98 for Ni(II) and -0.25 and 1.06 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.

### **Kurtosis**

It is a measure of the peakedness of the error distribution near a modal 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.

### 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, metal and volume (Table 2). The order of the ingredients that influence the magnitudes of stability constants due to incorporation of errors is alkali > acid > ligand > metal. Some species were even rejected when errors were introduced in the concentrations. The rejection of some species and increased standard deviations in the stability constants on introduction of errors confirm the appropriateness of the experimental conditions and choice of the best fit models.

**Table 2: Effect of errors in influential parameters on the stability constants of Co(II)-Cys complexes in 0.0% w/v SLS-water medium.**

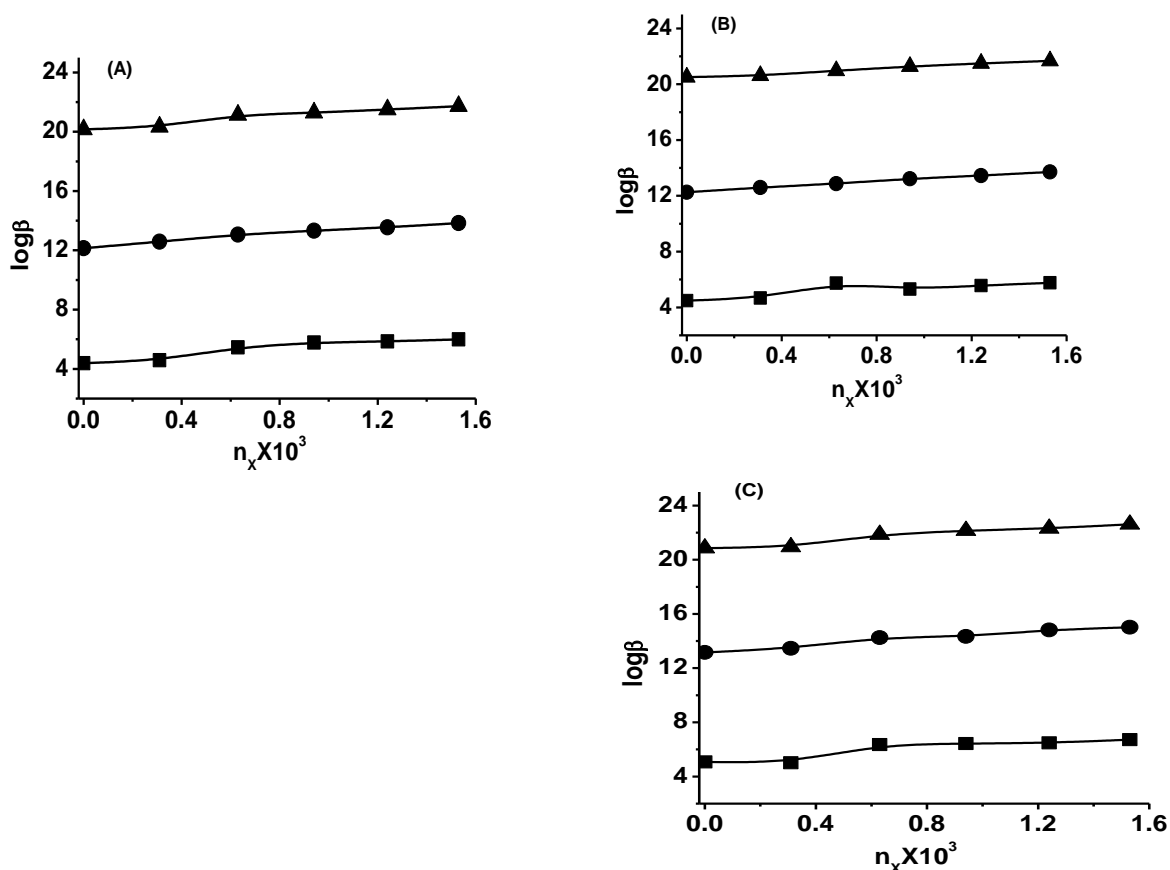
Ingredient	% Error	Log $\beta_{mlh}$ (SD)		
		120	121	122
Acid	0	4.69(27)	12.14(25)	20.35(16)
	-5	5.28(30)	13.76(40)	21.60(25)
	-2	4.81(23)	13.40(29)	21.30(21)
	+2	4.14(27)	12.95(27)	20.95(17)
	+5	Rejected	12.68(50)	20.97(24)
Alkali	-5	Rejected	12.60(67)	21.60(25)
	-2	4.14(27)	12.97(25)	20.97(25)
	+2	4.80(23)	13.38(29)	21.28(27)
	+5	5.28(28)	13.72(46)	21.57(25)
Ligand	-5	4.47(22)	13.12(26)	21.08(17)
	-2	4.49(21)	13.15(24)	21.10(16)
	+2	4.50(21)	13.19(23)	21.14(15)
	+5	4.52(21)	13.23(23)	21.17(15)
Metal	-5	4.48(21)	13.17(24)	21.12(16)
	-2	4.49(21)	13.17(24)	21.12(15)
	+2	4.50(22)	13.17(24)	21.12(15)
	+5	4.50(21)	13.18(24)	21.12(15)

