Binary Complexes of Aspartic Acid with Some Metal Ions in Aqueous Solution and Water-dioxane Mixtures

Ebrahim Ghiamati[1*], Maliheh Baniasadi[1] and Alireza Farrokhi[1]

[1]*Department of Chemistry, University of Birjand, P.O. Box 414, Birjand, Iran.*

Authors' contributions

Article Information

Original Research Article

ABSTRACT

The reaction between amino acids and metal ions has been used as a model for transferring the metallic ions to body tissues. How stable these complexes are formed is our concern. The stability constants of the complexes between L-aspartic acid (Asp) and Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Ce(III) metal ions were determined by potentiometric titrations in aqueous solution and in 25-75%(v/v) of water-dioxane mixtures(I = 0.1 M, NaNO$_3$) at 15, 20, 25, 35, and 45°C. The observed increasing stability order for the corresponding complexes in water and water-dioxane mixtures were as follows:

$$K_{f\ Cd\ (II)-Asp} < K_{f\ Zn\ (II)-Asp} < K_{f\ Co\ (II)-Asp} < K_{f\ Ni\ (II)-Asp} < K_{f\ Ce(III)-Asp} < K_{f\ Cu(II)-Asp}$$
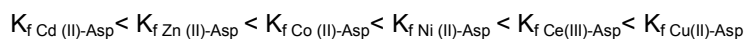
Mixed solvents such as water-dioxane provide a better model for in vivo reactions. It was observed that with increase in the percentage of dioxane, the stability constants were elevated. The results showed that the reactions were exothermic and spontaneous.

Corresponding author: E-mail: eghiamati@birjand.ac.ir;

1. INTRODUCTION

Amino acids are by far the most important low-molecular–weight ligands in biological systems. Proteins are constructed from 21 different α-amino acids including selenocysteine. The amino acid-metallic ion interactions are found to be responsible for enzymatic activity and stability of protein structures [1-3]. The mono acids form stable five-membered chelates with various metal ions through the amine and carboxylate moieties (N, O chelation). Several amino acids have an additional metal binding site in the side chain and thus form metal complexes with a variability in their structures. Metal complexes play a vital role in nature, they have been extensively used in clinical applications as enzyme inhibitors [4], anti-bacterial [5], anti-viral [6] and anti-cancerous [7].

L-Aspartic acid (Asp) is one of the naturally occurring α-amino acids, with an additional carboxylic group in the side chain [8]. Asp takes part in the thermogenic processes induced by prostaglandin E1, it is a component of the active center of some enzymes [9] and forms complexes with almost all the metal ions through its three binding sites. Chelation through the amino and α-carboxylate groups occurs readily, and study of metal binding has been devoted to attempts to deduce whether side chain carboxylates are also coordinated to the metal ion. Three chelate rings are formed, because Asp is a tridentate ligand [10,11].

Determination of the stability constants of these complexes have been carried out in aqueous media. This may be attributed to the widespread belief that "in vivo" media are represented by the aqueous system [12]. In recent years, however, it has been reported that aqueous media are not suitable for biological in vivo reactions, on the contrary the biological one show lipophilic character and is better represented by non-aqueous system [13-23]. Also, it is well established that the "effective" or "equivalent solution" dielectric constants in proteins [24,25] or active site cavities of enzymes [26] are small compared to that in bulk water. Estimates for the dielectric constants in such locations range from 30 to 70 [27,28]. Hence by using aqueous solutions containing ∼10–50% dioxane, one may expect to simulate to some degree the situation in active site cavities and therefore to extrapolate the data to physiological conditions [28,29].

Metal with the most interaction with Asp creates a very strong bond and has the largest stability constants. Several literature reports have been devoted to the study of stability constants of metal-Asp complexes. Bukietynska et al. [30] carried out potentiometric studies on complexes of vanadium (III) with L-alanine and Asp. Bottari [31] studied copper (II) complexes with aspartate and glutamate. Stability constants of mixed ligand complexes of bio-molecules and amino acids with Ni(II) by the potentiometric titration method were studied by Phase et al. [32].

Potentiometric study of Mo(VI) complexation with Asp was conducted by Gharib and Zare [33]. Asp complex of Al(III) was examined by Djurdjevic et al. [34]. Interactions of Asp and glutamic acid with some metal acetates through volumetric behavior over the temperature range were probed by Banipal et al. [35]. Metal ion-binding properties of the Asp in aqueous solution were investigated by Sajadi [36,37]. Interactions in copper (II) complexes of guanidinoacetic acid with Asp were studied by Miranda and Felcman [38] Bregier-Jarzebowska et al. [39] examined the interaction and coordination of copper (II) ions with Asp. Kroczewska et al. [40] investigated the role of the carboxylic group in the copper (II) mixed ligands. Our group probed the interactions of phenylalanine with some metal ions, coordination of histidine and glutamine with nickel(II) and vanadium (V) nanoparticles and the interactions of histidine with transition metal ions in water and water-dioxane mixtures [41-43].

Regarding the Job's method, it provides qualitative and quantitative insights into the stoichiometry's underlying association of m molecules of A and n molecules of B to form A_mB_n. Job plots in which reaction rates are monitored give the relative stoichiometry in rate-limiting transition structures [44]. In this study the different aspects of stabilization of metal ion complexes regarding to Irving-Williams sequence [45] for binary complexes of Asp with pertinent metal ions in water and water-dioxane mixtures have been investigated. In addition, thermodynamic parameters of the corresponding complexes have been evaluated and their degrees of stabilities have been examined.

2

2. MATERIALS AND METHODS

2.1 Reagents

Perchloric acid, sodium hydroxide, nitrate salts of Co(II), Ni(II), Cu (II), Zn(II), Cd (II) and Ce (III), dioxane and L-aspartic acid (Asp) were obtained from Merck as analytical reagent grade materials and were used without further purification. The NaOH solutions were prepared and their concentrations were determined by several titrations with standard $HClO_4$ solution. All dilute solutions were prepared from double-distilled water (DDW). Carbonate-free sodium hydroxide 0.10 M was prepared and standardized against Titrisol hydrochloric acid 0.10 M. M(II) nitrate solutions (0.10 M) were prepared by dissolving the appropriate amount of nitrate salts in water and were standardized with standard solution of EDTA 0.10 M (triplex).

2.2 Procedure

All pH titrations were performed using a Metrohm 794 basic automatic titrator (Titrino), coupled with a thermo-stating bath Hero (± 0.1°C). The pH meter was calibrated using Merck standard buffer solutions with pH of 4.0, 7.0 and 9.0. A combination of calomel and glass electrode was used. To compensate for acid error (pH 2-4) of the electrode, 30 mL solution containing KCl, 0.1 M was titrated with standardized perchloric acid, following a plot of calculated pH versus read pH, and producing the equation for pH correction. To account for alkaline error (pH 10-12) the same volume of KCl, 0.1 M solution was titrated with standardized sodium hydroxide and the pH correction equation was acquired [46]. For determination of the stability constant of Asp complexes, 10 mL each, solution of 1.0×10^{-3} M of the metal ion, 5.0×10^{-3} M of Asp, and 0.01690 M of $HClO_4$ were poured inside a double-walled glass reactor, stirred well, and was titrated with standardized NaOH solution under the nitrogen gas bubbling. Each titration was repeated five times in order to check the reproducibility of the data. Potentiometric pH titrations were conducted in aqueous solution and in mixtures of water and dioxane at 15, 20, 25, 35, and 45°C. The calculation of the stability constants of the complexes were performed using our developed computer programme. Spectrophotometric measurements were done with a UV-Vis Specord 210 plus with a GDU computer and using thermostated matched 10 mm quartz cells. IR spectra were acquired as KBr disc by Avatar, USA 370 FT-IR.
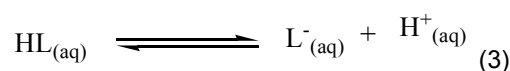
2.3 Synthesis of Asp-metal Ions

