Int. J. Electrochem. Sci., 7 **(2012)** 7699 - 7711

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Mixed-Ligand Complex Formation of Cu(II) with 1,2-Diphenylethylenediamine as Primary Ligand and Amino Acids as Secondary Ligands

Siham A. Lahsasni[1], *Reda A. Ammar*[1,2], *Mona F. Amin*[2] *and Eman M. Shoukry*[2]

[1] Department of Chemistry, Faculty of Science, King Saud University, Riyadh, Saudi Arabia
[2] Department of Chemistry, Faculty of Science, Al Azhar University, Cairo, Egypt
*E-mail: dr_reda06@yahoo.com

Received: 19 March 2012 / *Accepted:* 3 July 2012 / *Published:* 1 August 2012

The binary and ternary systems of Cu(II) complexes with 1,2-diphenylethylenediamine (A) as primary ligand and amino acids (L) as secondary ligands are investigated. The stability constants of the complexes were determined potentiometrically in 50% (*v/v*) dioxane-water media at 25 ºC and $I = 0.10$ mol/L $NaClO_4$. The relative stabilities of the ternary complexes are determined and compared with those of the corresponding binary complexes in terms of their $\Delta \log K$ values. Species distribution of all complexes in solution was evaluated.

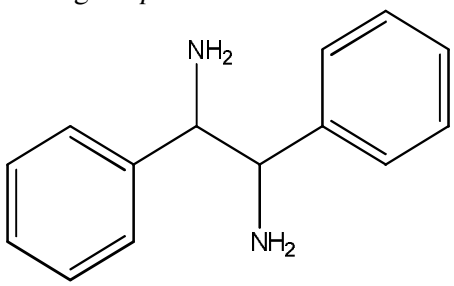
Keywords: Mixed-ligand; Amino acids; Potentiometric studies; Stability constants.

1. INTRODUCTION

Copper, among other transition metals, is essential for many enzymes, particularly those catalyzing physiologically important reactions. It has strong ability to form complexes with proteins, peptides, amino acids as well as other organic substances in the living organisms [1, 2]. Examples of copper-containing compounds include electron-transfer (ET) proteins (e.g., azurin, plastocyanin, laccase), oxygen-binding enzymes (e.g., ferroxidase, tyrosinase, ascorbate oxidase), and in oxygen-transport proteins (hemocyanin) [1]. Similarly, amino acids have special importance compared to other chemical compounds in the sense that they are regarded as the foundation stones of living organisms. Fostered by the crucial role of amino acids in our life, studying their structural, chemical and physical properties becomes very necessary to explain their behavior and potential applications. Among these properties are protonation and stability constants of complexes they form with various metal ions and copper in particular. Importantly, elucidation and understanding of the various phenomena in the

biological systems requires the determination of the protonation constants of the bio-ligands along with their stability constants with various metal ions in media similar to those of biological systems [3-6]. In this context, it is generally believed that "*in vivo*" media can be represented by aqueous solution. However, in recent years, it has been shown that aqueous media are not totally suitable for in vivo biological reactions, and consequently, nonaqueous media have been introduced as an alternative on the basis that biological media have lipophilic character [7-9].

Historically speaking, ternary Cu(II)-amino acids complexes have attracted considerable attention because of their occurrence and involvement in the transport of copper(II) ions in biological systems [10]. In this venue, several binary and ternary amino acid complexes containing copper (II) and other divalent transition metal ions have been synthesized in solution as well as in the solid state [11-16]. Of particular relevance, in the present investigation, efforts have been made to study the stability constants of copper (II) complexes with 1,2-diphenylethylenediamine (Fig. 1) as primary ligand and vast array of amino acids as secondary ligands via potentiometric titrations in 50 % (*v/v*) dioxane-water mixture, having ionic strength (I) of 0.10 mol/L $NaClO_4$ at ambient temperature. The reactions associated with formation of these complexes have been undertaken in different pHs. Species distribution in solution over a wide range of *pHs* was also evaluated.



1,2-diphenylethylenediamine

Figure 1. Chemical structure of 1,2-diphenylethylenediamine.

