

TERNARY COMPLEX FORMATION OF ISONIAZID WITH SOME TRANSITION METALS AND AMINO ACIDS

D.Bilgic Alkaya^{*}, S. Karaderi, G.Erdogan

Marmara University, Faculty of Pharmacy, Department of Analytical Chemistry,

P.O. Box 34668, Haydarpasa, Istanbul, TURKEY

phone: (+90) 2164142962-1165, Fax: (+90) 2163452952, e-mail: dbilgic@marmara.edu.tr

ABSTRACT

In this article mixed ligand complex formation equilibria of copper(II) and zinc(II) with isoniazid and two amino acids have been determined by potentiometric titration method, involving the use of Irving Rossotti procedure. The formation constants of mixed ligand complexes have been found to be Cu(II)-isoniazid-L-asparagine $\log K = 7.29$; Zn(II)-isoniazid-L-asparagine $\log K = 4.09$ and Cu(II)-isoniazid-L-glutamic acid $\log K = 6.70$; Zn(II)-isoniazid-L-glutamic acid $\log K = 6.30$, at $25.0 \pm 0.1^\circ \text{C}$ and $I = 0.11 \text{ mol dm}^{-3}$ ionic strength (NaClO_4) in aqueous solution. The maximum values of the conditional formation constants were found to be in accordance with the mixed-ligand complex formation constants in a given pH region. In addition, the mole fractions of different species from mixed complexes were calculated by means of formation constants.

Keywords: Isoniazid, L-asparagine, L-glutamic acid, metal complex, stability constant

İSONIAZİD VE BAZI AMİNO ASİTLERİN GEÇİŞ METALLERİ İLE OLUŞTURDUĞU ÜÇLÜ KOMPLEKSLERİN TAYİNİ

ÖZET

Bu çalışmada isoniazid ve iki aminoasitin bakır(II) ve çinko(II) metalleri ile oluşturduğu karışık kompleks oluşum dengeleri Irving Rossotti metodu ile potansiyometrik olarak tayin edildi. Üçlü komplekslerin stabilite sabitleri Irving Rossotti yöntemi ile $25 \pm 0.1^\circ \text{C}$ de ve iyonik kuvvet (0.11M) NaClO_4 ile sabit tutularak sulu çözeltide incelendi. 25°C de karışık ligand komplekslerin oluşum sabitleri;

Cu(II)-isoniazid-L-asparajin $\log K = 7.29$; Zn(II)-isoniazid-L-asparajin $\log K = 4.09$; Cu(II)-isoniazid-L-glutamik asit $\log K = 6.70$; Zn(II)-isoniazid-L-glutamik asit $\log K = 6.30$ olarak bulundu. Oluşan komplekslerin koşullu oluşum sabitleri pH'a bağlı olarak hesaplandı ve belirli bir pH bölgesinde koşullu oluşum sabitinin maksimum olduğu değerin deneysel olarak bulunan kararlılık sabiti ile uyum içinde olduğu gözlemlendi. Ayrıca karışık kompleksten türeyen türlerin bağıl bollukları oluşum sabitlerinden hesaplandı.

Anahtar Kelimeler: İsoniazid, L-asparajin, L-glutamik asit, metal kompleks, potansiyometri, kararlılık sabiti

INTRODUCTION

Isoniazid (INH) (pyridine-4-carboxylic acid hydrazide) is an extremely important antituberculosis drug whose rediscovery in the early 1950's resulted in a virtual revolution in the treatment of that disease. The chemical structure of INH is shown in Figure 1. The importance of this drug is now widely used together with other antituberculostatic agents for chemotherapy of tuberculosis and its efficiency in the treatment of pulmonary tuberculosis. Many analytical methods have been reported for the analysis of INH, such as spectrophotometry (Safavi et al., 2004; Epinosa 2001), high performance liquid chromatography (Seifart et al., 1995; Sadeg et al., 1996), capilar electrophoresis (You and et al., 1999) and fluorimetry (Lapa et al., 2000).

