

Effect of denaturants on the speciation of amino acid complexes—Computer augmented modelling studies - IV: Cobalt(II), copper(II) and zinc(II) complexes of L-glutamic acid in water-N,N'-dimethylformamide mixtures†

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A computer assisted investigation has been made on the nature of complexes of cobalt(II), copper(II) and zinc(II) with L-glutamic acid. The distribution of these metal ions amongst the complexes formed with the above amino acid has been computed. The formation constants required for this calculation have been determined experimentally by monitoring H^+ concentration. The formation constants have been refined with the computer program, MINQUAD 75 using the primary alkalimetric data. The predominant complexes formed are CoL , CoL_2^{2-} , CuL , CuL_2^{2-} , $CuLH^+$, CuL_2H^- , ZnL , ZnL_2^{2-} , and $ZnLH^+$. The distribution patterns of different species varies with the relative concentrations of the metal ion and the ligand.

Glutamic acid has multiple biological roles. It links the metabolism of carbon and nitrogen in the animal world. Depending on the need, glutamic acid serves as a source component in the synthesis of both small molecules like glutathione and large molecules like peptides and proteins. In the central nervous system glutamate has two additional functions; it is a neurotransmitter and a precursor of γ -aminobutyric acid (GABA). Because of its capacity to induce depolarising effects, it acts as an excitatory transmitter in cerebral cortex. In many animals glutamate is the most abundant intra-cellular amino acid.

Cobalt(II), copper(II) and zinc(II) ions act as structure promoters or associated with active centres of enzymes. Glutamic acid acts as a powerful tridentate ligand and the interaction of the carboxylate side chain with the metal ions is of importance in metalloenzymes and metal catalyzed reactions. Hence, the biomimetic study of

enzyme-substrate interaction was undertaken using glutamic acid and the metal ions, cobalt(II), copper(II) and zinc(II) as model systems. N,N'-Dimethylformamide-water mixtures were chosen as media to mimick the low dielectric constant active sites in the hydrophobic environments of the enzymes.

Experimental

Solutions of L-glutamic acid, cobalt(II), copper(II) and zinc(II) chlorides were prepared by dissolving AnalaR grade (E. Merck, Germany) salts in triply distilled water. N,N'-Dimethylformamide (DMF) of 99.5% purity (E. Merck) was used. To assess the errors that might have crept into the determination of the concentrations, the data were subjected to analysis of variance of one way classification (ANOVA). The strength of alkali was determined using the Gran plot method¹.

The titrations were carried out in the medium containing varying concentrations of DMF, maintaining an ionic strength of 0.16 mol dm^{-3} with potassium chloride at $303 \pm 0.05 \text{ K}$. An ELICO (Model LI-120) pH meter (readability 0.01) was used to monitor changes in H^+ concentration. The glass electrode was equilibrated in a well stirred water-DMF mixture containing inert electrolyte. The effect of variations in asymmetry, liquid junction potential, activity coefficient, sodium ion error and/or dissolved carbon dioxide on the response of glass electrode is to be considered for accurate determinations. The simulated acid-base titration data (PHCi) calculated by SCPHD program² was used to compute correction factor ($\log F$) for each of the solvent compositions. The $\log F$ value is used to convert pH meter dial reading into logarithm of reciprocal of hydrogen ion concentration (PHEi) according to the equation.

$$\log F = \text{PHCi} - \text{PHEi}$$

Titration of strong acid with alkali was carried out at regular intervals to check whether complete equilibration was achieved. The calomel electrode was refilled with water-DMF mixture of equivalent composition as that of the titrand. In each of the titrations, the titrand consisted of mineral acid of approximately 1 mmol in a total volume of 50 cm^3 . Titrations with different ratios (1:1.5-1:5.3) of metal-ligand were carried out with 0.4 mol dm^{-3} potassium hydroxide. Other experimental details are given elsewhere³.

†Part III : Ref. 7.

Modelling strategy

The approximate complex stability constants of metal-glutamic acid were calculated with the computer program SCPHD. The best fit chemical model for each system investigated was arrived at using a non-linear least squares computer program MINQUAD 75 (ref. 4). The variation of step-wise equilibrium constants of proton-ligand as well as metal-ligand species was analysed on electrostatic

grounds using the program ESOCE⁵ and on the basis of solute-solute and solute-solvent interactions. Some of the heuristics that are to be followed in the refinement of the stability constants were listed in our earlier publication⁶.

