



Equilibrium study and Stability constants of mixed Ligand complexes of Bio-molecules and Amino acids with Metal ions by Potentiometric method

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Abstract

Research of metal complexes with biologically active ligand is one of high scientific and practical interest. Formation of binary and ternary complexes of metal ions such as Cu(II), Co(II), Pb(II), Zn(II) and Cd(II) with biologically important ligand were investigated. Where nucleic acid Adenine (A) used as primary ligand and amino acid Histidine (B) used as secondary ligand. Potentiometric technique was applied for determination of complexation behavior of binary and ternary species in aqueous media at $37 \pm 1^\circ \text{C}$ under ionic strength $I = 0.1 \text{ M NaNO}_3$. Stability constant have been determined through the method suggested by Irving & Rossetti and further refined through SCOGS computer program. The mixed ligand ternary complexes were occurring simultaneously in most cases generally at high pH with gradually decline of binary complexes of particular metal complexes. The percentage of ternary species of 56 at 6.4 pH for A-Cu(II)-B, 67.7 at 6.3 pH for A-Co(II)-A, 65.56 at 7.2 pH for A-Pb(II)-B, 48.5 at 7.4 pH for A-Zn(II)-B and 56.5 at 7.3 pH for A-Cd(II)-B. The overall stability constant of mixed ligand Adenine(A)-M(II)-Histidine(B) ternary system are obtained in the following order A-Cu(II)-B > A-Co(II)-B > A-Pb(II)-B > A-Zn(II)-B > A-Cd(II)-B.

Keywords: Potentiometric studies, bio-molecules, trace metals, SCOGS.

Introduction

The coordination behaviors of essential metal ions have great interest in the area of analytical, environmental, electrochemical and biological chemistry¹. The functional group of amino acid have potential to interactions of divalent metal ions which play an important role in promoting and maintaining their functionalities^{2,3}. Bioaccumulation and detoxification of the metal in living organism are most important role of coordination of metal to reduce their toxicity that highlighted the area of chelation therapy. Chelation is an intravenous treatment, designed to bind heavy metals in the body, in order to treat heavy metals toxicity^{4,5}. When the two or more donor atoms of ligand bind single metal ion and form a heterocyclic structure, it is said to be a chelating ligand and the complex compound itself is termed as metal chelate⁶. There have been now links between chelation therapy and potentiometry of ternary chelates as mixed chelation occurs commonly in biological fluids i.e. a million of potential ligands are likely to compete for the metal ions found in vivo. The essential metal ions like Cu(II), Co(II), Cd(II), Zn(II), have significant role in complexation with amino acid and peptides in living system which act as model for many complexes metal-amino acid equilibria occurring in enzymatic process⁷. Copper mainly found in Cu(II) form in biological systems but in some rare cases Cu(I) and Cu(II) form were also investigated in deoxyhemocyanine. Except histidine various α amino acid formed similar Copper complexes⁸. The role of cobalt in biological systems was investigated in series of coenzyme and vitamin B₁₂. The complexes of cobalt with cysteine methionine, lysine, and serine are plays significant role in bacteriostatic and inhibition of virus replication⁹. Due to

contraction and manufacturing cellular mechanism Zn(II) considered one of the most important essential metal in biological systems¹⁰.

Adenine is a derivatives of purine and component of nucleic acids that are used by living cells as adenosine monophosphate (AMP), adenosine diphosphate (ADP) and Adenosine triphosphate (ATP) which play vital roles in many metabolic processes. Adenine has four nitrogen atom labeled N¹, N³, N⁷, N⁹ suitable for metal binding. In previous studies it has been reported that adenine work as anti-inflammatory, antitumor activity when administered as metal complexes¹¹. Like other amino acids histidine is one of essential naturally occurring amino acid containing weakly basic imidazole group and plays an important role in the binding of the metal ions by proteins¹². Histidine has been formed six member ring when the metal attached with nitrogen atom of imidazole and the α amino group but this type chelation occurs due to exceptional ability of metal ions in which carboxylate oxygen of histidine also participate in coordination and thus it show tridentate behavior¹³. At physiological pH, the tridentate chelation of histidine ligand has been established for bis-metal complexes of cobalt (II), cadmium (II), lead (II). Copper (II)-histidine is most stable in comparison with the other metal (II)-histidine complexes¹⁴. Biochemical process in living system kept maintains their stability due to interaction of mixed metal mixed ligand complex formation. The formation of various simple as well as ternary (mixed-ligand) complex is always possible, depending on the affinity of the metal ions towards the various ligand present and the relative coordination sites¹⁵.