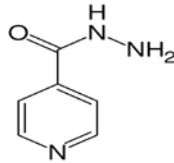


Figure 1. Isoniazid

Metal ions have vital importance on the metabolism and they catalyses transamination reaction of aminoacids. The metal coordination in metal aminoacid complexes has received much attention because they are simple systems to study the coordination of the ions in metalloproteins (Brill S, 1977; Sakar et al., 1966). Nitrogen requirements of the mycobacteria in the metabolism have been reviewed by Bernheim (Bernheim 1951), Darzins (Bowles A et al., 1965) and Long (Long 1958). Richard H. Lyan et al. (Lyan 1974) have documented asparagine controls the utilization of other aminoacids by *Mycobacterium tuberculosis*. Other authors have observed the toxicity of INH in humans, in particular, upon the liver and central nervous system, to take advantage of the supposed protective action of glutamic acid (Francis et al., 1960).

Due to involved in oxidant damage copper and zinc, which are serum transition metals, bear important roles in the immune system. *Mycobacterium tuberculosis* need to transition metals to protect itself against oxidant substances that are required to synthesize the antioxidant substances. It was shown that the investigations on complex formation equilibria between metal ions and tuberculostatics have received attention for a long time and their stabilities have been determined several times (Bogden 1977; Bogden 1978). Because of the limited data on the serum level of copper and zinc in patients with adult pulmonary tuberculosis. M. Rieber(Rieber Mand Bemski 1969) have investigated ability of INH to interact with Cu(II) ions and it was concluded that forming a complex has enhanced mycobactericidal activity compared with that of isoniazid or copper alone (Sorkin et al., 1952; Hanson 1981). Copper and zinc bonding geometry is square planar with the isoniazid carbonyl oxygen and hydrazide amino nitrogen atoms. Complexing with copper(II) and zinc(II) don't significantly alter the isoniazid molecular conformation.

Ternary complexes formed between metal ions and two different types of bioligands, namely heteroaromatic nitrogen bases and aminoacids may be considered as models for substrate metal ion–enzyme interactions and other metal ion mediated biochemical interactions. Much attention has been paid recently to the study of ternary complexes of transition metals with molecules of biological and pharmaceutical interest (Ammar 2011; Haropriya et al.,2005). As a matter of fact that in spite of large amount of clinical results only a few chemical works exist on the stability of Cu(II) and Zn(II) complexes with INH and aminoacids (Magare et al., 2011).

In the present paper, we thoroughly characterized M-INH-aminoacid system by a combined potentiometric and spectrophotometric procedure because of their advantages such as simple, less time consuming and economical viable method.

MATERIALS AND METHODS

SPECTROPHOTO METRIC

APPARATUS: All spectrophotometric measurements were performed using a Shimadzu UV-160 double beam spectrophotometer with a fixed slit width (2 nm) and its recorder were used. The absorbance of solutions were measured in wavelength range 190 nm to 700 nm, in 10mm quartz cells. All experiments were performed at 25° C. UV spectra of INH (2.5×10^{-4} mol dm⁻³ pH= 2.5), L-asparagine (ASP)(2.5×10^{-4} moldm⁻³ pH= 2.5) and L-glutamic acid (GLUT) (2.5×10^{-4} mol dm⁻³ pH= 2.5) in 0.1 mol dm⁻³ NaClO₄ ionic medium, were recorded

using in doubly distilled water as a reagent blank. Potentiometric measurements were made with a TIM 800 titration Manager Radiometer with ABU 901 automatic burette. The pH-meter, which was accurate to 0.01 pH unit was standardized before each titration using buffer solutions of citrate-hydrochloric acid (pH=4.00 at 20°C) and phosphate (pH=7.00 at 20°C). INH (1.0×10^{-2} mol dm⁻³), ASP (1.0×10^{-2} mol dm⁻³), GLUT (1.0×10^{-2} mol dm⁻³), metal salts (1.0×10^{-2} mol dm⁻³) were prepared in potentiometric titrations.

STANDART SOLUTIONS: Isoniazid was obtained from Merck. All reagents such as sodium perchlorate, perchloric acid, sodium hydroxide (titrisol) were analytical reagent grade from Merck at least % 99,55 pure. Triply distilled water was used throughout the study. A stock solution of isoniazid (2.5×10^{-4} mol dm⁻³; 1.0×10^{-2} mol dm⁻³) was prepared by dissolving water and kept in a refrigerator at about 4°C. The stock solutions of metal ions were prepared in doubly distilled CO₂ free water. The aqueous solutions of metal salts (2.5×10^{-4} mol dm⁻³, 1×10^{-2} mol dm⁻³) and standardized by titration with ethylenediamine tetraacetic acid (EDTA).

