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Rao, G. Nageswara; Balakrishna, M.; Rao, G. Srinivasa; Ramanaiah, M.; Ramaraju, Bendi

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## Chemical Speciation of Binary Complexes of Co(II), Ni(II) and Cu(II) with 5-Sulfosalicylic acid in Urea-Water Mixtures

M.Balakrishna<sup>1,2</sup>, G. Srinivasa Rao<sup>2\*</sup>, M. Ramanaiah<sup>1</sup>, B.Ramaraju<sup>3</sup>  
and G. Nageswara Rao<sup>4</sup>

<sup>1</sup>Department of Chemistry, Aditya Institute of Technology and Management, Tekkali, A.P India.

<sup>2</sup>Department of Chemistry, GITAM Institute of Science, GITAM University, Visakhapatnam, A.P, India

<sup>3</sup>School of Material Science and Engineering, Nanyang Technological University, Singapore

<sup>4</sup>Department of Inorganic & Analytical Chemistry, Andhra University, Visakhapatnam, A.P India

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### ABSTRACT

*Speciation of Co(II), Ni(II) and Cu(II) complexes with 5-Sulfosalicylic acid (5-SSA) in the presence of urea-water mixtures at an ionic strength of 0.16 mol dm<sup>-3</sup> and temperature 303 K were investigated pH metrically. The existence of different binary complex species was established from modeling studies using the computer program MINQUAD75. The increased stability of the complexes with increasing urea was explained by electrostatic forces. The influence of the urea on the chemical speciation is discussed based on the dielectric constant of the medium. Distribution diagrams of various species of the complexes in relation to pH are presented.*

**Keywords:** 5-Sulfo salicylic acid; Speciation; Urea-Water; Binary complexes; Dielectric constant.

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### INTRODUCTION

The toxicity, bioavailability, bioaccumulation, biodegradability, persistence, mobility, solubility, extractability and many other critical properties depend on the form and nature of the chemical species [1-3]. Bioavailability of metal ions depends on either in free State or in binding state or in complexation state with various constituents present in the requisite amounts during biological reactions. The changes in various constraints like change in pH, temperature and ionic strength cause change in complexation behavior of metals and binding state. So complexation can signify the bioavailability of the metal ions in various biosystems [4, 5].

Speciation analysis, the determination of the concentrations of separate and unique atomic and molecular forms of an element instead of its total concentration in a sample is important in human biology, nutrition, toxicology and in clinical practice [6-8]. On the other hand, speciation profoundly influences both the toxicity and bioavailability of an element. The speciation study of toxic and essential metal ion complexes is useful to understand the role played by the active site cavities in biological molecules and the bonding behavior of drug residues with the metal ion [9, 10]. The species refined and their relative concentrations under the experimental conditions represent the possible forms of drugs in bio-fluids.

Cobalt is essential for the production of red blood cells. It acts as coenzyme in several biochemical processes. Cobalt in the form of vitamin B12 is essential for animals. Vitamin B12 is synthesized only by microorganisms, in

particular anaerobic bacteria. Nickel is associated with several enzymes [11-13] and any variation in its concentration leads to metabolic disorders [14]. Copper is largely rejected from cells but outside the cell, it is essential for the metabolism of many hormones and connective tissue. The biological functions include electron transfer, dioxygen transport, oxygenation, oxidation, reduction and disproportionation [15, 16].

The aim of the present study is to understand the role of metal ions at active site cavities in bioactive molecules like drugs, enzymes and proteins to know the effect of dielectric constant of the medium on the chemical speciation of the title systems. 5-SSA has been taken as a model compound for drug residues, since the dielectric constant at the active site cavities is very small compared to that at bio-fluids, low dielectric constant is mimicked by using a water soluble organic solvent like urea.