Present study aims to understand the order, nature and extent of coordination in physiological system of Cu(II), Co(II), Cd(II), Zn(II), Pb(II) bio metal ions at active site cavities in bioactive molecule like adenine and histidine as these made a model for many complex metal-purine derivative occurring in enzymatic process.

Material and Methods

Materials and Reagents: Both the ligands i.e. adenine and histidine was analytical grade reagent commercially available and used without further purification. The solution of metal nitrate and ligands were prepared as described in previous reported literature¹⁶. All the solutions were prepared in double distilled water. The aqueous solutions of metals nitrate were standardized by sodium salt of EDTA in presence of suitable indicator, where as the stock solutions of each ligand standardized against a standard oxalic acid solution. The potentiometric titration were carried out by carbonate free sodium hydroxide with an electric digital pH meter (Eutech-501) with a glass electrode at $37 \pm 1^\circ\text{C}$ and $I = 0.1 \text{ M NaNO}_3$.

Apparatus and Measuring Techniques: All the Potentiometric titrations were carried out at room temperature through the electric digital pH meter with a glass electrode working on 220 V/50 cycles stabilized by A.C. mains. With reproducibility of $\pm 0.01 \text{ pH}$, the electrode of pH meter was conditioned monthly by saturated potassium chloride (BDH) solution. An ultra thermostat type U10 (VEB MLW Sitz, Freital, Germany) was used to maintain a constant temperature in all the experiments. For all binary and ternary systems following solution mixture have been titrated against standardized NaOH (0.1 M) solution, keeping the total volume 50 ml in following manner.

5 ml NaNO_3 (1.0M) + 5 ml HNO_3 (0.02 M) + H_2O

5 ml NaNO_3 (1.0M) + 5 ml HNO_3 (0.02 M) + 5 ml M (0.01) + H_2O

5 ml NaNO_3 (1.0M) + 5 ml HNO_3 (0.02 M) + 5 ml M (0.01) + 5 ml A (0.01M) + H_2O

5 ml NaNO_3 (1.0M) + 5 ml HNO_3 (0.02 M) + 5 ml M (0.01) + 5 ml A (0.01M) + 5 ml B (0.01M) H_2O

Where: M is Cu(II)/Co(II)/Cd(II)/Zn(II)/Pb(II) metal ions and A is Adenine and B is histidine. The titration curves of pH of solution verses volume of NaOH for each set of solutions were plotted for potentiometric behavior of solution at different pH. The species distribution curves were obtained by plotting percentage concentration of the species obtained through Stability Constant of any Generalize Species (SCOGS) computer programme against pH.

Results and Discussion

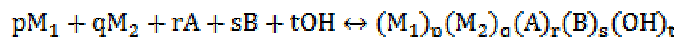
The protonation constants of the ligand were calculated from the potentiometric pH titrations data of solutions according to Irving

and Rossetti's method¹⁷. The pH value of solution regulate the equilibria of metal-ligand complex formation due to protonation of respective ligand which participate in complexes formation, At high pH it was observed that 1:1:1 ternary complex titration curves strongly overlap with the titration curves of the 1:1 binary complexes due to dissociation of binary complexes which also depend on the nature of both metal and ligand. The protonation constants of ligand and stability constant of selected various essential metal ions were calculated shown in table-1 at quite convincing with previous reported literature¹⁸. For the calculation of stability constants of binary complexes using the potentiometric titration data of the solutions and according to Irving and Rossetti's method. The order of the stability seems to be quite convincing on the basis of their ionic radii, electronic structure and the concept that the soft acids prefer to form complex with soft bases. The ionization equilibria of adenine and histidine have been extensively studied in different kinds of background electrolyte and the following equilibria were studied:



Where: A^{2-} represents the fully dissociated amino acid anion, the ionization constants of the amino K_1 and the second carboxylic acid groups K_2 , of the side chain of the amino acid have been determined using potentiometric techniques and have been calculated using SCOGS¹⁹ computer programme.

