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Equilibrium Studies of Pyrimidine and Aminoacids with Bivalent Toxic Metals: Computational Analysis

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Abstract: ***Interaction of Bivalent Toxic metal ions Pb(II), Cd(II), Hg(II), with 4,6 – Dimethyl–2–Pyrimidinol in presence of Alanine/Phenylalanine has been investigated pH metrically at 35° C and at three ionic strengths (μ=0.05M, 0.10M and 0.15M) in aqueous medium. The values of proton dissociation constants of ligands and formation constants of complexes were calculated by algebraic method of Chaberek and Martell as modified by Dey et. al. pH-metric data was subjected to SCOGS computer program. Thermodynamic formation constants were obtained by extrapolating the values to zero ionic strength in log β vs.$\sqrt{\mu}$ curve. The stability constants of binary and ternary complexes with respect to metal ion is found to follow the trend Cd(II) < Pb(II) < Hg(II).***

Keywords: 4, 6–dimethyl–2- pyrimidinol, formation constant, toxic metal, SCOGS

1. Introduction

Pyrimidine molecules are very important in biological systems and are essential components of the nucleic acids. Metal ions are also required for the incorporation of pyrimidine nucleosides in the DNA and RNA molecules. The presence of metal ions like Ca(II),Mg(II) and Fe(III) enhances the stability of double helix of DNA where as Cu(II) destablises the double helix of DNA by forming cross links between the strands of unwound DNA and facilitates the separation of strands from each other [1]-[2]. Therefore, to aid in the understanding of these important biological systems, much work has been concentrated as the study of metal ion interaction with isolated nucleic bases, nucleosides and nucleotides [3]-[7]. Substituted pyrimidines are also proved biologically active. The divalent ions forms binary system with pyrimidines in which pyrimidines coordinate to the metal ion through N(3) position. The proton dissociation and its release is an important factor in determining the extent and nature of the metal ligand interaction in a metal nucleoside complex and its process of dissociation is reported in literature [8]-[10]. It is of great interest to study the metal ligand interaction in a metal nucleoside complex since metal ions plays important role in several biological reactions[11]. It is evident from the literature that most of the earlier investigations have been confined to spectroscopic methods on metal nucleoside complexes and less work has been reported [12]-[16] an effect of the stability of the systems in solutions and also observed that ternary complexes act as good models for metalloenzyme reactions, is remarkably absent with respect to the physiochemical properties such as stability of the complexes with metal ions.

The speciation study of toxic metal ion complexes is useful to understand the role played by the active site cavities in biological molecules and the bonding behaviour of protein residues with the metal ion. The species refined and their relative concentrations under the experimental conditions represent the possible forms of complexes in bio fluids. Due to its numerous uses and high persistence, lead is a major environmental contaminant. Lead is toxic even at low concentrations for living organisms, which can absorb it in various ways. Lead intake by humans can be due to the consumption of crop plants grown on soils with high plant-available metal concentrations [17]-[19]. Cadmium causes iron deficiency by binding to cysteine, glutamate, aspartate, and histidine ligands. Cadmium inhibits enzymes that participate in bilirubine conjunction. It increases urine Ca^{2+} excretions which can cause severe bone pathology [20]-[22]. Mercury is one of the most toxic elements and has negative health effects in human populations, highly dependent on fish consumption [23]-[24].

Based on the importance of pyrimidine and aminoacids and their involvement in physiological reactions, it was considered an important to study in detail the formation and stability of mixed ligand complexes of 4,6-dimethyl-2-pyrimidinol with Pb(II), Cd(II) and Hg(II) in aqueous medium.

2. Experimental

All the systems were investigated in equimolar condition at three different ionic strengths (i.e., μ= 0.05M, 0.10M, 0.15M) at 35±1ºC in aqueous medium. $NaNO_3$ was used as background electrolyte. For each set of titration moles of alkali required per mole of ligand / metal. 'a' was determined and curves were obtained by plotting pH vs 'a'.

2.1 Solution

All the reagents used were highest purity Merck/Aldrich products. Solutions of metal and ligand (each 0.01M) were prepared by dissolving accurately weighed amounts in CO_2-free deionized double distilled water.

2.2 Instrument

pH-measurements were made by using Elico digital pH-meter model LI-127 with ATC probe and combined electrode type (CL-51B- Glass Body; range 0-14 pH unit; 0-100˚C(Automatic/Manual) with accuracy ±0.01 and standardized by using buffer solution of pH=4.0 and 9.2.