SAMPLE PREPARATION: Spectrophotometric measurements were performed with the aim to examine the possibility of complex formation between INH - ASP, INH - GLUT and M (Cu(II), Zn(II)). UV spectra were presented in figure 4 and 5. For this purpose 2.5×10^{-4} mol dm⁻³ isoniazid and 2.5×10^{-4} mol dm⁻³ M(NO₃)₂ + 0.1 mol dm⁻³ HClO₄ + 0.1 mol dm⁻³ NaOH solution were mixed and diluted to 5.0 mL. Absorbion spectra of INH +HClO₄ + NaOH were recorded between 190 and 700 nm. The solutions of INH-Cu(II) and INH-Zn(II) solutions, and have shown two peaks: INH-Cu(II) 256.0 nm .and INH-Zn(II) 262.5 nm.

The stoichiometries of INH-M complexes were determined by continuous variation method; in order to select convenient pH values and composition of complexes. The method is simple and widely used for elucidating the composition of complexes and is based on the variation of both isoniazid and metal of equal molar concentrations. Absorbion spectra of isoniazid and its ternary complexes were shown in figure 2 and figure 3.

STABILITY OF THE COMPLEXES: The stability of the complexes formed between the isoniazid and zinc, copper was evaluated. Although the ion pairs were obtained instantaneously, the constant absorbance readings were obtained after not less than 10min of standing at room temperature. The complexes were stable for at least 24 h without any change in color intensity or in λ_{\max} .

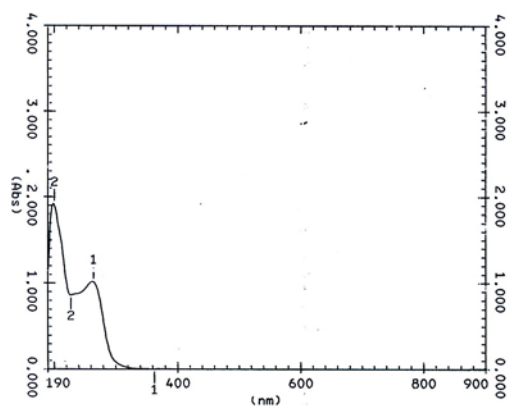
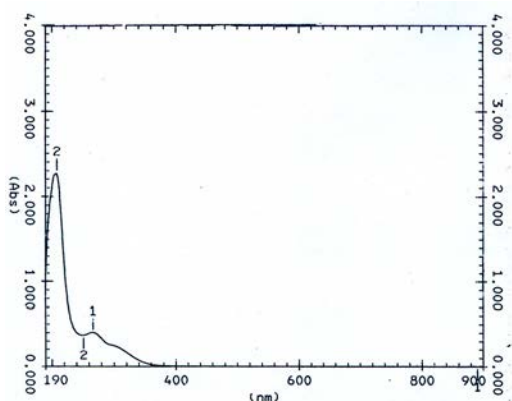
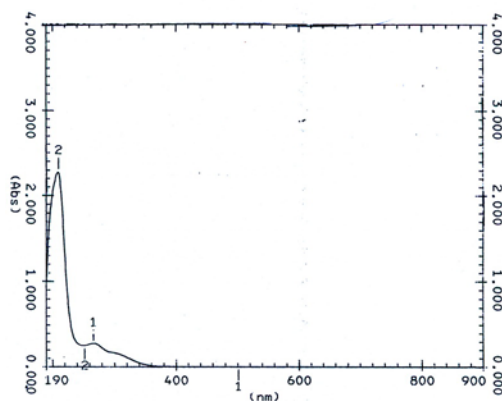