The formation of ternary complexes in an aqueous solution may be conveniently expressed by the equilibrium:



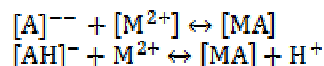
The overall stability constant is given by:

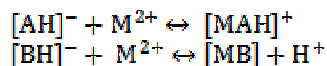
$$\beta_{pqrst} = \frac{(\text{M}_1)_p(\text{M}_2)_q(\text{A})_r(\text{B})_s(\text{OH})_t}{[\text{M}_1]^p[\text{M}_2]^q[\text{A}]^r[\text{B}]^s[\text{OH}]^t}$$

Where A, stands for the primary and B, for the secondary ligand and the stoichiometric number p, q, r, s are either zero or positive integer and t is a negative integer for a protonated species, positive values for a hydroxo or deprotonated species and zero for a neutral species.

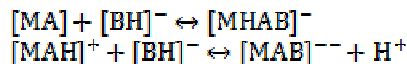
The overall stability constant (β_{pqrst}) defined may be used to calculate the species distribution curves that provides the clues for the formation equilibria of the complexes.

After the description of species distribution curve through SCOGS, following equilibria have been proposed:

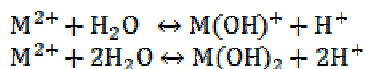




The formation of ternary complex may be explained as per the following equilibria:



Other general hydrolytic equilibria as:



where M = Metal ions, A represents the primary ligand (adenine) and B represents the secondary ligand (histidine). The Ternary (1:1:1) A: M: B complexes have been used in this study to ensure the exclusive formation of the A-M-B type complex, considering protonation constants of the ligand (A and B) hydrolytic constants of the metal (M^{2+}) aqueous ions, the following species have been considered to exist in the equilibria

A precondition for any study of a metal ion ligand interaction is the detailed knowledge of the basicity properties of the ligand. Therefore, we have first determined the protonation constant of Adenine and Histidine and formation constant of their binary complex under experimental condition at room temperature and $I = 0.1 \text{ M HNO}_3$. In ternary complexes the obtained value of binary complexes were used and result are in good agreement with the literature data. Equilibrium and formation constant study of 1:1:1 ternary systems of Cu(II), Pb(II), Cd(II), Zn(II), and Co(II) ions were carried out. In many cases it's has been shown that the ternary complex formation may proceed either through the step wise or simultaneous mechanism depending on chelating potentiality of primary or secondary ligand.

The speciation curve of A-Cu(II)-B complexes shown in figure-1 clearly reveals that at the starting of reaction as the pH increase protonated species HL is increases with decrease of H_2L species. Another protonated species H_3B increases sharply up to pH 3.6 and shows a gradual fall in concentration and

become constant⁵. The binary species Cu(II)-A and Cu(II)-B increased with pH as the concentration of protonated species is decreased. At pH 4.42 the complexes of Cu(II)-B obtain a maximum 91.8% meanwhile ternary complex species formation is found to be start with reducing the Cu(II)-B concentration. When the pH increases up to pH 6.4 the concentrations of ternary species obtain maximum concentration²⁰.

