Research Article

A Study on Speciation and Coordination Tendency of Glutamic Acid and Uracil for Ternary Complexation towards some toxic metal ions

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Abstract

A potentiometric study has been carried out in aqueous medium to reveal the speciation and coordination tendencies of Glutamic Acid and Uracil which have been used as primary and secondary ligands respectively. The ternary complexation of the said bio-ligands with toxic and heavy metal ions Pb^{II}, Hg^{II}, Cd^{II}, Cu^{II}, Zn^{II}, Co^{II}, and Ni^{II} investigates the stability constants of ternary (1:1:1) complexes at37±1°C and in ionic strength I = 0.1 M $NaNO_3$. The predominant species detected are H_3A, H_2A, HA, HB, MA, MB, and MAB. The species distribution diagrams and the plausible equilibria for the formation of the species are also presented and discussed. Calculations have been done and the models containing different numbers of species were refined through the 'Stability Constant of Generalized Species' (SCOGS) computer program. Attempts have been made to explain the enhanced stability of the ternary coordination complexes as reflected in species distribution plots.

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Introduction

In continuation to our studies in the area of Chelation Therapy [1] and diversified and extensive application of bio-ligands with an increased affinity and sensitivity of metal detoxification [2], present work throws light on the speciation of toxic metal ion complexes which is useful to understand the role played by the active site cavities in biological molecules and the bonding behavior of protein residues with the metal ion. The species refined and their relative concentrations under the experimental conditions represent the possible forms of amino acids in bio fluids.

Mercury is one of the most toxic elements and has negative health effects in human populations, highly dependent on fish consumption [3,4]. Humans are exposed to Hg primarily as mono-methylmercury [5], which is the form of Hg that accumulates readily in organisms and biomagnifies in food webs [6]. Cadmium causes iron deficiency by binding to cysteine, glutamate, aspartate, and histidine ligands [7]. Cadmium inhibits enzymes that participate in bilirubine conjunction [8]. It increases urine Ca^{2+} excretion which can cause severe bone pathology [9]. The possible effects of long term low-level exposure to cadmium are of concern because it is readily distributed to tissues of liver and kidney, which are the main target organs in acute and chronic cadmium exposure [10,11]. Other tissues involved in cadmium toxicity include the testis, heart, bone, eye, and brain [12]. Due to its numerous uses and high persistence, lead is a major environmental contaminant [13]. Lead is toxic even at low concentrations for living organisms, which can absorb it in various ways [14]. Lead intake by humans can be due to the consumption of crop plants grown on soils with high plant-available metal concentrations [15]. It can, however, migrate through the soil with dissolved organic matter [16] or mobilized by certain plants [17]. Moreover, carried from the air to the soils as fine particles, lead could be released more easily in soil solution [18]. Studies in individuals with acrodermatitis enteropathica, a genetic disorder with zinc malabsorption resulting in severe deficiency, have provided much insight into the functional outcomes of zinc deficiency [19]. In the form of Vitamin B_{12} (cobalamin), Co metal plays a number of crucial roles in many biological functions and Nickel plays numerous roles in the biology of microorganisms and plants [20].

L-Glutamic acid (Glu) plays a vital role in various biochemical processes at the molecular levels. In the animal world, it links the metabolism of carbon and nitrogen. In the central nervous system (CNS) glutamate has two functions: it is a neurotransmitter and a precursor of α-aminobutyric acid [21,22]. It is the main excitatory transmitter of the CNS of vertebrates [23] being involved in fundamental mechanisms of learning, memory, formation, and plasticity of the synaptic endings and in the development of the CNS. In many animals Glu is the most abundant intra-cellular amino acid. The protonation constants of Glu [24], their binary complexes with Pb(II), Cd(II), Hg(II), Co(II), Ni(II), Cu(II) and Zn(II) and ternary complexes with Pb(II), Cd(II) and Hg(II) in 1,2-propanediol (PG)-water mixtures were reported earlier [25-27]. The ternary complexes containing Glu have also been studied [28-30]. Mixed ligand complexes of Cr(II) with Met and Glu and Cd(II) with Glu, formic acid and ornithine have been reported in the literature [31,32].

