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An analytical study on complexation equilibria of non -essential amino acid and pyrimidine with heavy elements followed by specific computer programme

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ABSTRACT

The interaction of non - essential amino acid 2-Amino-3-(4-hydroxyphenyl) propanoic acid (2–AHPPA) and pyramidine base 2, 4-dihydroxypyrimidine (2, 4-DHP) with heavy element or heavy metal ions Hg (II) and Cd (II) have been studied in the biologically relevant conditions by potentiometric technique at silver- silver chloride electrode and at a fixed ionic strength I = 0.1M $NaNO_3$ and 37 ± 1^0C temperature in aqueous solution with the aid of SCOGS computer programme analysis. The complexation equilibria and formation constant (log $ß_{pqrst}$) of biologically significant ternary and quaternary metal chelates formed by above metal-ligand interaction have been described on the basis of resulting speciation curves sketched with the help of ORIGIN 4.0.

Key words: Formation Constants, Metal Chelates, SCOGS, Speciation Curves

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INTRODUCTION

The co-ordination chemistry is a very valuable and fascinating branch of chemical science. It provides the great view to study in the formation of various type complexes. In complex formation the co-ordination number or ligancy plays most important role which is the total number of atoms of the ligand that can co-ordinate the central metal ion or total number of chemical bond formed between the central metal ion and donor atoms of the ligands**.** The ligands whose structure permit the attachment of their two or more donor atoms to the central metal ion simultaneously and thus produce a heterocyclic ring structure said to be a chelating ligand and the complex compound is termed as metal chelate. The formation of such rings said to have chelation or cyclisation and resulting structure is called as chelate ring or simply chelate by Margan and Drew [1].Chelates are the results of the sharing of electrons between a metal and a ligand and was used to designate such cyclic structures which arise from the union of metallic ions with organic or inorganic ligands. The toxic metal ions interaction with the numerous molecules possessing groupings capable of complexation or chelation. Mixed chelation is an important phenomenon[2] in the coordination chemistry of living organisms. The peculiar interest of polydentate chelating ligands is connected with their importance [3] in agricultural industry and medicine. It has also been suggested that chelation play a vital role in cure of cancer [4]. Metal ion complex formations are among the prominent interaction in nature[5]. Heavy elemental Hg (II) and Cd (II) toxicity is a dangerous and worldwide problem. These are the cause of various disease and disorder in living beings. Metals in an oxidation state abnormal to the body may also become toxic in another oxidation state, chromium (III) is an essential trace element, but chromium (VI) is a carcinogen. We know that insoluble compounds as well as the metallic forms often exhibit negligible toxicity and this is possible by the aid of suitable chelating agents.

The 2- Amino-3-(4-hydroxyphenyl) propanoic acid (2-AHPPA) is a non-essential amino acid with a polar side group having the condos UAC and UAU. It is a precursor to neurotransmitters and increases plasma neurotransmitter levels particularly dopamine and nor epinephrine. A number of studies have found that it would be useful during conditions of stress, cold, fatigue[6] loss of a loved one such as in death or divorce, prolonged work and sleep deprivation[7]. It is used as a dietary supplement and has promise as a valuable precursor compound for various industrial and pharmaceutical applications. The 2, 4-dihydroxypyrimidine is a pyrimidine base which is common and naturally occurring and are used in many biological, medicinal and agricultural applications.The complexes of pyramidine play important role in catalysis of drug interaction. It was isolated the hydrolysis of yeast nuclein [8] that was found in bovine thymus and spleen, herring sperm, and wheat germ[9] It is a planar, unsaturated compound that has the ability to absorb light[10].

EXPERIMENTAL SECTION

2.1- Theoretical Principle:

The study of complexation equilibria of complexes has kinetic and thermodynamic approach. The scope of this fascinating field has become so broad that a comprehensive survey of literature has become almost an impossible task. An immense amount of equilibrium data is available in different volumes published by Martell [11,12] and Smith[13]. The study of chelate formation which is due to complexation provides information about stability and existence of different species. Abegg and Bodlander [14,15] introduced the concept of stability constant in chemical equilibrium between a metal ion and a ligand .