Results and discussion

The protonation constants of L-glutamic acid in varying water-DMF mixtures (10-60% v/v DMF)

Table 1—Results of an exhaustive modelling study performed on Zn(II)-glutamic acid system in 30.0% DMF
pH range = 3.5-8.5; NP = 237

| Model No. | log β_{mlh} (SD) | | | U_{corr} | χ^2 | R | Skewness | Kurtosis |
|-----------|------------------------|----------|-----------|------------|----------|--------|----------|----------|
| | 110 | 120 | 111 | | | | | |
| 1 | 5.65(8) | — | — | 38.351 | 332.13 | 0.0374 | 1.12 | 7.49 |
| 2 | — | 9.99(5) | — | 56.233 | 155.64 | 0.0242 | 0.58 | 8.83 |
| 3 | — | — | 11.21(75) | 73.621 | 613.53 | 0.1164 | 0.08 | 6.20 |
| 4 | 5.33(4) | 9.84(4) | — | 63.574 | 230.24 | 0.0152 | 5.74 | 6.70 |
| 5 | 5.76(9) | — | 11.27(22) | 37.102 | 497.76 | 0.0360 | 1.15 | 7.81 |
| 6 | — | 10.16(6) | 11.25(14) | 14.885 | 266.70 | 0.0230 | 0.59 | 9.29 |
| 7 | 5.49(2) | 10.08(3) | 11.28(5) | 4.871 | 251.83 | 0.0087 | 4.48 | 0.63 |

110 = ML; 120 = ML_2^{2-} ; 111 = MLH^+

Table 2—Best fit chemical models of Co(II), Cu(II) and Zn(II)-glutamic acid complexes in water-DMF, mixtures
Temp. = 303 K, Ionic strength = 0.16 mol dm⁻³, No. of titrations in each percentage = 9