As an example, for preparation of Co (II)-Asp complex, in two separate beakers, 10 mmol Asp, and 10 mmol of Co $(NO_3)_2$, $6H_2O$ were dissolved in 80 and 30 mL of DDW respectively, and they were mixed. The solution pH was reached to 8.4 by 0.1 M NaOH and stirred for an hour. Then the volume of the purple solution was reduced to 15 mL and let it aside to dryness, and washed with ethanol, following the crystallization. The crystallized complex was dissolved ion 50 mL DDW and its UV-Vis spectrum was taken. Similar procedures were used for synthesis of the other metal ion complexes, except pH, which for Cu, Cd and Ce were maintained at 6.15 and 8.20 and 8.50 respectively.

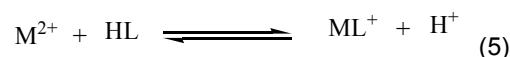### 2.4 The Method for Determination of Stability Constant and Thermo-dynamic Parameters

In considering the reacting species H2L+ was used as di-protonated amino acid; HL, the mono-protonated amino acid; L,- the amino acid anion, and M, the metal. Indeed HL is the neutral amino acid. Then the following chemical equations could be occurred in the solution.

$$H_2L^+ \rightleftharpoons HL_{(aq)} + H^+_{(aq)} \quad (1)$$

$$K_{a_1} = \frac{[HL][H^+]}{[H_2L^+]} \quad (2)$$

$$HL_{(aq)} \rightleftharpoons L^-_{(aq)} + H^+_{(aq)} \quad (3)$$

$$K_{a_2} = \frac{[L^-][H^+]}{[HL]} \quad (4)$$

$$M^{2+} + HL \rightleftharpoons ML^+ + H^+ \quad (5)$$

$$K_{f1} = \frac{[ML^+][H^+]}{[M^{2+}][HL]} \quad (6)$$

$$ML^+ + L^- \rightleftharpoons ML_2 \quad (7)$$
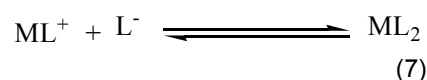
$$K_{f2} = \frac{[ML_2]}{[ML^+][L^-]} \quad (8)$$

Here K_{f1} and K_{f2} are the first and the second stability constants of the complexes. We define $\bar{n}$ as the average number of ligands bound per metal ion concentration which can be expressed as:

$$\bar{n} = \frac{\text{bound ligand}}{\text{total metal ion concentration}} = \frac{L\,\text{bound}}{CM} = \frac{L_{\text{total}} - L_{\text{free}}}{CM} \tag{9}$$

The concentration of free ligand is the sum of concentration of contain species at different form

$$L_{\text{free}} = [H_2L^+] + [HL] + [L^-] \tag{10}$$

The bound ligand concentration (Lbound) was then estimated as:

$$L_{\text{bound}} = L_{\text{total}} - L_{\text{free}} \tag{11}$$

After rearrangement and substitutions we have:

$$\bar{n} = \frac{T_{H_2L^+} - [H_2L^+] - [HL] - [L^-]}{T_{M^{2+}}} \tag{12}$$

Then:

$$\bar{n} = \frac{[ML^+] + 2[ML_2]}{[M^{2+}] + [ML^+] + [ML_2]} \tag{13}$$

According to mass balance relation we have:

$$T_M = [M^{2+}] + [ML^+] + [ML_2] \tag{14}$$

$$T_{HL} = [HL] + [L^-] + [ML^+] + 2[ML_2] \tag{15}$$

$$[ClO_4] = T_{HClO_4} + 2T_M \tag{16}$$

$$[ML^+] + 2[ML_2] = [Na^+] - T_{HClO_4} + [H^+] \tag{17}$$

$$\bar{n} = \frac{[Na^+] - [HClO_4] + [H^+]}{T_M} \tag{18}$$

$$[HL] = \frac{K_a(T_{H_2L^+} - \bar{n}T_M)}{k_a + [H^+]} \tag{19}$$

From plot of $_{p[HL]}$ versus $\bar{n}$ the stability constants could be calculated.

$$K_{f1} = \frac{1}{[HL]_{\bar{n} = \frac{1}{2}}} \tag{20}$$

$$K_{f2} = \frac{1}{[HL]_{\bar{n} = \frac{3}{2}}} \tag{21}$$

All our calculations in this work were executed by GRCβeta computer-programme developed in our lab. The software inputs are: I) the initial volume of solution containing the amino acid, metal ion, and perchloric acid, II), III) and IV), the concentration of perchloric acid, sodium hydroxide, and amino acid, V) pKa1 and pKa2 of the amino acid in the specified medium and at desired ionic strength. After insertion of the pertinent values, The software output is a plot of calculated pH (corrected pH) of the titrand solution versus the concentration of added standardized NaOH, plus plot of two curves, one for a $\bar{n}$ =0.5 and the other for $\bar{n}$ =1.5. The intersection of the potentiometric titration curve with these two curves produces two points whose corresponding pHs will be used to evaluate the respective stability constants of the metallic ion-amino acid complexes. Additionally the software is capable of plotting first and second derivative of d-pH versus d-V_{NAOH} to clarify the end points. For each potentiometric titration approximately 12-20 mL of standardized sodium hydroxide was used.

Fig. 1 illustrates the software output, i.e. the potentiometric titration curve for Zn(II)-Asp with standardized sodium hydroxide in water at 25°. The value of the calculated stability constants of respective Asp complexes are given in Table 1. Thermodynamic calculations were conducted as follows:

The Gibb's free energy change, ΔG°, can be calculated from the equation below:

$$\Delta G^0 = -RT \ln K_f \tag{22}$$

$$\ln K_f = \frac{-\Delta G^0}{RT} \tag{23}$$

By taking the derivative with respect to 1/T from both side of the equation 23 we have:

$$\frac{d \ln K_f}{d\frac{1}{T}} = -\frac{1}{R}\left\{ \frac{d}{d\frac{1}{T}}\frac{\Delta G^0}{T} \right\} \tag{24}$$

$$\frac{d\ln K_f}{d\frac{1}{T}} = -\frac{1}{R}\left\{\Delta G^0 + \frac{d\Delta G^0}{Td\frac{1}{T}}\right\} \quad (25)$$

$$\frac{d\ln K_f}{d\frac{1}{T}} = -\frac{1}{R}\left\{\Delta H^0 - T\Delta S^0 + T\Delta S^0\right\} = -\frac{\Delta H}{R} \quad (26)$$

So:

$$\frac{d\log K_f}{d\frac{1}{T}} = -\frac{\Delta H^0}{2.303R} \quad (27)$$

Regarding equation 27, the plot of log K_f versus 1/T produces straight line with slop equals:

$$\text{slope} = \frac{-\Delta H^0}{2.303R} \quad (28)$$

Using Equation 28 enables us to calculate Enthalpy change. For calculating ΔS^0 we have:

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad (29)$$

Knowing Gibbs free energy change and enthalpy change, ΔS^0 could be evaluated.

$$\Delta S^0 = \frac{\Delta H^0 - \Delta G^0}{T} \quad (30)$$

3. RESULTS AND DISCUSSION

Metal ion hydrolysis, if occurs in the pH range of complex formation, would result in higher than the true values of stability constants. Use of high ligand-metal ratio has been recommended to depress the pH range of complex formation if necessary. Perchloric acid prevents hydrolysis of metal ions, otherwise significant errors in the calculation could be emerged. Sodium nitrate is added as supporting electrolyte to stabilize the ionic strength. Asp has one amino and two carboxylic groups as potential chelating centers. It may coordinate either via the two carboxylate groups or by the amino and one carboxylate group (Scheme 1). Asp forms a five, six and seven membered chelate ring. The formation of the seven membered chelate ring in binary complexes causes a weaker bond.