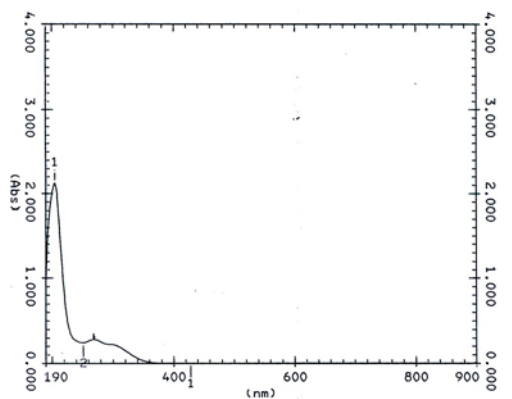
Figure 2: The spectrum of isoniazid



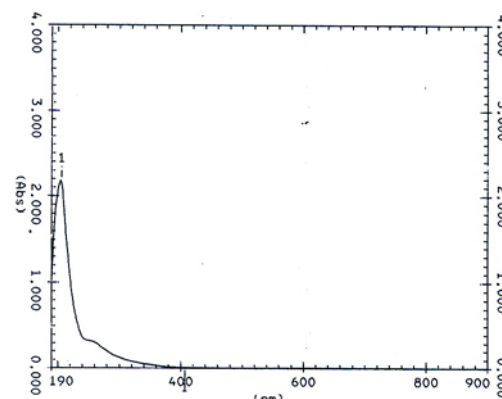
a. Zn(II)-isoniazid-L-Asparagine



b. Zn(II)-isoniazid-L- Glutamic acid



c. Cu(II)-isoniazid-L-asparagine



d. Cu(II)-isoniazid-L- glutamic acid

Figure 3: The spectra of ternary complexes

POTENTIOMETRIC METHOD

THE DETERMINATION OF STABILITY CONSTANTS: The protonation constants of pKa of the ligands and stability constants of mixed ligand complexes (M-INH-ASP; M-INH-GLUT) were calculated from the potentiometric pH titrations data according to Irving

Rossotti method which is used in our previous studies (Karaderi and Bilgic 2006; Karaderi and et.al 2007). In order to determine the stability constants of mixed ligand, the solutions including $\text{HClO}_4:(\text{Y}+\text{HClO}_4) : (\text{Y}+\text{HClO}_4+\text{L}+\text{M})$ solutions were titrated potentiometrically using NaOH solutions (0.1 mol dm^{-3}). For each mixture, the volume was made up to 50,0ml with deionized water before the titration was performed.

The average \bar{n}_A values were calculated from the titration curves. The following equation was used for this calculation:

$$\bar{n}_A = y + \frac{(V_1 - V_2) (N + E^0)}{(V^0 + V_1) T_L^0}$$

Where:

V^0 = volume at the beginning	: 50.00 mL
N = normality of base	: $0.100 \text{ mol dm}^{-3}$
T_L^0 = total ligand concentration	: $0.002 \text{ mol dm}^{-3}$
E^0 = concentration of acid	: $0.010 \text{ mol dm}^{-3}$
y = the number of protons given for isoniazid	: 0

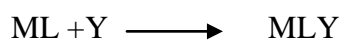
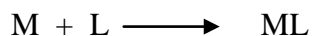
The mixtures which also contain metal ions were titrated with 0.1000 N NaOH solution potentiometrically and the titration curves were plotted in (Figure 4 and Figure 7). The \bar{n}_A values corresponding to several pH values for ligands were calculated by the use of V_1 and V_2 volumes from figure 4. The figure of $\bar{n}_A = f(\text{pH})$ was plotted by using the values obtained (Figure 5). The protonation constants for the corresponding acid constants were found. The dissociation constants of ligands were calculated via MS Excel programme at $\bar{n}_A = 0.5, 1, 5, \dots$

Isoniazid	$\log K_1 = 4.39$		
L-glutamic acid	$\log K_1 = 10.27$;	$\log K_2 = 4.33$;	$\log K_3 = 2.42$
L-asparagine	$\log K_1 = 9.26$;	$\log K_2 = 2.44$	

Using the potentiometric titration data of the solutions, the metal ligand average formation numbers \bar{n}_L at various were calculated. The ionic strength of the reaction media was kept constant at 25°C ($I=0.1$) using NaClO_4 solution. In order to establish the stability constants of ternary complexes Irving-Rossotti method was used. The stability constants derived from the complexes of all ligands and the ligand which has a lower stability constant was selected as the second ligand.

First ligand : L (L-GLUT) or L (L-ASP)

Second ligand : Y (INH)



The mixtures consisted of metal and ligands were titrated potentiometrically. The separation between (HClO₄) ; (Y+HClO₄) ; and (Y+HClO₄+L+M) all potentiometric titration curves, showed the formation of a mixed compound. The $\bar{n}_L = f(pL)$ graphics were plotted using \bar{n}_L and pL values which were calculated from titration curves (Figure 6 , Figure 8). Formation constants are calculated via MS Excel at $\bar{n}_L = 0.5$ and 1.5. The approach in binary system of Irving-Rossotti method was applied for the mixed system which has a higher stability constant was behaved as the metal in the binary system when it was binding to the second ligand. In this condition, the free metal equation and the second ligand (Y) according to equation can formed this complex.