| % v/v DMF | log β_{mlh} (SD) | | | | pH range | NP | U_{corr} | χ^2 | R | Skewness | Kurtosis |
|----------------------|------------------------|----------|-----------|-----------|----------|-----|------------|----------|--------|----------|----------|
| | 110 | 120 | 111 | 121 | | | | | | | |
| Co(II)-glutamic acid | | | | | | | | | | | |
| 00.0 | 4.62(1) | 8.06(1) | — | — | 5.0-8.5 | 76 | 3.466 | 30.60 | 0.0038 | -0.29 | 3.65 |
| 10.0 | 4.40(2) | 7.71(4) | — | — | 5.0-8.5 | 89 | 3.581 | 127.30 | 0.0113 | 0.21 | 3.77 |
| 20.0 | 4.73(1) | 8.31(2) | — | — | 5.0-8.5 | 95 | 1.262 | 22.28 | 0.0067 | 0.25 | 3.35 |
| 30.0 | 5.10(1) | 9.05(2) | — | — | 5.0-8.5 | 109 | 2.006 | 57.02 | 0.0087 | 0.26 | 5.88 |
| 40.0 | 5.29(1) | 9.42(2) | — | — | 5.0-8.5 | 120 | 8.028 | 62.98 | 0.0060 | -0.27 | 5.83 |
| 50.0 | 5.16(1) | 9.38(2) | — | — | 5.0-8.5 | 143 | 1.829 | 42.85 | 0.0079 | 0.61 | 3.91 |
| 60.0 | 5.13(5) | 9.28(8) | — | — | 5.0-8.5 | 131 | 2.891 | 101.03 | 0.0314 | -0.03 | 5.20 |
| Cu(II)-glutamic acid | | | | | | | | | | | |
| 00.0 | 8.54(3) | 15.36(2) | 12.89(1) | 20.36(8) | 1.5-6.0 | 447 | 0.526 | 120.12 | 0.0039 | -1.93 | 8.06 |
| 10.0 | 8.57(2) | 14.94(2) | 12.78(1) | 20.05(1) | 1.5-6.0 | 430 | 3.196 | 88.60 | 0.0027 | -0.96 | 7.58 |
| 20.0 | 8.69(5) | 15.03(4) | 12.79(3) | 20.03(8) | 1.5-6.0 | 422 | 3.757 | 174.67 | 0.0126 | 0.55 | 7.39 |
| 30.0 | 9.42(6) | 16.28(3) | 13.71(2) | 21.28(9) | 1.5-6.0 | 450 | 4.123 | 38.52 | 0.0043 | 0.30 | 4.81 |
| 40.0 | 9.71(7) | 16.98(7) | 14.50(3) | 22.24(13) | 1.5-6.0 | 451 | 4.023 | 54.39 | 0.0105 | -0.01 | 5.71 |
| 50.0 | 10.89(9) | 17.02(7) | 14.52(3) | 21.67(12) | 1.5-6.0 | 446 | 3.759 | 74.67 | 0.0133 | -0.34 | 4.86 |
| 60.0 | 10.61(9) | 16.55(9) | 14.72(4) | 21.35(12) | 1.5-6.0 | 436 | 2.359 | 149.80 | 0.0130 | -1.05 | 4.38 |
| Zn(II)-glutamic acid | | | | | | | | | | | |
| 00.0 | 4.89(3) | 9.01(3) | 10.63(11) | — | 3.5-8.5 | 212 | 7.089 | 201.87 | 0.0105 | 3.29 | 9.53 |
| 10.0 | 4.96(7) | 9.28(5) | 10.81(9) | — | 3.5-8.5 | 213 | 0.976 | 208.37 | 0.0142 | 2.50 | 1.09 |
| 20.0 | 5.26(6) | 9.67(5) | 10.95(8) | — | 3.5-8.5 | 215 | 3.199 | 402.72 | 0.0334 | 3.70 | 9.27 |
| 30.0 | 5.49(2) | 10.08(3) | 11.28(5) | — | 3.5-8.5 | 237 | 4.871 | 251.83 | 0.0087 | 4.48 | 0.63 |
| 40.0 | 5.93(4) | 10.65(5) | 11.69(5) | — | 3.5-8.5 | 246 | 5.062 | 158.35 | 0.0122 | 2.96 | 4.35 |
| 50.0 | 5.17(4) | 9.89(4) | 9.79(7) | — | 3.5-8.5 | 263 | 7.492 | 185.75 | 0.0116 | 4.09 | 8.42 |
| 60.0 | 4.23(7) | 8.92(6) | Rej | — | 3.5-8.5 | 258 | 6.756 | 133.82 | 0.0391 | 1.19 | 4.25 |

Table 3—Effect of TLO to TMO on the pH at which maximum concentration of the species is observed for the Cu(II)-glutamic acid system in 30% DMF

| TLO/TMO | pH (% concentration of species) | | | |
|---------|---------------------------------|-------------------------------|------------------|--------------------------------|
| | ML | ML ₂ ²⁻ | MLH ⁺ | ML ₂ H ⁻ |
| 1.6 | 5.33(61) | 5.94(42) | 3.57(69) | 4.97(14) |
| 2.0 | 4.88(52) | 5.67(49) | 3.47(70) | 4.88(18) |
| 2.4 | 4.63(46) | 5.72(64) | 3.43(76) | 4.04(25) |
| 2.7 | 4.75(49) | 5.81(67) | 3.47(71) | 4.90(22) |
| 3.0 | 4.63(41) | 5.75(71) | 3.48(76) | 4.83(28) |
| 3.2 | 4.43(39) | 5.96(83) | 3.43(80) | 4.83(32) |
| 4.0 | 4.45(36) | 5.82(79) | 3.34(81) | 4.85(34) |
| 5.3 | 4.36(35) | 5.93(84) | 3.38(81) | 4.76(36) |

The values in parentheses indicate the maximum percentage concentrations of species.

