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pH-Metric Evidence on Metal-Peptide Nitrogen Linkage in Some Oligopeptide Complexes

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Already reported pH-metric experimental data by Martell *et al.* on metal (Cu^{2+}, Ni^{2+}, and Co^{2+}) complexes of oligopeptide derivatives (H_3L), diglycine-N, N-diacetic acid (2GDA), triglycine-N, N-diacetic acid (3GDA), tetraglycine-N, N-diacetic acid (4GDA) has been analysed, for obtaining the structural information on the complexes formed at higher pH, using the previously described procedure with little modifications, Comparing the results obtained in this work with those of IR study (by Martell *et al.*), participation of peptide nitrogen in coordination with metal ions has been confirmed. Along with the earlier reported protonated complexes, formation of $NiH_{-1}L^{2-}$ instead of $NiL(OH)^{2-}$ [with 2GDA], mixed protonated hydroxo complexes $NiH_{-1}L(OH)^{3-}$ [with 3GDA] and $CuH_{-2}L(OH)^{4-}$, $CoH_{-2}L(OH)^{4-}$ [with 4GDA] has been characterized.

INTRODUCTION

Elucidation of structures of metal peptide complexes in aqueous sloution is of considerable attraction in view of their importance as model systems in biological studies. Martell *et al.*[1] investigated the complexes of some oligopeptide derivatives [H_3L, H-dissociable protons under the experimental conditions of pH, 2-II], diglycine-N,N-diacetic acid (2GDA), triglycine-N,N-diacetic acid (3GDA), tetraglycine-N,N-diacetic acid (4GDA) with metal ions, Cu^{2+}, Ni^{2+} and Co^{2+} in aqueous solution using pH-metric measurements in combination with IR studies[1]. From 1 : 1 metal-ligand titration curves (pH vs. a, a = moles of alkali used per mole of ligand), they suggested the formation of MHL (with terminal unionized carboxylate) and ML^- species (below $a = 3$) ($M^{2+} = Cu^{2+}$, Ni^{2+} and Co^{2+}) involving the coordination through amino nitrogen-diacetic acid carboxylates-amido carbonyl in Ni^{2+} and Co^{2+} and amino nitrogen-diacetic acid carboxylates in Cu^{2+} complexes, which was further supported by IR results.

An increase in a values beyond 3.0 may be expected due to either complex hydrolysis or proton dissociation from peptide nitrogen (free dissociation or complex formation). However, on the basis of IR studies only, Martell *et al.*[1] suggested metal-peptide nitrogen bonding beyond $a = 3$, except in Ni^{2+}-2GDA system (where existence of $MLOH^{2-}$ was inferred), while association of OH^- ion with the complexes appears to be more reasonable because of high pK values of the peptide hydrogen. Thus, utilizing the data and results reported by these authors[1], a pH-metric evidence has been presented to confirm IR results on the complexes formed at higher pH (above $a = 3$) employing the previously described method[2–4] with some modifications. Also, in certain systems where IR studies could not be applied by Martell and coworkers[1], due to incomplete formation of the complex species (much less than 100%) or the other reasons, the possible reactions have been worked out.

EXPERIMENTAL

Experimental data and results already reported by Martell and coworkers[1] have been used. With the help of titration curves, proton complexes association constants for Cu^{2+}-2GDA, Ni^{2+}-3GDA, Co^{2+}-2GDA (beyond $a = 4$) and Cu^{2+}-4GDA, Co^{2+}-4GDA (beyond $a = 5$) systems were evaluated applying usual methods. Further, the equilibrium constant data, including those already reported by Martell *et al.*[1], were used to calculate free ligand concentrations, $[L^{3-}]$ in 1 : 1 metal-ligand mixtures and 4GDA solution (keeping total initial concentration of the ligand 0.0025 M in each case).

