# Interaction of acidic Amino acids with Bivalent Metal ions

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THE interaction of L-aspartic and L-glutamic acids with bivalent metal ions has been studied using potentiometric (pH titrimetric) method. The formation constants are calculated using the method of Irving and Rossotti. The alkaline earth metal ions form 1 : 1 complexes with both the amino acids, except in the case of magnesium which forms a 1 : 2 complex with aspartic acid. The stability constants of these complexes follow the order $Mg^{2+} > Ca^{2+} > Sr^{2+} > Ba^{2+}$. However, in the case of transition metal ions which form 1 : 2 complexes with these two amino acids the stability constants increase from $Cu^{2+}$ to $Mn^{2+}$ in the order, $Cu^{2+} > Ni^{2+} > Hg^{2+} > Zn^{2+} \approx Co^{2+} > Fe^{2+} > Cd^{2+} > Ag^{+} > Mn^{2+}$. The $\alpha-NH_2$ and $\alpha-COO^-$ groups of the amino acids are involved in the formation of five membered chelates, without the participation of the side chain carboxyl groups.

THE study of metal-amino acid complexes is of great importance from biological stand point of view as it is likely to contribute to a better understanding of the metal-protein interactions. An insight into this can be obtained by studying the equilibria involving the metal ions and amino acids which are the building units of proteins. The stability constants of L-amino acids with a number of bivalent metal ions have been determined and reported in literature[1-4]. Aspartic and glutamic acids are present at the active site of lysozyme[5], an enzyme present in hen egg white, which has been investigated exhaustively for its physicochemical characteristics and for its structure-function[6-12]. With a view to study the metal-lysozyme interaction, it was felt to take up a preliminary study of the interaction of these acidic amino acids with metal ions. The present communication describes the determination of formation (stability) constants of complexes of aspartic and glutamic acids with alkaline earth and transition metal ions, potentiometrically following the Calvin-Bjerrum pH titration techniques as adopted by Irving and Rossotti[13].

## EXPERIMENTAL

Systronics digital pH meter 335 in conjuction with glass and calomel electrodes was used for titrations carried out at 25±0.1°C. The pH meter was calibrated using suitable buffers as described by Bates[14]. All chemicals except amino acids were of analytical grade. The amino acids were from Sigma Chemical CO (U.S.A.). All solutions were prepared using double distilled water. Carbon-dioxide free potassium hydroxide was used in the investigation. $1 \times 10^{-3}$M aqueous solutions of amino acids and $5 \times 10^{-3}$M metal ion solutions except in the case of $Fe^{2+}$ were used. Constant ionic strength was maintained at 0.1 M throughout the titrations using $KNO_3$.

## RESULTS AND DISCUSSION

The titration curves for L-aspartic and L-glutamic acids in the presence and absence of metal ions were drawn and they were used to calculate the formation constants. The calculation of formation constants was based on the method of Irving and Rossotti[13]. The stability constants are given in tables 1 and 2. The formation curves (Fig. 1-2)

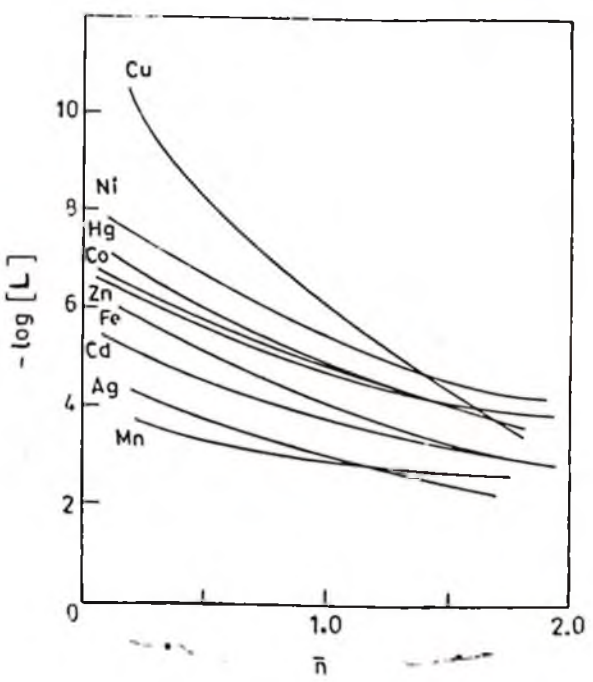



FIG.1

Fig. 1. Formation curves for L-aspartic acid.