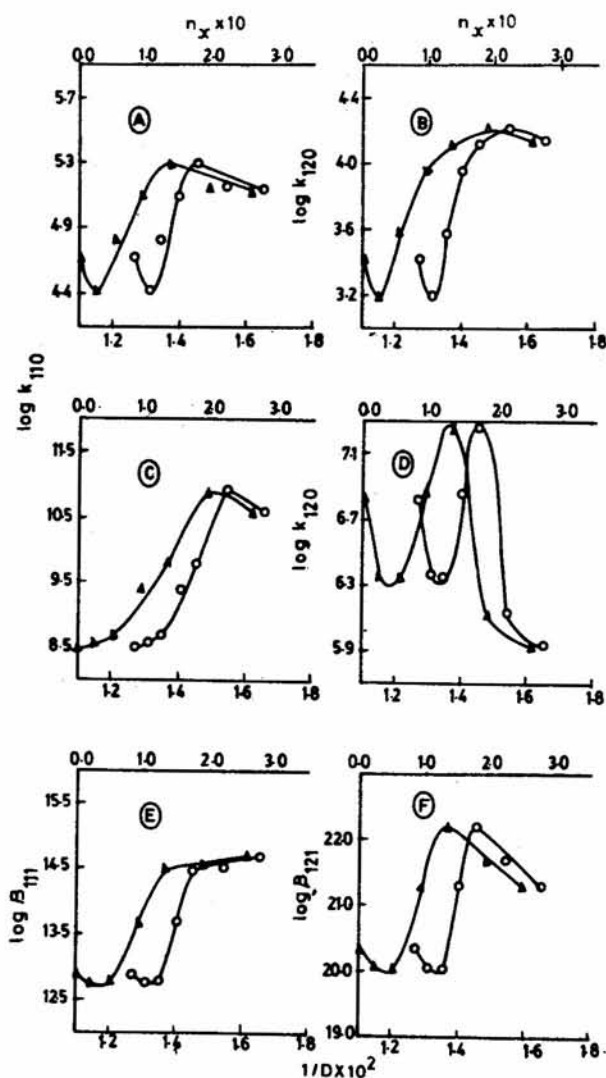


Fig. 1—Variation of $\log K^M$ of Co(II)-GLU(A,B) and Cu(II)-GLU(C,D,E,F) with mole fraction (Δ - Δ) and reciprocal of dielectric constant (\circ - \circ) in water-DMF mixtures

were reported elsewhere⁷. Existence of species was determined by performing exhaustive modelling and the results of one such system are given in Table 1. The models are evaluated assuming the simultaneous existence of different combinations of the species. For the system Zn(II)-glutamic acid in 30% water-DMF mixture solution, the models gave better statistics as the number of species increased, conforming the better fit. There is no further improvement in the fit on inclusion of some more species in the model containing ZnL, ZnL₂²⁻, and ZnLH⁺. This indicates that the final model appropriately fits the experimental data. Such exhaustive modelling is performed for all the systems and the consolidated data of the finally selected models are given in Table 2.

Effect of ligand-metal ratio

As the ratio of the total ligand concentration (TLO) to the total metal ion concentration (TMO) increases from 1.6 to 5.3, the concentration of ML is decreased whereas that of MLH⁺, ML₂²⁻ and ML₂H⁻ increased as shown for Cu(II)-GLU system in Table 3. This is quite obvious since as the ligand concentration is increased the formation of the higher species will be favoured. An interesting conclusion can be drawn from the data in Table 3 that the pH corresponding to the maximum percentage concentration of the species, ML and MLH⁺ shifted to lower side with increase in the ratio of TLO/TMO. However, for ML₂²⁻ and ML₂H⁻ species the values remained constant.

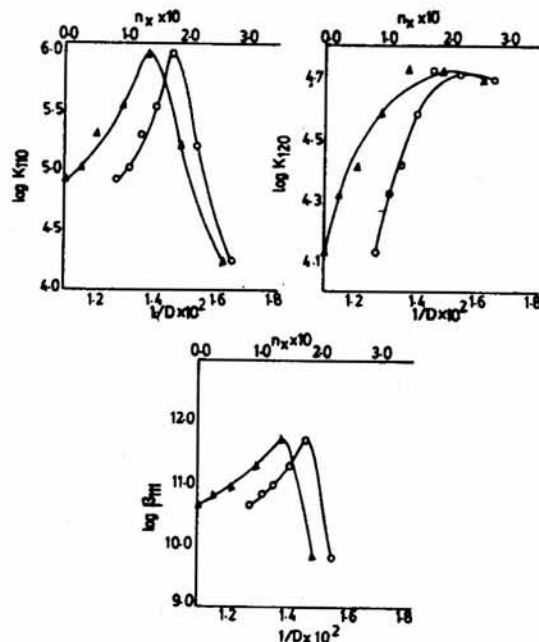
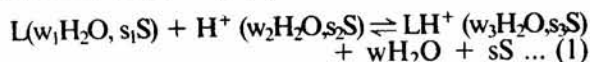


