Short communication

# Chemical Speciation of Binary Complexes of Embelin With Some Biologically Important Metal Ions

**Vaddadi Usha Rani,[1] Ghanta Jyothi,[2] Gollapalli Nageswara Rao[1] and Budati Bala Venkata Sailaja[1,*]**

[1] *Department of Inorganic and Analytical Chemistry, Andhra University, Visakhapatnam-530 003, India*

[2] *St. Theresa's College for women, Eluru, India*

* *Corresponding author: E-mail: sailaja_bbv@yahoo.co.in*

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## Abstract

Protonation and complexation equilibria of embelin were investigated in 86% v/v dimethyl sulphoxide – water mixture electrometrically at an ionic strength of 0.17 mol $dm^{-3}$ and at a temperature of 303K. The best fit chemical models were arrived at based on statistical grounds employing crystallographic R factor, $\chi^2$, skewness and kurtosis. The existence of binary complex species with Co(II), Ni(II), Cu(II) and Zn(II) were established from modeling studies using the computer program MINIQUAD75. Distribution diagrams of various species of the complex in relation to pH are presented.

**Keywords:** Embelin, speciation, DMSO, binary complexes

## 1. Introduction

Embelin is produced by plants of the family myrsinaceae. The myrsinaceae are shrubs or climbers comprising about 30 genera and 1000 species, with high ethnomedical application in the East and Central African geographical region and the Asian continent. The dried fruits have been in use in India for a long time in the treatment of ringworm and other skin diseases and as an anti-helmintic,[1–5] antimicrobial[6–9] and antifertility.[10–12] The seeds of embelia were used in mordant dyeing.[13] The myrsinaceae are chemotaxonomically associated with long alkyl side chain benzoquinones which are found to constitute 10% of the dry weight of their berries and 7% of the root bark.[14] It has also been used as an analytical reagent.[15–17] Synthesis and characterization of copper(II) complexes of embelin in the cavities of zeolite Y have been conducted[18] and found to have good catalytic activity for the reduction of molecular oxygen. Embelin complexes of different metals were also studied.[19,20]

Speciation studies of essential metal ion complexes of embelin are useful for the understanding of the role played by active site cavities in biological molecules and the binding behavior of protein residues with the metal ions. Cobalt in the form of vitamin $B_{12}$ is essential for animals. Vitamin $B_{12}$ is synthesized only by micro-organisms, in particular anaerobic bacteria. Nickel is associated with several enzymes[21–23] and any variation in its concentration leads to metabolic disorders.[24] Copper is largely rejected from the biological cells but outside the cell, it is essential for the metabolism of many hormones and connective tissue. The biological functions include electron transfer, dioxygen transport, oxygenation, oxidation, reduction and disproportionation.[25,26] Zinc is the second most abundant essential trace metal after iron and it plays vital roles in biological systems.[27–30] Based on solubility considerations speciation studies of embelin with some essential metal ions such as Co, Ni, Cu and Zn in Dimethyl sulphoxide(DMSO)-water mixture have been reported in this paper.

## 2. Experimental

### 2. 1. Materials

A solution of 0.05 mol $dm^3$ of embelin was prepared in DMSO. Aqueous solutions of Co(II), Ni(II), Cu(II) and Zn(II) chlorides (0.05 mol $dm^{-3}$) were prepared in 0.05 mol $dm^{-3}$ HCl, to suppress the hydrolysis of the metal salts. Sodium chloride was used to maintain the ionic strength in the titrand. The strengths of alkali and mineral

acid were determined using the Gran plot method.[31] 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).