The order of stability of the binary complexes in the system under investigation, in terms of the nature of metal ion, follows the sequence of Cu(II) >Ce(III) >Ni(II) >Co(II) > Zn(II) > Cd(II). For the transition metal ions, this behavior is consistent with the order of stability of such metal ion complexes as revealed in the Irving-Williams series (Table 1). The stability constants of the binary complex of the Ce (III)has higher value than Cu(II), Ni(II), Co(II), Zn (II) and Cd(II). This is due to the fact that Ce(III) ion is harder Lewis acid than the other transition metal ions and constitute the stronger bonds to oxygen and nitrogen atoms of Asp ligand.
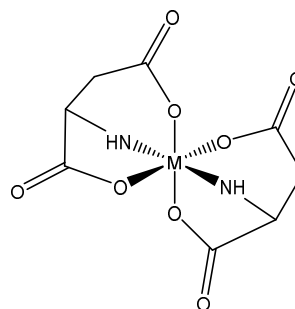
Scheme 1. Asp complexes with a di-metal ion

Table 1. Stability constant values for binary Asp complexes in water at 25°C and I = 0.1 M

Complex	Log K_{f1}
Co(II)-Asp	6.68±0.06
Ni(II)-Asp	7.45±0.06
Cu(II)-Asp	8.80±0.03
Zn(II)-Asp	6.39±0.05
Cd(II)-Asp	5.17±0.06
Ce(III)-Asp	8.34±0.07

Although CFSE of copper (II) is less than nickel (II), but the copper octahedral complexes have been stabilized by Jahn–Teller distortion, as a result the stability constant of copper (II) is more than nickel (II). Cd(II) is a member in group Zn(II), but the ionic radius of Cd (II) is larger than Zn(II), so the stability constant of Zn(II) complex is higher. In General the stability of the different binary complexes containing the dicarboxylic amino acid (Asp) is high. This could be ascribed primarily to the effective high basicity of Asp, i.e., a good σ-donor, where it can probably act as a tridentate ligand giving rise to dramatic increase in entropy and Gibbs free energy upon complex formation.

On the other hand, the 1,4-dioxane – water mixture is a well-defined solvent of the class of binary mixtures of water and organic solvents. These systems are important in fundamental research and technology, because properties

such as viscosity, relative permittivity (dielectric constant), molar volume, etc., can be continuously changed with solvent composition. Changes in stability constants upon addition of 1, 4-dioxane to aqueous solutions are due to increasing ion-ion interactions resulting from the decreasing dielectric constant and from changes in solvent-ion and solvent-solvent interactions (Table 2). As the dielectric constant decreases, the ion interactions involving the proton and anionic oxygen on the acid decrease to a greater extent than the ion dipole interaction between the proton and the solvent molecules. Co-solvent influences the protonation-deprotonation equilibrium in solution by changing the dielectric constant of the medium, which varies the relative contributions of electrostatic and non-electrostatic interactions.

Linear variation in the logarithm of the stability constant (log K_{f1}) values of Asp complexes of Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) versus the variation of 1/D (reciprocal of the dielectric constant of the medium), as demonstrated in Fig.

2 indicates the dominance of electrostatic interactions. Fig. 3 exhibits the plot of log k_{f1} of Cd (II)-Asp complex versus 1/T in 50/50 (v/v) water-dioxane mixture. By examining the plot we are capable of calculating the stability constant for Cd(II) complex at any temperature. The stability constant of the pertinent complexes increase with increasing the percentage of dioxane due to base strength enhancement (Table 2).

Job's method was used to acquire the identity of the predominant form of the respective complexes in each solution. It involves the preparation of a series of solutions in which the sum of the total molar concentration of M and L is constant while the ligand mole fraction, X, is varied from 0-l. The concentration of the complex, or its proportional quantity is then measured and plotted against X. The method showed that, if certain assumptions were valid, such a plot would exhibit a maximum at a value of X = n/(m + n).

Table 2. Log of the Stability constant (Log K_{f1}) of the respective Asp complexes in dioxane-water mixtures, I= 0.1 M (NaNO₃) at 25°C

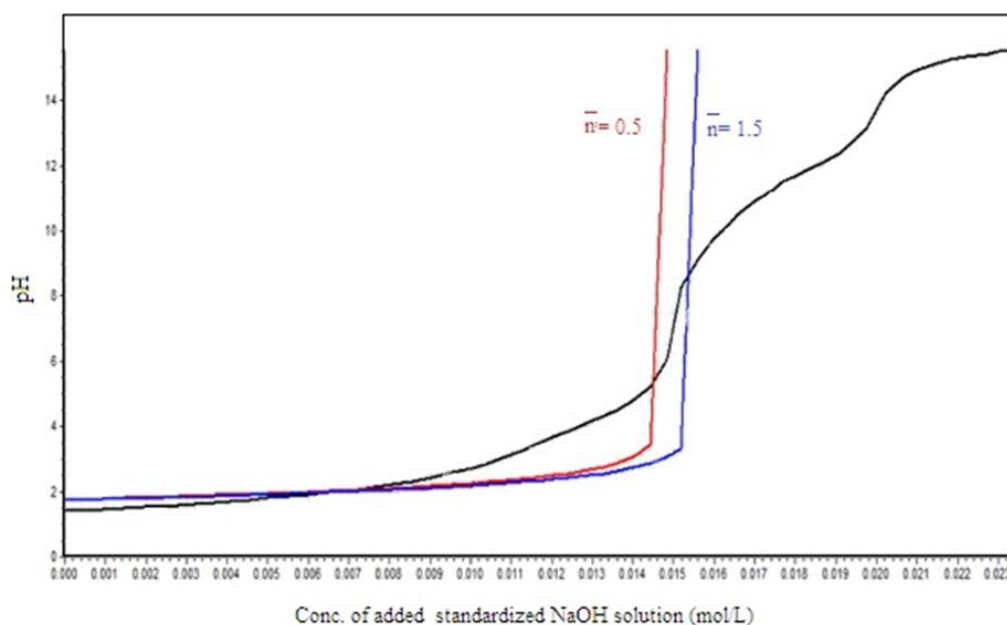| Dioxane %(v/v)	Cd(II)-Asp	Zn(II)-Asp	Co(II)-Asp	Ni(II)-Asp	Ce(III)-Asp	Cu(II)- Asp
0.0	5.17	6.39	6.68	7.45	8.34	8.80
25	5.32	6.54	6.84	7.61	7.40	8.96
50	5.59	6.70	7.18	7.75	8.28	9.17
75	6.43	7.54	8.77	8.50	8.46	9.81

