Chemical speciation and biological activity of bivalent metal complexes of polydentate amide ligands

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Stability constants of N,N'-bis-(3-carboxy-1-oxopropanyl)-1,2-ethylenediamine(L1), N,N'-bis-(3-carboxy-1-oxopropanyl)-1,2-phenylenediamine(L2), N,N'-bis-(3-carboxy-1-oxophenelenyl)-1,2-phenylenediamine(L3) have been determined in aqueous medium (for L1 & L2) and 75% (v/v) methanol-water medium for L3 pH-metrically. The formation constants (log β) have been calculated using the Bjerrum half π integral method and weighted least squares method. Species distribution curves of complexes have been plotted as a function of pH which shows at lower pH values 1:1 complexes are formed and 1:2 complexes are formed at neutral or higher pH. The biological study indicates that ligands and their complexes are better fungicidal than bactericidal. Copper complexes of all the ligands showed potential antifungal and antibacterial activities. Ligand L3 and its copper(II) complexes are most effective against A. niger, E. coli and S. aureus.

The importance and significance of amide groups containing ligands are growing day-by-day as potential models for naturally occurring and biologically important complexes such as metal carrierc proteins, metalloenzymes or antibiotic peptides and as an angiotensin I/II receptor antagonists. Literature survey reveals that little potentiometric and biological work has been done on the bivalent metal complexes of amide ligands. So the present study was undertaken to determine the stability constants of Cu(II), Ni(II), Co(II) and Mn(II) with L1, L2 and L3 at ionic strength 0.1 M NaClO4 and temperature, 25±0.5°C and nature of complexes was ascertained by plotting species distribution curves as a function of pH and their biological activity against E. coli, S. aureus (bacterial cultures) and A. niger (fungal culture) has been studied.

Materials and Methods

A digital (pH 5651) pH-meter with combined glass electrode assembly was used for pH measurements. The pH-meter was standardized with potassium hydroxphthalate and phosphate buffers before performing the titrations.

The solution of ligand (L1 & L2) was prepared in double distilled water and L3 in methanol. All the metal ion solutions were prepared and standardized by conventional procedures. A solution of tetramethylammonium hydroxide (TMAH) (E. Merck) in double distilled water for L1 and L2 and 75% (v/v) methanol-water for L3 was used as the titrant. It was standardized with a standard solution of oxalic acid. All other chemicals used were of reagent grade. The titrations were carried out in an atmosphere of nitrogen, which was presaturated with double distilled water. All measurements were made at a definite temperature which were kept constant by using a Julabo F20 (West Germany) thermostat.

The method of Bjerrum and Calvin as modified by Irving and Rossotti, has been used to determine π and pL values.

pH-Titratiion procedure

The experimental procedure involved the potentiometric titrations of the following solutions against standard TMAH solution in water (For L1 and L2) and 75% (v/v) methanol-water for L3 at 25±0.5°C and at 0.1 M NaClO4 ionic strength to determine π and pL values of the complexes.

For L1 and L2

(i) 0.8 mL HClO4 (0.05 M) + 1.0 mL NaClO4 (2.0 M) + 0.5 mL K2SO4 (0.02 M) + 17.7 mL H2O.
(ii) 0.8 mL HClO4 (0.05 M) + 1.0 mL NaClO4 (2.0 M) + 0.5 mL K2SO4 (0.02 M) + 10.0 mL ligand (0.01 M) + 7.7 mL H2O.
(iii) 0.8 mL HClO4 (0.05 M) + 1.0 mL NaClO4 (2.0 M) + 0.5 mL MSO4 (0.02 M) + 10.0 mL ligand (0.01 M) + 7.7 mL H2O.

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For $L_3$

(i) $0.8$ mL $\text{HClO}_4$ ($0.05$ M) + $1.0$ mL $\text{NaClO}_4$ ($2.0$ M) + $0.5$ mL $\text{K}_2\text{SO}_4$ ($0.02$ M) + $15.0$ mL $\text{MeOH}$ + $2.7$ mL $\text{H}_2\text{O}$.

(ii) $0.8$ mL $\text{HClO}_4$ ($0.05$ M) + $1.0$ mL $\text{NaClO}_4$ ($2.0$ M) + $0.5$ mL $\text{K}_2\text{SO}_4$ ($0.02$ M) + $10.0$ mL ligand ($0.01$ M) in $\text{MeOH}$ + $5.0$ mL $\text{MeOH}$ + $2.7$ mL $\text{H}_2\text{O}$.