## 2. 2. Apparatus

The titrimetric data were obtained using a calibrated CONTROL DYNAMICS pH-meter (readability 0.01). The glass electrode was equilibrated in a well-stirred 86% v/v DMSO-water mixture containing an inert electrolyte. All the titrations were performed at 303.0 ± 0.1 K in aqueous medium containing 86.0 % v/v DMSO, maintaining an ionic strength of 0.17 mol $dm^{-3}$ with sodium chloride. The effect of variations in asymmetry, liquid junction potential, activity coefficient, sodium ion error and dissolved $CO_2$ on the response of glass electrode was taken into account in the form of a correction factor.[32]

## 2. 3. Procedure and Modeling Strategy

For the determination of the protonation constants of embelin and stability constants of the binary metal-ligand species, initially titrations of a strong acid with alkali were performed at regular intervals to check whether complete equilibrium had been achieved. Then the calomel electrode was refilled with 86%v/v DMSO-water mixture. In each of the titrations, the titrand consisted of approximately 1 mmol mineral acid in a total volume of 50 $cm^3$. Titrations with different ratios of metal to ligand were performed with 0.20 mol $dm^{-3}$ sodium hydroxide.

The computer program SCPHD[33] was used to calculate the correction factor. The binary stability constants were calculated from pH-metric titration data using the computer program MINIQUAD75[34] which exploits the advantage of a constrained least- squares method in the initial refinement and reliable convergence of the Marquardt algorithm. During the refinement of the binary systems, the correction factor and the protonation constants of embelin were fixed.

# 3. Results and Discussion

## 3. 1. Protonation Constants

Secondary formation functions like average number of protons bound per mole of ligand ($\bar{n}H$) and number of moles of alkali consumed per mole of ligand (**a**) are useful to detect the number of equilibria. Plots of $\bar{n}H$ versus pH for different concentrations of the ligand should overlap if there is no formation of polymeric species. Overlapping formation curves for embelin (Figure 1) rule out the polymerization of the ligand molecules. The pH values at half integral values of $\bar{n}H$ correspond to the protonation constants of the ligand shown in Figure 1 which can detect the number of equilibria.

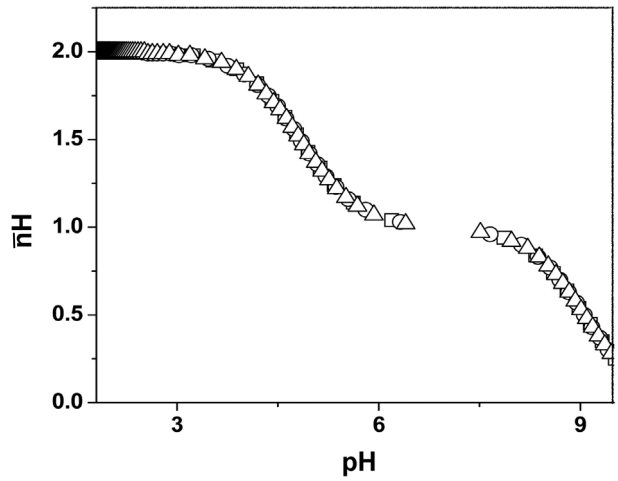


**Figure 1:** Plots of $\bar{n}H$ versus pH of embelin: (□)0.199, (○)0.299, and (△)0.399 mmol

A plot of **a** versus pH is given in Figure 2. The negative values of **a** correspond to the number of moles of free acid present in the titrand and the number of associable protons. The positive values of **a** indicate the number of dissociable protons in the ligand molecule. The maximum value of **a** in Figure 2 is two, which clearly infers that embelin has two dissociable protons.

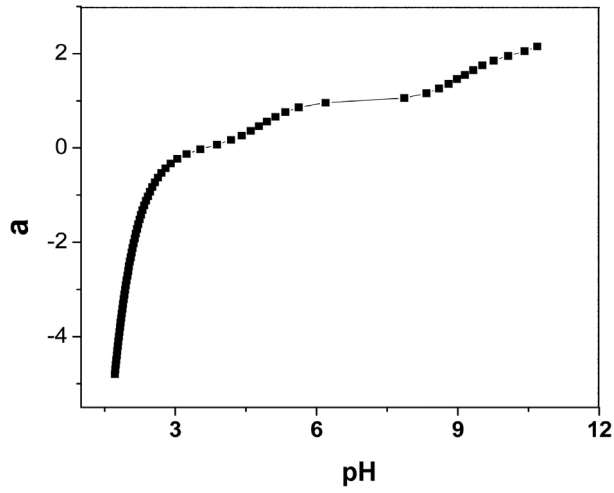


**Figure 2:** Variation of **a** with pH of embelin

Embelin has two dissociable protons and its various forms are $LH_2$, and $LH^-$ in the pH ranges 2.0–4.0 and 6.0–8.0, respectively. The species distribution diagram of embelin shown in Figure 3 represents the acido-basic equilibria which are active in the pH range 2.0–10.0. The protonation equilibria of embelin are as follows