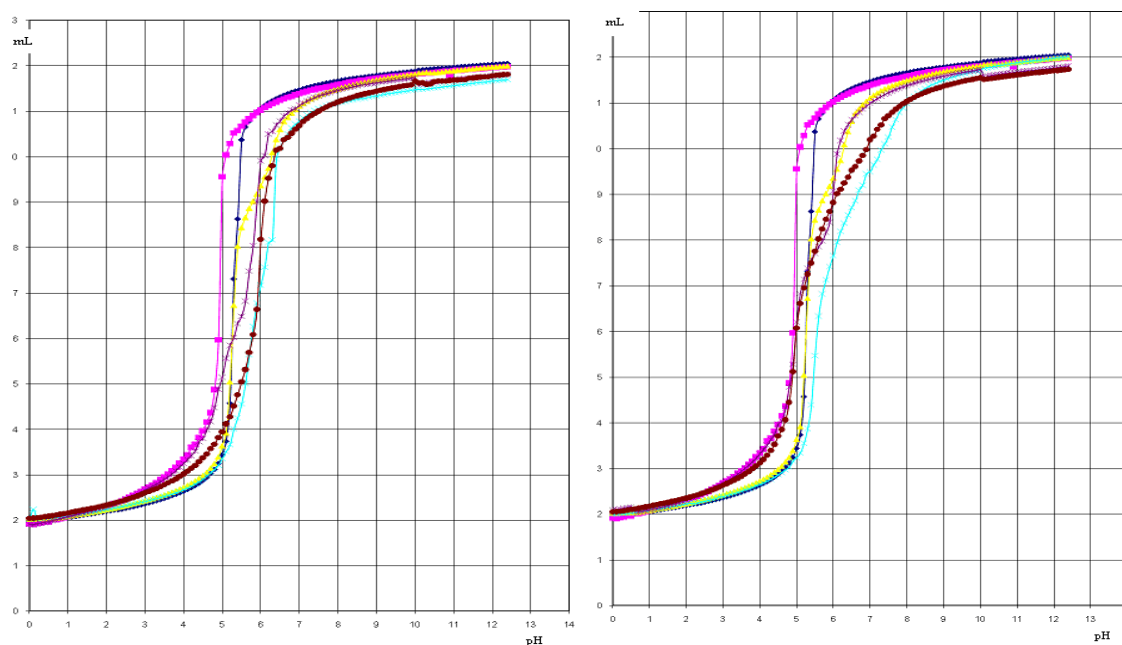
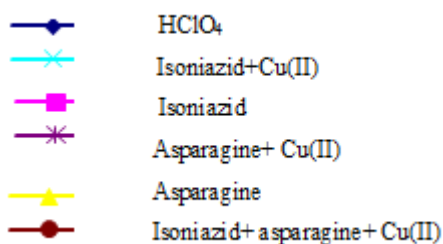


Figure 4. Potentiometric titration curves for binary and ternary complexes for L-asparagine



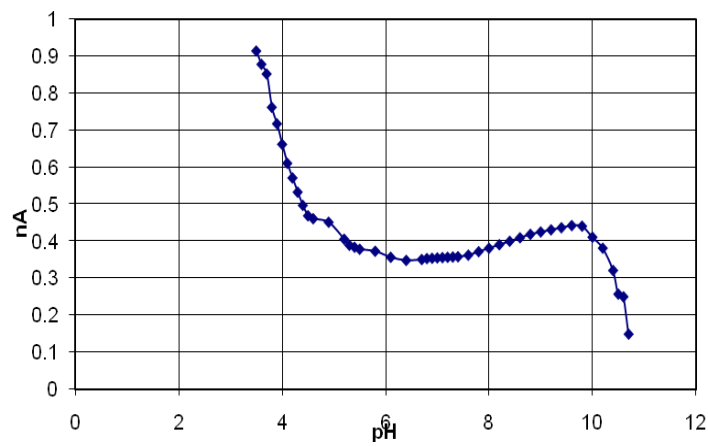


Figure 5. Isoniazid $\bar{n}_A = f(\text{pH})$ ($I = 0.11$; $t = 25^\circ\text{C}$)