***Formation of Mixed - ligand ternary complexes*:**

$$L_1 + M + L_2 \rightleftharpoons ML_1L_2$$

$$\beta_{ML1L2} = \frac{[ML_1L_2]}{[L_1]\,[M]\,[L_2]} \qquad \text{.......(1)}$$

***Formation of Multi metal-multi ligand quaternary complexes*:**

$$M_1L_1L_2 + M_2 \rightleftharpoons M_1M_2L_1L_2$$

$$\beta_{M1M2L1L2} = \frac{[M_1M_2L_1L_2]}{[M_1L_1L_2]\,[M_2]} \qquad \text{.........(2)}$$

$$L_1 + M_1 + L_2 + M_2 \rightleftharpoons M_1M_2L_1L_2$$

$$\beta_{M1M2L1L2} = \frac{[M_1M_2L_1L_2]}{[L_1]\,[M_1]\,[L_2]\,[M_2]} \qquad \text{.........(3)}$$

2.2 - pH- Metric Titration:

In the study of electrochemical [16] behavior of complexation equilibria of interaction of metal ions and ligands, potentiometry is one of the most important methods used in these type of analysis. In such cases there is release of hydrogen ions accompanying the complexation reaction where a glass electrode furnishes information on the H^+ ion concentration and thereby on the extent of complex formation. This paper contains analytical study of mixed - ligand ternary and multi metal-multi ligand quaternary chelates of heavy metals or toxic metal Hg (II) and Cd (II) with non– essential amino acid 2-Amino-3-(4-hydroxyphenyl) propanoic acid (2–AHPPA) used as primary ligand (A) and pyramidine base 2, 4-dihydroxypyrimidine (2, 4-DHP) used as secondary ligand (B) in the biologically relevant conditions by potentiometry in aqueous medium using Bjerrum's[17] method modified by Irving and Rossotti[18]. All the solutions were prepared in double distilled water. The pH measurements were done by an electric digital pH meter (Eutech 501) with a glass electrode supplied with the instrument and working on 220V/50 cycles stabilized by A.C. mains. The pH meter has a reproducibility of ±0.01 pH. The pH meter calibrated with buffer solutions of pH (4.0) and pH (9.2) respectively which were prepared by dissolving buffer tablets (BDH) in double distilled water in appropriate concentrations. A carbonate free sodium hydroxide solution [19] was prepared for completion of this work. All the metal salts used were of A R Grade and were standardized volumetrically by titration with the disodium salt of EDTA [20] is presence of suitable indicators and were used without further purification. An ultra thermostat type U_{10} (VEB MLW Sitz, Freital, Germany) was used to maintain a constant temperature in all the experiments. A SONAR magnetic stirrer 2 MLH was used for constant stirring of the solution mixtures throughout the experiment.

Following set of solution mixtures were prepared for analytical study:

Set I: ***Acid Solution:*** 5 ml $NaNO_3$ (1.0 M) + 5ml HNO_3 (0.02M) + 40 ml water

Set II: ***Ligand - Solution:*** 5ml $NaNO_3$ (1.0M) + 5ml HNO_3 (0.02M) + 5ml A (2-AHPPA) / B (2, 4-DHP) (0.01M) + 35 ml. water

Set III: ***Binary system (1: 1) (M: A) / (M: B):-*** 5 ml $NaNO_3$ (1.0 M) + 5ml HNO_3 (0.02M) + 5ml A (2-AHPPA) / B (2, 4-DHP) (0.01M) + 5 ml M (II) (0.01M) Hg (II) / Cd (II) + 30 ml water.