$$L^{2-} + H^+ \leftrightarrow LH^- \quad (1)$$

$$LH^- + H^+ \leftrightarrow LH_2 \quad (2)$$

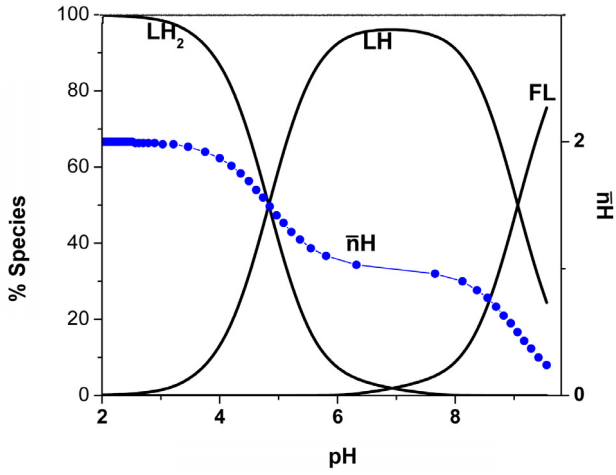


**Figure 3:** Formation function (●) and species distribution diagram of embelin

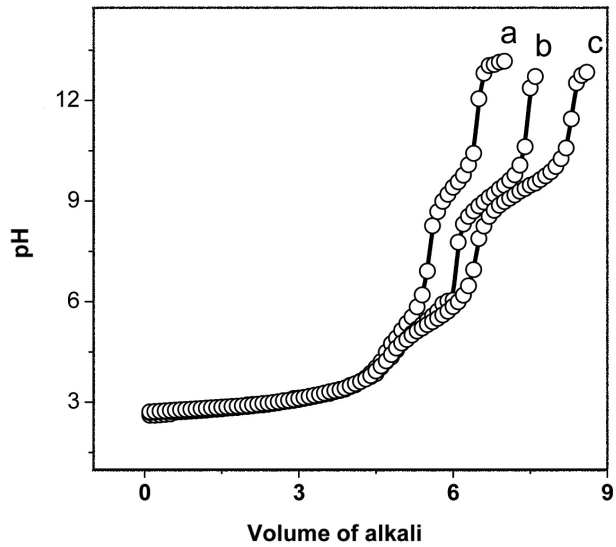


**Figure 4:** Simulated (o) and experimental (solid line) alkalimetric titration curves in 86.0% DMSO-water mixture of embelin: (a) 0.199, (b) 0.299 and (c) 0.399 mmol.

The best fit models that contain the type of species and overall protonation constants of embelin along with some important statistical parameters are given below in Table 1. A very low standard deviation in log β values indicates the precision of these parameters. The small values of $U_{corr}$ (the sum of the squares of deviations in concentrations of ligand and hydrogen ion at all experimental points) corrected for degrees of freedom, indicate that the experimental data can be represented by the model. Small values of mean, standard deviation and mean deviation for the system corroborate that the residuals are around a zero mean with little dispersion. For an ideal normal distribution, the values of kurtosis and skewness should be three and zero, respectively. The kurtosis values in the present study indicate that the residuals form leptokurtic patterns. The value of skewness given in Table 1 evinces that the residuals form a part of normal distribution; hence, least squares method can be applied to the present data. The sufficiency of the model is further evident from the low crystallographic R-values. The statistical parameters thus show that the best fit models portray the acido-basic equilibria of embelin in DMSO-water mixtures.

The primary alkalimetric titration data are simulated and compared with the experimental alkalimetric titration data, to verify the sufficiency of the model. The overlap of the typical experimental and simulated titrations data given in Figure 4 indicates that the proposed models represent the experimental data.

