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# Speciation of Co(II), Ni(II) and Cu(II) Complexes with L-Glutamic Acid in Dioxan–Water Mixtures

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ABSTRACT

**Chemical speciation of Co(II), Ni(II) and Cu(II) complexes of L-glutamic acid in the presence of Dioxan-Water mixtures at an ionic strength of 0.16 mol $L^{-1}$ at 303 K was studied pH– metrically. Glu is taken as a model compound for amino acid residues and dioxan is used to mimic the low dielectric constant at the active site cavities in bioactive molecules like enzymes and proteins. The active forms of the ligand were $LH_3^+$, $LH_2$, $LH^-$ and $L^{2-}$. The models containing different numbers of species were refined using the computer program, MINIQUAD75. The predominant species detected were $ML_2H_2$, $ML_2H^-$, $ML_2^{2-}$, $MLH^+$ and ML. The best fit chemical models were arrived at based on statistical parameters. The trend in variation of complex stability constants with the medium composition was explained on the basis of changes in the dielectric constant of the solution. Effect of errors in the ingredients on the stability constants was also studied. Chemical speciation is discussed based on the distribution diagrams.**

KEYWORDS

**Complex equilibria, chemical speciation, L-glutamic acid, essential metals, dioxan.**

## 1. Introduction

Speciation studies of essential metal ion complexes of L-glutamic acid (Glu) are useful[1–4] for the understanding of the role played by active site cavities in biological molecules and the binding behaviour of protein residues with metal ions.

Cobalt in the form of vitamin $B_{12}$ or one of the cobinamides is essential for the production of red blood cells. It acts as a coenzyme in several biochemical processes. Although cobalt is an essential element for life in minute amounts (10 mg $day^{-1}$), at higher levels of exposure it shows mutagenic and carcinogenic effects.[5] Minot and Murphy[6] discovered that pernicious anaemia can be treated by feeding patients with large amounts of liver which contains vitamin $B_{12}$.

Nickel is also present in specific environments of nucleic acids (or nucleic acid-binding proteins), since nickel activates the gene for hydrogenase[7]. Nickel plays numerous roles in the biology of microorganisms and plants[8–9]. Nickel is also found in enzymes, such as urease, which is a dinuclear Ni(II)-containing metalloenzyme. This enzyme accounts for 6 % of the soluble cellular proteins[10–12] and catalyzes the hydrolysis of urea to yield ammonia and carbamate.

Copper is distributed widely in the body and occurs in the liver, muscle and bones. The Recommended dietary allowance for copper in normal healthy adults is 0.9 mg $day^{-1}$(ref. 13). The biological functions include electron transfer, dioxygen transport, oxygenation, oxidation, reduction and disproportionation. The blue copper proteins that participate in electron transport include azurin and plastocyanin. Copper deficiency can often produce anaemia-like symptoms. Copper is essential for the production of insulin.

Diabetic patients with vascular complications have higher plasma copper levels than diabetic patients without complications or normal controls[14]. High accumulation of copper in the liver, kidney and brain causes Wilson's disease[15,16], which arises due to a genetic disorder in copper metabolism. The alterations in its cellular homeostasis are related to neurodegenerative diseases, like Parkinsonism, Alzheimer's disease[17], familial amyotrophic lateral scelerosis[18,19] and prion disease[20].

1,4-Dioxan[21] (Dox) is miscible with water and most organic solvents. It is a non-polar solvent capable of acting as hydrogen bond acceptor with random structure. Dox is primarily used as a solvent in the manufacturing sector, but it is also found in fumigants and automotive coolant. It is also a contaminant in cosmetics and personal care products such as deodorants, shampoos, toothpastes and mouthwashes.[22]

Glu is a non-essential amino acid, interconvertible to glutamine, which is important in preventing ammonia intoxication. Adults may ingest 20–35 mg $day^{-1}$ of this amino acid without any apparent ill-effects. Glu is a ubiquitous amino acid present in many foods either in free form or in peptides and proteins.[23] It is an excitatory neurotransmitter for the central nervous system, the brain and spinal cord. It is important in the metabolism of sugars and fats. It aids in the transportation of potassium into the spinal fluid. It acts as fuel for the brain and helps correct personality disorders. It is used in the treatment of epilepsy, mental retardation, muscular dystrophy and ulcers.[24–29] The interaction of the side chain of Glu with metal ions is of importance in metalloenzymes.