## MATERIALS AND METHODS

### Materials

5-SSA (TCI, India) solution ( $0.05 \text{ mol L}^{-1}$ ) was prepared in triple-distilled deionised water by maintaining  $0.05 \text{ mol L}^{-1}$  hydrochloric acid concentration to increase the solubility. Urea (Qualigens, India) was used as received.  $2 \text{ mol L}^{-1}$  sodium chloride (Qualigens, India) was prepared to maintain the ionic strength in the titrand.  $0.1 \text{ mol L}^{-1}$  aqueous solutions of Co(II), Ni(II) and Cu(II) chlorides were prepared by dissolving G.R. Grade (E-Merck, India) salts in triple-distilled water maintaining  $0.05 \text{ mol L}^{-1}$  hydrochloric acid to suppress the hydrolysis of metal salts. All the solutions were standardized by standard methods. 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 [17, 18]. The strengths of alkali and mineral acid were determined using the Gran plot method [19, 20]

### Apparatus

The titrimetric data were obtained using EQUIPTRONICS (Model EQ 614 A) pH meter (readability 0.01), which was calibrated with  $0.05 \text{ mol L}^{-1}$  potassium hydrogen phthalate in acidic region and  $0.01 \text{ mol L}^{-1}$  borax solution in basic region. The glass electrode was equilibrated in a well stirred urea-water mixture containing the inert electrolyte. All the titrations were carried out in the medium containing varying concentrations of urea-water mixtures (0.0-42.47% w/v) by maintaining an ionic strength of  $0.16 \text{ mol L}^{-1}$  with sodium chloride at  $303.0 \pm 0.1 \text{ K}$ . 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 [21].

### Procedure

For the determination of stability constants of metal-ligand binary species, initially titrations of strong acid with alkali were carried out at regular intervals to check whether complete equilibration was achieved. In each of the titrations, the titrand consisted of approximately 1 mmol mineral acid in a total volume of 50 mL. Titrations with different ratios (1:2.5, 1:3.75 and 1:5.0) of metal-to-ligand were carried out with  $0.4 \text{ mol L}^{-1}$  sodium hydroxide. Other experimental details are given elsewhere [22, 23].

### Modeling Strategy

The computer program SCPHD [24, 25] was used to calculate the correction factor. By using the pH-metric titration data, the binary stability constants were calculated with the computer program MINQUAD75 [26, 27], which exploit the advantage of the constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm. During the refinement of binary systems, the correction factor and the protonation constants of 5-SSA were fixed. The variation of stability constants with the dielectric constant of the medium was analyzed on electrostatic grounds on the basis of solute-solute and solute-solvent interactions.

## RESULTS AND DISCUSSION

The results of the final 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  $U_{\text{corr}}$  (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 [28].

**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 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, the residuals are tested for normal distribution. Such tests are  $\chi^2$ , Skewness, Kurtosis and R-factor [29]. These statistical parameters show that the best-fit models portray the metal-ligand species in urea-water mixtures, as discussed below.

**Table 1. Parameters of best fit chemical models of Co(II), Ni(II) and Cu(II) – 5-SSA complexes in urea-water mixtures. Temp= 303 K, Ionic strength=0.16 mol dm<sup>-3</sup>**