## 3. 2. Speciation of Binary Complexes

The overall stability constants of binary complexes of embelin with Co(II), Ni(II), Cu(II) and Zn(II) are given in Table 2.

**Table 1:** Best fit chemical model of acido-basic equilibria of embelin in 86% v/v DMSO- water mixture

| Log β (SD) | | pH-range | NP | $U_{corr}$ | $\chi^2$ | Skewness | kurtosis | R – factor |
|---|---|---|---|---|---|---|---|---|
| LH | $LH_2$ | | | | | | | |
| 9.06(2) | 13.89(4) | 3.5–10.0 | 63 | 10.015 | 22.57 | –1.85 | 10.94 | 0.031761 |

**Table 2:** Best fit chemical models of Metal-Ligand complexes in 86% v/v DMSO- water mixture

| Metal | Log β (SD) | | | pH-range | NP | $U_{corr}$ | $\chi^2$ | Skewness | kurtosis | R –factor |
|---|---|---|---|---|---|---|---|---|---|---|
| | MLH | $ML_2H$ | $ML_2H_2$ | | | | | | | |
| Co | 13.93(1) | 25.06(1) | 26.88(1) | 1.8–2.0 | 45 | 2.788 | 5.50 | 0.58 | 4.66 | 0.0068 |
| Ni | 14.37(2) | – | 29.33(1) | 1.6–1.9 | 68 | 3.976 | 13.65 | –0.04 | 4.39 | 0.0066 |
| Cu | 7.64(1) | 30.56(1) | 32.85(1) | 1.6–1.9 | 86 | 9.8 | 33.60 | –0.21 | 4.83 | 0.0117 |
| Zn | 15.26(2) | – | 28.35(1) | 1.7–2.0 | 75 | 6.696 | 17.60 | –0.03 | 4.51 | 0.0097 |

$U_{corr}$ = U/ (NP-m) X $10^8$; NP = Number of points m = number of constants; SD = Standard deviation

## 3. 3. Residual Analysis[35]

In data analysis with least squares methods, the residuals (the differences between the experimental data and the data simulated based on the model parameters) are assumed to follow Gaussian or normal distribution. When the data are fit into the models, the residuals should be ideally equal to zero. Further, a model is considered adequate only if the residuals do not show any trend. Respecting the hypothesis of the least squares analysis, the residuals are tested for normal distribution. Such tests are $\chi^2$, skewness, kurtosis and R-factor. These statistical parameters of the present data show that the best fit models portray title systems, as discussed below.

### 3. 3. 1. $\chi^2$ test

$\chi^2$ is a special case of gamma distribution whose probability density function is an asymmetric function. This distribution measures the probability of residuals forming a part of standard normal distribution with zero mean and unit standard deviation. If the $\chi^2$ calculated is less than the table value, the model is accepted.

### 3. 3. 2. Crystallographic R-test

Hamilton's R factor ratio test is applied in complex equilibria to decide whether inclusion of more species in the model is necessary or not. In pH-metric method the readability of pH meter is taken as the $R_{limit}$ which represents the upper boundary of R beyond which the model bears no significance. When there are different numbers of species the models whose values are greater than R-table are rejected. The low crystallographic R-values given in Table 2 indicate the sufficiency of the model.

### 3. 3. 3. Skewness

It is a dimensionless quantity indicating the shape of the error distribution profile. A value of zero for skewness