### Effect of surfactant

The variations of stability constants ( $\log \beta$ ) with mole fraction of different micellar media are shown in Figure 1. The stabilities of complexes varied almost linearly with the mole fraction of the surfactant. This linear variation, due to the dielectric constant of the medium, decreases with increasing concentration of the surfactant. The non-linear variation depends upon the polarity of the medium, charge on the micellar surface, and on the non-electrostatic forces/hydrophobic interactions operating between the complex species and micellar surface. The species formed by the metals are either positive charged or neutral. The species should be stabilized in the micellar medium with opposite charges due to electrostatic interactions, but these charged species should be destabilized due to the decreased dielectric constant of the medium. The non-linear variation depends upon the polarity of the medium, charge on the micellar surface, and on the non-electrostatic forces/hydrophobic interactions operating between the complex species and micellar surface. The stabilities of these species in SLS–water medium do not show a particular trend. one of the reasons for such behavior is the accumulation of metal ions and ligands on the surface of micelles with an increased concentration of surfactant, and species with lower charge or high hydrophobicity are stabilized in the micellar pseudophase. The second reason that the species with lower charge or high hydrophobicity are stabilized in the micellar pseudophase. So metal ligand combinations resulting in neutral complexes will be favoured. Highly hydrophobic ligands are expected to partition significantly between the surface of the micelle and its interior, which would lead to the reduction in  $\log \beta$ . on the other hand, more hydrophobic ligands will

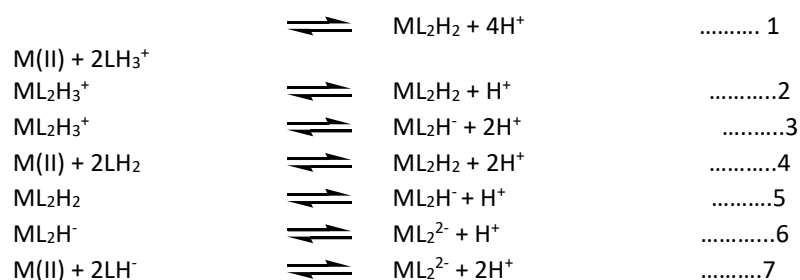
distribute between micelle surface and bulk water leaving less of the ligand available in the surface region of the micelle for reaction. Thus in either case, the  $\log \beta$  may decrease. Amino acids exist either as charged species or as zwitter ions, and so the probability of inserting them into the inner core is minimal. Since there is alkyl chain between the polar groups of Cys may not completely go into bulk water. Thus depending on the charge on amino acids and nature of the complex species, the stability varies. This trend reflects in all Cys-M(II) complexes (Figure 1).

**Fig. 1: Variation of stability constants of Cys complexes of (A) Co(II), (B) Ni(II) and (C) Cu(II) with mole fraction of SLS-water mixtures: ( $\square$ )  $\log \beta_{ML_2}$ , ( $\circ$ )  $\log \beta_{ML_2H}$  and ( $\Delta$ )  $\log \beta_{ML_2H_2}$ .**