Fig. 2—Variation of $\log K^M$ of Zn(II)-GLU with mole fraction (Δ - Δ) and reciprocal of dielectric constant (\circ - \circ) in water-DMF mixtures

Solute-solvent interactions

A plot of step-wise formation constant, $\log K^M$ versus $1/D$ (D is the dielectric constant of the medium) should be linear if Born's classical treatment⁸ holds good. The change in $\log K^M$ values of complexes of L-glutamic acid with Co(II), Cu(II) and Zn(II) with variation of n_x and $1/D$ are depicted in Figs 1 and 2. In most of the cases a segmented linear relationship has been observed which indicates that the dielectric constant or long range interactions alone are not responsible for the stability trend⁹. Since complex formation can be viewed as a competition between the pure and solvated forms of the ligand¹⁰ and the metal ion, solute-solvent interactions, relative thermodynamic stabilities and kinetic labilities are expected to play an important role. Different types of electrostatic forces dominate in different ranges of concentration of water-DMF mixtures. In some cases, there is deviation from linearity even in the segments indicating the cumulative effect of both electrostatic and non-electrostatic interactions.

In another model proposed by Marshall¹¹ and extended by Mui and McBryde¹², both water and co-solvent are considered to be involved in the solvation, as per Eq. (1).



and $K^M = K^{M'} [H_2O]_M$ where $K^{M'}$ is the complete equilibrium constant, K^M is the observed equilibrium constant and $[H_2O]_M$ is the concentration of water at each of the co-solvent contents. The number of water

molecules released in the equilibria are calculated by the linear least squares analysis of Eq. 2.

$$j \log[S] + \log K^M = \Delta G^1/2.303 RT + \log K^w - w \log [(H_2O)_M/[S]] + j \log [H_2O]_M \dots (2)$$

where, s and w are number of solvent and water molecules released and j is equal to $w + s$. This equation has two unknown parameters j and w and as such w is calculable from a plot of $\{j \log[S] + \log K^M\}$ versus $\log [(H_2O)_M/[S]]$ provided j is assumed. A linear plot of $j \log[S] + \log K^M$ versus $\log [H_2O]/[S]$ implies that total change of water and solvent molecules associated with an equilibrium is invariant over the composition range investigated. Since the variation of $\log K^M$ of different metal complex species in the best fit chemical model with solvent parameters (n_x and functions of dielectric constants) is non-linear, linearization of a function of $\log K^M$ ($j \log[S] + \log K^M$) versus $\log [H_2O]/[S]$ was tried for different values of j (-5 to $+5$) employing the computer program ESOCE⁵. The linearity has been judged by inspection of Fig. 3, residuals in $\log K^M$ and statistical parameters like corrected correlation coefficient, Exner ψ (ref. 13) and Ehreusen (FEHR)¹⁴ parameters. This approach is preferable to conventional statistics in the case of chemical data with a few (less than 10) observations. The non-linearity in the n_x plots is almost eliminated in $\log [S]$ plots. The change in the slopes of the plots indicates the variation in the number of water molecules released. This leads to a tentative conclusion that species of same stoichiometric