| % w/v Urea | log $\beta_{mlh}$ (SD) |           |                 | pH-Range   | NP | U <sub>corr</sub> | $\chi^2$ | Skewness | Kurtosis | R-factor |
|------------|------------------------|-----------|-----------------|------------|----|-------------------|----------|----------|----------|----------|
|            | ML                     | MLH       | ML <sub>2</sub> |            |    |                   |          |          |          |          |
| Co (II)    |                        |           |                 |            |    |                   |          |          |          |          |
| 0.0        | 4.03(90)               | 13.93(32) | 7.52(96)        | 1.80-9.80  | 81 | 53.38             | 44.24    | 0.25     | 4.14     | 0.03885  |
| 5.8        | 4.15(50)               | 13.72(24) | 7.82(89)        | 3.0-10.80  | 28 | 132.9             | 52.76    | 0.10     | 4.80     | 0.12908  |
| 11.52      | 4.21(41)               | 13.62(46) | 8.12(25)        | 1.97-9.50  | 30 | 10.37             | 11.69    | 0.11     | 4.85     | 0.01661  |
| 20.31      | 4.33(43)               | 13.81(57) | 8.28(93)        | 3.60-9.95  | 19 | 74.94             | 34.37    | 0.06     | 4.49     | 0.07797  |
| 29.64      | 4.42(32)               | 13.65(53) | 7.73(60)        | 3.50-10.0  | 19 | 123.3             | 14.44    | 0.07     | 3.47     | 0.08865  |
| 36.83      | 4.59(72)               | 13.75(88) | 7.99(54)        | 3.60-10.8  | 34 | 34.29             | 20.04    | 0.13     | 4.48     | 0.05839  |
| 42.47      | 4.72(61)               | 13.89(60) | 8.15(62)        | 6.85-10.8  | 18 | 87.47             | 7.93     | -0.34    | 2.02     | 0.09445  |
| Ni (II)    |                        |           |                 |            |    |                   |          |          |          |          |
| 0.0        | 4.12(49)               | 13.57(32) | 7.25(94)        | 1.90-10.10 | 73 | 30.36             | 16.80    | 0.25     | 3.86     | 0.11338  |
| 5.8        | 4.29(47)               | 13.70(59) | 7.47(52)        | 1.80-10.00 | 93 | 38.94             | 106.6    | -0.16    | 4.35     | 0.03562  |
| 11.52      | 4.45(32)               | 13.92(34) | 7.63(40)        | 3.00-10.50 | 18 | 86.40             | 100.8    | 0.16     | 3.58     | 0.18963  |
| 20.31      | 4.37(70)               | 13.74(31) | 7.82(58)        | 5.00-10.60 | 29 | 73.70             | 22.52    | -0.03    | 4.78     | 0.06494  |
| 29.64      | 4.50(52)               | 13.48(93) | 7.94(42)        | 4.40-10.90 | 09 | 45.98             | 20.83    | 0.01     | 8.88     | 0.03736  |
| 36.83      | 4.53(28)               | 13.49(33) | 8.03(21)        | 3.90-10.50 | 19 | 50.34             | 36.05    | 0.06     | 6.49     | 0.07979  |
| 42.47      | 4.77(46)               | 13.93(68) | 8.25(84)        | 3.90-10.80 | 30 | 82.40             | 23.60    | 0.01     | 5.47     | 0.08848  |
| Cu (II)    |                        |           |                 |            |    |                   |          |          |          |          |
| 0.0        | 4.74(91)               | 13.97(30) | 7.70(42)        | 1.80-9.90  | 58 | 44.02             | 27.56    | 0.23     | 3.84     | 0.32120  |
| 5.8        | 4.86(75)               | 13.64(56) | 7.84(56)        | 2.0-10.20  | 70 | 22.71             | 14.00    | 0.13     | 3.78     | 0.03297  |
| 11.52      | 4.98(74)               | 13.81(32) | 8.01(34)        | 2.0-9.60   | 48 | 17.01             | 23.00    | 0.22     | 3.33     | 0.03285  |
| 20.31      | 5.15(21)               | 13.66(16) | 8.14(23)        | 3.80-10.10 | 19 | 105.2             | 28.75    | 0.02     | 4.86     | 0.08333  |
| 29.64      | 5.33(62)               | 13.76(59) | 8.28(43)        | 3.80-10.40 | 32 | 53.13             | 25.00    | 0.11     | 4.29     | 0.07300  |
| 36.83      | 5.65(28)               | 13.42(87) | 8.43(21)        | 4.40-10.80 | 31 | 17.66             | 54.87    | 0.04     | 8.59     | 0.04399  |
| 42.47      | 5.88(60)               | 14.02(50) | 8.65(37)        | 4.90-10.20 | 22 | 37.37             | 82.36    | -0.01    | 6.57     | 0.05709  |

$$U_{corr} = U / (NP - m); m = \text{number of species}; NP = \text{number of experimental points}; SD = \text{standard}$$

**Deviation**

In the present study, the  $\chi^2$  values are less than the table values, and so the models are accepted. The kurtosis values in this study indicate that the residuals form leptokurtic pattern. The values of skewness recorded in Table 1 are between -0.34 and 0.25 for Co(II), -0.16 and 0.25 for Ni(II) and -0.01 and 0.23 for Cu(II). These data evince that the residuals form part of a normal distribution. Hence, least square method can be applied to the present data. The sufficiency of the model is further evident from crystallographic R-values. These statistical parameters thus show that the best-fit models portray the metal-ligand species in urea media.