RESULTS AND DISCUSSION

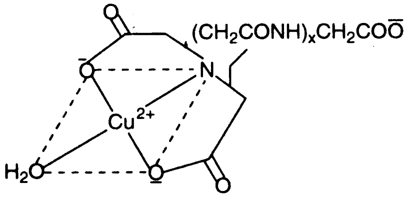
The pH-metric titration curves reported by Martell *et al.*[1] showed buffer regions between $a = 0$ and 2 (below pH ~ 4.5) and between $a = 2$ and 3 (below pH ~ 8.0) for the ligands leading to dissociation of three protons. The curves for 1 : 1 metal : ligand complex systems indicated buffer region between $a = 0$ and 3 (below pH ~ 5.0) with close similarities for all metal ions. Analysing the experimental data and evaluating the equilibrium constants, they established the following equilibria:

Between $a = 0$ and 3:

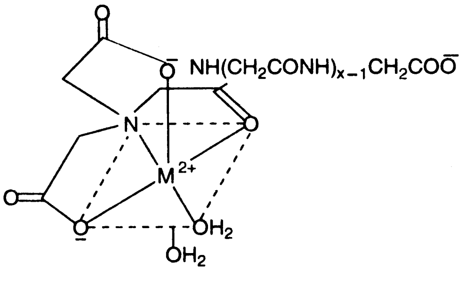
$$M^{2+} + L^{3-} \rightleftarrows ML^{-}, \quad K_{ML} \tag{1}$$

$$ML^{-} + H^{+} \rightleftarrows MHL, \quad K_{MHL} \tag{2}$$

and suggested structure I for Cu^{2+} and II for Ni^{2+} and Co^{2+} complexes involving the coordinations through amino nitrogen-diacetic acid carboxylates and amino nitrogen-diacetic acid carboxylates-amido carbonyl, respectively. These structures were further confirmed by IR studies.



I (CuL^{-})



II (ML^{-}, Ni^{2+}; Co^{2+})

pH vs *a* curves further show liberation of protons beyond $a = 3$ which indicates either association of OH^- ion with the complex species, ML^- or the proton dissociation from the peptide nitrogen or displacement of hydrogen ion due to metal-peptide nitrogen bonding. The last two possibilities will always remain till all the peptide hydrogens are removed. The equilibrium reactions lying between $a = 3$ and 4, 4 and 5, etc. for complex hydrolysis and for proton liberation from peptide nitrogen may be represented by:

Between $a = 3$ and 4:

$$MH_{-1}L^{2-} + H^+ \rightleftarrows ML^-,\ K_{A1} \qquad (3)$$

$$ML^- + OH^- \rightleftarrows ML(OH)^{2-} \qquad (4)$$

Between $a = 4$ and 5:

$$MH_{-2}L^{3-} + H^+ \rightleftarrows MLH_{-1}L^{2-},\ K_{A2} \qquad (5)$$

$$MH_{-1}L^{2-} + OH^- \rightleftarrows ML_{-1}L(OH)^{3-} \qquad (6)$$

$$ML(OH)^{2-} + OH^- \rightleftarrows ML(OH)_2^{3-} \qquad (7)$$

Between $a = 5$ and 6:

$$MH_{-3}L^{4-} + H^+ \rightleftarrows MH_{-2}L^{3-},\ K_{A3} \qquad (8), \text{etc.}$$

Martell *et al.*[1] evaluated K_{A1} and K_{A2} considering dissociation of peptide hydrogen due to coordination and suggested the structures of the complexes by IR studies. For obtaining the necessary pH-metric support regarding the bonding of OH^- ion with complexes or proton dissociation from peptide nitrogen, the previously described method[2–4], as modified below, has been applied.