2. EXPERIMENTAL

2.1. Materials and reagents

All the chemicals used were of analytical grade reagents. 1,2-diphenylethylenediamine, amino acids and related compounds (glycine, α-alanine, β-phenylalanine, valine, leucine, asparagine, histidine• HCl, histamine• 2HCl, imidazole, lysine, ornithine. HCl and glutamine) were obtained from the Sigma Chem. Co. All stock solutions of $Cu(ClO_4)_2$, sodium perchlorate and perchloric acid (analytical reagent grade, Merck) were prepared in deionized water. Stock solution of $Cu(ClO_4)_2$ was standardized by EDTA titrations [17]. Furthermore, no supporting electrolyte was used in these mixed solvents. Carbonate-free sodium hydroxide solution was prepared and standardized against a potassium hydrogen phthalate solution. The ionic strength of each solution was adjusted to 0.10 mol/L

by addition of $NaClO_4$. Acid solutions prepared from perchloric acid were titrated against standardized sodium hydroxide [18].

2.2. Apparatus

Potentiometric titrations were followed with a Metrohm 686 titroprocessor equipped with a 665 dosimat (Switzerland). The titroprocessor and the combined glass electrode were calibrated with standard buffer solutions, KHphthalate, pH = 4.008 and a mixture of KH_2PO_4 and Na_2HPO_4, pH = 6.865 under the same experimental conditions. The temperature was maintained constant (± 0.1) by a Colora ultra thermostat.

2.3. procedure

The acid dissociation constants of the ligands were determined potentiometrically by titrating the ligand (40 ml) solution (1.25×10^{-3} mol/L) of constant ionic strength 0.1 mol/L, adjusted with $NaClO_4$. The stability constants of the binary complexes were determined by titrating 40 ml of a solution mixture of metal ion (1.25×10^{-3} mol/L), the ligand (2.5×10^{-3} mol/L) and 0.1 mol/L $NaClO_4$. The stability constants of mixed ligand complexes were determined by titrating 40 ml of solution containing Cu(II), A and amino acids, all of concentration (1.25×10^{-3} mol/L) and 0.1 mol/L $NaClO_4$.

The above solutions were virtually titrated against 0.1 mol/L NaOH in an atmosphere of pure N_2 gas.

For all the titrations, $HClO_4$ solution was added, so that they were fully protonated at the beginning of the titrations. The pK_w in 50% dioxane-water solutions was determined as described previously [19].

2.4. Data processing

The stoichiometries and stability constants of the complex species formed in solution were determined by examining various possible composition models for the systems studied. About 110 to 150 experimental data points were available for evaluation in each run. All the dissociation and the complex formation constants were determined using the HYPERQUAD program [20] and the speciation as a function of pH using the HYSS program [21].

3. RESULTS AND DISCUSSION

The proton dissociation constants of the amino acids studied have been re-determined by means of the data obtained from potentiometric titrations in 50% (*v/v*) dioxane-water mixtures at 25 °C and I = 0.10 mol/L $NaClO_4$ in order to obtain values under the same experimental procedures employed for binary and ternary systems. Importantly, our estimated values are in a good agreement with data reported in literature [22, 32].

3.1. Acid-Base Equilibria of 1,2-diphenylethylenediamine

The calculated acid dissociation constants of the 1,2-diphenylethylenediamine, expressed as pK_a values, were given in Table 1. In acid medium both amine groups are protonated. The pK_{a1} and pK_{a2} values were found to be 7.88 and 4.45, respectively. These results are consistent with previous investigations undertaken for related systems [24].

3.2. Stability constants of binary complexes

The potentiometric titration curves of Cu(II)-leucine, taken as a representative, and Cu(II) 1,2-diphenylethylenediamine systems are shown in Figs. 2, 3. In all titration curves, the Cu(II)-ligand complex is lower than that of the free ligand curve, indicating formation of Cu(II) complex by displacement of protons. The copper(II)-complexes of amino acids have been extensively investigated in a medium containing 50% (*v/v*) dioxane–water mixture [11]. The formation constants of Cu(II)-A and Cu(II)-L complexes were determined by fitting potentiometric data on the basis of possible composition models. The selected model that gives the best statistical fit consists of Cu(L)(1010), Cu(L)2(1020), Cu(A) (1100), Cu(A)2 (1200) and Cu(A)H(1101) complexes respectively.