Set IV: Mixed ligand ternary system (1: 1: 1) (M:A: B) :- 5 ml $NaNO_3$ (1.0 M) + 5ml HNO_3 (0.02M) + 5ml A (2-AHPPA) (0.01M) + 5 ml M (II) (0.01M) Hg (II) / Cd (II) + 5 ml B (0.01M) (2,4-DHP) + 25 ml water.

Set V: Mixed metal mixed ligand quaternary system (1: 1: 1: 1) (M_1: M_2:A: B)
5 ml $NaNO_3$ (1.0 M) + 5ml HNO_3 (0.02M) + 5ml A (0.01M) (2-AHPPA) + 5 ml M_1 (II) (0.01M) Hg (II) + 5ml B (0.01M) (2, 4-DHP) + 5ml M_2 (II) (0.01M) Cd (II) + 20 ml water

All the experiments were carried out in an atmosphere of purified nitrogen by bubbling it thrown the solution in which the electrode was dipping. The nitrogen gas thus served to prevent atmospheric oxidation and also to stir the solution. Each set of solution was then titrated against alkali (NaOH). The pH meter reading with progressive addition of alkali to the titration mixtures were noted, when the reading of pH meter stabilized. The pH values were plotted against the volume of NaOH and the titration curves were obtained. The titrations were continued until the appearance of turbidity. The ionic strength of all mixture solutions was kept 0.1M $NaNO_3$. The free acid concentration was kept equal in each case is 0.02M.

2.3 - Calculation of Formation or Stability Constant
The earliest computer applications to chemical equilibria studies were concerned with gas mixtures initially using mass balance equations after that designed to seek minimum values for the free energies or chemical potentials. Programs for the treatment of equilibria in aqueous system developed independently. Now various types of computer programs have been developed for calculating the stability constants of metal complexes, from pH titration data.

Anderegg [21] published a program that could deal with protonated, hydrolysed or polynuclear metal complexes, hydrolysed metal ions and protonated ligands. In 1962 Ingri and Sillen [22] described the program KUSKA for calculating the compositions of solutions containing one kind of metal ion and one kind of ligand. I.G.Sayce [23-25] developed a new computer program named as SCOGS (Stability constant of generalized species) which employs the conventional non linear least square approach. The program is written in FORTRAN IV. It is capable of calculating simultaneously or individually , association constants for any of the species formed in the system containing up to two metals and two ligands, provided that the degree of complex formation is pH-dependent. Thus, SCOGS may be utilized to analyse appropriate pH titration data to yield metal-ion hydrolytic constants, stability constants of simple complexes (MA, MB and MA_2 etc.). SCOGS may also be used to calculate constants for "mixed" complexes containing two different metals and two different ligands resulting the formation of MAB and M_1M_2 AB types of complexes.

RESULTS AND DISCUSSION

In this study the calculation of overall stability constant (β_{pqrst}) of mixed - ligand ternary and mixed metal – mixed ligand quaternary complexes of biological significances were completed with the help specific computer programme SCOGS given by I.G.Sayce .

$$pM_1 + qM_2 + rA + sB + t\,(OH) \rightleftharpoons (M_1)_p\,(M_2)_q\,(A)_r\,(B)_s\,(OH)_t$$

$$\beta_{pqrst} = \frac{[(M_1)_p\,(M_2)_q\,(A)_r\,(B)_s\,(OH)_t]}{[M_1]^p\,[M_2]^q\,[A]^r\,[B]^s\,[OH]^t} \qquad \ldots\ldots\ldots\ldots(4)$$