The aim of the present study is to mimic the role of the metal ions, Co(II), Ni(II), Cu(II), at the active site cavities in bioactive molecules like enzymes and proteins. Glu is taken as a model compound for amino acid residues. Since the dielectric constant at the active site cavities is small compared to biofluids, the authors tried to mimic low dielectric constant by using a water soluble organic solvent like dioxan.

## 2. Experimental

### 2.1. Materials and Methods

1,4 Dioxan (Qualigens, India) was used as received. Aqueous solutions of L-glutamic acid of 0.05 mol $L^{-1}$ (Merck, Germany) and hydrochloric acid of 0.2 mol $L^{-1}$ (Qualigens, India) were prepared in triple distilled, deionized water. Sodium chloride

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(Merck, India) of 2 mol $L^{-1}$ was prepared to maintain the ionic strength in the titrand. Solutions of Co(II), Ni(II) and Cu(II) chlorides were prepared. To increase the solubility of glutamic acid 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 (Merck, India) of 0.4 mol $L^{-1}$ was prepared. Sodium hydroxide was standardized by titration against oxalic acid and potassium hydrogen phthalate. HCl was standardized against borax solution and the standardized sodium hydroxide solution. The end points of the titrations were determined using the Gran plot Method.[31–32] The concentrations of the metal ions were determined complexometrically by titrating against a standard solution of EDTA using xylenol orange as indicator and hexamine powder as buffer to maintain the pH at 6.0 for Co(II), murexide indicator and $NH_3/NH_4Cl$ buffer to maintain the pH at 7.0–10.0 for Ni(II), fast sulphone black-F as indicator and ammonia to maintain basic pH for Cu(II). To assess the errors that might have crept into the determination of the concentrations, the data were subjected to analysis of variance using a one way ANOVA.[30]

### 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 Dox-water mixture containing the inert electrolyte. 10 mL Corning microburettes of 0.02 readability were used in the titrations . All titrations were carried out at 303 ± 0.1 K in a medium containing varying concentrations of Dox-water mixtures (0–60 % v/v) at an ionic strength of 0.16 mol $L^{-1}$ (sodium chloride). 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 factors.[33]

### 2.3. Procedure

Initially strong acid was titrated against alkali at regular intervals to check the complete equilibration of the glass electrode. The calomel reference electrode was refilled with Dox-water mixture of equivalent composition to that of the titrand. In each of the titrations, the titrand consisted of approximately 1 mmol mineral acid in a total volume of 50.0 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 mol $L^{-1}$ sodium hydroxide[34].

### 2.4. Modeling Strategy

The computer program SCPHD[35] was used to calculate the glass electrode correction factor. By using pH-metric titration data, the binary stability constants were calculated with the computer program MINIQUAD75[36] which exploits the advantage of constrained least-squares method in the initial refinement and the reliable convergence of the Marquardt algorithm. The correction factor and protonation constants[37] (Table 1) of Glu were fixed during the refinement of the binary systems.

## 3. 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 2. 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 the mean standard deviation and mean deviation for the systems are validated by the residual analysis. The stability constants of the binary complexes in aqueous solution are comparable with those reported in the literature[38,39] (Table 3).

**Table 1** Best-fit chemical models of protonation equilibria of L-glutamic acid in Dox-water mixtures.

| % v/v Dox | Dielectric constant of Dox-water mixture | $\log \beta_1$ | $\log \beta_2$ | $\log \beta_3$ |
|---|---|---|---|---|
| 00.0 | 78.48 | 9.78 | 14.01 | 16.19 |
| 10.0 | 70.33 | 9.80 | 14.18 | 16.43 |
| 20.0 | 61.86 | 9.95 | 14.57 | 17.17 |
| 30.0 | 53.28 | 10.09 | 14.98 | 17.75 |
| 40.0 | 44.54 | 10.20 | 15.31 | 18.29 |
| 50.0 | 35.85 | 10.23 | 15.51 | 18.71 |
| 60.0 | 27.21 | 10.47 | 16.04 | 19.47 |

### 3.1. Residual Analysis

In data analysis with the least squares method, the residuals (the differences between the experimental data and the data simulated based on model parameters) are assumed to follow a Gaussian or normal distribution. 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. Furthermore, a model is considered adequate only if the residuals are normally distributed and do not show any trend. The hypothesis that the errors are random, following a normal distribution is best gauged in terms of $\chi^2$, skewness, kurtosis and the R factor. These statistical parameters are given in Table 2 and show that the best fit models are a good portrayal the metal-ligand species in Dox-water mixtures.