The calculated stability constants of binary complexes of amino acids and 1,2-diphenylethylenediamine with Cu(II) are presented (Table 1). The *pKa* of the protonated form is 3.43. The lower value of *pKa* can be attributed to coordination of A with the metal ion. Species distribution diagram of Cu(II)-A system is shown in Fig. 4. The concentration of the 1100 species increases with increasing pH, attaining a maximum of 49.4% at pH 4. Further increase in pH is accompanied by a decrease in the concentration of the1100 species and an increase in the concentration of the 1200 species. 1101 complex species has been found to be most favored at lower pH values.

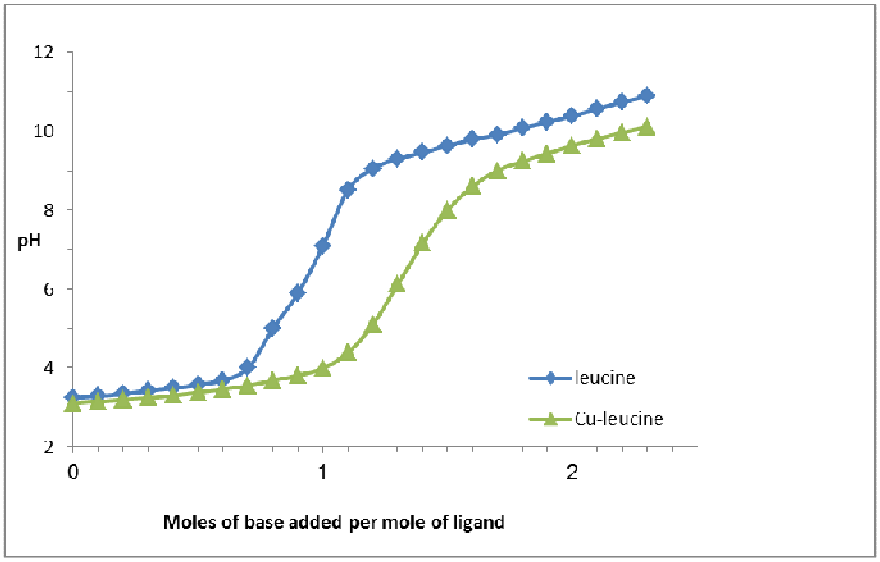


Figure 2. Potentiometric titration curves of free leucine and Cu(II)-leucine obtained from 50% (*v/v*) dioxane–water mixture.

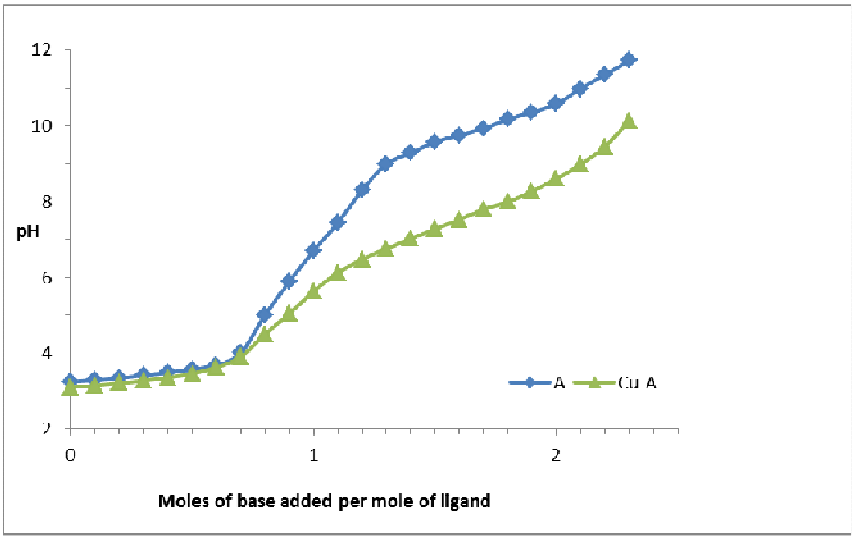


Figure 3. Potentiometric titration curve of the free ligand (A) and the Cu(II)-A system obtained from 50% (*v*/*v*) dioxane–water mixture.