Stability constant for mixed- ligand ternary system (1: 1: 1) (M: A: B)

$$pM_1 / qM_2 + rA + sB + t(OH) \rightleftharpoons (M_1)_p / (M_2)_q\,(A)_r\,(B)_s\,(OH)_t$$

$$p(Hg^{2+})/q(Cd^{2+}) + r(2\text{-AHPPA}) + s(2,4\text{-DHP}) + t(OH) \rightleftharpoons (Hg^{II})_p/(Cd^{II})_q(2\text{-AHPPA})_r(2,4\text{-DHP})_s(OH)_t$$

$$\beta_{pqrst} = \frac{[(Hg^{II})_p/(Cd^{II})_q(2\text{-AHPPA})_r(2,4\text{-DHP})_s(OH)_t]}{[Hg^{2+}]^p/[Cd^{2+}]^q[2\text{-AHPPA}]^r[2,4\text{-DHP}]^s[OH]^t} \quad \ldots\ldots\ldots\ldots (5)$$

Stability constant for **mixed metal –mixed ligand quaternary system (1: 1: 1: 1) (M_1: M_2:A: B**:

$$p(Hg^{2+}) + q(Cd^{2+}) + r(2\text{-AHPPA}) + s(2,4\text{-DHP}) + t(OH) \rightleftharpoons (Hg^{II})_p(Cd^{II})_q(2\text{-AHPPA})_r(2,4\text{-DHP})_s(OH)_t$$

$$\beta_{pqrst} = \frac{[(Hg^{II})_p(Cd^{II})_q(2\text{-AHPPA})_r(2,4\text{-DHP})_s(OH)_t]}{[Hg^{2+}]^p[Cd^{2+}]^q[2\text{-AHPPA}]^r[2,4\text{-DHP}]^s[OH]^t} \quad \ldots\ldots\ldots\ldots (6)$$

In above equation the p, q, r and s are either the zero or positive integer and t is a negative integer for a protonated species like H_3A, H_2A, HA and BH, positive integer for a hydroxo or a deprotonated species like $[M(OH)^+]$, $[M(OH)_2]$ and zero for a neutral or normal species like [M A] [M B] and [M A B] and $[M_1M_2AB]$ etc.

3.1- Potentiometric Titration Curve

The pH titration curves for the ternary and quaternary complexes were drawn by plotting pH Vs volume of alkali. A representative pH titration curves for the studied systems is given in fig.1 which shows the binary, ternary and quaternary complexes by individual lines. Each lines of the curve were denoted by alphabetic word which are A, B, C, D. and E. In the curves, “A” represents the Acid, “B” represent the Ligand, “C” represents the binary complex, “D” represents the mixed ligand ternary complex and “E” represents the mixed metal - mixed ligand quaternary complex.

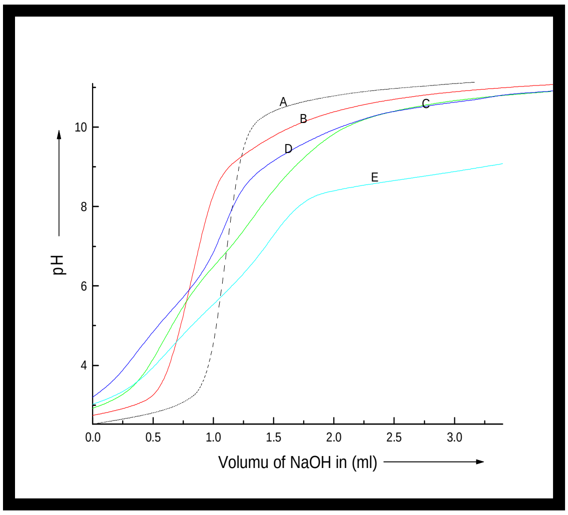


Fig.1. Titration Curves of 1:1:1:1 Hg(II)-Cd(II) -2-AHPPA(A) - 2,4-DHP(B) system
(A) Acid (B) Ligand (C) Hg(II)- 2-AHPPA (D)Hg(II)- 2-AHPPA - 2,4-DHP (E)Hg (II)-Cd (II) - 2-AHPPA - 2,4-DHP

3. 2 - Speciation Curves:

The speciation curves were obtained by plotting percent (%) concentration of the species obtained through SCOGS computer programme against pH which are represented in (fig. 2 and fig. 3) respectively. The pH titration curves and speciation curves are finally sketched by running the computer program ORIGIN 4.0.