Ternary complexes of metal (II) containing nitrogen and oxygen-donor ligands have received much attention recently as they can display exceptionally high stability [33-35]. Ternary metal complexes have been recently studied due to their ability as metal systems for metal–protein complexes such as metallo-enzymes. They received particular attention and have been employed in mapping protein surfaces [36], as probes for biological redox centers [37] and in protein capture for both purification [38] and study [39,40]. Study of the structure of model ternary complexes provides information about how biological systems achieve their specificity and stability, as well as strategies to improve these features for biotechnological applications. Hence, the aim of present research work is to assess the nature and extent of coordination of Glutamic Acid and Uracil bio-ligands with bio metal ions viz., Hg(II), Pb(II), Cd(II), Zn(II), Co(II) and Ni(II) using potentiometric technique. Acid dissociation constants of the ligands and the formation constants of the binary and ternary complexes were determined at 37 ± 1^0C and in ionic strength I = 0.1 M $NaNO_3$ adopting the Irving Rossotti technique [41]. The stability and complexation tendency of the formed ternary complexes have been examined and discussed in relation to that of the corresponding binary complexes as well as the nature of metal ion.

Experimental

The entire chemical used were of analytical grade products. A stock solution (0.01 M) of Glutamic acid was prepared by constant stirring in double distilled water. Metal Nitrate solutions were prepared and standardized by EDTA titrations [42]. A 0.01 M solution of Uracil was prepared by dissolving it into one equivalent of alkali (NaOH), which itself was prepared in double distilled water and had been standardized against a standard oxalic acid solution. Carbonate free sodium hydroxide (E. Merck) and Nitric Acid (A.R.) were prepared in double distilled water and standardized as usual.

The titrations were performed at (30 ± 0.1) °C in a double-walled cell fitted with an Ultra Thermostat type U10 (VEB MLW Sitz, Freital, Germany) which was used to maintain a constant temperature in all the experiments. The free acid concentration was kept equal in each case i.e., 0.02 M. pH measurements were done by an electric digital pH meter with a glass electrode supplied with the instrument and working on 220 V/50 cycles stabilized by A.C. mains. The pH meter has a reproducibility of ±0.01 pH. The electrode of pH meter was conditioned monthly by saturated potassium chloride (BDH) solution. The titrant (CO_2-free standard NaOH) was added to the titration cell, and the pH changes were monitored through the pH meter. The pH meter was calibrated with standard buffer solutions (pH 4.0 and 10.0) before the pH measurements. The ionic strength was kept constant (0.10 mol dm^{-3}) using a $NaNO_3$ solution, and a total volume of 50 cm^{-3} was used for each titration. For the study of ternary (1:1:1) complexes, the different solutions titrated were as follows:

Solution A: 5 ml $NaNO_3$(0.1 mol dm^{-3}) + 5 ml HNO_3(0.02 mol dm^{-3}) + H_2O
Solution B: 5 ml $NaNO_3$(0.1 mol dm^{-3}) + 5 ml HNO_3(0.02 mol dm^{-3}) + 5 ml A(0.01 mol dm^{-3}) + H_2O
Solution C: 5 ml $NaNO_3$(0.1 mol dm^{-3}) + 5 ml HNO_3(0.02 mol dm^{-3}) + 5 ml A(0.01 mol dm^{-3}) + 5 ml M(II)(0.01 mol dm^{-3}) + H_2O
Solution D: 5 ml $NaNO_3$(0.1 mol dm^{-3}) + 5 ml HNO_3(0.02 mol dm^{-3}) + 5 ml B(0.01 mol dm^{-3}) + 5 ml M(II)(0.01 mol dm^{-3}) + H_2O
Solution E: 5 ml $NaNO_3$(0.1 mol dm^{-3}) + 5 ml HNO_3(0.02 mol dm^{-3}) + 5 ml A(0.01 mol dm^{-3}) + 5 ml M(II)(0.01 mol dm^{-3}) + 5 ml B(0.01 mol dm^{-3}) + H_2O

Where, M(II) is Cu/Pb/Hg/Zn/Ni/Co metal ion; A=Glutamic acid and B=Uracil. Each set of solution was then titrated against standard alkali (NaOH). The pH values were plotted against the volume of NaOH and the titration curves were obtained. The titration curves were discontinued at the appearance of turbidity.