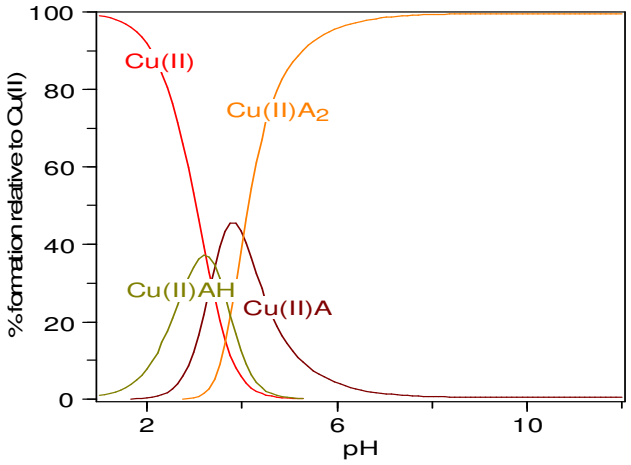


Figure 4. Variation of complex species concentration with pH in the binary system Cu(I1)-A.

Table 1. Stability constants of binary systems Cu(II):A, Cu(II):L and proton-association constants at 25 °C and I = 0.10 mol/L $NaClO_4$.

System	M	A	L	H^a	$log\ \beta^b$
1,2-diphenylethylenediamine (A)	0	1	0	1	7.88(0.02)
	0	1	0	2	12.33(0.01)
	1	1	0	0	8.46(0.07)
	1	2	0	0	15.99(0.02)
	1	1	0	1	11.89(0.03)
Glycine	0	0	1	1	9.88(0.02)
	0	0	1	2	16.54(0.01)
	1	0	1	0	8.67(0.01)
	1	0	2	0	16.33(0.02)
α- Alanine	0	0	1	1	9.79(0.01)
	1	0	1	0	8.71(0.03)
	1	0	2	0	16.22(0.01)
β-phenylalanine	0	0	1	1	9.12(0.01)
	0	0	1	2	12.24(0.01)
	1	0	1	0	8.16(0.01)
	1	0	2	0	16.44(0.02)
Valine	0	0	1	1	9.62(0.02)
	1	0	1	0	8.28(0.01)
	1	1	2	0	16.62(0.03)
Leucine	0	0	1	1	9.84(0.01)
	1	0	1	0	8.36(0.01)
	1	0	2	0	15.85(0.01)
Asparagine	0	0	1	1	8.95(0.001)
	1	0	1	0	8.26(0.03)
	1	0	2	0	15.01(0.02)
Glutamine	0	0	1	1	9.31(0.01)
	1	0	1	0	8.13(0.02)
	1	0	2	0	14.87(0.01)
Lysine	0	0	1	1	10.79(0.01)
	1	0	1	2	20.12(0.02)
	1	0	1	0	10.99(0.01)
	1	0	2	0	15.76(0.01)

Table 1. (Continued)

System	*M*	*A*	*L*	*H*[a]	*log β*[b]
Ornithine	0	0	1	1	10.55(0.01)
	0	0	1	2	19.56(0.03)
	1	0	1	0	11.17(0.02)
	1	0	2	0	15.36 (0.05)
Imidazole	0	0	1	1	6.10(0.02)
	1	0	1	0	4.20(0.03)
	1	0	2	0	7.14(0.02)
Histidine	0	0	1	1	9.69(0.01)
	0	0	1	2	15.53(0.02)
	1	0	1	0	10.67(0.01)
	1	0	2	0	18.41(0.02)
Histamine	0	0	1	1	9.43(0.01)
	0	0	1	2	15.98(0.05)
	1	0	1	0	10.32(0.02)
	1	0	2	0	16.13(0.01)

[a]*M*, *A* and *L* are the stoichiometric coefficient corresponding to Cu(II), 1,2- diphenylethylenediamine (or amino acids) and H^+, respectively. [b]standard deviations are given in parentheses.