3.2.1 - Hg (II)-2-AHPPA (A) - 2, 4- DHP (B) (1:1:1) Ternary System:

Speciation curve for this system given in fig.2 in which the binary complex of Hg B shows ~ 98% concentration at start of the titration but gradual decreases with increase in pH and now the ternary complex increases gradually from pH ~ 5.8. Another binary complex Hg A shows its maximum concentration ~ 52% at the pH ~ 9.8 and may be assumed to follow the equilibrium:

$$H_2A + Hg^{2+} \rightleftharpoons [Hg(II)-A] + 2H^+$$
$$BH^- + Hg^{2+} \rightleftharpoons [Hg(II)-B] + H^+$$

From the speciation curves it is very clear that maximum concentration attaining species is mixed ligand ternary complex Hg AB its maximum concentration is ~ 68% at the pH ~ 9.0 and this complex formation equilibrium follows the given order in simultaneous formation.

$H_2A + Hg^{2+} + BH^- \rightleftharpoons [Hg (II) – A - B] + 3H^+$

The stepwise formation of mixed ligand complex may be explained as per equilibrium:

$[Hg (II) - A] + BH^- \rightleftharpoons [Hg (II)– A - B] + H^+$

Protonated ligand species H_3A, H_2A, HA and HB shows their remarkable presence in this system. It may be concluded that both the ligand undergo the process of complexation with Hg (II) metal ion at the result of which binary and ternary complexes were formed. Concomitant decline in all other species shows the formation of mixed ligand chelates.

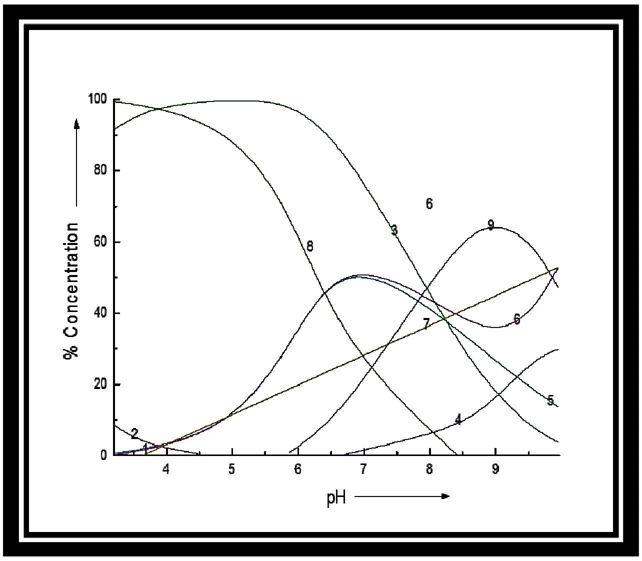


Fig. 2. Speciation Curves of 1:1:1 Hg (II) - 2-AHPPA (A) -2,4-DHP(B) System
(1) Hg^{2+} (2) H_3A (3) H_2A (4) HA(5) BH (6) Hg $(OH)_2$(7)Hg A(8)HgB (9) HgAB

3.2.2 - Hg (II)-Cd (II) -2-AHPPA (A) - 2, 4-DHP (B) (1: 1:1:1 Quaternary System

The graphical representation reveals that mixed-metal mixed- ligand complex is the dominant species, attaining maximum concentration ~78% at the ~6.2pH value. Cd^{2+} (aq.) attain maximum concentration ~69% at the start of titration which shows decline trend with increase in pH while Hg^{2+} (aq.)have low existence ~8.0% at the start of titration. H_2A have the maximum existence ~72% at the start of titration which shows decline trend with increase in pH. Binary complex of Hg with ligand B have lower existence while another binary complex Hg A is not present in this system. Cd- A complex have maximum concentration ~68% at the start of titration shows decline trend with increase in pH while the Cd B complex attain the maximum concentration ~26% at the ~6.0 pH. The equilibria of binary complexation may be assumed as in this system.