**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 undertaken by introducing pessimistic errors in the influential parameters like concentrations of alkali, mineral acid, ligand, metal, log F and volume in Table 2. The order of the ingredients that influence the magnitudes of stability constants due to incorporation of errors is alkali > acid > metal > ligand > volume > log F. 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 suitability of the experimental conditions (concentrations of ingredients) and choice of the best-fit models.

**Table 2. Effect of errors in influential parameters on Ni(II) and 5-SSA complex stability constants in 36.83% w/v urea-water mixture**

| Ingredient | % Error | Log $\beta_{\text{min}}$ (SD) |            |          |
|------------|---------|-------------------------------|------------|----------|
|            |         | 110                           | 111        | 120      |
| Acid       | 0       | 4.53(28)                      | 13.49(33)  | 8.03(21) |
|            | -5      | 4.61(45)                      | 13.70(1.1) | Rejected |
|            | -2      | 4.50(30)                      | 13.53(30)  | 8.09(40) |
|            | +2      | 4.43(41)                      | 13.47(35)  | 8.08(46) |
|            | +5      | 4.40(76)                      | 13.59(52)  | 8.12(52) |
| Alkali     | -5      | 4.51(30)                      | 13.52(31)  | 8.00(32) |
|            | -2      | 4.54(29)                      | 13.56(42)  | 8.01(33) |
|            | +2      | 4.23(87)                      | 13.12(90)  | 8.03(22) |
|            | +5      | 4.23(95)                      | 12.78(1.2) | 7.59(77) |
|            | -5      | 4.40(65)                      | 13.20(87)  | 7.89(66) |
| Ligand     | -2      | 4.49(66)                      | 13.38(58)  | 8.21(87) |
|            | +2      | Rejected                      | 13.11(85)  | 8.01(35) |
|            | +5      | 4.48(44)                      | 13.50(62)  | 8.03(25) |
|            | -5      | 4.29(78)                      | 13.25(99)  | 7.98(60) |
|            | -2      | 4.45(65)                      | 13.45(25)  | 8.03(35) |
| Metal      | +2      | 4.50(60)                      | 13.50(71)  | 8.06(36) |
|            | +5      | 4.52(22)                      | 13.60(58)  | 8.10(61) |
|            | -5      | 4.43(12)                      | 13.44(16)  | 8.16(21) |
|            | -2      | 4.38(29)                      | 13.36(38)  | 8.12(82) |
|            | +2      | 4.32(38)                      | 13.52(22)  | 8.06(28) |
| Volume     | +5      | 4.43(23)                      | 13.26(89)  | 8.13(49) |
|            | -5      | 4.43(24)                      | 13.41(30)  | 8.15(52) |
|            | -2      | 4.66(65)                      | Rejected   | 8.22(55) |
|            | +2      | 4.45(22)                      | 13.45(19)  | 8.05(19) |
|            | +5      | 4.28(66)                      | Rejected   | 7.51(66) |

### Effect of Solvent

The dielectric constant is one of the characteristics of liquid. The metal-ligand stability constants are strongly affected by the dielectric constant of the medium because of the fact that at least one of the constituents is charged and other is either changed or has a dipole. Variations in the relative strengths of acids and bases with changing solvents should be a function of the charge, the radius of the ion and the dielectric constants of the medium [30].

When the ionization of an acid gives a net increase of ions, a decrease in the dielectric constant of the solvent should be accompanied by an increase in the stability constant of a weak acid dissolved in it. The variation of stability constant or change in free energy with co-solvent content depends upon two factors, viz, electrostatic and non-electrostatic forces. Born's classical treatment holds good in accounting for the electrostatic contribution to the free energy change [31]. According to this treatment, the energy of electrostatic interaction or stability constants should vary linearly as a function of the reciprocal of the dielectric constant (1/D) of the medium. Such linear variation of stability constants of 5-SSA in urea-water mixtures shows the dominance of electrostatic interactions and the trend is showed in Figure 1. Urea acts as a denaturant of macromolecules [32] by interacting with peptide groups through its amido group. In the same way, urea also interacts with drugs thereby decreasing the chelating power of the ligands. In addition, urea competes with the ligands for complexation with the metals. Hence, the stability of the species decreased with increasing urea content [33].