The values of skewness recorded in Table 2 are between –0.72 and 0.60 for Co(II), –1.68 and 1.97 for Ni(II) and –0.23 and 0.22 for Cu(II). The kurtosis values in the present study indicate that the residuals form a leptokurtic pattern. The $\chi^2$ values are in the range 1.75 to 38.36 for different number of points and all of them are less that the table values.[30] The pH meter readability (0.01) was taken as the $R_{lim}$ and all the Crystallographic R values given in Table 2 are less than the $R_{lim}$. These data evince that the residuals form a normal distribution and hence, the least squares method can be applied to the present data.

### 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, concentration and titration volume (Table 4). The sensitivity of the measured stability constants to errors in concentration are in the order: 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 conform the appropriateness of the chosen best fit models. This study also indicates the relative sensitivities of model parameters.

### 3.3. Effect of Solvent

The Dox-water mixtures are the combination of aprotic and protic solvents with a wide range of dielectric constants and with good solubility for polar as well as non-polar solutes. The cosolvent induced increased basicity of Dox-water mixtures,

**Table 2** Parameters of best fit chemical models of Co(II), Ni(II) and Cu(II)-Glu complexes in Dox-water mixtures.

| %v/v Dox | $\log \beta_{mlh}$ (SD) | | | | | pH range | NP | $U_{corr} \times 10^8$ | $\chi^2$ | Skewness | Kurtosis | R-factor |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 111 | 110 | 122 | 121 | 120 | | | | | | | |
| | | | | | | Co (II) | | | | | | |
| 00.0 | — | 4.53(29) | 23.31(79) | 16.82(35) | 8.22(16) | 5.0-10.0 | 28 | 0.09 | 18.10 | -1.23 | 4.82 | 0.0096 |
| 10.0 | — | 4.64(28) | 23.73(60) | 16.81(46) | 8.55(20) | 5.2-10.0 | 29 | 0.10 | 9.46 | 0.65 | 4.82 | 0.0099 |
| 20.0 | — | 5.01(27) | 23.99(29) | 17.15(44) | 9.20(12) | 5.0-10.0 | 31 | 0.12 | 3.95 | 0.54 | 2.56 | 0.0106 |
| 30.0 | — | 5.06(16) | 24.09(18) | 16.69(91) | 8.85(8) | 5.0-10.3 | 38 | 0.07 | 10.63 | 0.22 | 3.09 | 0.0082 |
| 40.0 | — | 5.57(18) | 25.02(9) | 17.48(73) | 9.74(9) | 5.0-10.2 | 37 | 0.09 | 16.21 | 0.63 | 3.10 | 0.0087 |
| 50.0 | — | 6.13(23) | 25.38(8) | 18.45(43) | 10.89(10) | 5.0-10.0 | 36 | 0.11 | 10.81 | 0.89 | 4.02 | 0.0091 |
| 60.0 | — | 5.97(20) | 25.65(7) | 18.43(46) | 10.65(8) | 5.0-10.0 | 43 | 0.09 | 14.94 | 1.68 | 8.91 | 0.0080 |
| | | | | | | Ni (II) | | | | | | |
| 00.0 | — | 5.83(5) | 22.98(26) | — | 10.32(7) | 3.5-9.0 | 46 | 0.07 | 8.90 | -0.26 | 3.49 | 0.0067 |
| 10.0 | — | 6.02(5) | 23.56(10) | — | 10.62(6) | 4.0-9.0 | 38 | 0.05 | 8.95 | 0.13 | 2.40 | 0.0062 |
| 20.0 | — | 6.53(5) | 24.63(5) | — | 11.33(7) | 4.0-9.0 | 40 | 0.06 | 8.53 | 0.12 | 2.80 | 0.0065 |
| 30.0 | — | 6.72(5) | 25.07(5) | — | 11.41(8) | 4.0-9.0 | 46 | 0.08 | 6.12 | 0.11 | 3.17 | 0.0071 |
| 40.0 | — | 7.00(4) | 25.46(4) | — | 11.93(7) | 4.0-9.0 | 45 | 0.06 | 10.60 | -0.12 | 3.48 | 0.0058 |
| 50.0 | — | 7.43(11) | 25.71(14) | — | 13.05(17) | 4.0-9.0 | 47 | 0.05 | 12.59 | -0.40 | 3.61 | 0.0175 |
| 60.0 | — | 7.45(4) | 26.31(5) | — | 13.11(7) | 4.0-9.0 | 54 | 0.08 | 13.51 | -0.28 | 3.77 | 0.0067 |
| | | | | | | Cu (II) | | | | | | |
| 00.0 | 12.80(13) | — | 24.95(17) | 20.73(8) | 15.30(8) | 3.0-8.0 | 52 | 0.16 | 38.36 | 0.10 | 3.50 | 0.0094 |
| 10.0 | 12.76(6) | — | 25.20(6) | 20.96(3) | 15.31(4) | 3.0-8.0 | 51 | 0.03 | 5.59 | -0.14 | 2.61 | 0.0043 |
| 20.0 | 13.60(2) | — | 26.00(5) | 21.80(2) | 16.05(2) | 3.0-8.0 | 52 | 0.14 | 11.79 | -0.67 | 4.02 | 0.0027 |
| 30.0 | 14.13(4) | — | 26.78(11) | 22.52(4) | 16.39(7) | 3.0-8.0 | 56 | 0.06 | 5.52 | -0.05 | 3.37 | 0.0054 |
| 40.0 | 14.68(3) | — | 27.52(8) | 23.04(3) | 16.71(5) | 3.0-8.0 | 53 | 0.03 | 1.75 | -0.26 | 3.29 | 0.0038 |
| 50.0 | 15.25(3) | — | 28.59(5) | 23.70(4) | 17.59(5) | 3.0-8.0 | 57 | 0.04 | 27.41 | -0.58 | 3.94 | 0.0040 |
| 60.0 | 15.95(2) | — | 29.87(3) | 24.55(3) | 18.05(3) | 3.0-8.0 | 63 | 0.16 | 22.43 | -0.48 | 4.43 | 0.0026 |