$H_2A + Hg^{2+}/ Cd^{2+} \rightleftharpoons [Hg (II) / Cd (II) A] + 2H^+$
$BH^- + Hg^{2+}/ Cd^{2} \rightleftharpoons [Hg (II) / Cd (II) - B] + H^+$

The ternary complex Hg AB existed with very low amount attaining maximum concentration ~5.0% at the ~ 8.0 pH value and ternary complex of Cd AB existed with maximum concentration ~89% at the ~ 9.0 pH. The formation of both ternary complexes takes place in two ways, stepwise and simultaneous.

If stepwise complex formation then it explained as:
$[Hg (II) - A] + BH^- \rightleftharpoons [Hg (II)– A - B] + H^+$
$[Cd (II) - A] + BH^- \rightleftharpoons [Cd (II)– A - B] + H^+$

When the complex formation takes place simultaneously then it may be explained as per equilibria:
$H_2A + Hg^{2+} + BH^- \rightleftharpoons [Hg (II) – A - B] + 3H^+$
$H_2A + Cd^{2+} + BH^- \rightleftharpoons [Cd (II) – A - B] + 3H^+$

Hydroxo species formation assumed the equilibria showing very good existence in the present system.
$Hg^{2+}/Cd^{2+} + H_2O \rightleftharpoons$ Hg (II) / Cd (II) $(OH)^+ + H^+$
$Hg^{2+}/Cd^{2+} + 2H_2O \rightleftharpoons$ Hg (II) / Cd (II) $(OH)_2 + 2H^+$

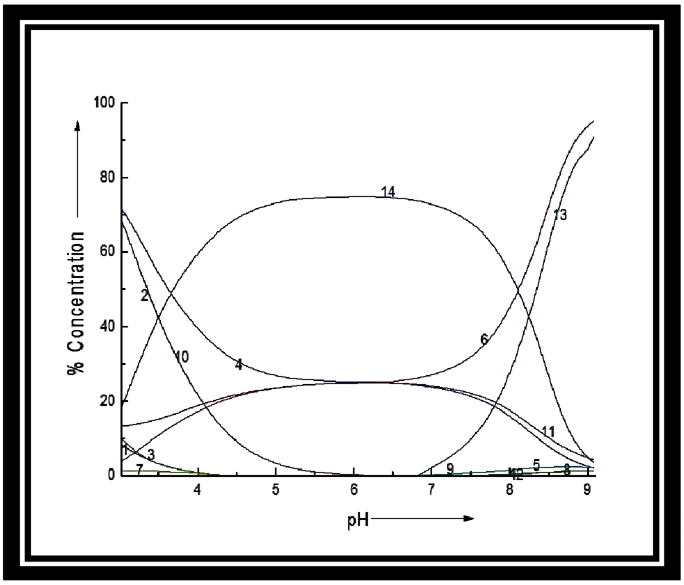


Fig.3. Distribution Curves of 1:1:1:1Hg (II) -Cd (II)-2-AHPPA(A) -2,4-DHP(B) system
(1) Hg^{2+}(II) (2) Cd^{2+}(II) (3) H_3A (4) H_2A (5) HA (6)$Hg(OH)_2$(7) Hg $(OH)^+$(8)Cd $(OH)_2$ (9) Hg B(10) Cd A (11)Cd B (12)Hg AB (13)Cd A B(14)Hg Cd AB

The mixed-metal mixed- ligand quaternary complex formation may takes place in two way one of them is stepwise formation of mixed - ternary complex and then addition of bivalent Hg^{2+} or Cd^{2+} ions, for which alternative equilibrium is given as :

Hg (II) - A $BH^- + Cd^{2+} \rightleftharpoons$ [Hg (II) - Cd (II) - A -B] + H^+
Cd (II) - A $BH^- + Hg^{2+} \rightleftharpoons$ [Cd (II) – Hg (II) - A -B] + H^+