### Distribution Diagrams

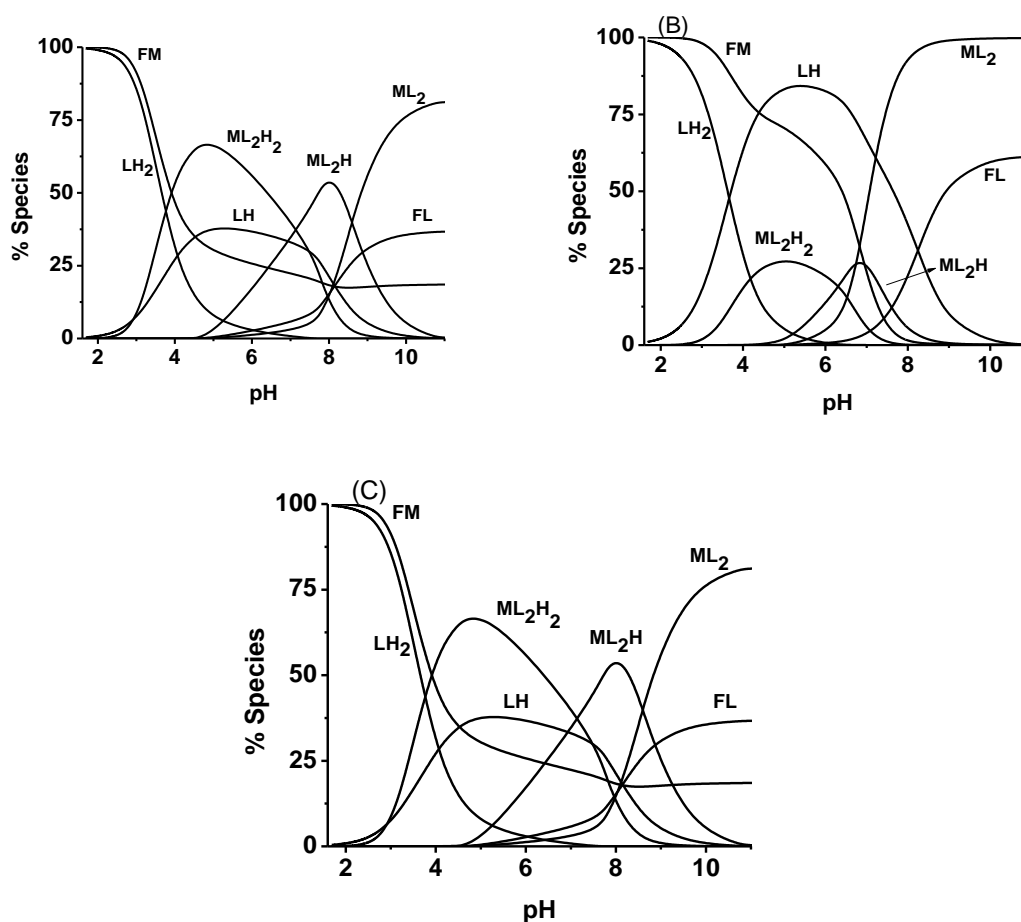
Cys is a tridentate ligand that has one dissociable (carboxylate group) and two associable (amino, thiol group) protons. The different forms of Cys are  $LH_3^+$ ,  $LH_2$  and  $LH^-$  in the pH ranges 2.0-4.0, 4.0-10.0 and 10.0-12.0 respectively. Hence, the plausible binary metal-ligand complexes can be predicted from these data. The present investigation reveals the existence of  $ML_2$ ,  $ML_2H$ , and  $ML_2H_2$  forms for Co(II), Ni(II) and Cu(II). The formation of various L-cysteine complex species is shown in the following equilibria



They indicate that the binary complexes of Co(II), Ni(II) and Cu(II) are formed in the pH range 2.0-10.5. The species  $ML_2H_2$  may be formed either from FM and  $LH_3^+$  /  $LH_2$  (Equilibria 1) or deprotonation of  $ML_2H_3^+$  (Equilibrium 2). The interaction of free metal ion with  $LH_3^+$  or  $LH_2$  at lower pH (Equilibria 1 and 4) and deprotonation of  $ML_2H_3^+$  at higher pH (Equilibrium 3) result in the formation of  $ML_2H_2$  species. The species  $ML_2H^-$  may be formed from deprotonation of either  $ML_2H_3^+$  or  $ML_2H_2$  (Equilibria 3 and 5). However the chances of formation of  $ML_2H^-$  from deprotonation of  $ML_2H_2$  are more than, since the concentrations of  $ML_2H_3^+$  is negligible in the pH range of formation of  $ML_2H^-$ . Hence it is concluded that the species  $ML_2H^-$  is formed from deprotonation of  $ML_2H_2$  rather than  $ML_2H_3^+$ .