$U_{corr}$ = U/(NP-m); NP = Number of points; m = number of stability constants; SD = Standard deviation

**Table 3** Comparison of stability constants of binary complexes of glutamic acid in aqueous solution with the literature values.

| %v/v Dox | $\log \beta_{mlh}$ | | | | | Reference |
|---|---|---|---|---|---|---|
| | 111 | 110 | 122 | 121 | 120 | |
| | | | **Co(II)** | | | |
| 00.0 | — | 4.53 | 23.31 | 16.82 | 8.22 | Present work |
| | | 4.58 | 22.94 | | 7.75 | 39, 40 |
| | | | **Ni(II)** | | | |
| 00.0 | — | 5.83 | 22.98 | — | 10.32 | Present work |
| | | 5.14 | | 16.47 | 9.56 | 39, 40 |
| | | | **Cu(II)** | | | |
| 00.0 | 12.80 | — | 24.95 | 20.73 | 15.30 | Present work |
| | 12.52 | 7.99 | 24.11 | 19.50 | 14.49 | 39, 40 |

increases the stabilization of protons. At the same time the coordinating solvent (Dox) competes with the ligands for coordination with the metals. This decreases the stability of the complexes. Hence, the stability of the complex is expected to either increase or decrease.

The variation of overall stability constant values with cosolvent content depends upon electrostatic and non-electrostatic factors. The variation of log $K^M$ or change in free energy ($\Delta G^M$) with cosolvent content is separable into two terms, *viz.* electrostatic ($\Delta G_{el}^M$) and non-electrostatic ($\Delta G_{nel}^M$) as given in Equation 1.

$$\Delta G^M = -RT \ln K^M = \Delta G_{el}^M + \Delta G_{nel}^M \quad (1)$$

Born's[40] classical treatment holds good in accounting for the electrostatic contribution to the free energy change. In this model, an ion of charge $Z_i e_o$ is considered to be a rigid sphere of radius, r. The free energy of Ion-Solvent (or electrostatic) interaction ($G_{el}$) is assumed to be equal to the sum of work done to discharge the ion in vacuum and to charge it again in a medium of dielectric constant, $D_s$ (Equation 2).