(iii) $0.8$ mL $\text{HClO}_4$ ($0.05$ M) + $1.0$ mL $\text{NaClO}_4$ ($2.0$ M) + $0.5$ mL $\text{K}_2\text{SO}_4$ ($0.02$ M) + $10.0$ mL ligand ($0.01$ M) in $\text{MeOH}$ + $5.0$ mL $\text{MeOH}$ + $2.7$ mL $\text{H}_2\text{O}$.

The pH meter readings were plotted against the volume of TMAH used for each titrations.

Calculation of $\beta_k$

The complexation takes place in the stepwise manner as follows:

$\text{M}^{2+} + \text{H}_2\text{L} \rightleftharpoons [\text{MHL}]^+ + \text{H}^+; \quad K_1 = \frac{[\text{MHL}]^+ [\text{H}^+]}{[\text{M}^{2+}] [\text{H}_2\text{L}]}$

$[\text{MHL}]^+ \rightleftharpoons [\text{ML}] + \text{H}^+; \quad K_2 = \frac{[\text{ML}] [\text{H}^+]}{[\text{MHL}]^+}$

and $\beta_2 = K_1 K_2$, i.e. $\beta_2 = \frac{[\text{ML}] [\text{H}^+]^2}{[\text{M}^{2+}] [\text{H}_2\text{L}]}$

In view of the low concentration of metal ion ($2 \times 10^{-2}$ M) used in the titration, it has been assumed that the possible formation of polynuclear complexes is negligible

From the titration curves of solution (i) and (ii) which are known as acid blank and ligand blank, respectively, values of $\bar{n}_H$ were calculated at various pH values using following formula:

$\bar{n}_H = Y \cdot T_1 + \frac{[V'' - V'''] [N_H + E'' + T_0 (Y - \bar{n}_H)]}{(V'' + V''') \bar{n}_L T_M}$

where $\bar{n}_H =$ average number of protons bound to each ligand molecule (which is not bound to metal), $Y =$ number of dissociable hydrogen atoms, $T_1 =$ total concentration of ligand, $V' =$ volume of acid blank, $V'' =$ volume of ligand blank, $N_H =$ concentration of TMAH (base), $E'' =$ total concentration of $\text{HClO}_4$ (acid), $V''' =$ initial volume of the set solution.

Since ligand has two ionizable hydrogen atom, it has two $pK$ values. The $pK_1$ of the ligand was obtained by plotting $\log (\bar{n}_H - 1)/(2 - \bar{n}_H)$ versus pH and the intercept of the plot of $\log \bar{n}_H (1 - \bar{n}_H)$ versus pH gave $pK_2$.

From curves of acid blank, ligand blank and a plot of $\bar{n}_H$ values against pH reading, $\bar{n}$ values of metal complexes were determined at various pH values using the following expression:

$\bar{n} = \frac{(V'' - V''') [N_H + E'' + T_0 (Y - \bar{n}_H)]}{(V'' + V''') \bar{n}_L T_M}$

where $\bar{n} =$ average number of ligand molecule attached to the metal, $V'' =$ volume of base and all other symbols have their usual significance.

From $pK_1$ and $\bar{n}$ values at different pH, the corresponding values of free ligand exponent, $PL$ were calculated using the equation:

$\text{PL} = -\log [L] = \log \left[ \frac{1 + 10^{pK_1 - pH} + (10^{pK_1 - pH}) [N_H + E'' + T_0 (Y - \bar{n}_H)]}{(V'' + V''') \bar{n}_L T_M} \right]$

The formation curves have been plotted from the $\bar{n}$ and $PL$ values which gives $logK_1$ and $logK_2$ and overall stability constants ($log \beta_2$) calculated from them.

From the above titration curves of solutions (i), (ii) and (iii) the values of $\bar{n}$ and $PL$ which gives $logK_1$ and $logK_2$ and overall stability constants ($log \beta_2$) have been calculated using the weighted least squares method of Sullivan et al. $^7$. The weighted least squares treatment determines that the set of $\beta_n$ values which makes the function:

$U \left[ U = \sum_{n=1}^{N} (y - x - nz) \beta_n x^n \right]$}

The corresponding values of stability constants have been calculated using the weighted least squares method of Sullivan et al. $^7$. The weighted least squares treatment determines that the set of $\beta_n$ values which makes the function:

$U \left[ U = \sum_{n=1}^{N} (y - x - nz) \beta_n x^n \right]$}

The above equations, $y$ is the total ligand concentration, $x$ is the total concentration of unbound ligand, $z$ is the total metal ion concentration and $\beta_n$ denotes stability constants.

We report the $S_{min}$ values for the different metal complexes. $S_{min}$ has the same statistical distribution as
**Studies at higher pH values could not be equated to** $\chi^2$ with $K$ degrees of freedom and with weights defined in accordance with Sullivan. $S_{\text{min}}$ can be equated to $\chi^2$. The stability constants calculated are given in Table 1.