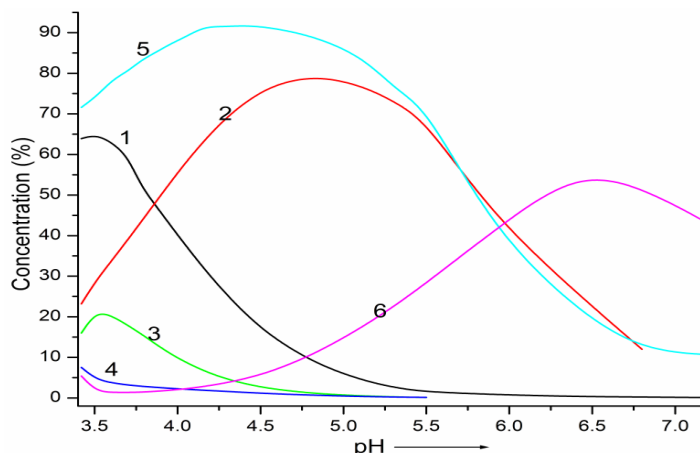


Figure-1
Species distribution curve of 1:1:1 ternary Adenine-Cu(II)-Histidine system: (1) H_2A , (2) HA , (3) H_3B , (4) $Cu-A$, (5) $Cu-B$, (6) $A-Cu-B$

Speciation curve of A-Pb(II)-B (1:1:1) ternary complex is shown in figure-2. With increasing pH the concentration of protonated species formation $H_2A < HA$ increases. After at pH 5.1 the obtained 93.5 % HA concentration going to decreased. Protonated species of secondary ligand i.e. H_3B is also decrease with increasing pH. The concentration of binary species of Pb(II)-B was found maximum than Pb(II)-A. The curve demonstrate that at the starting point ternary complex formation is slow and both the binary species is dominated that time but when the concentration of binary complex decrease ternary complex attained maximum concentration of 69.23% at pH 7.9 which closely related to the literature value²¹.

Table-1

Proton ligand formation constant, Stability constant and other related constants of the binary and ternary species of some essential metal complexes with adenine as a primary ligand and Histidine as secondary ligand

Species	Protonation constant (Log β pqrst)						
	Ligands		Metal Hydrolytic		Metal Complex		
	Primary	Secondary	$M(OH)^+$	$M(OH)_2$	MA	MB	A-M-B
H_2A	10.88	-	-	-	-	-	-
HA	7.02	-	-	-	-	-	-
H_3B	-	8.96	-	-	-	-	-
$Cu(II)$	-	-	-6.29	-13.10	6.18	10.30	16.12
$Pb(II)$	-	-	-9.84	-15.54	4.05	6.96	9.91
$Zn(II)$	-	-	-7.89	-14.92	4.06	6.52	9.30
$Co(II)$	-	-	-8.23	-17.83	6.84	9.05	14.95
$Cd(II)$	-	-	-6.89	-14.35	5.88	5.39	8.10

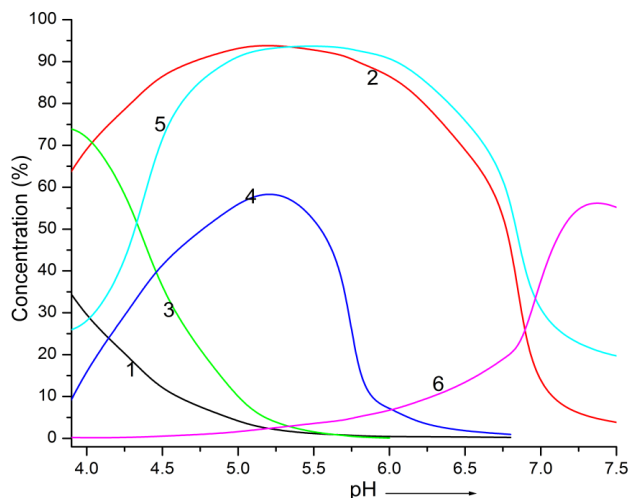


Figure-2

Species distribution curve of 1:1:1 ternary Adenine-Pb (II)-Histidine system: (1)H₂A, (2)HA, (3) H₃B, (4)Pb-A, (5)Pb-B, (6)A-Pb-B