pH vs pL curves for Cu^{2+}-4GDA 1 : 1 complex system was plotted (Fig. 1, curve A′) using the values of K_{MHL}, K_{ML}, K_{A1}, K_{A2} and K_{A3} ($\log K_{A3} = 10.71$ as evaluated here), proton dissociation constants, equilibrium constants of Cu^{2+} hydrolysis[5] (in the formation of $Cu(OH)^+$, $Cu(OH)_2$ and $Cu(OH)_3^-$). Curve A′ was extended beyond $a = 3$ (between $a = 3$ and 4) by B″ such that the vertical distance between B″ and A″ (pH vs. pL curve for ligand alone obtained in absence of metal ion) remains the same. Curve B″ and other similar curves, C″ and D″ plotted between $a = 4$ and 5,5 and 5 and 5 and 6, respectively, instead of coniciding with curve A′ (indication of OH^- association with the complex) or moving below curve A′ (condition for peptide hydrogen dissociation)[2–4] move upwards showing decomposition of the complexes, which is not favoured by the experimental data. A parallel trend is followed in all systems, except in Cu^{2+}-2GDA system, between $a = 3$ and 4, where proton liberation from the peptide nitogen is indicated. The increasing tendency of free ligand concentration, during metal-ligand or metal-ligand-OH^- association, as evident from the deviation of the extended curves above curve A′, appears due to no consideration of metal hydrolysis in curve A″. Moreover, such behaviour of the extended curves in previous studies[2–4] could not be observed because in those systems only lower metal hydroxo species were introduced for calculating free ligand concentration.

Thus, better results can be achieved if deviations of curves B′, C′, D′ obtained considering $MHL + ML^-$, between $a = 3$ and 4; $MHL + ML^- + MH_{-1}L^{2-}$, between $a = 4$ and 5; $MHL + ML^- + MH_{-1}L^{-2} + MH_{-2}L^{3-}$, between $a = 5$ and 6, respec-