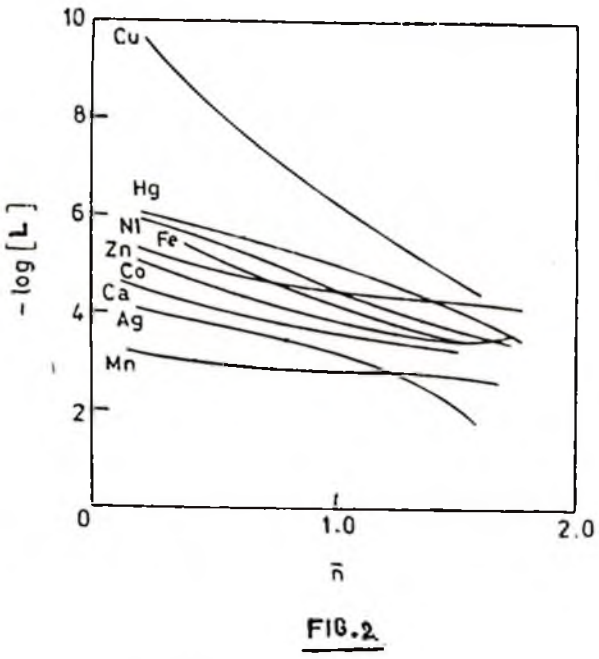



Fig. 2. Farmation curves for L-glutamic acid

were obtained by plotting $\bar{n}$ vs. pL values where $\bar{n}$ is the average ligand number and pL = $-\log [L]$. From these curves, stepwise formation constants log $K_1$ and log $K_2$ were obtained for pL values correspoding to $\bar{n} = 0.5$ and $\bar{n} = 1.5$ respectively.

It is observed that the alkaline earth metal form 1:1 complexes with both the acidic amino acids except in the case of magnesium which forms a 1:2 complex with aspartic acid. The values of the chelate stability constants of these complexes indicate that their stability increases with decrease in the basicity of the metal ions : $Mg^{2+} > Ca^{2+} > Sr^{2+} > Ba^{2+}$. The stabilities also decrease with increase of ionization potential or polarizing power of the metal ion[15]. Therefore it was concluded that asparate ion has a greater affinity for the less basic metal ions, while glutamate ion is more effective in complexing with more basic metal ions.

TABLE I

*Stability constants of aspartic and glutamic acids with alkaline earth metal ions.*

| $M^{2+}$ | ASPARTIC ACID | | | GLUTAMIC ACID | | |
|---|---|---|---|---|---|---|
| | $pK_{NH_2} = 9.95$ $pK\alpha\text{-}_{COOH} = 2.10$ $pK\beta\text{-}_{COOH} = 3.90$ | | | $pK_{NH_2} = 9.90$ $pK\alpha\text{-}_{COOH} = 2.20$ $pK\gamma\text{-}_{COOH} = 4.40$ | | |
| | log $K_1$ | log $K_2$ | log $K_s$ | log $K_1$ | log $K_2$ | log $K_s$ |
| $Mg^{++}$ | 2.82 | 2.52 | 5.35 | 2.79 | — | 7.29 |
| $Ca^{++}$ | 2.77 | — | 2.77 | 2.63 | — | 2·63 |
| $Sr^{++}$ | 2.68 | — | 2.68 | 2.41 | — | 2.41 |
| $Ba^{++}$ | 2.57 | — | 2.57 | 2.30 | — | 2.30 |

TABLE II

*Stability constants of aspartic and glutamic acids with transition metal ions.*

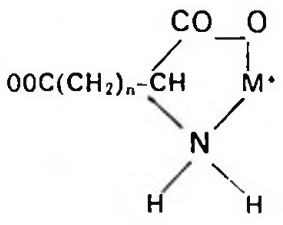
| $M^{2+}$ | ASPARTIC ACID | | | GLUTAMIC ACID | | |
|---|---|---|---|---|---|---|
| | log $K_1$ | log $K_2$ | log $K_s$ | log $K_1$ | log $K_2$ | log $K_s$ |
| $Mn^{2+}$ | 3.45 | 2.34 | 5.79 | 2.98 | 2.55 | 5.53 |
| $Fe^{2+}$ | 5.34 | 3.23 | 8.57 | 5.08 | — | 5.08* |
| $Co^{2+}$ | 5.81 | 4.08 | 9.89 | 4.56 | 3.11 | 7.67 |
| $Ni^{2+}$ | 6.74 | 4.57 | 11.31 | 5.42 | 3.69 | 9.11 |
| $Cu^{2+}$ | 8.61 | 5.11 | 13.72 | 6.22 | 5.10 | 13.32 |
| $Zn^{2+}$ | 5.77 | 4.05 | 9.82 | 4.89 | 4.07 | 8.96 |
| $Ag^{2+}$ | 3.94 | 2.09 | 6.03 | 3.80 | 2.07 | 5.87 |
| $Cd^{2+}$ | 4.62 | 3.07 | 7.69 | 4.15 | 2.99 | 7.14 |
| $Hg^{2+}$ | 6.10 | 4.08 | 10.18 | 5.60 | 4.06 | 9.66 |

* log $K_1$ value, the insolubility of the complex prevented determination of log $K_2$

In the case of transition metal ions it is observed that L-aspartic and L-glutamic acids form 1:2 complexes and the stability constants follow the order :

$Cu^{2+} > Ni^{2+} > Hg^{2+} > Zn^{2+} \approx Co^{2+} > Fe^{2+} > Cd^{2+} > Ag^{2+} > Mn^{2+}$ which is in agreement with the order of Mellor and Maley[16]. This order is followed by log $K_1$, log $K_2$ and the overall formation constant log $K_s$ (log $K_1 K_2$).