$$G_{el} = \frac{Ne^2}{2} \times \frac{1}{D_s} \times \frac{1}{r} \quad (2)$$

From Equations 1 and 2, it can be concluded that a plot of log $K^M$ versus 1/D should be linear. The trends of stability constant ($\log\beta$) values of complexes of Glu with 1/D in Dox-water mixture are given in Fig. 1. The linear trend indicates that the dielectric constant or long range interactions are responsible for the stability trend. This linear increase indicates the dominance of the structure-forming nature of Dox over its complexing ability.

**Table 4** Effect of errors in influential parameters on Cu-Glu complex stability constants in 20 % v/v Dox-water mixture.

| % Error | | $\log\beta$ (SD) | | | |
|---|---|---|---|---|---|
| | | MLH | $ML_2H_2$ | $ML_2H$ | $ML_2$ |
| 0 % | | 13.60(2) | 26.00(5) | 21.80(2) | 16.05(2) |
| Alkali | –5 | 12.48(8) | Rejected | 19.39(7) | Rejected |
| | –2 | 13.14(6) | 25.36(12) | 20.96(4) | 14.40(8) |
| | 2 | Rejected | 26.97(5) | 21.99(14) | 7.23(8) |
| | 5 | Rejected | 27.65(9) | 21.96(219) | 18.69(12) |
| Acid | –5 | Rejected | 27.64(7) | 22.66(28) | 18.46(9) |
| | –2 | Rejected | 26.97(5) | 22.03(12) | 17.08(8) |
| | 2 | 13.14(5) | 25.41(10) | 21.08(3) | 14.81(6) |
| | 5 | 12.52(9) | Rejected | 19.96(6) | 11.83(31) |
| Ligand | –5 | 13.57(3) | 26.06(6) | 21.88(2) | 16.44(3) |
| | –2 | 13.59(2) | 26.02(5) | 21.84(2) | 16.21(2) |
| | 2 | 13.60(3) | 25.99(5) | 21.76(2) | 15.88(3) |
| | 5 | 13.61(3) | 25.98(6) | 21.69(3) | 15.59(4) |
| Metal | –5 | 13.62(2) | 26.11(4) | 21.89(2) | 16.27(2) |
| | –2 | 13.61(2) | 26.04(4) | 21.84(2) | 16.14(2) |
| | 2 | 13.59(2) | 25.96(6) | 21.77(2) | 15.97(3) |
| | 5 | 13.57(3) | 25.90(7) | 21.72(2) | 15.84(3) |
| Volume | –5 | 13.55(2) | 25.95(5) | 21.74(2) | 15.99(2) |
| | –2 | 13.58(2) | 25.98(5) | 21.78(2) | 16.03(2) |
| | 2 | 13.61(2) | 26.02(5) | 21.83(2) | 16.07(2) |
| | 5 | 13.64(2) | 26.04(5) | 21.86(2) | 16.11(2) |

### 3.4. Distribution Diagrams

Glu has two dissociable protons and one amino group which can associate with a proton. It exists as $LH_3^+$ at low pH and on deprotonation forms the species, $LH_2$, $LH^-$ and $L^{2-}$ successively in the pH ranges 2.0–6.0, 3.0–11.0 and above 8.0, respectively[37]. Hence, the plausible binary metal-ligand complexes can be predicted from these data. The present investigation reveals the existence of ML, $ML_2H_2$, $ML_2H$ and $ML_2$ for Co(II), ML, $ML_2H_2$ and $ML_2$ for Ni(II) and MLH, $ML_2H_2$, $ML_2H$ and $ML_2$ for Cu(II). The formation of various binary complex species is shown in the following equilibria. Some typical distribution diagrams in Dox-water mixtures are shown in Fig. 2. They indicate that the binary complexes of Co(II), Ni(II) and Cu(II) are formed in the pH range 3.0–10.3.