A plot of  $\log \beta$  versus 1/D (D is dielectric constant) should be linear if Born's classical treatment holds good indicating that electrostatic forces alone operate. The values of Co(II), Ni(II) and Cu(II) complexes of 5-SSA with 1/D reveals that the electrostatic forces are dominating the equilibrium process under the present experimental conditions. The solute-solvent interactions, relative thermodynamic stabilities and kinetic liabilities are also expected to play an important role.

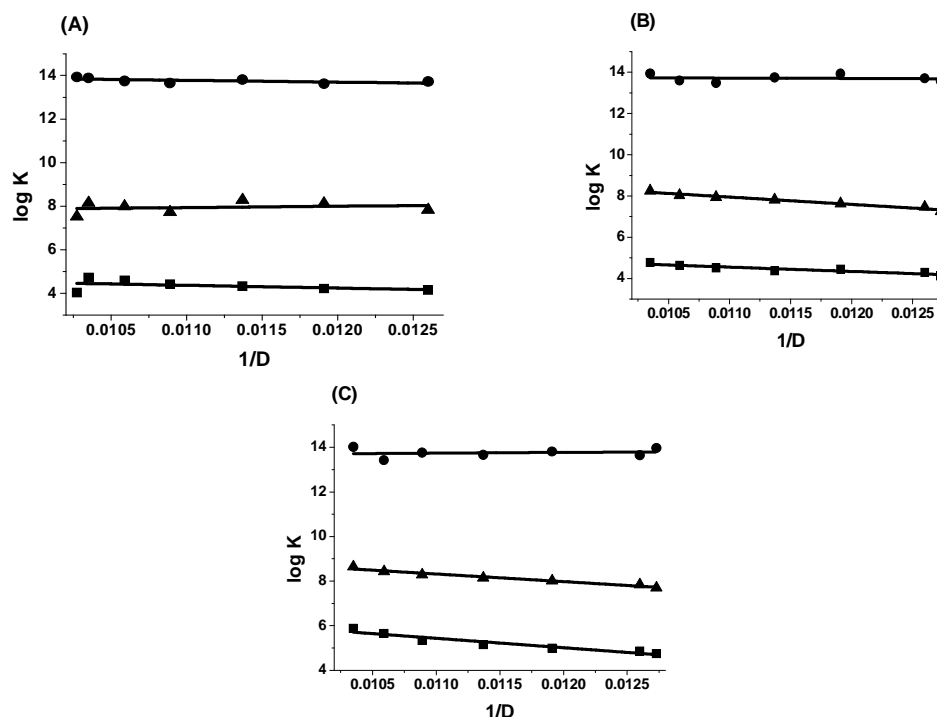
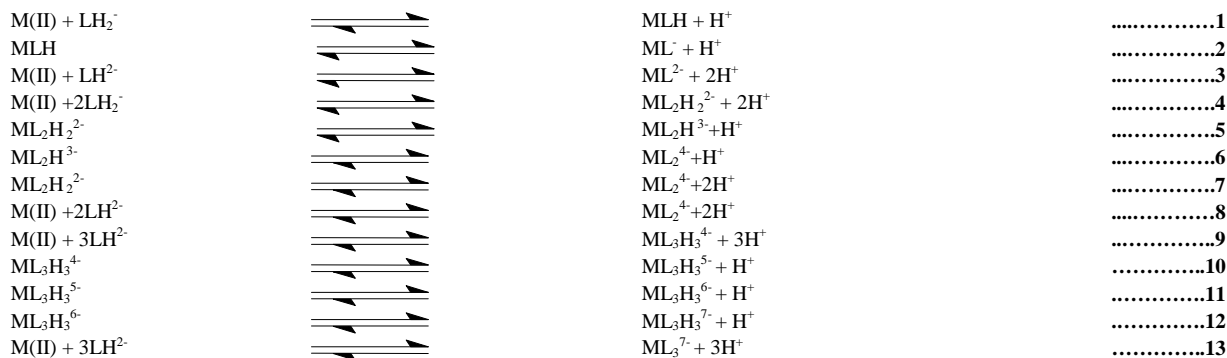