In the case of L-amino acid-metal complexes an increase in the chainlength of the ligand side chain decreases the stability of the corresponding complexes[17]. This is found to be true with both the acidic amino acids from the present studies. The pKa of the $\beta/\gamma$-carboxyl groups of these amino acids is intermediate between those for the two $\alpha$-groups (i.e $\alpha$-amino and $\alpha$-carboxyl groups). This introduces a difficulty in the interpretation of the titration data when the end point coincides with the addition of two equivalents of alkali[18]. In such cases it is not clear, whether the $\beta/\gamma$ carboxyl group is involved in the chelaion, because it must be completely titrated in order to deionize any appreciable amount of the $\alpha$-amino group which is essential for chelation. From the above reasons it is evident that the co-ordination is either by two $\alpha$—groups or by $\beta/\gamma$—carboxyl and an amino group or even by to carboxyl groups of the amino acids. In last two cases it results in the formation of six or seven membered rings, both of which are known to be unstable.[18] Hence the only other alternative is between $\alpha$—amino and $\alpha$—carboxyl groups resulting in the formation of five membered rings. This conclusion was further supported by Bjerrum[19] that in the rings of equal size two carboxyl groups do not bind the metals so avidly as a carboxyl and an amino group. Hence the aspartate or glutamate chelate can be represented as




When n = 1, Aspartate chelate
n = 2, glutamate chelate

Although, the $\beta/\gamma$—carboxylate ion probably is not involved in the binding to metal ion, it has an indirect influency on the stability of the chelate ring. In general, stability constants indicate that the inductive effect of the $\beta$-carboxylate group has imparted higher stability to aspartate chelates relative to gluamate chelates. Chebreck and Martell[20] have suggested that aspartic acid acts a tridentate ligand with respect to first row transition metal ions. Popa and Fisal[21] have studied the complexation of these transition metal ions with certain amino acids by ion exchange technique, and they have shown that these amino acids form 1.1, 1:2, 1:3 and also 1:4 metal/acid complexes.

Some initial irregularities were observed for copper complexes of glutamic and aspartic acids during the course of their titration with copper. These irregularities in the determination of log $K_1$ of glutamic (or aspartic) acid-copper complex can be attributed to the ionization of $\gamma/\beta$-carboxyl group of the amino acid. The lower affinty of other metal ions permits rhis group to ionize almost completly before the complex formation occurs at nigher pH-values.

If one considers the statistical effects of the availablity of the two carboxylate groups in each glutamate ion to the cation, one would expect the glutamate complex to be more stable than the glycinate. However, chelation of Cu (II) with amino and $\gamma$-carboxylate group would introduce a 7-membered ring and the resulting decrease in stability somewhat nullifies the statistical effect. In the copper (II)-aspartate complex investigated by Li and Doody[22], chelation of copper with amino and $\beta$-carboxylate group introduces a six membered ring, so that the aspartate complex is more stable than the glutamate.

Plots of log $K_s$ against atomic number, second ionization potential and electronegativity values of metal ions are shown in figures 3-5. Similar correlations were made by Calvin and Melchior[23] and Srivastava[24] et al for the complexes of 5-salicylaldehyde sulphonate and diaminoacetyl urea complexes respectively. The stability of manganese complex compared to those of the other divalent metals in the same series may be explained as due to the absence of ligand field stabilization energy in its complexes and the large ionic size of Mn (II). A correlation between the stability constants with the sum of the first and second ionization potentials for the complexes of alkaline earth ions have been observed by Schwerznbach[25] et al. An analogous