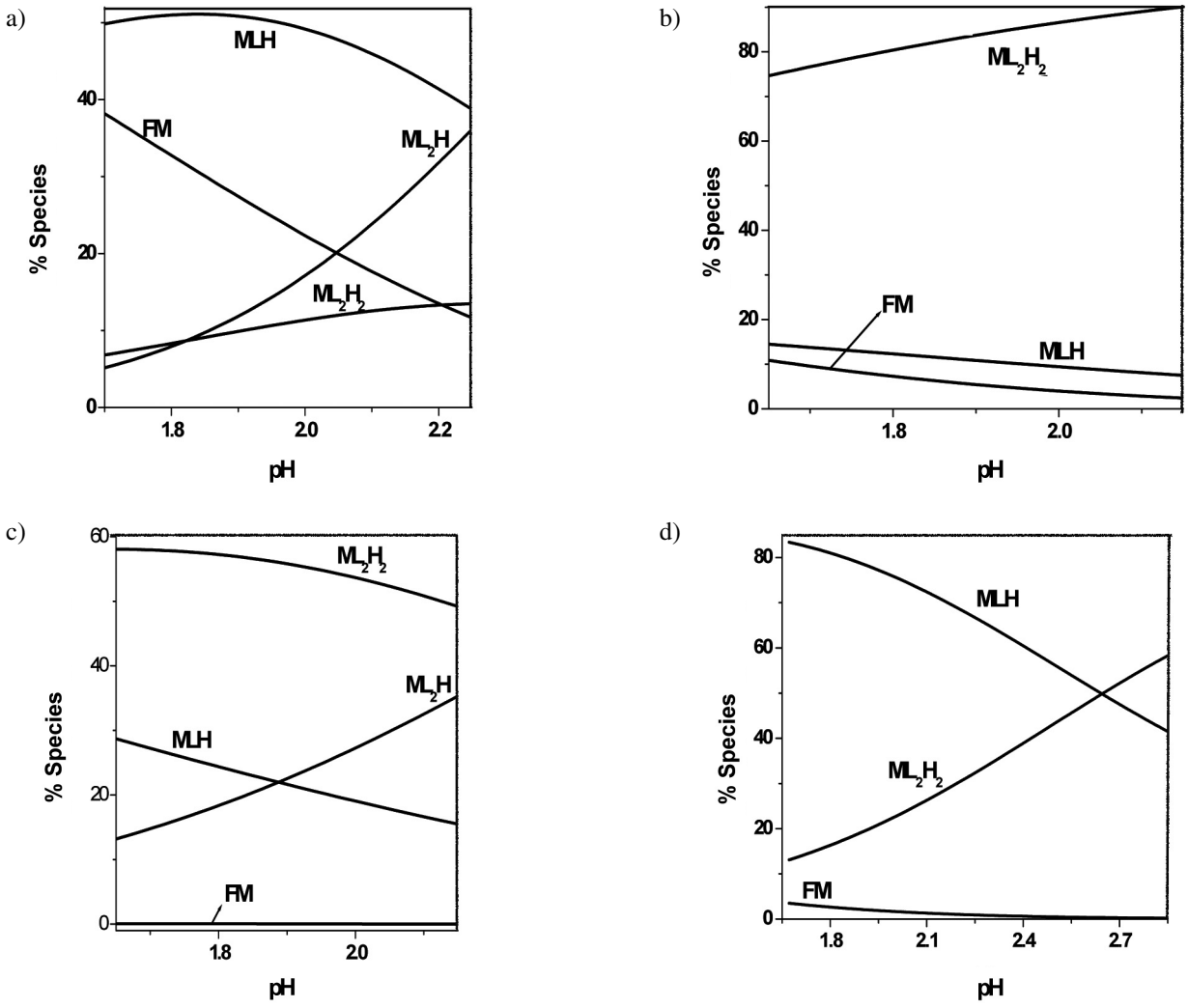


**Figure 5:** Species distribution diagrams of embelin complexes in 86.0% v/v DMSO-water mixture. a) Co(II), b) Ni(II), c) Cu(II) and d) Zn(II)

indicates that the underlying distribution is symmetrical. If the skewness is greater than zero, the peak of the error distribution curve is to the left of the mean and the peak is to the right of the mean if skewness is less than zero. The values of skewness recorded in Table 2 are between –0.21 and 0.58. These data evince that the residuals form a part of normal distribution; hence, least-squares method can be applied to the present data.

### 3. 3. 4. Kurtosis

It is a measure of the peakedness of the error distribution near a model value. For an ideal normal distribution kurtosis value should be three (mesokurtic). If the calculated kurtosis is less than three, the peak of the error distribution curve is flat (platykurtic) and if the kurtosis is greater than three, the distribution shall have sharp peak (leptokurtic). The kurtosis values in the present study indicate that the residuals form platykurtic pattern.