3.3. Stability constants of ternary complexes

The formation constants of 1:1 Cu (II) complexes with A or L are of the same order of magnitude, Table 2. Consequently the ligation of A and L will proceed simultaneously. The experimental and simulated titration curves of the Cu-A-glycine system are presented in Fig. 5. The titration data of the ternary complexes with A and L fit satisfactorily upon considering formation of the species: M(A), $M(A)_2$ M(L), $M(L)_2$, M(A)(L) and M(A)(LH). The stability constants, complex formation, between M, A, L are represented by the general equilibria (charges were omitted for simplicity):

$$l(Cu)+p(A)+q(L)+r(H) \rightleftharpoons (Cu)_l(A)_p(L)_q(H)_r \qquad (1)$$

Stability of the formed species is measured by the stoichiometric equilibrium constant β_{lpqr} expressed in terms of concentrations at constant temperature and ionic strength.

$$\beta_{lpqr} = \frac{[Cu_l(A)_p(L)_q(H)_r]}{[Cu^l(A)^p(L)^q(H)^r]} \qquad (2)$$

A comparison of the overall stability constants of Cu(II)-A-L ternary systems, (Table 2) reveals higher stabilities of the ternary complexes containing histidine or histamine compared to those of α-amino acids, thereby indicating that histidine and histamine ligands interact much strongly with the metal ion via the amino and imidazole nitrogen atoms.

Lysine and ornithine may bind to Cu(II) ion as α-amino acid (N,O- *donor set*) or via α- and ω-amino groups (N,N – *donor set*). Our results show that the stability constants of their ternary complexes are higher than those of α-amino acids. This behavior is consistent with the possibility that both lysine and ornithine coordinate through the two amino groups. Importantly, the data obtained from this work attest that

Phenylalanine forms less stable complexes than alanine. This could be attributed, in part, to the steric effect exerted by the phenyl group of phenylalanine and the two phenyl groups of A, in addition to the lower basicity of the amino group of phenylalanine compared to that of alanine. This, of course, will contribute to the decreased stability of the complex formed.

Furthermore, the stability of ternary complexes involving *α*-alanine are found to be lower than those containing glycine. This behavior does not follow their basicities as expected. However, it is suggested that steric hindrance caused by the presence of a methyl group on the carbon bearing the amino group (*α*-alanine), is responsible for the lower stability of its ternary complexes [25].

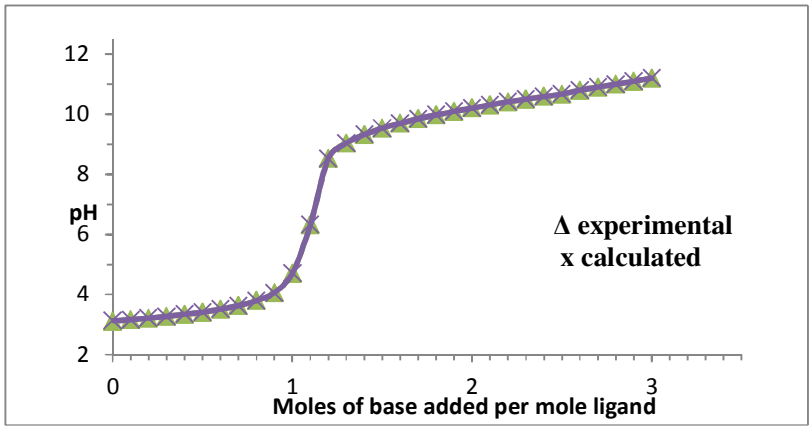


Figure 5. Potentiometric titration curve of the Cu(II)-A-glycine system.