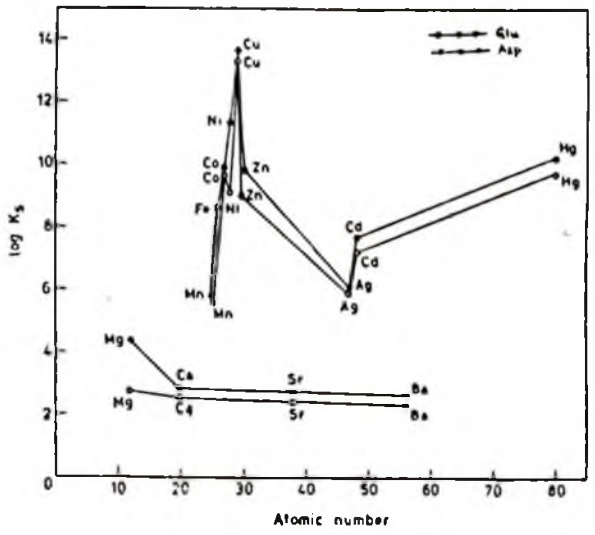



FIG 3

Fig. 3. The plot of log Ks Vs atomic number of divalent metalions

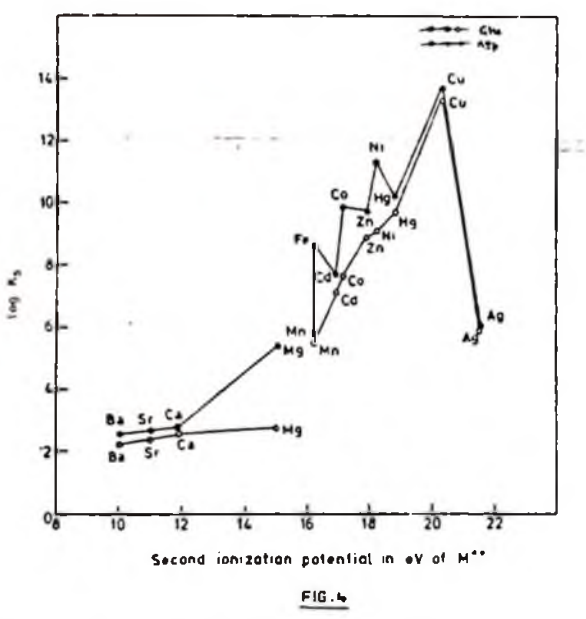



FIG.4

Fig. 4. The plot of log Ks Vs second ionization potential of divalent metal ions.

correlation of stability constants with the second ionization potentials of the metal ion for the transition metal series has been observed by Calvin and Martell[26]. Plots of log $K_s$ against reciprocal of the ionic radii of transition metal ions (Fig 6) show that the ligands from most unstable complexes with Mn (II) and stabler complexes with Cu (II) than with other divalent transition metal ions.

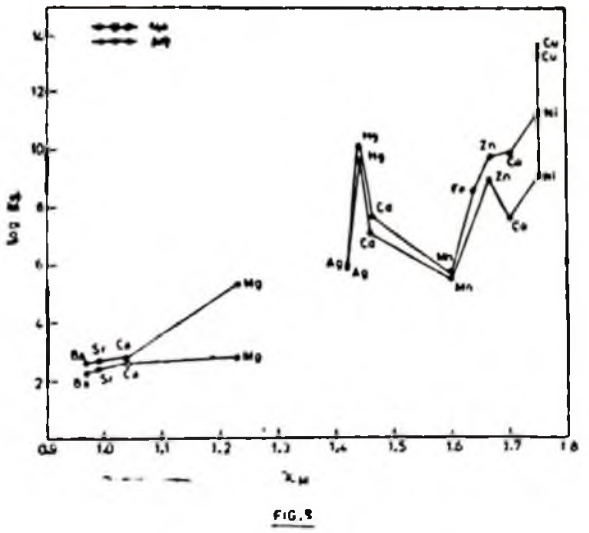



FIG.5

Fig. 5. The plot of log Ks Vs electronegatively ($X_M$) of divalent metalions

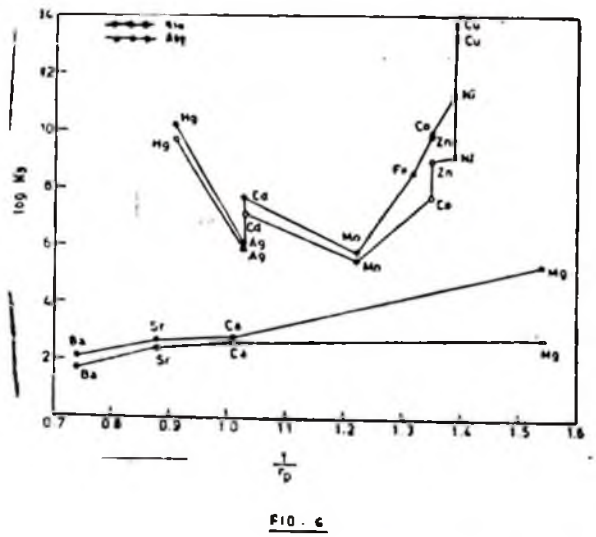



FIG. 6

Fig. 6. The plot of log Ks Vs reciprocal of ionic radii (1/rp) divalent metal ions.

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