2.3 Experimental condition

Various sets of titration mixture were prepared and titrated with carbonate free 0.10M NaOH solution.

1) Acid titration: $HNO_3(2.0\times10^{-3}M)$.
2) Ligand 'A' titration: $HNO_3(2.0\times10^{-3}M)$ + Ligand 'A'$(1.0\times10^{-3}M)$.
3) Ligand 'B' titration: $HNO_3(2.0\times10^{-3}M)$ + Ligand 'B'$(1.0\times10^{-3}M)$.
4) Metal-Ligand'A '(1:1) titration:$HNO_3(2.0\times10^{-3}M)$ + Ligand 'A' $(1.0\times10^{-3}M)$ + Metal nitrate $(1.0\times10^{-3}M)$.
5) Metal -Ligand'B'(1:1) titration: $HNO_3(2.0\times10^{-3}M)$ + Ligand 'B'$(1.0\times10^{-3}M)$ + Metal nitrate $(1.0\times10^{-3}M)$.
6) Metal - Ligand 'A' –Ligand 'B'(1:1:1) titration: $HNO_3(2.0\times10^{-3}M)$ + Ligand'A'$(1.0\times10^{-3}M)$ + Ligand 'B'$(1.0\times10^{-3}M)$ + Metal nitrate $(1.0\times10^{-3}M)$.

Where,
Ligand 'A' = 4,6 Dimethyl ,2- Pyrimidinol abbreviated as (DMP). It is used as their diprotonated form by adding one equivalent of acid in the course of titration.

Ligand 'B' = Alanine / Phenyl alanine abbreviated Ala/Phe.

M = Pb(II) , Cd(II) and Hg(II).

Representative pH vs. 'a' Titration Curves Cd(II)-DMP-Ala / Hg(II)-DMP-Phe (1:1:1) biligand systems at 35±1 °C [μ=0.1M(NaNO3)]

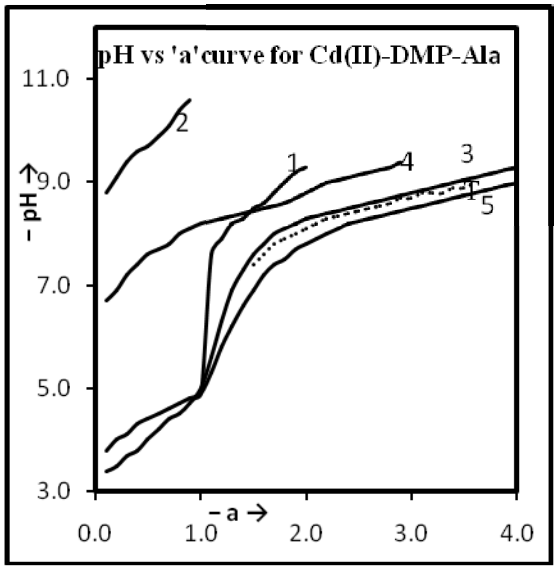

Figure 1(a)

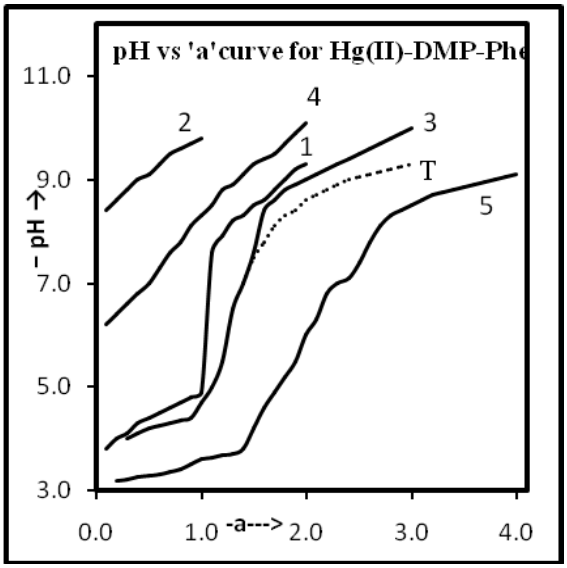

Figure 1(b)

Where,

- Curve 1; Ligand 'A' (DMP) Titration curve.
- Curve 2 : Ligand 'B' (Ala/Phe)Titration curve.
- Curve 3 : Metal-Ligand 'A' (1:1) Titration curve.
- Curve 4 : Metal-Ligand 'B' (1:1) Titration curve.
- Curve 5 : Mixed-Ligand (1:1:1) Titration curve.
- Curve T ;Theoretical composite curve.