Fig. 1. A potentiometric titration curve of corrected pH versus the concentration of added standardized NaOH for Zn(II)-Asp in aqueous solution at 25°C and n̄ =0.5, n̄ =1.5 curves
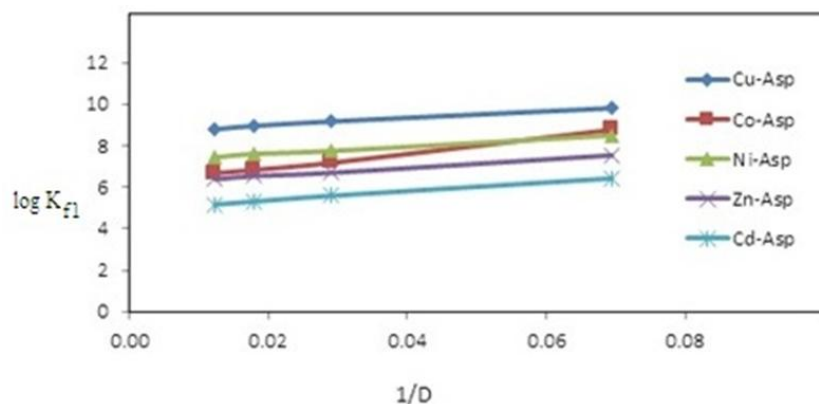
Fig. 2. Log K_{f1} variations of Asp-M(II) complexes versus 1/D in water-dioxane mixtures

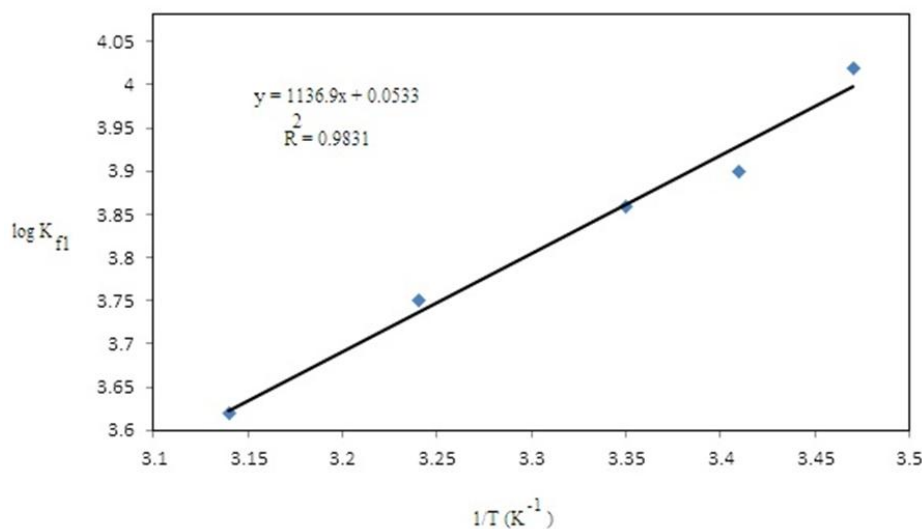$$y = 1136.9x + 0.0533$$
$$R^2 = 0.9831$$

Fig. 3. A Plot of log K_{f1} of Cd(II)-Asp complex versus 1/T in 50/50 (v/v) water-dioxane mixture

Here practically, different amounts of stock solutions of A and B are mixed, varying the mole ratio of reactants in such a compensatory manner that their total molar concentration is kept constant. Under given circumstances, the maximum amount of complex will form in the solution in which the two species are present in the correct combination ratio, provided that the mixing ratio has been varied from 0 to some value certain to be larger than n=m. Usually, this is achieved by preparing a series of mixture solutions such that the total moles of reactants and the total volume across the set of solutions are fixed. The great thing in complexation process of Asp is pH effect, since all sites of complexation in the ligand are strongly dependent on the pH of the solution. Asp in basic media can act as three dentate and in an acidic

media as a bidentate ligand. To recognize Cu (II) complex forms in acidic or basic media, Job's procedure was performed in two different pHs. Only one hydrogen in the acidic medium of the Asp is replaced by a metal ion (Fig. 4) but in the basic solution two protons of Asp separated and replaced by metal ions (Fig. 5). Therefore the complex formula in acidic solution is Cu(HAsp), and in basis medium is Cu (Asp)$_2$ imparting that coordination sites of Asp in different media depend on pH of the solution. All the thermodynamic parameters, i.e. $\Delta G°$ $\Delta H°$ and $\Delta S°$ were evaluated and are shown in Tables 5-8. They have negative values. Negative $\Delta G°$ means that the complex formation is a spontaneous reaction. Increase in dioxane percentage causes $\Delta G°$s to become more negative, because they have been under the

influence of entropy change. Hence the stability of complexes and release of ions and water molecules will be affected by decrease in dielectric constant of the medium. Negative $\Delta H°s$ conveys that the reactions are exothermic, and negative $\Delta S°$ are the indication that the complexes are more organized than the reactants. Some workers have conducted research on complex systems of mixed ligands as ternary complexes with transition metal ions which needed sophisticated softwares and more rigorous treatment of data [47-51].

3.1 The vibrational Modes Analysis of Asp Complexes

To confirm the formation of the complexes, their FT-IR spectra were examined. The vibrational modes of pure Asp and its complexes are summarized in Table 3. There are peaks with higher frequencies than that of 3300 cm^{-1},

indicating the presence of water or hydrogen bonds in their structures. Bending vibrations of amino group in the spectra of complexes convey that amino group coordinates to metal ions. The frequency of the M-O is observed at frequency of lower than 600 cm^{-1}. The asymmetrical stretching vibration frequency of COO^- in the complexes increases in comparing with pure Asp, but symmetric stretching vibration frequency of COO^- group has decreased. The increase in difference between the frequency $(COO^-)_{asym}$ and $(COO-)_{sym}$ in the complex with respect to pure Asp is indication of asymmetric bond formation of COO^- with metal ions. According to the results, copper bonding to the oxygen and nitrogen atoms in complex with Asp is stronger than the other metals. Since copper is a harder Lewis acid than the other first row of transition metal ions, prefers forms bond to oxygen and nitrogen atoms of Asp. Cerium is a harder Lewis acid and forms a stronger bond with the oxygen of Asp.
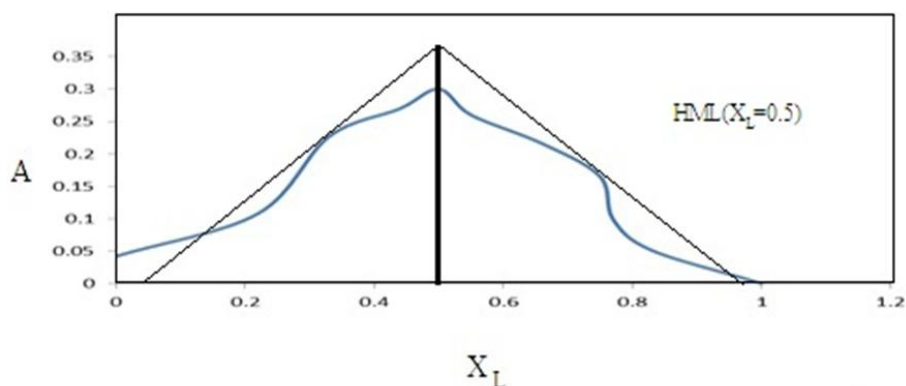