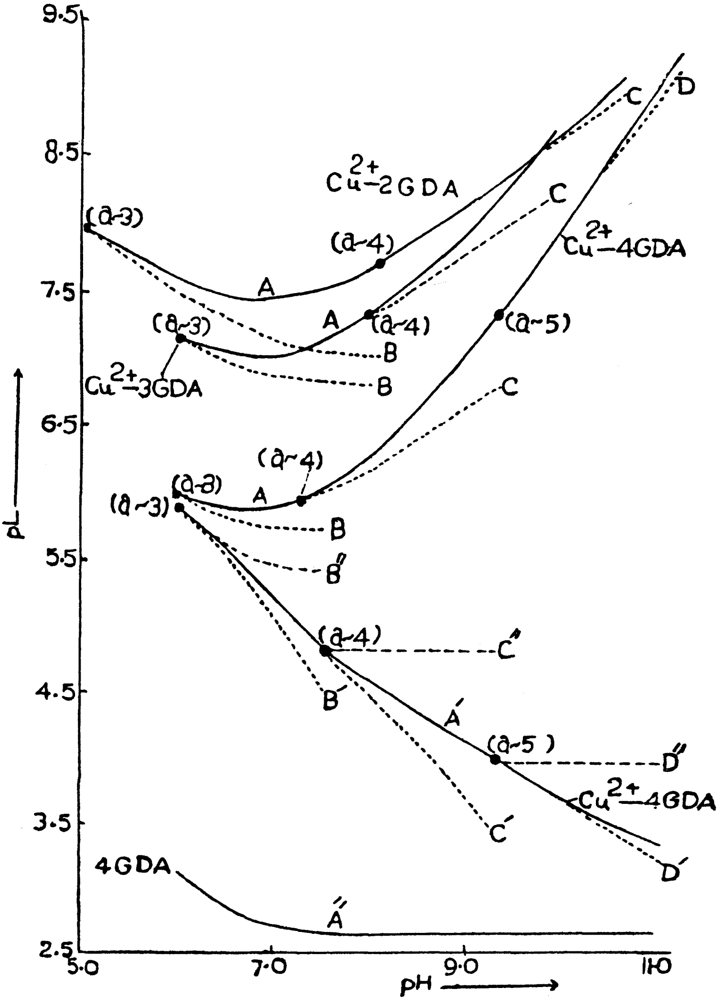


Fig. 1 pL vs pH curves for 1 : 1 Cu^{2+}-ligand complexes (total initial ligand concentration = 0.0025 M, $\mu = 0.10$ M KNO_3, 25°C): curves, A′ (4GDA); A′, B′, C′, D′ (Cu^{2+}-4GDA system, obtained introducing the concentrations of, all complexes, $MHL + ML^-$, $MHL + ML^- + MH_{-1}L^{2-}$, $MHL + ML^- + MH_{-1}L^{2-} + MH_{-2}L^{3-}$, respectively, and considering metal hydrolysis); B″, C″, D″ (extensions of curve A′); A, B, C, D (Cu^{2+}-ligand systems, obtained introducing the concentrations of, all complexes, $MHL + ML^-$, $MHL + ML^- + MH_{-1}L^{2-}$, $MHL + ML^- + MH_{-1}L^{2-} + MH_{-2}L^{3-}$, respectively).

tively along with metal hydroxo species, from curve A′ are examined (Fig. 1). The corresponding curves A, B, C, D, plotted ignoring the metal hydroxo species, again furnish the same result *i.e.* deviation in curves (vertical distance at any pH) remains the same in both the conditions (Fig. 1, Cu^{2+}-4GDA), which is also clear from the mass balance relations for a large metal-ligand association. Thus for obtaining the desired informations, pL vs pH curves, A, B, C etc. for all Cu^{2+} (Fig. 1) and Ni^{2+}, Co^{2+} (Fig. 2) complex systems were plotted and analysed.

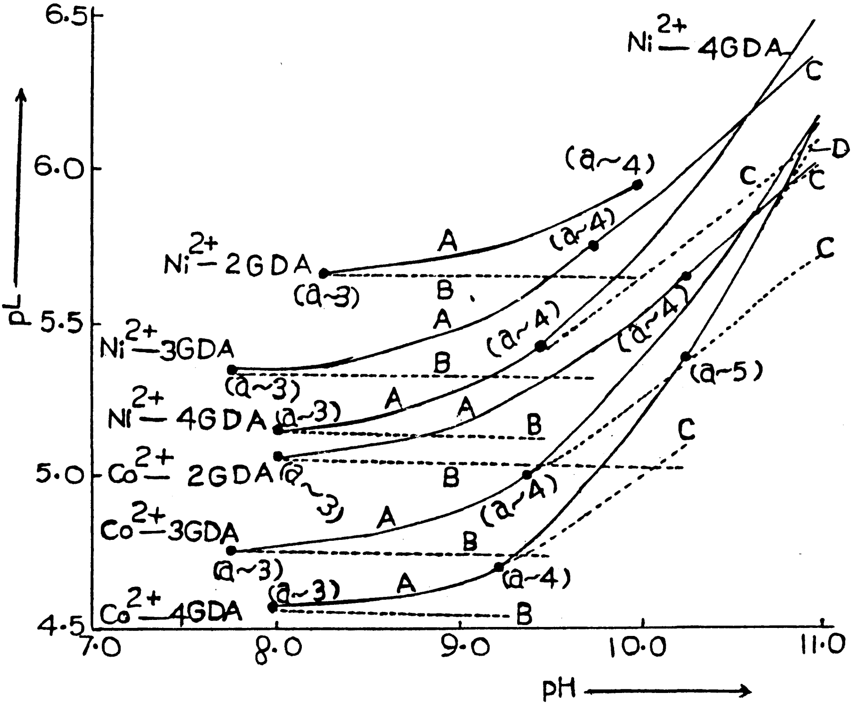


Fig. 2 pL vs pH curves for 1 : 1 metal-ligand complexes (total initial ligand concentration = 0.0025 M, $\mu = 0.10$ M KNO_3, 25°C): curves, A, B, C, D, (Ni^{2+}. Co^{2+}-ligand systems obtained introducing the concentradions of, all complexes, $MHL + ML^-$, $MHL + ML^- + MH_{-1}L^{2-}$, $MHL + ML^- + MH_{-1}L^{2-} + MH_{-2}L^{3-}$, respectively).