In A-Zn(II)-B complexes the protonated species concentration of primary ligand increased H₂A<HA with increasing 5.07 pH up to 86% shown in figure-3. Protonated species H₃B gradually increases and attain a maximum concentration at pH 4.1. The hydroxo species of Zn(OH)⁺ concentration was also found between pH 6.7 to 7.3 in a lesser extent. Complex of Zn(II)-A species start to form at pH 3.4 and with sharp increment attain maximum concentration at pH 4.8. But the Zn(II)-B complexes species increases with pH and obtain maximum concentration of 83% at pH 5.48. Ternary mixed ligand complex of Zn start to form sharply at lower pH but as the pH increase % concentration of ternary complex also increases gradually with the dissociation of binary complex²². The curve clearly reveals that at higher pH ternary complex is formed in higher concentration.

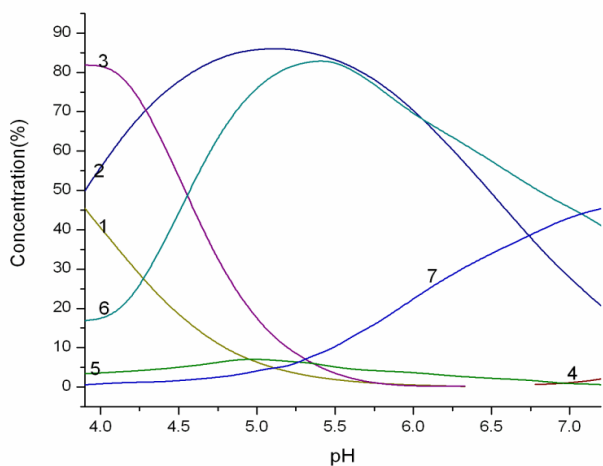


Figure-3

Species distribution curve of 1:1:1 ternary Adenine-Zn (II)-Histidine system: (1) H₂A, (2) HA, (3) H₃B, (4) Zn(OH)⁺, (5) Zn-A, (6) Zn-B, (7) A-Zn-B

Speciation curve of A-Cd(II)-B (1:1:1) ternary complex is shown in figure-4. The maximum 99.4% protonated species of H₃B were obtained at pH 3.84. The H₂A< HA species increases with increasing pH. HA protonated species attained a maximum concentration of 60.7% at pH 5.4 after that it shows a decline in concentration with increasing pH. The hydroxo species Cd(OH)⁺ is also formed with sharp increment as pH increase²³. Cd(OH)⁺ species is dominated among all the species formed. Ternary complex at pH 7.25 have maximum concentration of 56.5% due to dissociation of binary complexes. After which it also decreases and become constant.

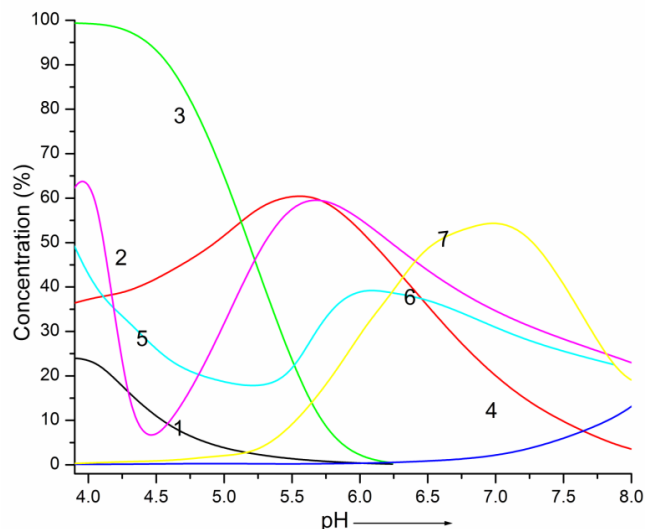


Figure-4

Species distribution curve of 1:1:1 ternary Adenine-Cd(II)-Histidine system: (1) H₂A, (2) HA, (3) H₃B, (4) Cd(OH)⁺, (5) Cd-A, (6) Cd-B, (7) A-Cd-B