3. Results and Discussion

The qualitative analysis of proton-ligand, metal-monoligand and metal-biligand equilibria were done by examination of titration curves in fig -1(a-b). Pattern of experimental curves for the systems M(II) –DMP-Alanine/Phenyl alanine [M=Pb,Cd,Hg] follow same trend respectively.The ligand titration curve 1 show that the deprotonation of ligand A occurs in two distinct steps and curve 2 indicates that the liberation of proton up to pH ≈ 9.0 showing the strong basic nature of ligand B. Further, the deviation of metal–ligand curves (curves 3 and 4) from ligand curves suggests the formation of binary complexes. Curve 5 in M(II)-DMP-Ala system is seen to be superimposed on 1:1 (MA) titration curve up to pH ≈ 4.5 followed by inflections at a≈1.0 and a≈2.0 whereas in M(II)-DMP-Phe system is seen to be displaced to the right of 1:1 MA / MB titration curve show a weak inflection at a≈2 to form ternary complex. It is also supported by non-superimposable nature of theoretical composite curve , obtained by plotting the theoretical addition of the values of 'a' corresponding to ligand titration curve of ligand B to the metal – ligand (1:1) titration curve of ligand A. The values of protonation and formation constants are in close agreement with literature values [25]-[26] in Table-1.

The observation leads to conclusion that metal ion coordinate with DMP (Ligand A) in the first step, at comparatively lower pH, and then mixed ligand complex is formed in the second distinct step in M(II)-DMP-Ala system while it interacts with both ligand simultaneously in M(II)-

DMP-Phe system, forming MAH, MA, MB type monoligand and MABH and MAB type biligand complexes. Hence, ligand A act as primary ligand and ligand B as secondary ligand. The equilibria (stepwise or simultaneous) involved in the complexation of investigated binary and ternary metal-ligand system are represented as follows:

M -DMP -Alanine/Phenyl alanine

Proton-ligand system:

DMP:

$$H_2A \underset{pK_1^H}{\overset{0 \le a \le 1}{\rightleftharpoons}} HA + H^+ \quad \text{---- 1.1}$$

$$HA \underset{pK_2^H}{\overset{1 \le a \le 2}{\rightleftharpoons}} A + H^+ \quad \text{---- 1.2}$$

Alanine / Phenyl alanine:

$$HB \underset{pK_1^H}{\overset{0 \le a \le 1}{\rightleftharpoons}} B + H^+ \text{------ 1.3}$$

Binary M-DMP system :

$$M + H_2A \underset{\log K^H_{MAH}}{\overset{0 \le a \le 1}{\rightleftharpoons}} MAH + H^+ \text{-----1.4}$$

$$M + HA \underset{\log K^M_{MAH}}{\overset{0 \le a \le 1}{\rightleftharpoons}} MAH \text{-------1.5}$$

$$MAH \underset{\log K^H_{MA}}{\overset{1 \le a \le 2}{\rightleftharpoons}} MA + H^+ \text{-------1.6}$$

$$M + A \underset{\log K^M_{MA}}{\overset{0 \le a \le 2}{\rightleftharpoons}} MA \text{--------1.7}$$

Binary M-Alanine/ Phenyl alanine system:

$$M + HB \underset{\log K^H_{MB}}{\overset{0 \le a \le 1}{\rightleftharpoons}} MB + H^+ \text{-----1.8}$$

$$M + B \underset{\log K^M_{MB}}{\overset{0 \le a \le 1}{\rightleftharpoons}} MB \text{-------1.9}$$

Ternary M- DMP –Alanine system :

Step-I: Interaction of metal and primary ligand A

$$M + H_2A \underset{\log K^H_{MAH}}{\overset{0 \le a \le 1}{\rightleftharpoons}} MAH + H^+ \text{---2.0}$$

$$M + HA \underset{\log K^H_{MA}}{\overset{0 \le a \le 1}{\rightleftharpoons}} MAH \text{------2.1}$$

$$MAH \underset{\log K^H_{MA}}{\overset{1 \le a \le 2}{\rightleftharpoons}} MA + H^+ \text{------2.2}$$

$$M + A \underset{\log K^M_{MA}}{\overset{0 \le a \le 2}{\rightleftharpoons}} MA \text{---------2.3}$$