Table 2. Stability constants of the ternary species in the Cu(II)-A-amino acid systems at 25 °C and I = 0.10 mol/L $NaClO_4$.

System	M	A	L	H^a	$Log\ \beta^b$	$\Delta\ log\ K$
Glycine	1	1	1	0	16.93(0.01)	-0.20
α- Alanine	1	1	1	0	16.89(0.07)	-0.28
β-phenylalanine	1	1	1	0	16.77(0.07)	0.15
Valine	1	1	1	0	16.61(0.02)	-0.13
Leucine	1	1	1	0	16.56(0.03)	-0.26
Asparagine	1	1	1	0	16.45(0.05)	-0.27
	1	1	1	1	22.89(0.02)	
Glutamine	1	1	1	0	16.35(0.01)	-0.24
	1	1	1	1	23.57(0.01)	
Lysine	1	1	1	0	18.13(0.02)	-1.32
	1	1	1	1	25.57(0.03)	
Ornithine	1	1	1	0	18.83(0.01)	-0.54
	1	1	1	1	25.55(0.05)	
Imidazole	1	1	1	0	10.78(0.03)	-1.88
	1	1	2	0	13.65(0.02)	
Histidine	1	1	1	0	19.09(0.01)	-0.04
	1	1	1	1	25.21(0.01)	
Histamine	1	1	1	0	19.55(0.03)	0.77
	1	1	1	1	24.45(0.02)	

[a] M, A and L and H are the stoichiometric coefficient corresponding to Cu(II), 1,2-diphenylethylenediamine, amino acids and H^+, respectively. [b] standard deviations are given in parentheses.

By analyzing the species diagram it can be concluded that the percentage of free metal ion decreases gradually upon increasing the pH of the solution. At lower pHs (pH < 4) binary Cu(II) complexes predominated whereas upon increasing the pH over the range from 5 to 10, the ternary

complexes appeared sequentially with 50% of Cu(II) in the form of Cu: A: L, as illustrated in Fig. 6, for the Cu-A- α-alanine system.

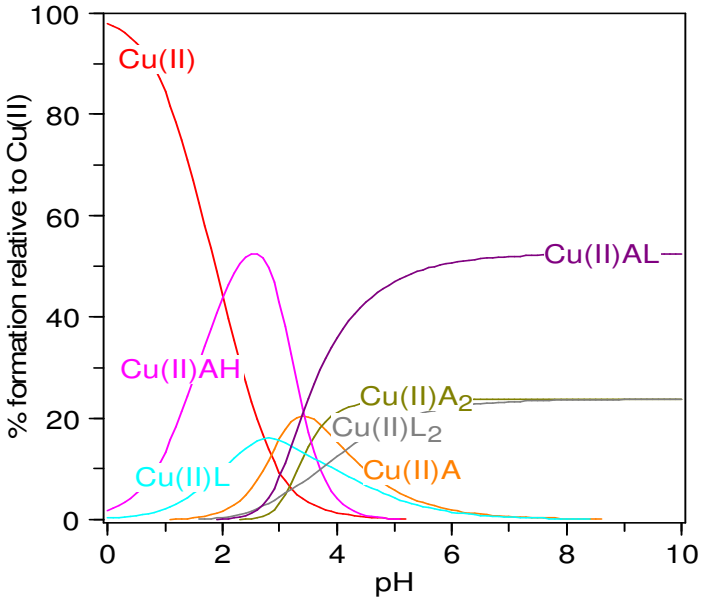


Figure 6. Variation of complex species concentration with pH in the ternary Cu(II)- A- α-alanine system.

The pk_a values of the protonated (1110) and (1111) complexes of histidine, ornithine, lysine asparagine, glutamine and histamine were calculated according to equation (3):

$$pka = \log \beta_{1111} - \log \beta_{1110} \quad (3)$$

In this context, the acid dissociation constant of the protonated complexes are: 6.44 for asparagines, and 7.22 for glutamine respectively. These values are lower than those obtained for the amino group of the α-amino acid ($pKa`s$ = (9.26-10.71), if the increase of acidity as a result of complex formation is considered. This observation is therefore indicative that α-amino acid coordinates by the carboxylic group at lower pH. As for the histidine complex, the measured pKa value is 6.12. Although this value is lower than that of the protonated amino group (NH^{3+}, pK_a = 9.69), it is closely similar to that of the protonated imidazole group (pKa = 6.10). This finding suggests that the proton in the protonated complex is most likely located on the imidazole group.