Figure 1. Variation of overall stability constant values of metal-5-SSA complexes with 1/D of Urea-water mixtures (a) Co(II); (b) Ni(II); (c) Cu(II); (■) log  $\beta_{ML}$ ; (▲) log  $\beta_{MLH}$ ; (●) log  $\beta_{ML_2}$ . Distribution Diagrams

5-SSA is a tridentate ligand, it has one carboxyl ( $-\text{COOH}$ ), one hydroxyl ( $-\text{OH}$ ) and one sulfo ( $-\text{SO}_3\text{H}$ ) groups. The different forms of 5-SSA are  $\text{LH}_2^-$ ,  $\text{LH}^{2-}$ , and  $\text{L}^{3-}$  in the pH range 1.0-8.0, 1.0-11.0, > 6.5-11.0, respectively. Hence, the plausible binary metal-ligand complexes can be predicted from these data. The present investigation reveals the existence of ML, MLH, and  $\text{ML}_2$  for Co(II), Ni(II) and Cu(II). The formation of various 5-SSA complex species is shown in the following equilibria.



Some typical distribution diagrams in urea-water mixtures are shown in Figure 2. They indicate that the binary complexes of Co(II), Ni(II) and Cu(II) are formed in the pH range 1.5-11.5. MLH, ML and  $\text{ML}_2$  are simultaneously formed with the increasing pH. ML,  $\text{ML}_2$  species percentage successively increases with increasing pH. Successive deprotonation of MLH forms ML beyond a pH 5.0. The percentage of the  $\text{ML}_2$  species increases successively with increasing in pH up to 11.5. The concentration of MLH species decreased, while the concentration of ML and  $\text{ML}_2$  increased in the pH range 4.0-11.5.  $\text{ML}_2$  formed at higher pH with high percentage in the case of Co(II), Ni(II) and Cu(II).

MLH species is formed at pH less than 2.0 (Equilibrium 1) by interacting  $\text{LH}_2^-$  with free metal, which might have been quickly deprotonated to ML (Equilibrium 2) at more than pH 4.0. Free metal reacts with  $\text{LH}^{2-}$  to form  $\text{ML}_2$

(Equilibrium 8) at a pH greater than 6.0. The deprotonation of  $ML_2H^{3-}$  and  $ML_2H_2^{2-}$  results to form  $ML_2$  at more pH (Equilibrium 6 and 7) in the case of Co(II), Ni(II) and Cu(II).