Step-II: Interaction of metal - primary ligand complex species with secondary ligand B

$$MAH + B \underset{\log K^M_{MABH}}{\overset{1 \le a \le 2}{\rightleftharpoons}} MABH \text{-----2.4}$$

$$MABH \underset{\log K^H_{MAB}}{\overset{2 \le a \le 3}{\rightleftharpoons}} MAB + H^+ \text{-----2.5}$$

$$MA + B \underset{\log K^M_{MAB}}{\overset{2 \le a \le 3}{\rightleftharpoons}} MAB \text{--------2.6}$$

Ternary M-DMP-Phenyl alanine system:

Simultaneous: Interaction of metal , primary ligand A and secondary ligand B

$$M + H_2A + HB \underset{\log K^H_{MABH}}{\overset{0 \le a \le 2}{\rightleftharpoons}} MABH + H^+ \text{-----2.7}$$

$$M + HA + B \underset{\log K^M_{MABH}}{\overset{0 \le a \le 2}{\rightleftharpoons}} MABH \text{--------2.8}$$

$$MABH \underset{\log K^H_{MAB}}{\overset{2 \le a \le 3}{\rightleftharpoons}} MAB + H^+ \text{------2.9}$$

$$MA + B \underset{\log K^M_{MAB}}{\overset{2 \le a \le 3}{\rightleftharpoons}} MAB \text{-----3.0}$$

(Charges have been omitted for the sake of simplicity)

The results were calculated by algebraic method of Chaberek and Martell [27]-[28] as modified by Dey et. al.[29] and refined by using SCOGS (stability constant of generalised species) computer program [30]-[32]. These values were plotted against μ and extrapolated to zero ionic strength to obtain thermodynamic formation constants of complexes.

Speciation curves (fig-2a) for Pb(II)/Cd(II)/Hg(II) -DMP-Alanine follow the same trend. Examination show the formation of MAH species occurs up to pH≈5.0 (Ref.eq.2.0 and 2.1) and then concentration of MAH and free metal decreasing continuously. Thereafter, MA species is formed by deprotonation of MAH complex (Ref eq.2.2). Simultaneously formation of protonated ternary species MABH (Ref.eq.2.4) also comes into existence. However the concentration of these two species is more than 50% in Cd(II),Hg(II) and less than 50% in Pb(II)-DMP-Alanine systems. Deprotonation of MABH species leads to the

formation of MAB in pH range above 5.0 and 2≤a≤3. Thereafter the MAB species is formed by the coordination of ligand B to MA complex. Formation of MAB species commences at a≈2 and attains a maximum value of ≈95% at a≈4. MAB ternary complex is the predominant species in all the systems. Formation of MAB occurs through two alternative equilibria (Ref. eq. 2.5 and 2.6). The equation 2.5 represents the formation of MAB by deprotonation of MABH species, whereas equation 2.6 shows the formation of MAB by coordination of ligand B (deprotonated form) to MA species.

Similarly , it is observed from the speciation curves (fig-2 b) that the interaction of metal ion with ligand leads to continuous decrease in the concentration of free metal ion, thereby indicating its association with the ligand, in the initial pH ≈ 3.0, hence leading to the formation of protonated species MABH (Ref.eq 2.8). The percentage of MABH species ranges from 10-40% between a≈ 0 and a ≈2 in different systems, which continuously decreases continuously decreases upto pH 5.5. The formation of non protonated MAB species commences simultaneously either with the deprotonation of MABH (Ref. eq.2.9) or coordination of MA to deprotonated B species (Ref.eq.3.0) and the maximum percentage of MAB species reaches nearly 95% in the system between a≈ 2 and a≈ 3 in the pH above 5.5.

The positive values of Δ log K obtained in present work can be considered as strong evidence for the occurrence of promoted stability of the mixed ligand complexes. Δ log K values indicates the amino acid is coordinated with great ease with the free metal ion than with the complexed metal ion. The reason for the extra stability of these complexes may be due to interaction outside the coordination sphere such as the formation of hydrogen bonds between the coordinated ligands, charge neutralisation, chelate effect and stacking interactions and a similar stabilizing effect may likewise be exerted by the electrostatic interactions between non-coordinated, charged groups of the ligands. Researcher's carried out extensive studies to establish the laws governing interactions of this nature [33]-[35]. The ternary formation constant along with the Δ log K and Relative Stabilisation (R.S.) energy values are listed in the Table- 2 & 3.