Fig. 4. Job's method-A plot of Absorbance vs. mole ratio for Cu (II)–Asp at pH=4.5

Table 3. IR vibrational frequency modes for the respective Asp complexes as compared with Asp

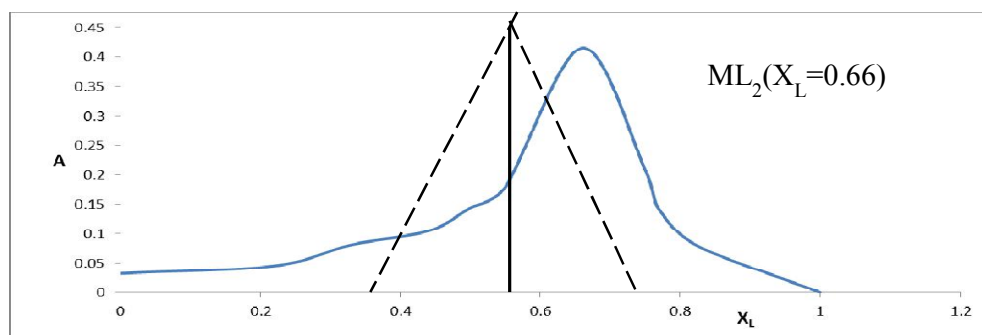| Cm^{-1}	H-bond	$COO^-_{asym.}$	$COO^-_{sym.}$	υ'_{NH2}	NH_2-bend	M-O or M-N
Asp	-	1618	1419	-	-	-
Cu(II)-Asp	3474, 3422	1622	1300	3267 (asym), 3150 (sym)	1599	<500
Co(II)-Asp	3300	1642	1374	3169 (asym)	1604	<600
Cd(II)-Asp	3521	1629	1368	3151 (asym)	hidden	<670
Zn(II)-Asp	3432, 3476	1631	1389	3278 (asym), 3174 (sym)	1585	<500
Ni(II)-Asp	3500	1629	1395	3364 (asym), 3003 (sym)	1544	<630
Ce(III)-Asp	3470	1640	1416	3359 (asym), 3018	1599	<600

Fig. 5. Job's method-A plot of Absorbance vs. mole ratio for Cu (II) –Asp complex at pH=8.5

Table 4. Absorbance data regarding different X_L for Cu(II)- Asp at pH=4.5 and 8.5 respectively

X_L	1.00	0.83	0.77	0.75	0.66	0.55	0.5	0.44	0.33	0.22	0.00
A(λ_{max}=690nm)	0.00	0.05	0.09	0.165	0.21	0.26	0.30	0.27	0.22	0.11	0.042
A(λ_{max}=645nm)	0.00	0.08	0.14	0.21	0.41	0.18	0.14	0.10	0.08	0.04	0.03

Table 5. $\Delta G°$ values for the respective Asp complexes in aqueous solution at different temperatures

Complex	ΔG	ΔG (KJ/mol)				
		15	20	25	35	45(°C)
Cd(II)-Asp	$-\Delta G_1$	15.2	15.5	15.7	16.0	16.3
	$-\Delta G_2$	13.7	13.9	14.2	14.6	14.9
Zn(II)-Asp	$-\Delta G_1$	15.4	15.5	15.6	15.9	16.1
	$-\Delta G_2$	14.2	14.3	14.5	14.6	14.9
Co(II)-Asp	$-\Delta G_1$	20.7	20.5	19.8	19.5	17.4
	$-\Delta G_2$	16.8	17.2	18.3	17.2	17.5
Ni((II)-Asp	$-\Delta G_1$	23.0	23.3	23.5	24.2	24.6
	$-\Delta G_2$	19.3	19.5	19.5	20.2	20.6
Ce(III)-Asp	$-\Delta G_1$	29.5	27.6	26.8	26.2	24.7
	$-\Delta G_2$	23.0	22.2	20.8	19.9	19.6
Cu(II)- Asp	$-\Delta G_1$	26.0	26.3	26.5	25.8	26.1
	$-\Delta G_2$	24.5	23.5	23.7	23.1	21.0

Table 6. $\Delta H°$ and $\Delta S°$ values for the respective Asp complexes in aqueous solution at different temperatures

Complex	ΔS	ΔS(J.mol-1.K) T(°C)					ΔH (KJ/mol)	
		15	20	25	35	45		
Cd(II)-Asp	$-\Delta S_1$	34.7	34.9	35.1	34.9	34.8	$-\Delta H_1$	5.23
	$-\Delta S_2$	40.1	40.2	40.2	40.3	40.1	$-\Delta H_2$	2.16
Zn((II)-Asp	$-\Delta S_1$	25.1	24.8	25.1	25.0	25.0	$-\Delta H_1$	8.18
	$-\Delta S_2$	22.7	22.8	23.0	22.5	22.8	$-\Delta H_2$	7.66
Co(II)- Asp	$-\Delta S_1$	103.0	101.8	102.5	100.3	103.8	$-\Delta H_1$	50.38
	$-\Delta S_2$	81.1	78.3	73.1	74.5	71.0	$-\Delta H_2$	40.13
Ni(II)-Asp	$-\Delta S_1$	56.0	56.2	56.0	56.3	55.9	$-\Delta H_1$	6.83
	$-\Delta S_2$	44.0	43.8	43.4	44.0	43.8	$-\Delta H_2$	6.62
Ce(III)-Asp	$-\Delta S_1$	138.0	142.2	142.4	139.7	140.3	$-\Delta H_1$	69.30
	$-\Delta S_2$	113.3	114.2	116.9	116.1	113.3	$-\Delta H_2$	55.66
Cu(II)-Asp	$-\Delta S_1$	123.9	120.6	117.8	116.3	111.7	$-\Delta H_1$	61.68
	$-\Delta S_2$	99.2	101.0	98.7	97.5	100.9	$-\Delta H_2$	53.13

Table 7. ∆G° values for respective the respective Asp complexes in 50/50 (v/v) water-dioxane solution mixtures at different temperatures

Complex	∆G	∆G (KJ/mol)				
		15	20	25	35	45(°C)
Cd(II)-Asp	-∆G$_1$	22.2	21.9	22.0	22.1	22.0
	-∆G$_2$	18.8	19.3	18.9	19.1	19.2
Zn(II)-Asp	-∆G$_1$	23.2	23.0	22.5	22.2	20.7
	-∆G$_2$	21.3	19.9	19.5	19.2	19.3
Co(II)-Asp	-∆G$_1$	25.7	25.5	21.9	21.8	20.0
	-∆G$_2$	22.1	20.8	19.0	18.5	17.8
Ni((II)-Asp	-∆G$_1$	25.0	24.0	23.4	23.0	22.7
	-∆G$_2$	20.8	20.8	20.0	19.3	18.9
Ce(III)-Asp	-∆G$_1$	26.8	25.7	25.5	25.6	25.0
	-∆G$_2$	22.6	22.3	21.8	22.2	21.9
Cu(II)- Asp	-∆G$_1$	31.8	31.2	30.7	29.4	28.9
	-∆G$_2$	22.9	22.2	21.6	21.3	20.1

Table 8. ∆H° and ∆S° values for the respective Asp complexes in 50/50 (v/v) water-dioxane solution mixtures at different temperatures