Cu^{2+} complexes

From the titration curve of Cu^{2+}-2GDA system, Martell *et al.*[1] reported the equilibrium constant for reaction (3) (lying between $a = 3$ and 4) and suggested the formation of $CuH_{-1}L^{2-}$ (at $a = 4$) on the basis of IR studies. Further increase in a values beyond $a = 4$ was not examined by them probably because the IR measurement could not be recorded for the species $CuH_{-1}L(OH)^{3-}$ (if the species at $a = 4$ is $CuH_{-1}L^{2-}$) which is *ca.* 60% of the total complex species at pH *ca.* 11.0. Similarly in 4GDA system they have ignored the species formed beyond $a = 5$.

Values of $K_{A2} = 10^{10.32}$ for Cu^{2+}-2GDA (beyond $a = 4$, pH *ca.* 8.0) and $K_{A3} = 10^{10.71}$ for Cu^{2+}-4GDA (beyond $a = 5$, pH *ca.* 9.2) were evaluated and were utilized to obtain the complet pL vs. pH curves. Curves, B for Cu^{2+}-2GDA, B, C for Cu^{2+}-3GDA and Cu^{2+}-4GDA systems deviate from curves A of their own systems indicating proton dissociation from the peptide nitrogen. Since the peptide hydrogens are not dissociable at the experimental pH, as indicated by the titration curves, the liberation of protons is most likely expected due to coordination of peptide nitrogen with the metal. The possible arrangements of coordinating groups based on these observations are shown in structures III, V-VIII, which are in conformity with the IR results reported by Martell *et al.*[1] In Cu^{2+}-2GDA system, after deprotonation of the single peptide proton due to co-ordination with metal (between $a = 3$ and 4), further change in a values is expected because of OH^- association forming $CuH_{-1}L(OH)^{3-}$ species (structure IV). An examination of pL vs. pH curves shows that curves A and C coincide indicating the existence of this species beyond $a = 4$. However, liberation of proton, beyond $a = 5$, in 4GDA system, is responsible for the formation of mixed protonated hydroxy species, $CuH_{-2}L(OH)^{4-}$ as curve D falls on curve A, while 3rd peptide nitrogen remains uncoordinated and protonated (structure IX).

Ni^{2+} complexes

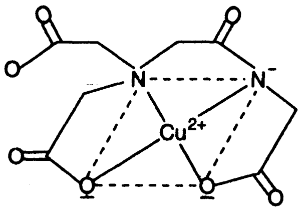
Ni^{2+}-2GDA mixture turns blue at $a = 4$, which shortly thereafter precipitated as $Ni(OH)_2$. Martell *et al.*[1] reported the formation of $NiL(OH)^{2-}$ species, on the support of IR studies, between $a = 3$ and 4. But, in the present study, curve B considerably deviates from curve A (Fig. 2) showing coordination of peptide nitrogen. The corresponding reaction in other complex systems, where $MH_{-1}L$ species are formed involving the peptide nitrogen, also justify the present result (structure X). However, few additional evidences are still desirable in favour of this reaction.

For obtaining the complete pL vs pH curve, $K_{A2} = 10^{11.14}$ for Ni^{2+}-3GDA system was evaluated beyond $a = 4$ (pH *ca.* 9.7). Curve B diverges from curve A between $a = 3$ and 4 pointing out the formation of $NiH_{-1}L^{2-}$ species (structue XII). Beyond $a = 4$, unlike Cu^{2+} or Co^{2+} systems (where curves C deviate from curves A) curves C and A overlap each other showing association of OH^- ion with $NiH_{-1}L^{2-}$ without disturbing the metal-ligand bonds (structure XIII).