Representative Speciation Curves (1:1:1) biligand systems at 35±1 °C [μ=0.1M(NaNO3)]

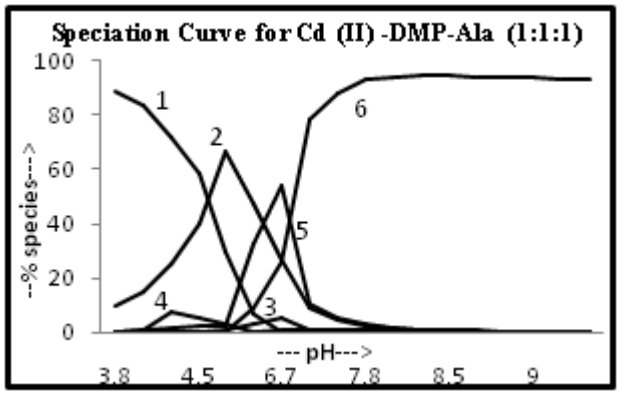

Figure 2(a)

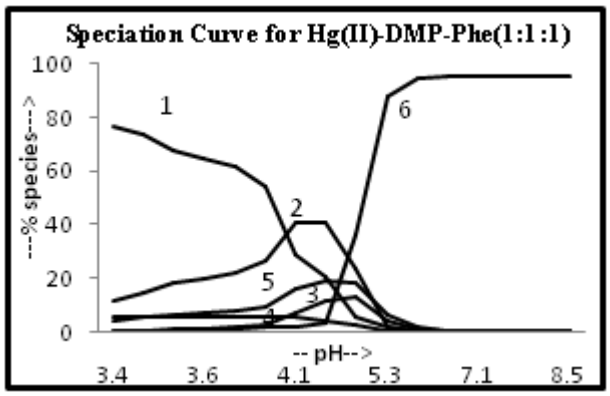

Figure 2(b)

Where, Curve 1: [M]; 2: [MAH]; 3 : [MA]; 4 : [MB]; 5: [MABH] ; 6 : [MAB]

Table 1: Protonation and Formation constants for M-DMP/Ala/Phe (1:1) monoligand systems at 35±1 °C in aqueous medium

Parameters	DMP	Alanine	Phenyl alanine
$\log\beta_1^{HA/HB}$	8.60	9.82	9.22
$\log\beta_2^{H2A}$	13.14	-	-
	Pb(II)-Ala	**Cd(II)-Ala**	**Hg(II)-Ala**
$\log\beta_{MB}$	6.16	6.48	4.85
	Pb(II)-Phe	**Cd(II)-Phe**	**Hg(II)-Phe**
$\log\beta_{MB}$	5.48	5.24	5.60
	Pb(II)-DMP	**Cd(II)-DMP**	**Hg(II)-DMP**
$\log K_{MAH}^{M}$	3.15	3.28	3.50
$\log K_{MAH}^{H}$	-4.15	-3.90	-3.70
$\log K_{MA}^{H}$	-6.22	-7.20	-7.24
$\log\beta_{MAH}$	10.65	9.30	11.05
$\log\beta_{MA}$	6.18	5.20	7.18

- $\log\beta_1^{HA} = pK_2^H$
- $\log\beta_1^{HB} = pK_2^H$
- $\log\beta_2^{H2A} = pK_1^H + pK_2^H$
- $\log\beta_{MA} = \log K_{MA}^M$
- $\log\beta_{MB} = \log K_{MB}^M$
- $\log\beta_{MAH} = \log K_{MAH}^M + \log\beta_1^{HA}$

Table 2: Formation constants for M-DMP-Ala / Phe (1:1:1) Biligand systems at 35±1 °C in aqueous medium

Parameters	Pb(II)-DMP-Ala	Cd(II)-DMP-Ala	Hg(II)-DMP-Ala	Pb(II)-DMP-Phe	Cd(II)-DMP-Phe	Hg(II)-DMP-Phe
$\log K^{M}_{MABH}$	27.30	21.02	31.50	27.22	27.20	30.25
$\log K^{MA}_{MABH}$	14.60	11.66	16.60	14.20	14.60	16.55
$\log K^{MAH}_{MABH}$	19.60	17.56	22.20	19.16	20.45	22.18
$\log K^{H}_{MABH}$	-5.34	-6.92	-6.72	-4.58	-3.84	-4.65
$\log K^{MA}_{MAB}$	18.16	17.05	18.02	17.88	18.50	17.80
$\log K^{MB}_{MAB}$	17.15	14.84	15.42	16.98	16.85	16.02
$\log K^{H}_{MAB}$	-6.42	7.84	-9.44	-6.65	-7.40	-7.52
$\log \beta_{MABH}$	35.70	29.54	39.80	35.98	35.70	38.74
$\log \beta_{MAB}$	17.65	13.55	18.40	17.95	17.05	23.30

- $\log \beta_{MABH} = \log K^{M}_{MABH} + \log \beta 1^{HA}$
- $\log \beta_{MAB} = \log K^{M}_{MAB}$
- Formation constants were obtained by extrapolating the log K vs.$\sqrt{\mu}$ plot to zero ionic strength
- Ala, Phe become the ligand 'B' in biligand systems.