## 3. 4. Distribution Diagrams

The species distribution diagrams of metal ligand complexes are shown in Figure 5 and their equilibria are represented as follows.

$$M(II) + LH_2 \leftrightarrow MLH^+ + H^+ \quad (1)$$

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

$$ML_2H_2 \leftrightarrow ML_2H^- + H^+ \quad (3)$$

$$MLH^+ + LH_2 \leftrightarrow ML_2H_2 + H^+ \quad (4)$$

$$MLH^+ + LH^- \leftrightarrow ML_2H^- + H^+ \quad (5)$$

## 3. 5. Structures of Complexes

Embelin acts as a bidentate ligand[19] by using two strong oxygen donor sites and results in the formation of five-membered chelate ring with the metal ions (Figure 6).

The authors have made some attempts to synthesize and characterize the biologically important divalent metal complexes of embelin based on their stability constant values.

# 4. Conclusions

1) Embelin has two dissociable protons. It exists as $LH_2$ at low pH and gets deprotonated with the formation of $LH^-$ with increasing pH. Secondary formation curves confirm the existence of two non-overlapping protonation-deprotonation equilibria to embelin.
2) Overlapping simulated and experimental titration curves indicate the sufficiency of the models.
3) The common binary species formed due to interaction of embelin with the studied metals are $MLH^+$, $ML_2H_2$ and $ML_2H^-$.
4) The metal ions form octahedral complexes with bidentate embelin molecules.

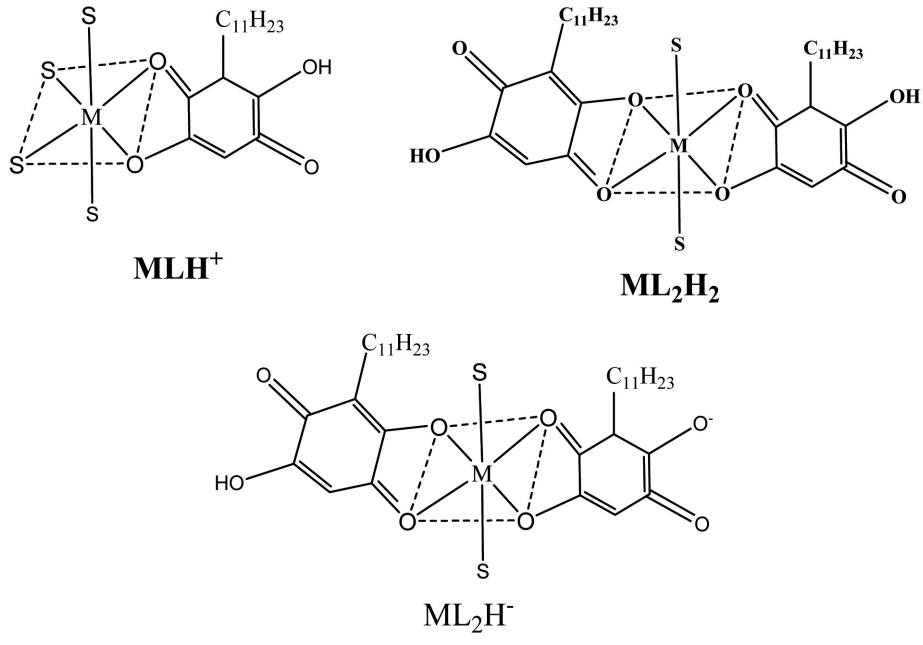


**Figure 6:** Suggested structures of embelin complexes where S is either solvent or water molecules M = Co(II), Ni(II), Cu(II) or Zn(II)

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## Povzetek

V 86 % v/v mešanici dimetilsulfoksida in vode smo pri 25 °C in ionski moči 0.17 mol $dm^{-3}$ proučevali ravnotežja protonacije in nastanka kompleksov embelina. Z uporabo modelov in uporabo programa MINIQUAD75 smo predvideli nastanek različnih binarnih kompleksov z Co(II), Ni(II), Cu(II) in Zn(II) in ugotovili, da je njihova prisotnost odvisna od pH medija.