$$M(II) + LH_3^+ \rightleftharpoons MLH^+ + 2H^+ \quad (3)$$

$$M(II) + 2LH_3^+ \rightleftharpoons ML_2H_2 + 4H^+ \quad (4)$$

$$MLH^+ + LH_2 \rightleftharpoons ML_2H_2 + H^+ \quad (5)$$

$$M(II) + 2LH_2 \rightleftharpoons ML_2H_2 + 2H^+ \quad (6)$$

$$ML_2H_2 \rightleftharpoons ML_2H^- + H^+ \quad (7)$$

$$ML_2H^- \rightleftharpoons ML_2^{2-} + H^+ \quad (8)$$

$$ML_2H_2 \rightleftharpoons ML_2^{2-} + 2H^+ \quad (9)$$

$$M(II) + LH_2 \rightleftharpoons ML + 2H^+ \quad (10)$$

$$ML + LH^- \rightleftharpoons ML_2^{2-} + H^+ \quad (11)$$

$$M(II) + LH^- \rightleftharpoons ML + H^+ \quad (12)$$

Figure 2A shows the formation of ML, $ML_2H_2$, $ML_2H$ and $ML_2$ species in Co-Glu system. Free metal reacts with $LH_2$ to form $ML_2H_2$ at low pH (Equilibrium 8). Above a pH of 5.0, $ML_2H_2$ is

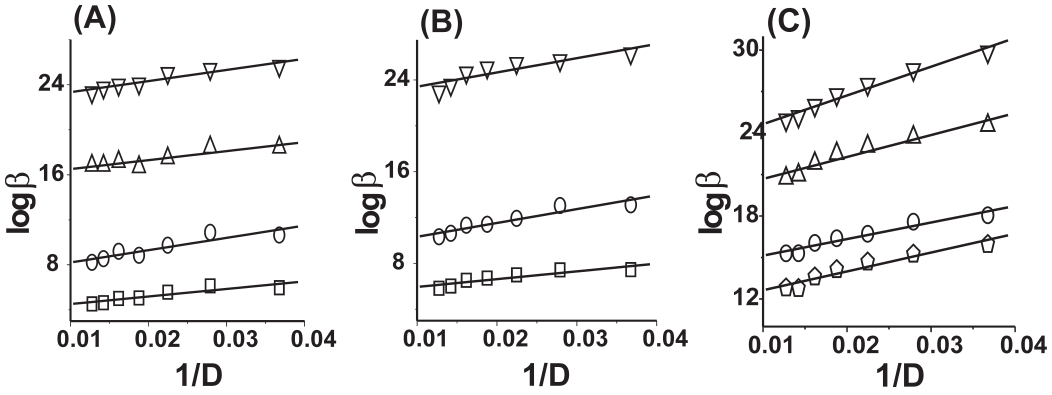



**Figure 1** Variation of stability constants of Glu complexes with reciprocal of dielectric constant (1/D) of Dox-water mixtures: (**A**) Co(II), (**B**) Ni(II) and (**C**) Cu(II); (□ $\log\beta_{ML}$, (⬠) $\log\beta_{MLH}$, (▽) $\log\beta_{ML2H2}$, (△) $\log\beta_{ML2H}$, (○) $\log\beta_{ML2}$.

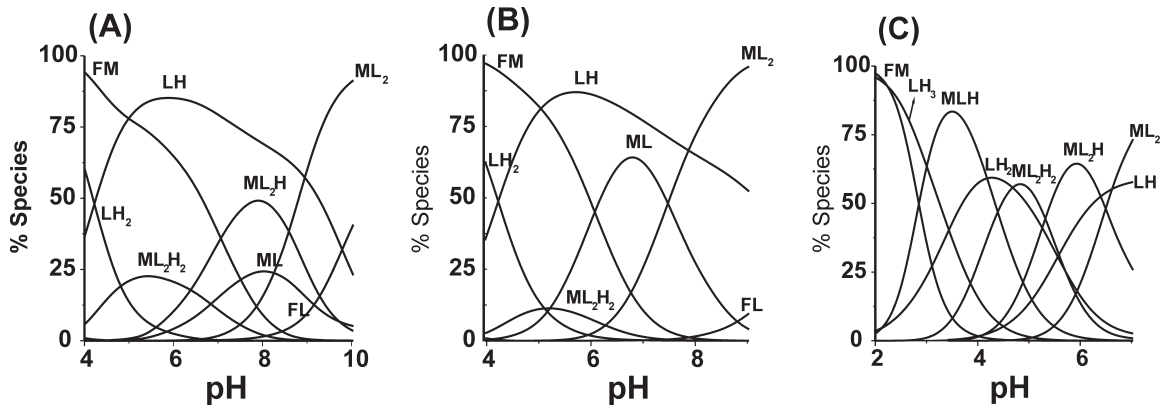



**Figure 2** Distribution diagrams of binary complexes of Glu in Dox-water mixtures in (**A**) 0.0 % v/v Co(II), (**B**) 0.0 % v/v Ni(II) and (**C**) 60.0 % v/v Cu(II).