Additionally, the speciation for lysine complex, taken as a representative, is given in Fig. 7. The protonated 1111 complex species predominates with a formation degree amounting to 19 % at pH 6.0, whereas the deprotonated species 1110 attains a maximum concentration of 98 % at pH 8.0. Therefore, the species 1111 predominates in the physiological pH range.

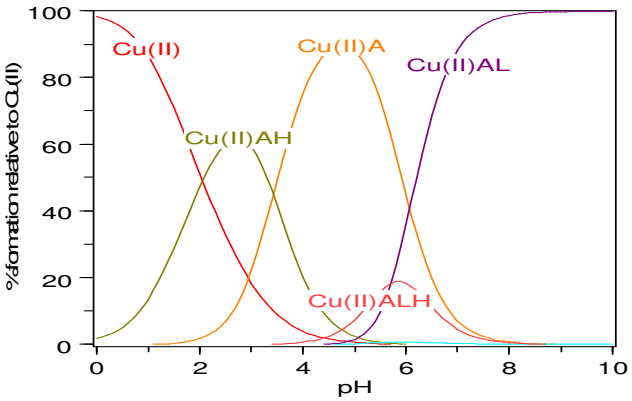


Figure 7. Variation of complex species concentration with pH in the ternary system Cu(II)- A- lysine.

The relative stability of the ternary complexes formed through simultaneous mechanism, as compared to those of the corresponding binary complexes, is expressed in terms of Δ *log K*, which represents the difference between the stability of the ternary and the two correspondent binary complexes, shows the tendency of the formation of ternary species [26]. This is expressed by the following equation:

$$\Delta \log K = \log \beta^{M}_{M(A)L} - (Log\beta^{M}_{M(A)} + Log\beta^{M}_{M(L)}) \qquad (4)$$

It is worthy to mention that negative Δ *log K* values (Table 2) imply that the ternary complexes are less stable than the binary ones, and therefore can be used to indicate that no interaction occurs between the ligands in the ternary complexes. However, this behavior does not mean that the complex is not formed. In this regards, the negative value may be interpreted in terms of higher stability of the binary complexes and/or reduced number of coordination sites in the ligand. Other electronic and structural factors such as steric hindrance [27, 28], bond type, and geometrical configuration are also expected to have an effect on Δ *log K* values. On the other hand, positive *Δ log K* value for the phenylalanine mixed-ligand complex (1110) indicates that the ternary complexes are more stable than the corresponding binary. This may be attributed to intramolecular aromatic-ring stacking, hydrogen bond, and π- π cooperative effect between ligands.

4. CONCLUSION

This study offers intensive investigation and mechanistic details associated with formation of binary and ternary Cu(II) complexes of the general formula Cu(II)-A-L, where A is 1,2-diphenylethylenediamine (primary ligand) and (L) represents a wide range of amino acids used as secondary ligands. The stability constants of all complexes were determined potentiometrically in 50 % (*v/v*) dioxane-water media at 25 ºC and *I* = 0.10 mol/L $NaClO_4$. The relative stabilities of the ternary

complexes are compared with those of the corresponding binary complexes and explained in view of their *Δ log K* values. Finally, species distributions in solution for all complexes were also evaluated.

ACKNOWLEDGEMENT

This project was supported by King Saud University, Deanship of Scientific Research, College of Science Research Center.