### Procedure for biological study

The biological activity of ligands $L_1$, $L_2$, $L_3$ and their Cu(II), Co(II) complexes in terms of their growth inhibitory property on specific known bacterial and fungal cultures were evaluated by standard disc diffusion method\(^8\)-\(^\text{10}\). The bacterial subcultures for *E. coli* and *S. aureus* and fungal subculture for *A. niger* were used as test organism and all sample (100 µg/mL$^\text{1}$ in DMF) where DMF acted as control. All apparatus used were sterilized by heating at 120°C in an oven fully wrapped in inert foil for 6-8 h. Nutrient agar slant used as bacterial culture and nutrient agar slant used as fungal culture were kept in an incubator maintained at 37°C for 24 h and the fungal culture were kept at room temperature for 48-72 h.

### Results and Discussion

#### (a) Protonation constants

The observed values of protonation equilibrium constants of the ligand are given in Table 1. These values for the logarithms of the stepwise protonation constants of the ligand indicate the protonation of the two carboxylic functional groups of the ligand, since the determination of the deprotonation constants involving $-\text{NH}$ of amide group by potentiometry is not reliable and requires very basic media\(^{11;12}\). These values are in good agreement with those reported earlier for such a ligand\(^9\) and are within the range observed for $\beta$-substituted carboxylic acids\(^{13}\). These high values are indicative of the fact that ligand behaves as a relatively strong base.

#### (b) Stability constants of the metal complexes

The complex forming ability of $H_2L$ with respect to bivalent metal ions, $M(\text{II})$ [$M(\text{II}) = \text{Mn(II)}, \text{Co(II)}, \text{Ni(II)}, \text{Cu(II)}$] has been investigated in the pH range of 3.0-10.6. Studies at higher pH values could not be carried out because of precipitation of complexes. The values of stability constants are given in Table 1 and are of the same order of the magnitude as those for 1:1 and 1:2, $M(\text{II})$: carboxylate ligand complexes in which the carboxylate group acts as a monodentate ligand\(^5\). This indicates the possibility of coordination of $L^2$ to $M(\text{II})$ through its carboxylate group to yield mononuclear 1:1 and 1:2 species.

The stability constants of the Cu(II) complex ($\log \beta_2 = 16.68$) is somewhat greater as expected according to higher stability of Cu(II) complexes. In fact, with respect to metal ions, the stability of complexes is in the sequence:

\[
\text{Mn(II)} < \text{Co(II)} < \text{Ni(II)} < \text{Cu(II)}
\]

which is in harmony with the Irving-Williams series\(^{16}\). The relatively higher stability constant values reveal the chelating character of the ligand.

### Table 1—Stability constants of bivalent metal complexes of amide ligands ($L_i$ & $L_2$) in aqueous medium and $L_3$ in 75% (v/v) methanol-water solution at ionic strength $\mu = 0.1 \text{ M NaClO}_4$ and at temperature = 25±0.5°C

<table>
<thead>
<tr>
<th>Metal ions</th>
<th>$\log K_1$</th>
<th>$\log K_2$</th>
<th>$\log \beta_2$</th>
<th>$S_{\text{min}}$</th>
<th>$\log K_1$</th>
<th>$\log K_2$</th>
<th>$\log \beta_2$</th>
<th>$S_{\text{min}}$</th>
<th>$\log K_1$</th>
<th>$\log K_2$</th>
<th>$\log \beta_2$</th>
<th>$S_{\text{min}}$</th>
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<tr>
<td>$H^+$</td>
<td>5.1</td>
<td>11.5</td>
<td>6.0</td>
<td>4.46</td>
<td>6.0</td>
<td>3.86</td>
<td>5.8</td>
<td></td>
<td>8.45</td>
<td>3.00</td>
<td>0.0034</td>
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<tr>
<td>Cu(II)</td>
<td>9.34</td>
<td>7.34</td>
<td>16.68</td>
<td>0.0077</td>
<td>4.49</td>
<td>3.44</td>
<td>7.93</td>
<td>0.0283</td>
<td>4.43</td>
<td>4.02</td>
<td>8.45</td>
<td>0.0034</td>
</tr>
<tr>
<td>Ni(II)</td>
<td>6.50</td>
<td>5.02</td>
<td>11.52</td>
<td>0.1342</td>
<td>4.26</td>
<td>3.59</td>
<td>7.85</td>
<td>0.0219</td>
<td>4.10</td>
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<tr>
<td>Co(II)</td>
<td>5.24</td>
<td>3.86</td>
<td>9.10</td>
<td>0.1061</td>
<td>3.72</td>
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<td>7.01</td>
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<td>3.57</td>
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<td>6.57</td>
<td>0.0016</td>
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<tr>
<td>Mn(II)</td>
<td>5.10</td>
<td>3.82</td>
<td>8.92</td>
<td>0.0382</td>
<td>3.70</td>
<td>2.92</td>
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<td>0.0241</td>
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</table>