deprotonated to $ML_2H$ and $ML_2$ (Equilibria 7–9). Free metal also reacts with the ligand to form ML (Equilibra 10 and 12). Figure 2B shows the formation of Ni-Glu complexes. The concentration of $ML_2H_2$ decreases, while the concentration of $ML_2$ increases above pH 6.0 (Equilibrium 9). $ML_2$ is also formed from ML and $LH^-$ (Equilibrium 11). Figure 2C shows the formation of Cu-Glu complexes in the pH range 2.0–8.0. The concentrations of MLH, $ML_2H_2$ and $ML_2H$ species are increased below a pH of 6.0 (Equilibria 3–7). The decreasing concentrations of $ML_2H_2$ and $ML_2H$ with increasing concentration of $ML_2$ support Equilibria 8 and 9.

### 3.5. Structures of Complexes

Amino nitrogen and carboxyl oxygen of Glu participate in bonding with metal ions. Amino nitrogen atoms can also associate with hydrogen ions in physiological pH ranges. Hence, there is often significant competition between hydrogen and metal ions for this donor site. This situation results in the simultaneous existence of a number of equilibria producing an array of protonated complexes as found in this study. Although it is not possible to elucidate or confirm the structures of complex species pH metrically, it is possible to postulate structures based on comparison with know structures for related complexes. Literature shows that, in aqueous solution, Co(II), Ni(II) and Cu(II) ions typically form octahedral complexes with Cu(II) normally being Jahn-Teller distorted[41,42] Thus octahedral structures have been proposed tentatively as given in Fig. 3. The structures are proposed based on the following argument.

Equilibrium constants for the formation of different species are calculated from the overall stability constants for some typical systems as given below.

**Cu(II)**

$$ML_2H_2 \rightleftharpoons ML_2H + H^+ \quad \Delta \log K^{ML_2H_2}_{ML_2H} = 4.22 \qquad (13)$$

$$ML_2H \rightleftharpoons ML_2 + H^+ \quad \Delta \log K^{ML_2H}_{ML_2} = 5.43 \qquad (14)$$

**Co(II)**

$$ML_2H_2 \rightleftharpoons ML_2H + H^+ \quad \Delta \log K^{ML_2H_2}_{ML_2H} = 6.49 \qquad (15)$$

$$ML_2H \rightleftharpoons ML_2 + H^+ \quad \Delta \log K^{ML_2H}_{ML_2} = 8.60 \qquad (16)$$

Equations 13 and 14 give the equilibrium constants for the deprotonation of $ML_2H_2$ to $ML_2H$ and $ML_2H$ to $ML_2$. Then the question is whether the deprotonation is from carboxyl group or from amino group of glutamic acid. The following structures represent the formation of $ML_2H_2$ either through both the carboxylates as shown in Structure A or through a carboxylate and the amino group as shown in Structure B.

If structure B were correct, during deprotonation of $ML_2H_2$, the carboxyl protons are lost. Their pKa value is 4.26 for the free ligand. The pH range of existence of $ML_2H_2$ is 3.0 to 7.0, in which pH range the carboxylic group has already lost its proton and hence the complex cannot exist as B. On the other hand the pKa value of amino proton is 9.67 and hence the complex can exist as shown in Structure A.

It is well known that the pKa values of functional groups of the ligands are lowered in the presence of metals. For this reason the pKa values of $NH_3^+$ group (9.67) are decreased to 4.22 and 5.43 on successive deprotonation of $ML_2H_2$ (Structure A). This argument supports Structure A rather than Structure B.