Results and Discussion

Identical bunches of titration curves are obtained for the different ternary systems under investigation according to the sequence described in the experimental section. With respect to the titration curves of the 1:1:1 ternary complexes, it may be deduced that these titration curves strongly overlap with the titration curves of the 1:1 binary M(II)-A at lower pH values. Generally, at higher pH's the divergence of the ternary titration curve from that of the corresponding binary ones. The pH value at which divergence occurred is largely dependent on the nature of both the metal ions and the two ligands.

Uracil, a pyrimidine undergoes keto enol tautomeric shifts because of its resonance structure due to –NH2 and –OH substituents. The keto tautomer is referred as the lactam structure while the enol tautomer is referred as lactim structure (**Figure 1**)

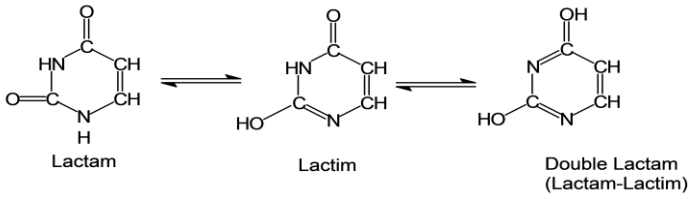

Figure 1 Structure of lactim

On the basis of UV measurements in aqueous solution, Uracil exists primarily in the diketo form where as in alkaline solution; it exists as approximately 1:1 mixture of the two possible deprotonated forms. Two overlapping absorption bands with max 260 and 284 nm have been reported for proton ionization from neutral Uracil with a conclusion that protons ionize simultaneously from both N_1H and N_3H groups.

Ultraviolet and ultrasonic absorption studies [43,44] indicate that U and T primarily exit in the lactam form in an aqueous solution. However, in the alkaline solution two ionizations were detected that of the α-hydroxyl group characterized by pK_1 and that of the γ-hydroxyl group for pK_2. The pKa values for T are slightly higher which is perhaps due to the presence of $–CH_3$ group at 5 positions. The possible stepwise ionizations involved in the cases of U and T are summarized below (**Figure 2**)

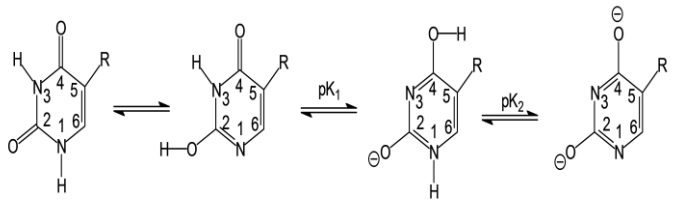

Figure 2 Stepwise ionizations of U and T (R = H, Uracil; R = CH_3, Thymine)

Glutamic acid is potentially a tridentate ligand towards metal ions, with three donor sites on the terminal amino acid and carboxyl groups as well as on the second carboxyl group on the side chain of the molecule. L-glutamic acid in its fully protonated form is designated as H_2L. In the most cases, glutamate is bidentate with the side chain extended. The carboxyl groups can be deprotonated twice and the $–NH_3^+$ is depotonated in a high pH aqueous solution.

The ionization equilibria of glutamic acid have been extensively studied in different kinds of background electrolyte and the following equilibria were studied:

$$H_2A \longleftrightarrow H^+ + HA^-$$
$$K_1 = [H^+][HA^-]/[H_2A] \quad \ldots\ldots(1)$$

$$HA^- \longleftrightarrow H^+ + A^{-2}$$
$$K_2 = [H^+][A^{2-}]/[HA^-] \quad \ldots\ldots(2)$$

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 SCOGS [45] computer program. Preliminary estimates of binary and ternary constants obtained by least-square method [46] were further by refined by SCOGS. These values are listed in **Table 1**, which are in good agreement with those reported in the literature [47].