Complex	∆S	∆S(J.mol^{-1}.K) T(°C)					Enthalpy	∆H (KJ/mol)
		15	20	25	35	45		
Cd(II)-Asp	∆S$_1$	1.426	0.416	0.896	1.158	0.889	-∆H$_1$	21.77
	∆S$_2$	4.154	5.771	4.480	5.060	5.133	-∆H$_2$	17.61
Zn(II)-Asp	-∆S$_1$	74.55	74.01	74.32	72.72	75.21	-∆H$_1$	44.65
	-∆S$_2$	66.06	69.98	70.16	68.64	66.25	-∆H$_2$	40.38
Co(II)-Asp	-∆S$_1$	190.8	188.1	196.7	191.0	190.7	-∆H$_1$	80.63
	-∆S$_2$	136.0	138.2	141.9	139.1	136.9	-∆H$_2$	61.32
Ni(II)-Asp	-∆S$_1$	68.79	71.11	71.99	70.93	69.62	-∆H$_1$	44.87
	-∆S$_2$	67.44	66.40	67.74	67.95	66.92	-∆H$_2$	40.23
Ce(III)-Asp	∆S$_1$	42.11	45.17	45.05	43.11	43.74	-∆H$_1$	38.89
	∆S$_2$	15.67	16.55	17.87	16.06	16.36	-∆H$_2$	27.14
Cu(II)-Asp	-∆S$_1$	99.44	99.88	99.86	100.8	99.37	-∆H$_1$	60.49
	-∆S$_2$	83.33	84.19	84.76	83.10	84.26	-∆H$_2$	46.91

Table 9. Comparison between our data and the literature

Complex	log K$_{\phi 1}$	Ref.	Our Data
Cd(II)-Asp	6.62, 4.85, 6.62	50, 51, 32	5.17
Zn(II)-Asp	5.87, 6.82, 5.35	11, 51, 36	6.39
Co(II)-Asp	6.65, 6.69, 6.74,5.81,6.14	47, 50, 32, 11, 48	6.68
Ni((II)-Asp	7.42, 7.41, 7.07, 7.35 7.20, 7.50	47, 50, 11, 51, 48, 49	7.45
Ce(III)-Asp	8.70, 8.46	32, 48	8.34
Cu(II)- Asp	8.78, 8.85, 8.92 8.83,8.64	47, 48, 32, 11, 49	8.80

Figs. 6-11 compare UV-Vis spectra of the pertinent Asp complexes. For the copper complex, d-d transition transfers to 745 nm. Cobalt complex has absorption bands in the 510, 465 and 275 nm. the first two peaks are related to the transfer of d-d electrons and the third peak corresponds to ligand to metal charge transfer. For the nickel complex, d-d transitions transfer to the visible region of 640 and 370 nm and 305 nm with regard to ligand to metal charge transfer. For the Asp complexes of Zn(II),Cd(II) and Ce(III) only charge transfer is observed, conveying complex formation.
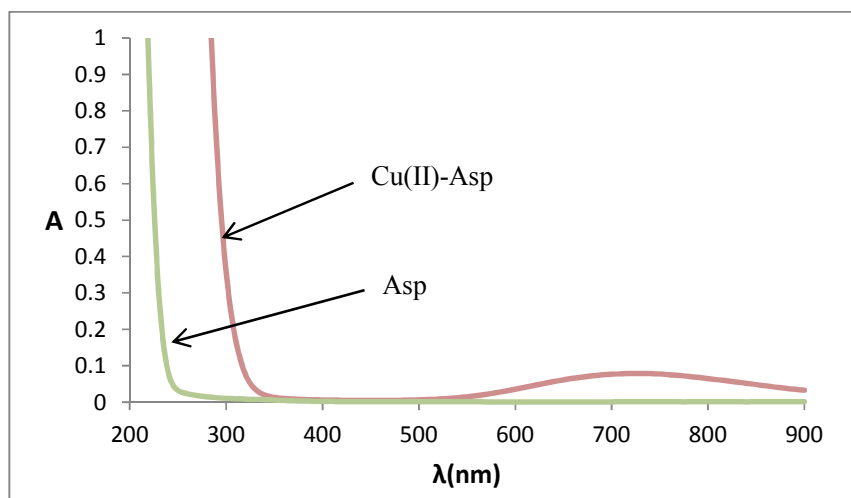
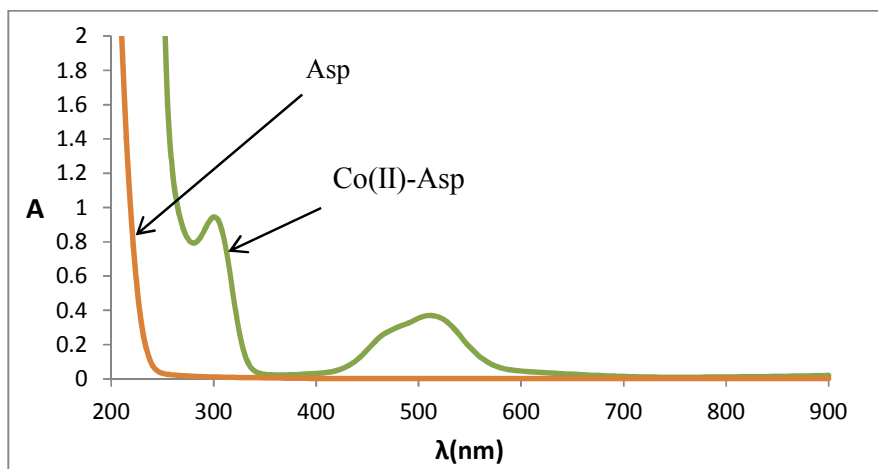**Fig. 6. UV-Vis spectra of Asp and Cu (II) – Asp complex**

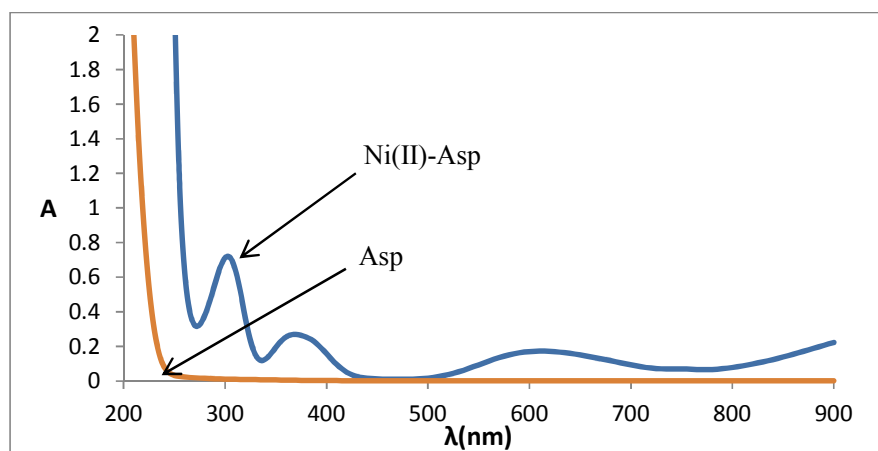**Fig. 7. UV-Vis spectra of Asp and Co (II) – Asp complex**