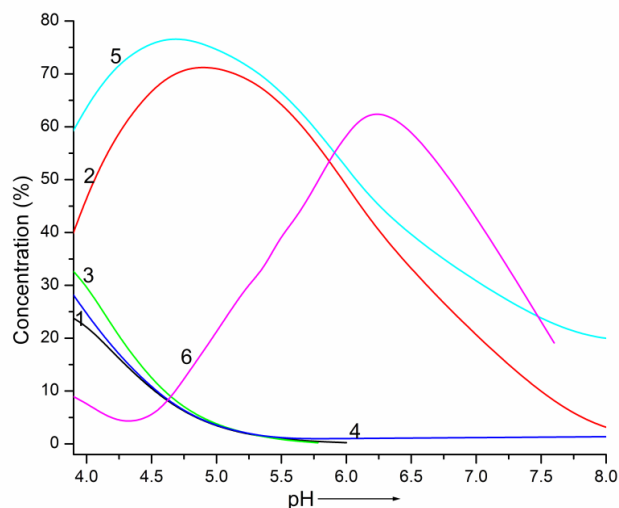


Figure-5

Species distribution curve of 1:1:1 ternary Adenine-Co(II)-Histidine system: (1) H₂A, (2) HA, (3) H₃B, (4) Co-A, (5) Co-B, (6) A-Co-B

Figure-5 shows speciation curve of A-Co(II)-B (1:1:1) ternary complex. First protonated species of adenine is decreased with increasing pH and become constant as the second protonated species begin to form. It attains a maximum concentration 71.3% at pH 4.96 after that its concentration decreases. Protonated species H_3B is also decrease with pH. Binary species Co(II)-A decreases with increases pH as concentration of another binary species i.e. Co(II)-B is increases and attain maximum concentration of 76.7% at pH 4.77 after that ternary complex begin to form with the dissociation of binary species³. As the pH increase concentration of ternary complex increase and attain a maximum concentration of 33% at pH 5.78. In

aqueous solution the three protonated species release from, N7, N9 binding site of adenine participate in complex formation. With three ionizable protons, adenine is naturally proton to complex formation shown in figure-6. Histidine has three binding site like, amino, imidazole and carboxylate which participate in octahedral complexes formation. In ternary system metal ligand formation the adenine coordinate to metal ion via N7 and N9 and histidine coordinate via either in histamine manner or in glycine like, form square planner complexes. Only Cu^{2+} ion form a square planner complex where as other metal ions taken in our study form octahedral complex.