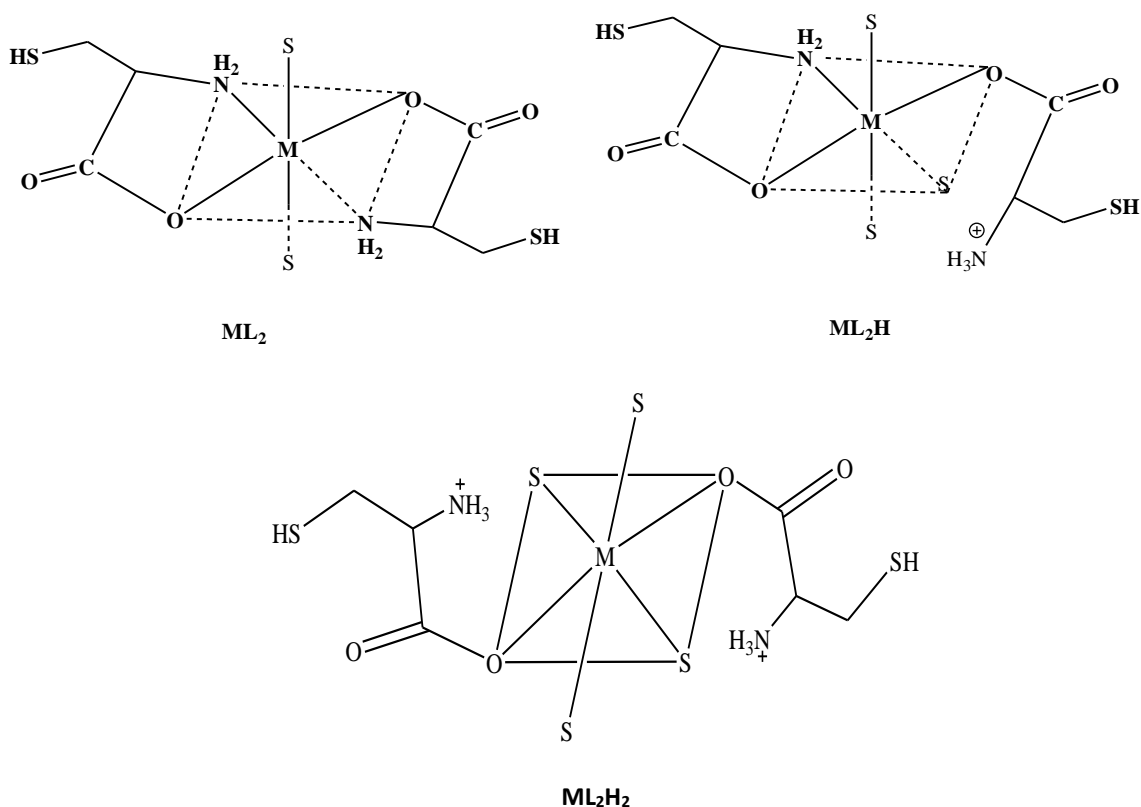
In the case of the species  $ML_2^{2-}$  may be formed from either  $M(II)$  and  $LH^-$  or deprotonation of  $ML_2H^-$  (Equilibria 7 and 6). From concentration considerations with increasing pH, the deprotonation of  $ML_2H^-$  is more prevalent than interaction of  $M(II)$  and  $LH^-$  form of ligand. Hence  $ML_2^{2-}$  is formed from deprotonation of  $ML_2H^-$ .

**Fig 2: Distribution diagrams of Cys complexes in 1.5% w/v SLS-water medium. (A)Co(II) (B)Ni(II) and (C)Cu(II).**



### Structures of binary complexes

Although it is not possible to elucidate or confirm the structures of complex species pH metrically, they can be proposed based on the literature reports and chemical knowledge. When the third donor site of the amino acid is a nitrogen atom, marked tridentate behavior is frequently found, more so when the additional chelation results in a five or six membered ring. Octahedral structures are proposed to the complexes of all the metal ions.



### CONCLUSIONS

The following conclusions have been drawn from the modeling studies of the speciation of binary complexes of Co(II), Ni(II), and Cu(II) with Cys in SLS-water mixture.

- L- Cysteine forms both protonated and unprotonated complexes in the pH range 2.5-10.5.
- The binary species detected are  $ML_2$ ,  $ML_2H$ , and  $ML_2H_2$ . These models are validated by statistical treatment of data.
- The linear variation of stability constants as a function of dielectric constant of the medium indicates the dominance of electrostatic forces over non-electrostatic forces.
- Some species are stabilized due to electrostatic interactions and some are destabilized due to the decreased dielectric constant.
- The order of ingredients influencing the magnitudes of stability constants due to incorporation of errors in their concentrations is alkali > acid > ligand > metal.

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