Fig. 8. UV-Vis spectra of Asp and Ni (II) – Asp complex
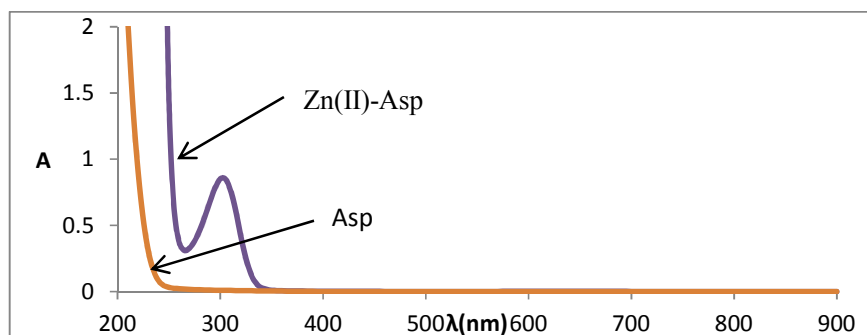
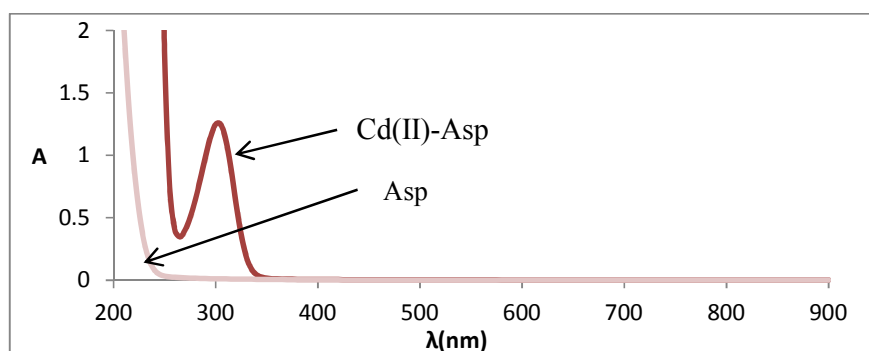**Fig. 9. UV-Vis spectra of Asp and Zn (II) – Asp complex**

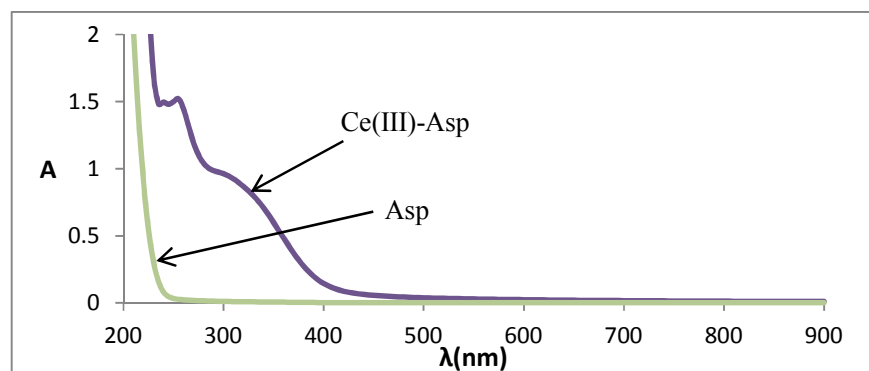**Fig. 10. UV-Vis spectra of Asp and Cd (II) – Asp complex**

Fig. 11. UV-Vis spectra of Asp and complex Ce (III) – Asp complex

4. CONCLUSION

We have measured the stability constants of L-aspartic acid with some divalent metal ions and Ce (III) in water and water-dioxane mixtures by potentiometric titration method in five different temperatures. In addition, thermodynamic parameters of $\Delta G°$ $\Delta H°$ and $\Delta S°$ have been estimated. In this systematic investigation we were able to measure the stability constants of the pertinent Asp complexes at any temperature and in water and water-dioxane mixtures. The obtained stability constants were in good agreement with the values of similar condition in the literature. The mechanism of the reactions and effect of the temperature and solvent on complex stability were discussed.

ACKNOWLEDGEMENT

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Laurie SH. Metal–ligand interactions in biological fluids. Bioinorganic Chemistry. 1st Edition, Marcel Dekker, New York. 1995;603.
2. Zitka O, Ryvolova M, Hubalek J, Eckschlager T, Adam V, Kizek R. From amino acids to proteins as targets for metal-based drugs. Curr. Drug Metab. 2012;13:306-320.
3. Szabo Z. Multinuclear NMR studies of the interaction of metal ions with adenine-nucleotides. J. Coord. Chem. Rev. 2008; 252:2362–2380.
4. Louie AY, Meade TJ. Metal complexes as enzyme inhibitors. J. Chem. Rev. 1999;99: 2711-2734.
5. Patel RN, Singh N, Shukla KK, Chauhan UK, Chakraborty S, Niclos-Gutierrez J, Castineiras AJ. X-ray, spectral and biological (antimicrobial and superoxide dismutase) studies of oxalato bridged Cu^{II}–Ni^{II} and Cu^{II}–Zn^{II} complexes with pentamethyldiethylenetriamine as capping ligand. J. Inorg. Biochem. 2004;98:231-237.
6. Balcarova Z, Kasparakova J, Zakovska A, Novakova O, Sivo MF, Natile G, Brabeck V. DNA interactions of a novel platinum drug, cis-$[PtCl(NH_3)_2(N7-acyclovir)]+$. Mol. Pharmacol. 1998;53:846-855.
7. Hammud HH, Nemer G, Sawma W, Touma J, Barnabe P, Bou-Mouglabey Y, Ghannoum A, El-Hajjar J, Usta J. Copper-adenine complex, a compound, with multi-biochemical targets and potential anti-cancer effect. Chem. Biol. Interact. 2008; 173:84-96.
8. Bregier-Jarzebowska R, Lomozik L. Noncovalent interactions and coordination reactions in the systems consisting of copper (II) ions, aspartic acid and diamines. J. Inorg. Biochem. 2009;103, 1228-1235.
9. Monda M, Viggiano A, Sullo A, De Luca V. Aspartic and glutamic acids increase in the frontal cortex during prostaglandin E_1 hyperthermia. Neuro Sci. 1998;83:1239–1243.
10. Ikhsan J, Wells JD, Johnson B, Angove MJ. The effect of aspartic acid on the binding of transition metals to kaolinite. J. Colloid Interface Sci. 2004;273:6-13.
11. Aydin R, Yirikogullari A. Potentiometric study on complexation of divalent transition metal ions with amino acids and adenosine 5′-triphosphate. J. Chem. Eng. Data. 2010; 55:4794-4800.
12. Dogan A, Koseoglu F, The Stability constants of copper (II) complexes with some α-amino acids in dioxan-water mixtures. J. Anal. Biochem. 2001;295:237-239.
13. Gharib F, Jabbari M, Farajtabar A, Shamel A. Solvent effects on protonation and complexation of glutamic and aspartic acids with molybdenum(VI) in different aqueous solutions of methanol. J. Chem. Eng. Data. 2008;53:1772-1778.
14. Leela AC, Rao GN, Stability of binary complexes of L-aspartic acid in dioxane-water mixtures. J. Indian Chem. Soc. 2014; 91:9-12.
15. Rani RS, Rao GN. Stability of binary complexes of l- aspartic acid in dioxane-water mixtures. Bull. Chem. Soc. Ethiop. 2013;27:367-376.
16. El-Sherif AA, Shoukry MM, Abd Elkarim AT, Barakat MH. Protonation equilibria of biologically active ligands in mixed aqueous organic solvents. Bioinorg. Chem. Appl. 2014;2014:1-18.
17. Reichardt C. Solvatochromic dyes as solvent polarity indicators. Chem. Rev. 1994;94:2319-2358.
18. Barbosa J, Barron D, Beltran JL, Buti S. On the role of solvent in acid–base equilibria of diuretics in acetonitrile–water mixed solvents. Talanta. 1998;45:817-827.
19. Kamlet MJ, Abboud JLM, Abraham MH, Taft RW. Linear Solvation Energy Relationships: A comprehensive collection of the solvatochromic parameters, π*, α, and β, and some methods for simplifying the generalized solvatochromic equation. J.Org. Chem.1983;48:2877-2887.
20. Roy MN, Sinha B, Dey R, Sinha A. Solute-solvent and solute-solute interactions of resorcinol in mixed 1,4-dioxanewater systems at different temperatures. Int. J. Thermophys. 2005;26:1549-1563.
21. Irving HM, Rossotti HS. The calculation of formation curves of metal complexes from pH titration curves in mixed solvents. J. Chem. Soc.1954;2904-2910.
22. Fazary AE, Mohamed AF, Lebedeva NS. Protonation equilibria studies of the standard a-amino acids in $NaNO_3$ solutions