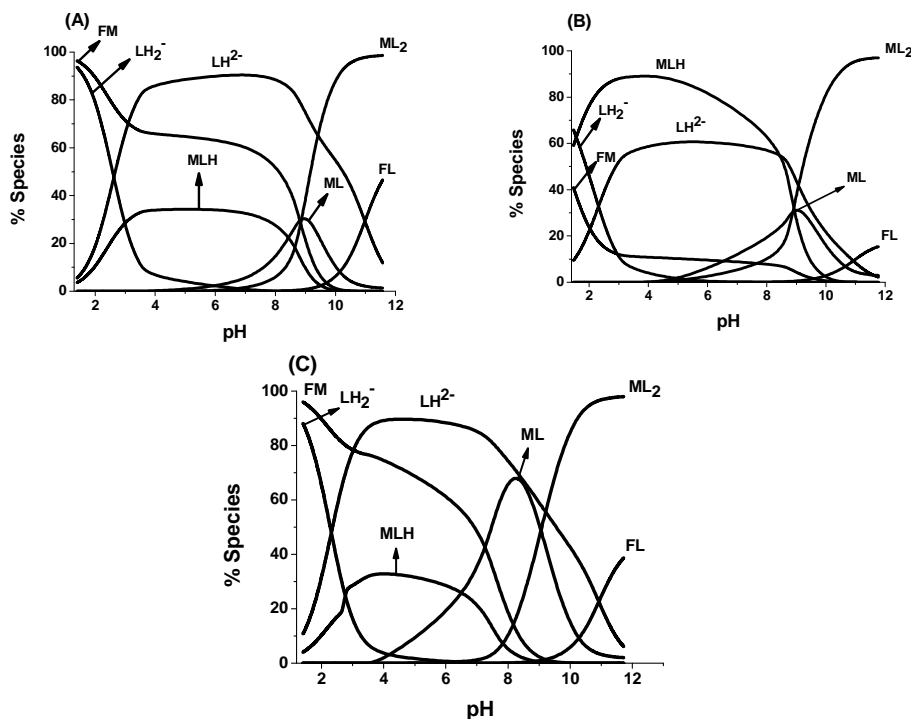
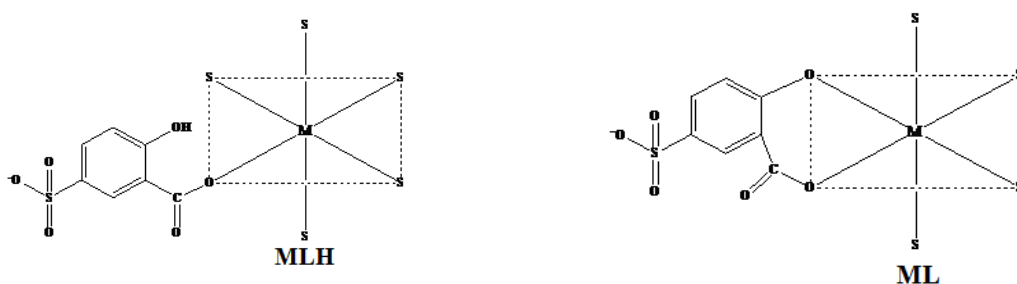


Figure 2: Distribution diagrams of binary complexes of 5-SSA in 5.8% w/v urea-water mixture: (a) Co(II), (b) Ni(II) and (c) Cu(II)

### Structures of complexes

5-SSA has three functional groups such as -OH, -COOH and  $SO_3H$  groups, and is helpful to form different complexes with Co(II), Ni(II) and Cu(II). Octahedral structures are proposed to the complexes of all the metal ions. The VSEPR theory suggests that Co(II), Ni(II) and Cu(II) complexes shall be octahedral because, there are six outer electron pairs. This argument supports the structures of complexes proposed in Figure 3.



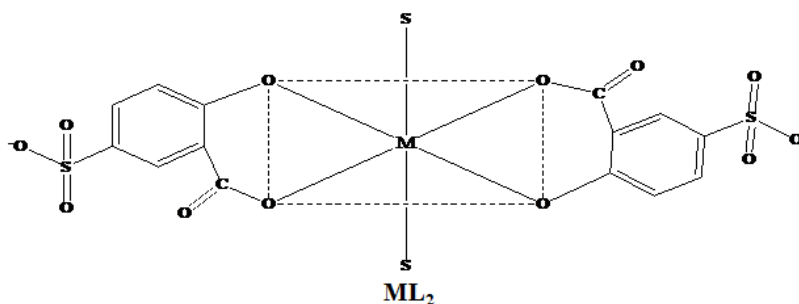


Figure 3: Structure of 5-SSA complexes, where S is either solvent or water molecules

### CONCLUSION

The following conclusions have been drawn from the modeling studies of 5-SSA complexes of Co(II), Ni(II) and Cu(II) in urea-water mixtures.

1. 5-SSA forms both protonated and unprotonated complexes under pH range 4.0–11.0
2. The complex species formed due to interaction of 5-SSA with the essential metals are ML, MLH and  $ML_2$ .
3. The linear variation of the stability constants with  $1/D$  of the urea indicates the dominance of electrostatic forces over non-electrostatic forces.
4. The order of the components in influencing the magnitudes of the stability constants due to incorporation of errors is alkali > acid > ligand > metal > volume > Log F.
5. Some species are stabilized due to electrostatic interactions and some are destabilized due to the decreased dielectric constant.

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