References

1. R. Alexandrova, G. Rasshkova, I. Alexandrov, W. Tsenova, R. Tudose and O. Costisor, *Experim. Pathol. and Parasitol.* 1311 (2003) 6851.
2. B.G. Malmström and J. Leckner, *Curr. Opin. Chem. Biol.* 2(19980) 286.
3. M. Robert, R. J. Motekaits and A. E. Martell, *Inorg. Chim. Acta* 103 (1985)73.
4. M. K. Singh and M. Srivastava, J. *Inorg. Nucl. Chem.* 35(1972) 2433.
5. B. R. Arbad and D. V. Sahaprdar, *Ind. J. Chem.* 25A(1986) 253.
6. A.Gergely, L. Nogypal and E. Farkas, *J. Inorg. Nucl. Chem.* 37(1975) 551.
7. L. D. Hughes, J. J. Bergan and J. E. Grabowski, *J. Org. Chem.* 51 (1986) 2579.
8. N. V. Rajashekar, G. Ganpa and R. K. Lomprakast, *Ind. J. Chem. A*25 (1986) 394.
9. J. Maslawska and L. Chruscinki, *Polyhedron* 5 (1986) 1135.
10. Sarkar, B., and Kruck, T. P. A. In J. Peisach, P. Aisen and W. E Blumberg (Eds.), Biochemistry of Copper, Academic Press, New York, 1966, p. 183; Sarkar, B. in H. Sigel (Ed.), Metal ions in biological system, Marcel Dekker, New York, 1981, vol. 12, p. 233.
11. A. Doĝan, F. Köseoĝlu and E. Kılıc¸, *Analytical Biochemistry* 295(2001) 237.
12. F. Dallavalle, G. Folesani, A. Sabatini, M. Tegoni and A. Vacca, *Polyhedron* 20 (2001)103.
13. M. M. Shoukry, *Transition Met. Chem.* 14 (1989) 69.
14. P. Deschamps, N. Zerrouk, I. Nicolis, T. Martens, E. Curis, M. F. Charlot, J.J. Girerd, T. Prange´, S. Bénazeth, J. C. Chaumeil and A. Tomas, *J. Inorg. Biochem.* 73(1999) 203.
12. T. Murakami, Z. Orihashi, Y. Kikuchi, S. Igarashi and Y. Yukawa, *Inorganica Chimica Acta* 303 (2000)148.
15. W. J. Puspita, A. Odani and O. Yamauchi, *J. Inorg. Biochem.* 73 (1999) 203.
16. T. Murakami, Z. Orihashi, Y. Kikuchi, S. Igarashi and Y. Yukawa, *Inorganica Chimica Acta* 303 (2000) 148.
17. Vogel, "Quantitative chemical analysis," 5th Edition, Longman, UK, pp. 326, 1989.
18. E. P. Serjeant, Potentiometry and potentiometric titrations," Wiley, New York, vol. 69, 1984.
19. R. J. Motekaitis, A. E. Martell and D. A. Nelson,, *Inorg. Chem.* 23 (1984) 275.
20. P. Gans, A. Sabatini and A. Vacca, *Talanta* 43 (1996) 1739.
21. P. Gans, A. Ienco, D. Peters, A. Sabatini and A. Vacca, *Coordination Chemistry Reviews* 184 (1999) 311.
22. W. Hosney, S. El-Medani and M. Shoukry, *Talanta* 48(1999) 913.
23. A. A. Al-Najjar, M. M. Mohamed, M. R. Shehata, M. M. Shoukry, *Annali di Chimica* 96 (2006)97
24. L. Lomozik, A. Gasowska, G. Krzysko and R. Bregier-Jarzebowska, *Bioinorganic Chemistry and Applications* 2010 (2010) 1.
25. A. E. Martell and L. G. Sillen, Stability Constants of Metal Ion Complexes, The Chemical Society: London, 1971.
26. H. Sigel, Metal Ions in Biological Systems: Mixed Ligand Complexes, vol. 2, Marcel Dekker, New York, 1973, p. 3.
27. M.M.Shoukry, M. Mohamed, M.R. Shehata and A.M. Mohmoud, *Mikrochim. Acta* 129 (1998) 107.

28. M.M. Shoukry, M.E. Khair and R.G. Khalid, *Transition Met. Chem.* 22 (1997) 465.