Table 3: Value of Δ log K and R.S for Biligand systems at 35±1 °C in aqueous medium

Parameters	Δ log K	R.S	Δ log K	R.S	Δ log K	R.S
	Pb(II)-DMP-Ala		Cd(II)-DMP-Ala		Hg(II)-DMP-Ala	
$\log \beta_{MABH}$	18.89	-	13.76	-	23.90	-
$\log \beta_{MAB}$	5.31	0.85	1.87	0.35	6.37	0.88
	Pb (II)-DMP-Phe		Cd (II)-DMP-Phe		Hg(II)-DMP-Phe	
$\log \beta_{MABH}$	21.39	-	21.20	-	22.09	-
$\log \beta_{MAB}$	6.29	1.01	4.33	0.82	10.52	1.46

$\Delta \log K_{MABH} = \log \beta_{MABH} - (\log \beta_{MB} + \log \beta_{MAH})$
$\Delta \log K_{MAB} = \log \beta_{MAB} - (\log \beta_{MA} + \log \beta_{MB})$
$R.S = \Delta \log K_{MAB} / \log K_{MA}$

4. Structure of Complex

Depending on the active sites in the ligand and the nature of the metal ions, the structure were proposed for the species as shown in fig -3(a-b)

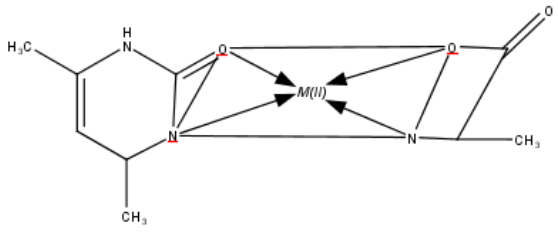

Figure 3(a)

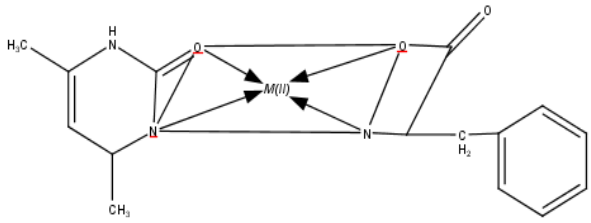

Figure 3(b)

Figure 3: Proposed structure (a) M-DMP-Ala (b) M-DMP-Phe complexes

5. Conclusions

The present biomimetic studies of metal ion complexes with 4,6-DMP indicates that the complexes are monoprotonated in acidic pH values and nonprotonated in higher pH region. The species detected are MAH, MABH and MAB respectively. The stability constants of binary and ternary complexes with respect to metal ion is found to follow the trend Cd(II) < Pb(II) < Hg(II) in concord with the electronegativity. The stability of mixed ligand complexes of Phenyl alanine is greater than alanine due to π back donation from the negatively charged aminoacid to the π system of the DMP.This is supported by the earlier investigation [36] . The positive values of ΔlogK and RS for all biligand system indicates that ternary complexes are more stable than the binary complexes which suggest that interligand interaction exist between Phe/Ala aminoacid ligand and DMP ligand.

The proton-ligand and metal-ligand stability constant logK decrease with an increase in ionic strengths. The knowledge of metal - ligand formation constant is essential for understanding of various vexed problems of biological, pharmaceutical and analytical chemistry because the metal - ligand interaction depends on the relative and absolute concentration of all the kinds of ligand present as well as on the relevant pK, formation constant and pH of the solution. The magnitude of log β values depend on the denticities of both ligand and co-ordination number of metal ions. It is

well known that mixed chelation is very strong way to remove the toxicity of heavy metals. The chelating agent must be of low toxicity and not metabolized so as to persist on changes in the biological system to perform their scavenging functions due to their interaction with metal ions to form metal chelates or dislodging the bound metals and excreting these as soluble chelates from the system.

The ligands 4,6-DMP (A) and Alanine / Phenylalanine (B) used in this study are very good chelating agent having very strong ability to grab onto toxic metals and dislodge them from the tissues so they can be removed. So the present work provides a very good and inexpensive method for the equilibrium study of ternary metal chelates of biological significance which protects living beings.

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