For the evaluation of stability constants and the determination of stability constants involving both the ligands by SCOGS, the complex formation has been assumed to take place according to:

$$_pM_1 + {}_qM_2 + {}_rA + {}_sB + {}_tOH \leftrightarrow (M_1)_p(M_2)_q(A)_r(B)_s(OH)_t$$

$$\beta pqrst = [(M_1)_p(M_2)_q(A)_r(B)_s(OH)_t] / [M_1]^p[M_2]^q[A]^r[B]^s[OH]^t$$

Where, 'A' stands for the primary and 'B' for the secondary ligand. p, q, and r are the respective stoichiometric coefficients.

When a solution contains two different ligands and a metal ion, they may exist in equilibria in which either (i) both the ligands may combine with the metal ion simultaneously or (ii) the two ligands may be combined one by one at different pH. As is evident from the titration curves in the present study, the addition of two ligands is stepwise. It was deduced that Glutamic acid interacts first with the M(II) ion, followed by the interaction with the Uracil; that is, the ternary complex formation could be considered in stepwise complexation equilibria, i.e., the formation of a ternary complex can be represented by the stepwise equilibria;

$$M + A \longleftrightarrow MA \quad \ldots\ldots\ldots\ldots\ldots\ldots(1)$$
$$MA + L \longleftrightarrow MAL \quad \ldots\ldots\ldots\ldots\ldots\ldots(2)$$
$$K_{MAL}^{MA} = [MAL] / [MA][L] \quad \ldots\ldots\ldots(3)$$

where M = M(II), A represents the primary ligand (Glutamic Acid) and L represents the secondary ligand (Uracil); for instance, examining titration curves, one may observe that the curves obtained for the different 1:1:1 ternary complex solutions overlap with the titration curve of the 1:1 binary at low pH values and a

divergence of the ternary complex titration curve from that of the binary is observed at higher pH.

Part of the information required for determining the stability constants of ternary complexes is the protonation constants of the ligands under study. Therefore, prior to determining the stability constants of mixed complexes, the protonation constants of the ligands under study are determined. The ligand species which occurred in solution under our experimental conditions are AH3, AH_2, AH, and BH. The experimental parameters and conditions used here are the same as those used to determine the stability constants. The distribution diagram of the ligand assists the determination of the experimental conditions where the coordination with metal ions would take place, especially in terms of pH.

The dominance of ternary complexes is traced at pH ranges between 5.0 and 9.5 which is about 70% of all the species. It is evident from the concentration profiles that the concentrations of AH_3, AH_2 and AH species are found to decrease within the pH range 3.0-9.5, representing the metal-ligand complexation. Binary complex species such as M(II)-A and M(II)-B exist in significant amounts at lower pH range. The incline in the concentration of ternary species with a concomitant decline in the concentration of binary species in most of the systems shows that there is a step-wise formation of ternary complexes. Ternary complex involving Hg(II) follows simultaneous complexation owing to the high stability of Hg(II) chelates. Speciation curves shows the similarity in the distribution diagram of A-Cu(II)-B system, with that of the A-Hg(II)-B system, of A-Ni(II)-B system, with that of the A-Co(II)-B system and of A-Cd(II)-B system with that of A-Pb(II)-B system with the species being tracked by the computer program. The % composition of A-Hg(II)-B and A-Cu(II)-B at pH □ 7.0 is about 90% out of the total concentration of M(II) ion, while that of A-Pb(II)-B and A-Cd(II)-B is about 60% at the same pH. Similary, the maximum abundance of ternary complex species of Ni(II) and Co(II) could be observed at pH □ 7.0 being 60% and 70% respectively. After this limiting pH, there is a gradual decline in the concentration of all these ternary complex species. In case of A-Zn(II)-B system, the dominance of mixed ligand species is evident at pH>9.0 i.e., □ 78%, while Zn(II)-A binary complex may be seen approaching 70% at pH □ 7.0.

There is a potential chance for the hydrolysis of the hydrated M(II) ion to take place in the pH range where the measurements were taken [48,49], which gives rise to hydrolyzed species in solution. The formation of this type of partially hydrolyzed species not only depends on the pH but on the availability of water molecules on the solvated metal ion [50]. The deprotonation, which includes the dissociation of hydroxyl groups, occurs as a result of the formation of the coordinate bond, which causes electron withdrawal toward the metal ion and therefore lessens the dissociation of the hydroxyl groups. In other words, the coordination of the ligand with the metal ion will intensify the dissociation of the hydroxyl group or in fact any other acidic hydrogen in the system due to an increase in the electron charge flow toward the metal ion, as a result of coordinate bond formation.