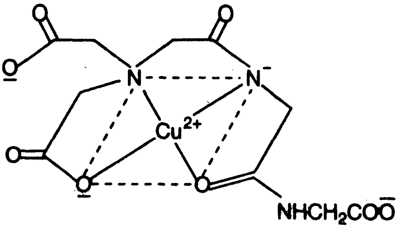
Both the curves B and C, in Ni^{2+}-4GDA system, deviate from curve A indicating metal-peptide linkage as suggested by previous workers by IR studies (structures XV, XVI).

Co^{2+} Complexes

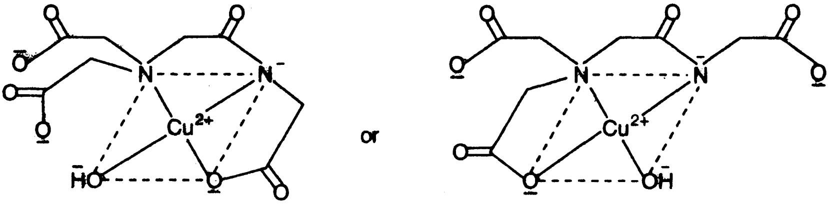
Values of K_{A2} and K_{A3} evaluated for 2GDA and 3GDA systems are $10^{11.33}$ and $10^{11.34}$ respectively. In Co^{2+}-2GDA system it is seen that curve B moves away from curve A (Fig. 2) due to metal-peptide nitrogen linkage (structure X). After displacement of the single peptide proton, forming $NiH_{-1}L^{2-}$, association of OH^- ion is expected beyond $a = 4$ which is evident from Fig. 2 as curve C coincides with curve A (structure XI). Deviations of curves B and C from curve A in Co^{2+}-3GDA system is an indication of the formation of the species $CoH_{-1}L^{2-}$ and $CoH_{-2}L^{3-}$ (structures XII, XIV) involving metal-peptide nitrogen linkage in each complex. Also, since in Co^{2+}-4GDA system curves B and C diverge from curve A and curve D almost rests upon it (Fig. 2) the species



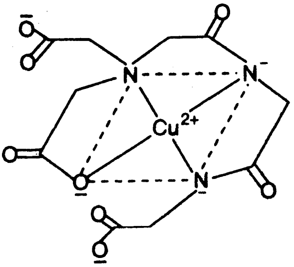
III ($CuH_{-1}L^{2-}$ 2GDA)



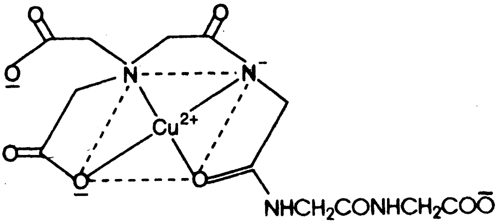
V ($CuH_{-1}L$, 3GDA)



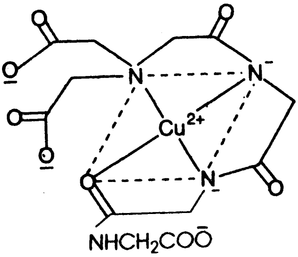
IV ($CuH_{-1}L(OH)^{3-}$, 2GDA)



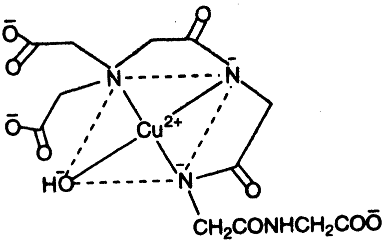
VI ($CuH_{-2}L^{3-}$, 3GDA)



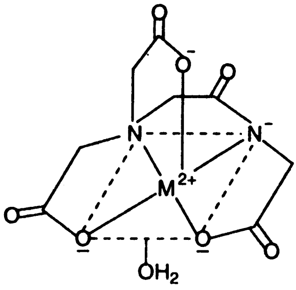
VII ($CuH_{-1}L^{2-}$, 4GDA)