Another way of heterobinuclear complexation equilibrium assumed as:

$H_2A + Hg^{2+} + BH^- + Cd^{2+} \rightleftharpoons$ [Hg (II) – Cd (II) - A -B] + $3H^+$

The binary constants of 2-AHPPA and 2, 4-DHP with heavy elements (Hg^{2+} and Cd^{2+}) taken under study were remeasured in biologically relevant condition at fixed ionic strength and temperature. The resulting values are in good agreement with literature values. Due to presence of both ternary species, occurring to its more involvement in the formation of quaternary complex species.

Table: 1 Overall stability constants and other related constants of binary, ternary and quaternary complexes for M_1(II)- M_2(II) 2-AHPPA (A) - 2,4-DHP(B) system

- Proton-ligand formation constant (log β_{00r0t}/ log β_{000st}) of 2-AHPPA(A) - 2,4-DHP(B) at 37 ± 1^0C I = 0.1 $NaNO_3$

Complex	log β_{00r0t}/ log β_{000st}
H_3A	21.35
H_2A	19.18
HA	10.14
BH	9.49

- Hydrolytic constants of M^{2+} (aq.) ions. (log β_{p000t}/ log β_{0q00t})

Complex	Hg	Cd
$M(OH)^+$	-3.84	-6.89
$M(OH)_2$	-6.38	-14.35

- Metal-Ligand constants (log β_{p0r00}/ log β_{0qr00}/ log β_{po0s0}/ log β_{0q0s0}) Binary System

Complex	Hg	Cd
MA	12.25	3.57
MB	13.08	11.45

- Metal-Ligand constants (log β_{p0rs0}/ log β_{0qrs0}) **:** Ternary System(1:1:1)

Complex	Hg	Cd
MAB	21.82	17.55

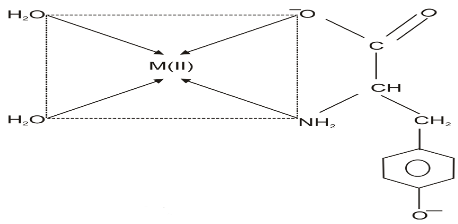
- Metal-Ligand constants (Log β_{pqrst}) : Quaternary System (1:1:1:1)

Complex	Hg-Cd
M_1 -M_2-A-B	28.95

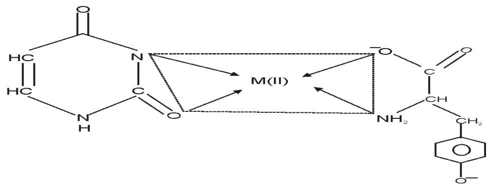
Overall Stability Order of Investigated Complex Species:

Hg (II) –Cd (II)-2-AHPPA (A) - 2, 4-DHP (B) > Hg (II)-2-AHPPA (A) - 2, 4-DHP (B) > Cd (II)- 2-AHPPA (A) - 2, 4-DHP(B) > Hg (II) -2, 4-DHP (B**)** **>** Hg (II) - 2-AHPPA (A) > Cd (II)- 2, 4-DHP (B) > Cd (II)-2-AHPPA (A)

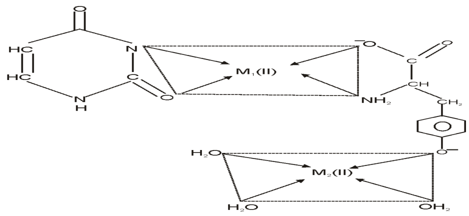
Proposed Structure of Investigated Complexes:



M (II)-AHPPA 4- Coordinated



M (II)-AHPPA-2,4DHP 4- Coordinated



M_1(II) - M_2 (II)-AHPPA-2,4DHP 4- Coordinated

CONCLUSION

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 element or 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 2-AHPPA (A) and 2, 4-DHP (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 and quaternary metal chelates of biological significance which protects living beings.

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