On the basis of the description of speciation curves obtained through SCOGS, following equilibria have been proposed:

$$[A]^{--} + M^{2+} \longleftrightarrow [MA]$$
$$[AH]^{-} + M^{2+} \longleftrightarrow [MA] + H^{+}$$
$$[AH]^{-} + M^{2+} \longleftrightarrow [MHA]^{+}$$
$$[BH]^{-} + M^{2+} \longleftrightarrow [MB] + H^{+}$$

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

$$[MA] + BH^{-} \longleftrightarrow [MHAB]^{-}$$
$$[MHA]^{+} + BH^{-} \longleftrightarrow [MAB]^{--} + H^{+}$$

Other general hydrolytic equilibria are:

$$M^{2+} + H_2O \longleftrightarrow M(OH)^{+} + H^{+}$$
$$M^{2+} + 2H_2O \longleftrightarrow M(OH)_2 + 2H^{+}$$

The two carboxylate groups and one deprotonated amino group of L-glutamate are proposed binding sites for metal ions, and are thought to form the mononuclear 1:1 species. These idea somewhat agrees with previously reported results [51,52]. Besides the monodentate and bidentate binding modes, the tridentate binding mode of the likely five - & seven-membered joint chelate also may be proposed, such chelates have been confirmed by multinuclear nmr measurements [51]. The magnitudes of equilibrium stability constants [52] suggest that in the ML and ML_2^{2-} complexes, glutamate is bidentate. In addition to the simple 1:1 complexes, various evidences for other species have been reported. In the formation of mixed-ligand complexes, the secondary ligand coordinates through N and carboxyl atoms with the tetra coordinated or hexa coordinated metal ions. The proposed structures of such types of mixed-ligand complexes are shown in **Figure 3 and 4**.

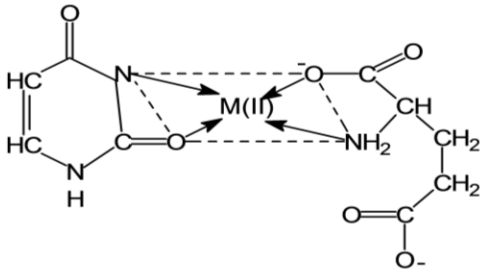

Figure 3 Proposed structure of ternary Glu-M(II)-Uracil (1:1:1) complex where M(II) = tetra coordinated metal ion

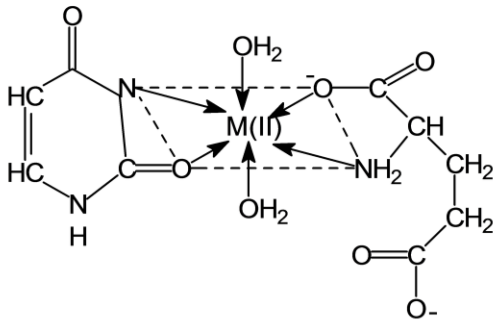

Figure 4 Proposed structure of ternary Glu-M(II)-Uracil (1:1:1) complex where M(II) = hexa coordinated metal ion

The protonation constants of ligands, overall stability constants of binary and ternary complexes determined have been compiled together in **Table 1**.

Conclusion

The ternary metal complex species detected are A-M(II)-B for all the metal ions understudy, where A = Glu and B = U. Only these species are refined due to the restricted pH ranges and the possible active forms of ligands like AH_3, AH_2, AH and BH for Glu and U, respectively. A perusal of Table-1 shows that the stability constants of mixed ligand [Glu(A)-M(II)-Uracil(B)] ternary systems are in the following order:

A-Hg(II)-B > A-Cu(II)-B > A–Pb(II)-B >A-Cd(II)-B > A-Zn(II)-B > A-Ni(II)-B > A-Co(II)-(B)

The marked stabilization of the ternary relative to the binary complexes is reflected in the species-distribution plots, (**Figure 5- 8**) i.e., the ternary complexes occur in larger concentrations than the binary complexes at higher pH in each of the systems studied. The extra stability of the ternary complexes is due to the interactions outside the coordination sphere. This may sometimes be due to the formation of hydrogen bonds between the coordinated ligands, charge neutralization; chelate effect, stacking interactions and a similar stabilizing effect may likewise be exerted by the electrostatic interactions between non-coordinated, charged groups of the ligands. Sakuri et al [53] carried out extensive studies to establish the laws governing interactions of this nature.