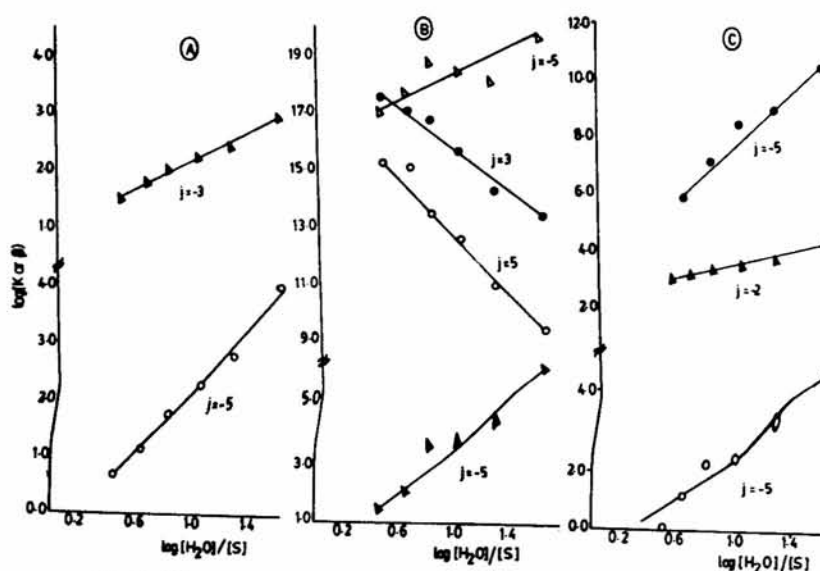


Fig. 3—The relation between $j \log[S] + \log K^M$ and $\log [H_2O]/[S]$ for (A) Co(II)-GLU, (B) Cu(II)-GLU and (C) Zn(II)-GLU in water-DMF mixtures ○—○: $\log K_{110}$, ●—●: $\log \beta_{111}$, △—△: $\log K_{120}$ and △—△: $\log \beta_{122}$

composition, M:L:H are formed but will be associated with different solvent shells.

Distribution diagrams

L-glutamic acid has two dissociable carboxyl protons and an amino group that can associate with a proton. As mentioned earlier⁷ the different forms of the ligand can be given as LH_3^+ , LH_2 , LH^- , and L^{2-} in the pH regions 1.5-3.5, 2.0-5.5, 4.0-9.5 and 8.5-11.0, respectively. Hence, the probable species that exist in different systems can be predicted from these data. The present study is confined to the pH ranges of 5.0-8.5 and 3.5-8.5 for Co(II) and Zn(II) systems, respectively. In the pH range 5.0-8.5, the ligand is expected to be in the form of LH^- . Hence, the plausible metal-ligand species are only ML and ML_2^{2-} which are confirmed by MINQUAD 75 for the Co(II) system. But the zinc(II) system was investigated in the pH region 3.5-8.5, where the expected ligand forms are LH_2 and LH^- . The complex species predicted in this pH range are ML, ML_2^{2-} and MLH^+ . The Cu(II) system is studied in the pH range, 1.5-6.0 which gave the species ML, ML_2^{2-} , MLH^+ and ML_2H^- because the ligand form also includes LH_3^+ . The equilibria in these pH ranges are given in Scheme 1.

| Equilibria | pH range |
|--|----------|
| 1) $\text{M(II)} + \text{LH}_3^+ \rightleftharpoons \text{MLH}_3^{2+} + \text{H}^+$ negligible | .5-3.5 |
| $\text{MLH}_3^{2+} + \text{H}^+ \rightleftharpoons \text{MLH}^+ + \text{H}^+$ negligible | |
| $\text{MLH}^+ + \text{LH}^- \rightleftharpoons \text{ML}_2\text{H}_3^+ + \text{H}^+$ negligible | |
| $\text{ML} + \text{LH}_3^+ \rightleftharpoons \text{ML}_2\text{H}_2 + \text{H}^+$ negligible | |
| $\text{M(II)} + \text{LH}_3^+ \rightleftharpoons \text{MLH}^+ + 2\text{H}^+$ major | |
| 2) $\text{M(II)} + \text{LH}_2 \rightleftharpoons \text{MLH}^+ + \text{H}^+$ minor | 3.5-5.0 |
| $\text{MLH}^+ + \text{LH}_2 \rightleftharpoons \text{ML}_2\text{H}^+ + 2\text{H}^+$ major | |
| $\text{M(II)} + \text{LH}_2 \rightleftharpoons \text{ML} + 2\text{H}^+$ major | |
| 3) $\text{ML}_2\text{H}^- \rightleftharpoons \text{ML}_2^{2-} + \text{H}^+$ major | 5.0-8.5 |
| $\text{ML} + \text{LH}^- \rightleftharpoons \text{ML}_2^{2-} + \text{H}^+$ major | |

Scheme 1

Distribution diagrams were obtained using the formation constants of the best fit model and are drawn in Fig. 4. The patterns of the distribution of species with pH show that the concentration of species are affected by the cosolvent, DMF. This trend depends on the relative solvation of DMF. Correlation of data in Table 4 and Fig. 4 infers that the concentrations of CoL, CuL_2^{2-} , CuL_2H^- , ZnL and ZnLH^+ decreased and the change in number of DMF molecules is around, -8 and total number of solvent molecules is -5. Due to decrease in solvent molecules the species may be destabilized. This can be confirmed

by observing increased concentration of CuL for which change in DMF molecules and total number of solvent molecules are +5 and +10.4, respectively.