in water and in mixtures of water and dioxane. J. Chem. Thermody. 2006;38: 1467-1473.

23. Durrani A. The Effect of solvent on stability constant of mixed ligand complexes. Rasyan J. Chem. 2011;4:554-556.

24. Nishi N, Takahashi S, Matsumoto M, et al. Hydrogen bonding cluster formation and hydrophobic solute association in aqueous solution of ethanol. J. Phys. Chem. 1995; 99:462–468.

25. Kramer-Schnabel UU, Linder PW. Substituent effects in the protonation and complexation with copper (II) ions of organic monophosphate esters. A potentiometric and calorimetric study. J. Inorg. Chem. 1991;30:1248–1254.

26. Rees DC. Experimental evaluation of the effective dielectric constant of proteins. J. Mole. Biol. 1980;141:323–326.

27. Rogers NK, Moore GR, Sternberg MJE. Electrostatic interactions in globular proteins: Calculation of the pH dependence of the redox potential of cytochrome c551. J. Mole. Biol. 1985; 182:613–616.

28. Akerlof G, Short OA. The dielectric constant of water dioxane mixtures between 0 and 80 degrees-correction. J. Am. Chem. Soc. 1953;75:6357–6357.

29. Mogopodi D, Torto N. Maximising metal ions flux across a microdialysis membrane by incorporating poly-l-aspartic acid, poly-l-histidine, 8-hydroxyquinoline and ethylenediaminetetraacetic acid in the perfusion liquid. Anal. Chim. Acta. 2005; 534:239–246.

30. Bukietynska K, Podsiadl H, Karwecka Z. Complexes of vanadium (III) with L-alanine and L-aspartic acid. J. Inorg. Biochem. 2003;94:317-325.

31. Bottari E. Copper (II) Complexes with Aspartate and Glutamate. Polyhedron. 1989;8:1019-1027.

32. Phase RP, Shankarwar AG, Shankarwar, SG, Chondhekar TK. Determination of the stability constants of mixed ligand complexes of bio-molecules and amino acids with Ni(II) by Potentiometric Titration Method. Der Pharmacia Sinica. 2013;4: 54-58.

33. Gharib F, Zare K. Ionic strength dependence of formation constants: Complexation of Mo (VI) with aspartic acid. J. Chem. Eng. Data. 2001;46:1140-1144.

34. Djurdjevic P, Jelic R, Joksovic L, Cvijovic M. Potentiometric and multinuclear magnetic resonance study of the solution equilibria between aluminum (III) ion and L-Aspartic acid. Monatshefte fur Chemie / Chemical Monthly. 2006;137:717-735.

35. Banipal TS, Singh K, Banipal PK, Kaur J. Study of interactions of L-aspartic acid and L-glutamic acid with some metal acetates through volumetric behavior over the temperature range (288.15 to 318.15) K. J. Chem. Thermody. 2008;40:1166-1185.

36. Sajadi SAA, Metal ion-binding properties of L-glutamic acid and L-aspartic acid, a comparative investigation. Nat. Sci. 2010; 2:85-90.

37. Sajadi SAA, Metal ion-binding properties of the aspartic acid, in aqueous solution. Int. J. Res. Inorg. Chem. 2011;1:1-5.

38. Miranda JL, Felcman J, Study on guanidine/carboxylate interactions in copper (II) ternary complexes of guanidinoacetic acid with glutamic and aspartic acids. Polyhedron. 2003;22:225-233.

39. Bregier-Jarzebowska R, Lomozik L, Noncovalent interactions and coordination reactions in the systems consisting of copper (II) ions, aspartic acid and diamines J. Inorg. Biochem. 2009;103:1228-1235.

40. Kroczewska D, Kurzak B, Jezierska J. The role of the carboxylic group in the copper (II) mixed-ligand and polyamines. Polyhedron. 2006;25:678-686.

41. Ghiamati E, Oliaei S. Thermodynamic studies on the interaction between phenylalanine with some divalent metal ions in water and water-dioxane mixtures. SOJ Biochem. 2017;3(1):1-6.

42. Ghiamati E, Lashkari M, Hasheminia M, Synthesis of metal oxide nanoparticles of nickel (II), vanadium (V) and potentiometric determination of the stability constants of their complexes with histidine and glutamine at different temperatures in aqueous solutions. Asian J. Chem. 2013; 25:1361-1365.

43. Ghiamati E, Jalaeian E. Determination of the formation constant of some transition metal ion-histidine complexes in water and water-dioxane and evaluation of their thermodynamic parameters. Asian J. Phys. Chem. Sci. 2017;2(2):1-10.

44. Renny JS, Tomasevich LL, Tallmadge EH, Collum DB. Method of continuous variations: Applications of job plots to the study of molecular associations in organometallic chemistry. J. Chem. Int. Ed. 2013;52:11998-2013.

45. Irving HM, Williams RJP, Stability of Transition Metal Complexes. J. Chem. Soc. 1953;3192-3210.

46. Chambers RA. Chemistry of complex equilibria. Van Nostrand Reinhold Company, London, UK; 1970.

47. Taha M, Khalil MM. Mixed- ligand complex formation equilibria of cobalt (II), nickel (II) and copper (II) with N, N–bis (2-hydroxyethyl) glycine (Bicine) and some amino acids. J. Chem. Eng. Data. 2005; 50:157-163.

48. Boraei AAA, Ibrahim SA, Mohamed AH. Solution equilibria of binary and ternary systems involving transition metal ions, adenosine 5-triphsphate, and amino acids. J. Chem. Eng. Data. 1999;44:907-911.

49. Khalil MM, Mahmoud RK, Solution equilibria of ternary systems involving transition metal ions, hydroxamic acids. and bioligands. J. Chem. Eng. Data. 2010; 55:789-797.

50. Ahmed IT, El-Roudi OM, Boraei AAA, Equilibrium studies of the ternary complex systems Mn+ + dipicolinic acid + N-(2-acetamido)iminodiacetic acid or amino acids. J. Chem. Eng. Data. 1996;4:386-390.

51. Khalil MM, Attia AE. Potentiometric studies on the formation equilibria of binary and ternary complexes of bsome metal ions with dipicolinic acid and amino acids. J. Chem. Eng. Data. 2000;45:1108-1111.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/19999