The dependence of the stability of the binary and ternary complexes studies on nature of metal ion is found to follow the trend: Co(II) < Ni(II) << Cu(II). This is in conformity with the Irving-Williams order. 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 equilibria has been found to be overlapping with the hydrolytic equilibria of Hg^{2+} aqueous metal ions. 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.

Table 1 Proton ligand formation constants of Glutamic acid (A) and Uracil (B).Stability constants and other related constants of binary ternary and quaternary complexes of Glutamic acid (A) and Uracil (B) with different metal ions in aqueous solution at 37±1°C I = 0.1M $NaNO_3$.

(a) Proton-ligand formation constant (Log β pqrst)							
H_3A		16.97					
H_2A		13.73					
HA		09.59					
HB		09.49					
(b) Hydrolytic constants (Log β pqrst)							
Complex	**Cu**	**Pb**	**Cd**	**Hg**	**Zn**	**Ni**	**Co**
$M(OH)^+$	**-6.29**	**-9.84**	**-6.89**	**-3.84**	**-7.89**	**-8.10**	**-8.23**
$M(OH)_2$	**-13.10**	**-15.54**	**-14.35**	**-6.38**	**-14.92**	**-16.87**	**-17.83**
(c) Metal-Ligand constants (Log β pqrst) Binary System							
Complex	**Cu**	**Pb**	**Cd**	**Hg**	**Zn**	**Ni**	**Co**
MA	**7.86**	**11.65**	**3.90**	**16.56**	**4.59**	**5.60**	**4.56**
MB	**8.25**	**12.77**	**11.45**	**13.08**	**6.61**	**6.80**	**6.28**
(d) Metal-Ligand constants (Log β pqrst) : Ternary System							
Complex	**Cu**	**Pb**	**Cd**	**Hg**	**Zn**	**Ni**	**Co**
M-A-B	**16.76**	**16.30**	**16.06**	**19.26**	**14.48**	**12.35**	**12.33**

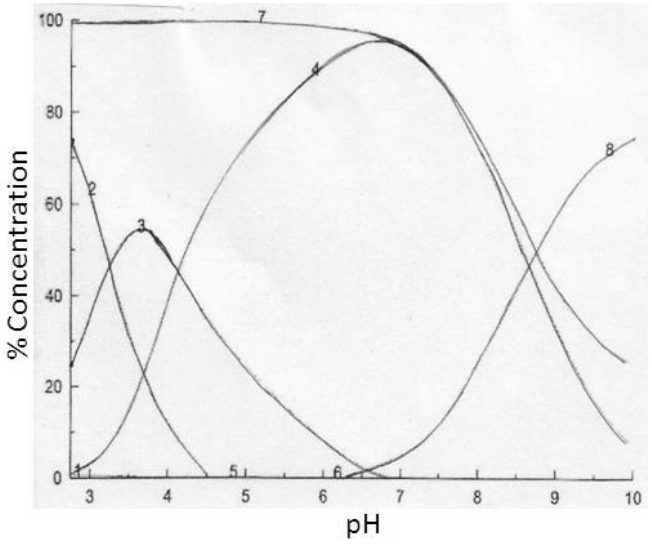

Fig. 5. Distribution curves of 1:1:1 Glu (A)-Pb(II)-Uracil(B) system: (1) Pb^{2+}, (2) AH_3, (3) AH_2, (4) AH, (5) BH, (6) Pb(II)-A, (7) Pb(II)-B, and (8) A-Pb(II)-B.