Although the pH metric data cannot provide any definite evidence regarding the binding sites they can be predicted with the aid of the data available in the literature. Glutamic acid acts as a tridentate ligand. Amino nitrogen and α -carboxyl oxygen of L-glutamic acid participate in bonding with cobalt(II), copper(II) and zinc(II). The γ -carboxylic group is protonated at pH less than 5 because its protonation constant is around 4.0. It is deprotonated at higher pH's and acts as a third binding site.

Table 4—Change in the number of water and DMF molecules in metal-glutamic acid equilibria in water-DMF media

| System | w | s | j |
|--------------------|------|------|----|
| CoL | 2.9 | -7.9 | -5 |
| CoL ₂ | 1.4 | -4.4 | -3 |
| CuL | -5.4 | 10.4 | 5 |
| CuL ₂ | 3.8 | -8.8 | -5 |
| CuLH | -3.8 | 6.8 | 3 |
| CuL ₂ H | 2.2 | -7.2 | -5 |
| ZnL | 3.6 | -8.6 | -5 |
| ZnL ₂ | 0.9 | -2.9 | -2 |
| ZnLH | 3.8 | -8.8 | -5 |

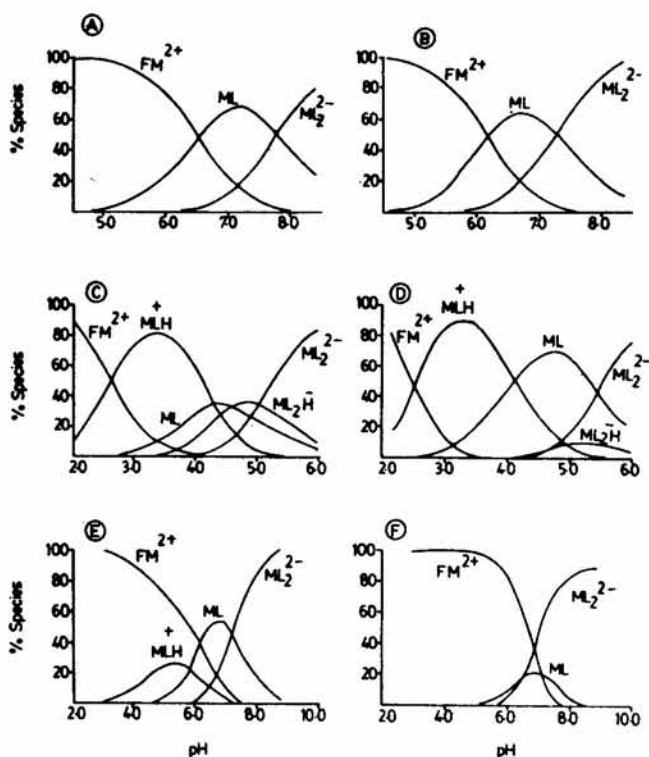


Fig. 4—Species distribution diagrams of (A) Co(II)-GLU in 30% DMF, (B) Co(II)-GLU, in 60% DMF, (C) Cu(II)-GLU in 30% DMF, (D) Cu(II)-GLU in 60% DMF, (E) Zn(II)-GLU in 30% DMF, (F) Zn(II)-GLU in 60% DMF

Cobalt(II) does not form protonated species because the pH range of study is confined to 5.0-8.5. In the case of copper(II) and zinc(II) the lower pH limit is less than 4.0 and so γ -carboxylic groups may be protonated which are in fact detected in this study.

On the basis of this study the following conclusions can be drawn. (i) Copper(II) and zinc(II) form protonated complexes with L-glutamic acid while similar ones are not observed with cobalt(II). (ii) The maximum percentage concentration of 1:1 species (ML and MLH^+) decreased with increasing TLO/TMO ratio whereas it remained constant for 1:2 species (ML_2^{2-} and ML_2H^-). (iii) The non-linear relationship between $\log K$ values of species and $1/D$ of the solvent indicated that non-electrostatic interactions dominated the electrostatic forces.

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