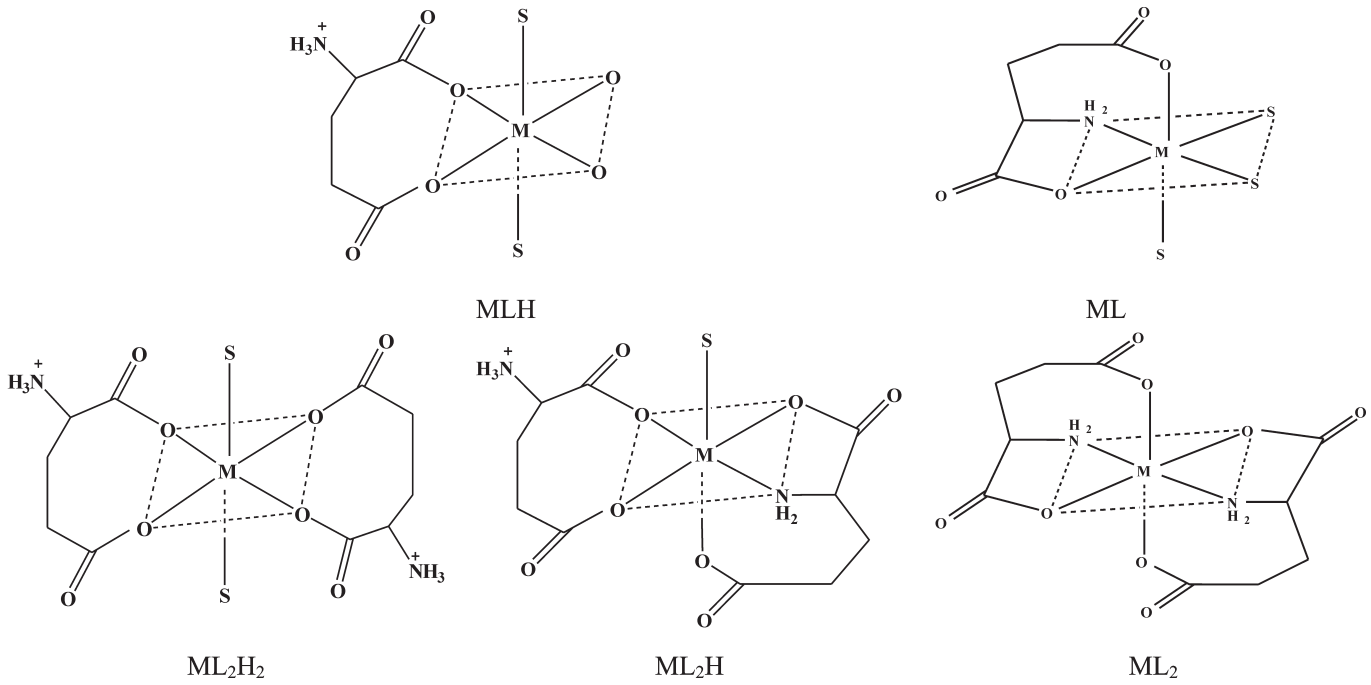
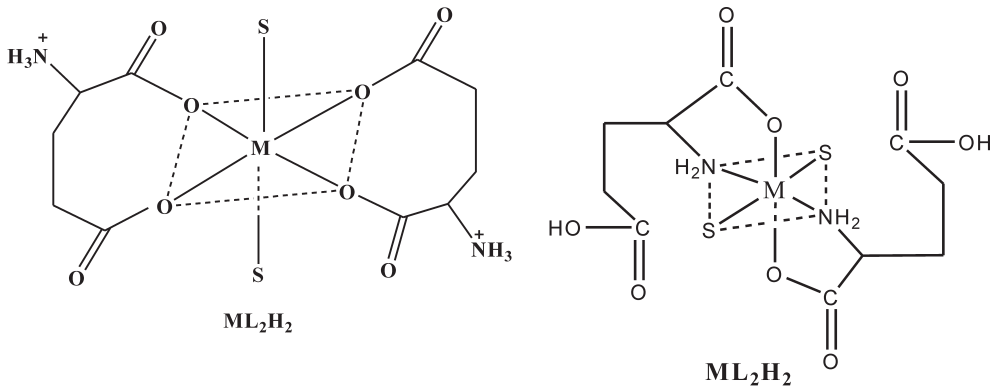



**Figure 3** Tentative structures of Glu complexes of Co(II), Ni(II) and Cu(II). S is either solvent or water molecule.




**Structures A and B**

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