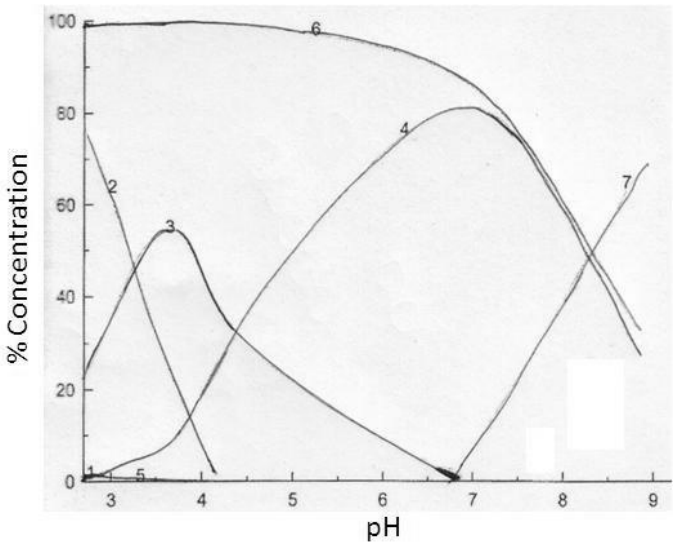

Fig. 6. Distribution curves of 1:1:1 Glu (A)-Cd(II)-Uracil(B) system: (1) Cd^{2+}, (2) AH_3, (3) AH_2, (4) AH, (5) BH, (6) Cd(II)-A, (7) Cd(II)-B, and (8) A-Cd(II)-B.

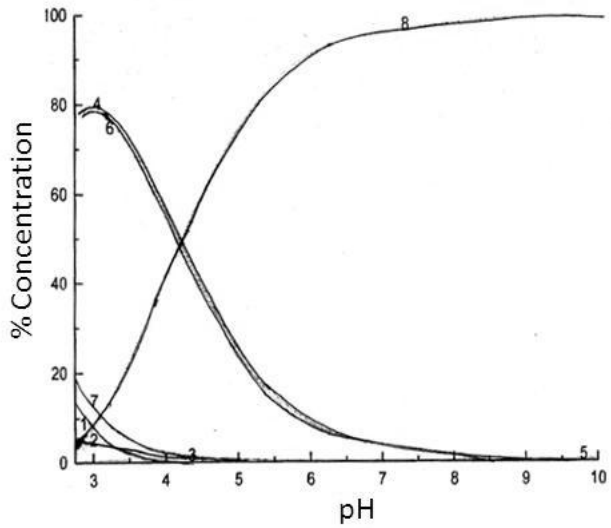

Fig. 7. Distribution curves of 1:1:1 Glu (A)-Hg(II)-Uracil(B) system: (1) Pb^{2+}, (2) AH_3, (3) AH_2, (4) AH, (5) BH, (6) Hg(II)-A, (7) Hg(II)-B, and (8) A- Hg(II)-B.

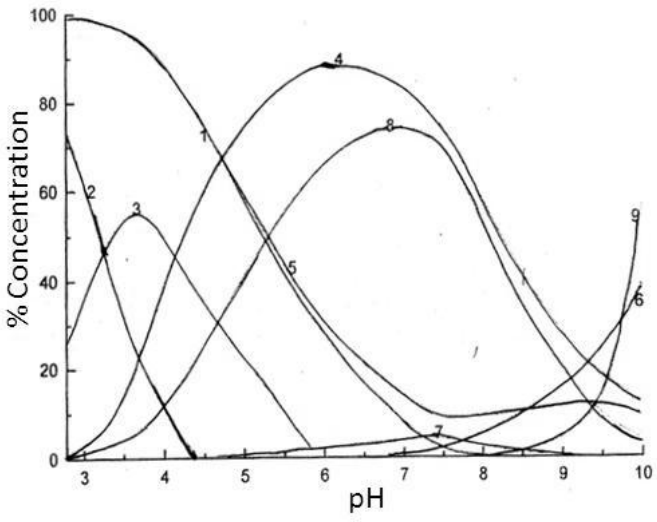

Fig. 8. Distribution curves of 1:1:1 Glu (A)-Zn(II)-Uracil(B) system: (1) Cd^{2+}, (2) AH_3, (3) AH_2, (4) AH, (5) BH, (6) Zn(II)-A, (7) Zn(II)-B, and (8) A- Zn(II)-B.

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