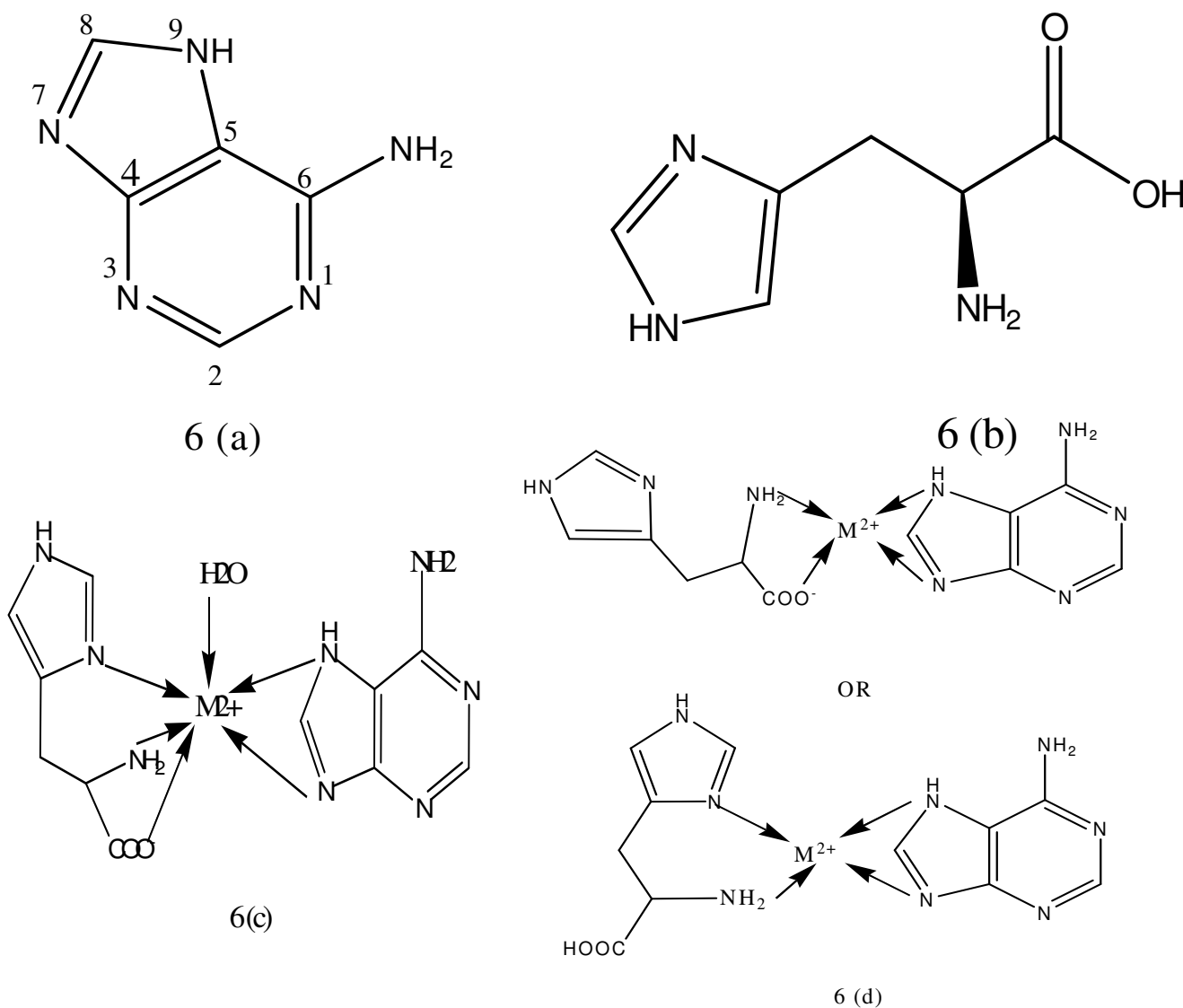


Figure-6

The structure 6 (a, b) are showing solution structure of Adenine and Histidine respectively where as structure 6(c,d) are showing the Proposed structure of ternary Adenine-M(II)-Histidine (1:1:1) system complexes where M^{2+} = metal ion

Conclusion

The formation equilibria, percentage of species composition, and relative stability of mixed ligand ternary complexes were investigated. The ternary complexes occur in larger concentration at higher pH in each of the system studied. At high pH it was observed that 1:1:1 ternary complex titration curves strongly overlap with the titration curves of the 1:1 binary complexes due to dissociation of binary complexes which also depend on the nature of both metal and ligand. In nature metal ions found to follow the trend $Cu > Pb > Zn > Cd > Co$. This is in conformity with the Meller and Maley and Irving-Williams order. The protonation constants of ligand and stability constant of selected various essential metal ions were calculated show the stability constant of ternary system are higher than binary system. The overall stability constant of mixed ligand adenine (A)-M(II)-Histidine(B) ternary system are in the following order.

$A-Cu(II)-B > A-Co(II)-B > A-Pb(II)-B > A-Zn(II)-B > A-Cd(II)-B$.

The additional high stability of the Cu(II) complex is attributed to the unique electronic configuration ($3d^9$) of Cu(II) ion which is capable of additional stabilization due to Jahn-Teller distortion. The dissociation of the ternary complex may also be attributed to the formation of hydroxo species as the buffer region corresponding to the complexation. The study also gives an insight into the metal availability/metal transport in biofluids. The ternary complexes are more amenable for "metal transport" because of their extra stability. The present study also helps to understand metal chelation behavior as a role model for detoxification of metal in living system and remediation of heavy metal from polluted site.

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