$log K$ & $log \beta_2$ and $(log K)$ and $(log \beta_2)$ are the values of stability constants calculated by using Bjerrum's half $\bar{n}$ integral method and weighted least squares method, respectively.
(c) Species study

Using the various equilibrium constants percentage species distribution of $L^2/M^{2+}$ system as a function of pH were calculated and same are depicted as representative for copper(II) complexes in Figs 1-3. Only the ML and ML$_2$ species are present in the pH range studied. In Cu(II)/L$_1$ system ML and ML$_2$ species are more predominant. Perusal of species distribution diagrams reveal that 1:1 complexes are formed in the pH range 7.0-9.0 (for Mn(II), Co(II), Ni(II)/L$_1$ systems) and in the pH range 4.0-8.0 for Cu(II)/L$_1$ system. The 1:2 complexes are predominant at pH > 7.0 (with very low percentage distribution) in Mn(II), Co(II)/L$_1$ systems and are significantly distributed in the pH range 6.0-12.0 in Ni(II), Cu(II)/L$_1$ systems. For L$_1$, at lower pH values H$_2$L species are dominant. At pH ~ 7.0, HL species have maximum abundance and L$_2^-$ species are maximum in the pH range 10.0-12.0.

Species distribution diagram reveals that for Mn(II), Co(II), Ni(II)/L$_2^-$/L$_3^-$ systems the 1:1 complexes are formed in the pH range 4.0-7.0 and 1:2 complexes are formed in the pH range 4.0-9.0 for L$_2$ and 5.0-7.0 for L$_3$. The ML and ML$_2$ species for Cu(II)/L$_2^-$/L$_3^-$ systems are predominant in the pH range 3.0-6.0 and 4.0-10.0 respectively. For L$_2$ and L$_3$, at lower pH values H$_2$L species are dominant. At pH range 2.0-8.0 HL species are more abundant and L$_2^-$ species are maximum at pH range 4.0-12.0.

(d) Biological study

The activity of the compounds against various microorganisms are presented in Table 2 and are depicted by positive signs (+) depending upon the diameter and clarity of zones of inhibition. Each (+) indicates a difference of 1 mm in diameter of the zone of inhibition, when there were no zones of inhibition, the results have been indicated by negative sign in the Table 2. The clearing zones around the discs indicated the inhibiting activity of the compound on the organism. These were measured and compared with that of control to evaluate the zone of inhibition to the test compounds.

All the ligands and copper(II) and cobalt(II) complexes of ligands L$_2$, L$_3$ and L$_4$ were screened for antifungal and antibacterial activities. Most of the compounds show significant inhibition against the microorganisms.

Copper(II) complexes of all the ligands are more potent fungicidal and bactericidal than ligands. Cobalt(II) complexes are less effective against these microorganisms than corresponding copper(II) complexes. Among the various compounds, the ligand...
complexes are formed at neutral or higher pH. The protonation constants of the ligands and formation constants of the complexes of the same ligand systems and trend is unexpected since several studies have shown that complexes show reversed activity compared to the corresponding metal ions, the stability constant is in accordance with Irving-Williams series of metal complexes to be more potent antimicrobial than their precursor ligands.

**Conclusion**

The protonation constants of the ligands and formation constants of the complexes of the same metal ion follow the order \( L_1^{2-} > L_2^{2-} > L_3^{2-} \). With respect to the metal ions, the stability constant is in the sequence: \( \text{Mn}^{2+} < \text{Co}^{2+} < \text{Ni}^{2+} < \text{Cu}^{2+} \). This is in accordance with Irving-Williams series of stability constant. Species distribution studies shows at lower pH values 1:1 complexes are formed and 1:2 complexes are formed at neutral or higher pH.

Ligands and their complexes are better fungicidal than bactericidal. Copper(II) complexes of all the ligands showed potential antifungal and antibacterial activities. Ligand L_1 and its copper(II) complexes are most effective against \( A. niger, E. coli \) and \( S. aureus \).

**Table 2—Antifungal and antibacterial activities of ligands and their Cu(II) and Co(II) complexes**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Antifungal Activity</th>
<th>Antibacterial Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{Cu}^{2+} \text{L}_1 )</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>( \text{Cu}^{2+} \text{L}_2 )</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>( \text{Cu}^{2+} \text{L}_3 )</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>( \text{Cu}^{2+} \text{L}_4 )</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>( \text{Cu}^{2+} \text{L}_5 )</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

References