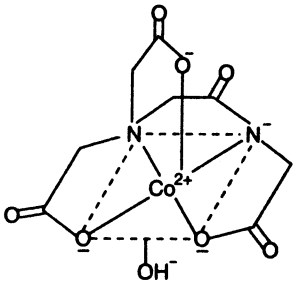
VIII ($CuH_{-2}L^{3-}$, 4GDA)



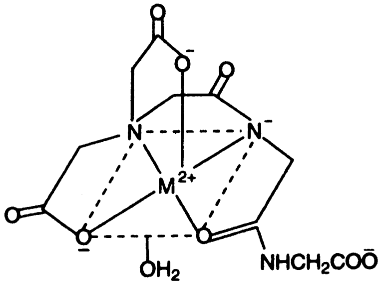
IX ($CuH_{-2}L(OH)^{4-}$, 4GDA)



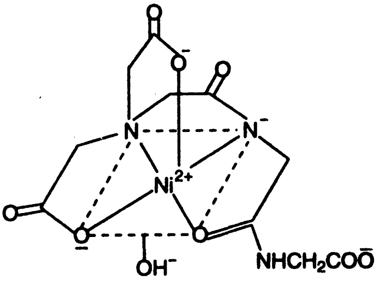
X (MH$_{-1}$L^{2-}; Ni^{2+}, Co^{2+}, 2GDA)



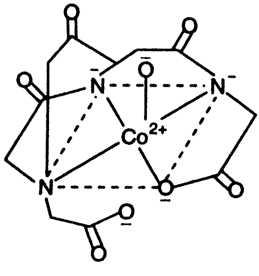
XI (CoH$_{-1}$L(OH)$^{3-}$, 2GDA)



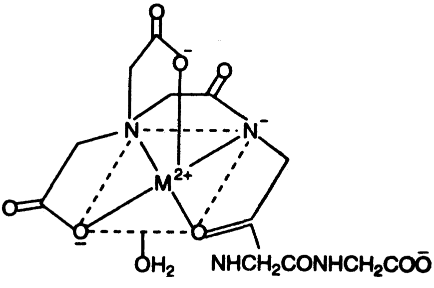
XII (MH$_{-1}$L^{2-}, Ni^{2+}, Co^{2+}, 3GDA)



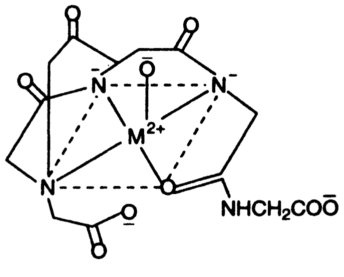
XIII (NiH$_{-1}$L(OH)$^{3-}$, 3GDA)



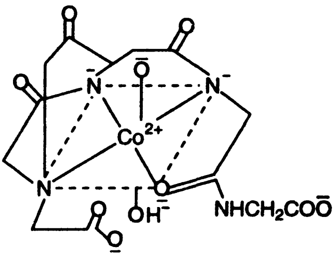
XIV (CoH$_{-2}$L^{3-}, 3GDA)



XV (MH$_{-1}$L^{2-}, Ni^{2+}, Co^{2+}, 4GDA)



XVI (MH$_{-2}$L^{3-}; Ni^{2+}, Co^{2+}, 4GDA)



XVII (CoH$_{-2}$L(OH)$^{4-}$, 4GDA)

$CoH_{-1}L^{2-}$,$CoH_{-2}L^{3-}$ and $CoH_{-2}L(OH)^{4-}$ (structures XV, XVI, XVII) must be formed in steps beyond $a = 3$.

REFERENCES

1. R.J. Motekaitis and A.E. Martell, *Inorg. Chem.*, **13**, 551 (1974).
2. R. Nayan and A.K. Dey, *Transition Metal Chem.*, **2**, 110 (1977).
3. R. Nayan, *J. Inorg. Nucl. Chem.*, **42**, 1743 (1980).
4. ________, *Indian J. Chem.*, **20A**, 382 (1981).
5. ________, *Indian J. Chem.*, **19A**, 786 (1980).

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