$$\bar{n}_A = 0.5; \log K_1 = 4.39$$

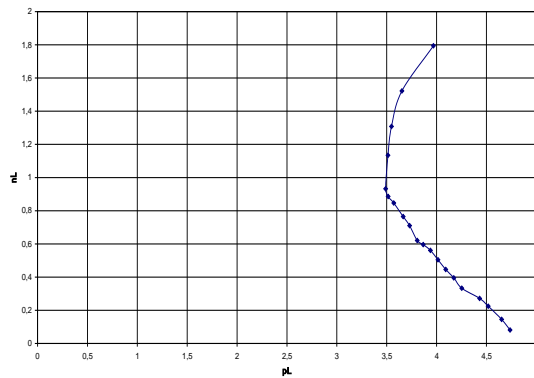
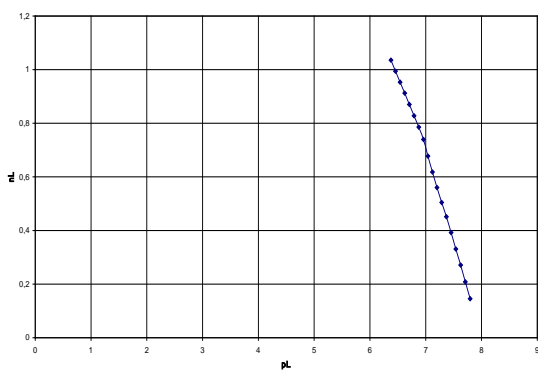


Figure 6. $\bar{n}_L = f(\text{pL})$ curves of mixed ligand complexes

a. Cu(II)-Isoniazid-L-asparagine

$$\bar{n}_L = 0.5; \log K = 7.29$$

b. Zn(II)-Isoniazid-L-asparagine

$$\bar{n}_L = 0.5; \log K = 4.09$$

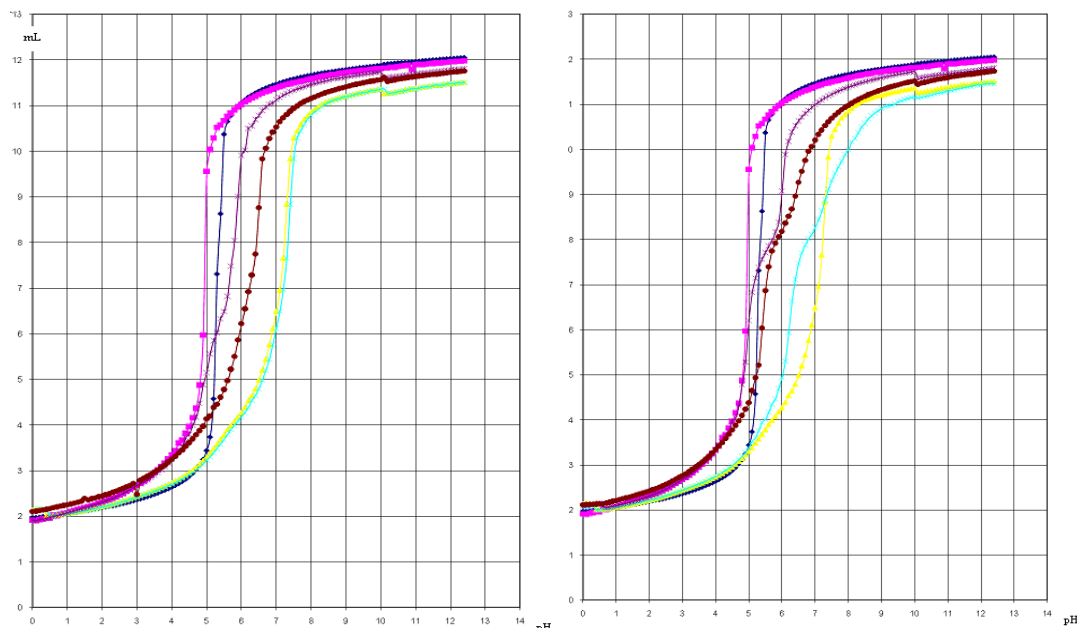


Figure 7. Potentiometric titration curves for binary and ternary complexes for L-glutamic acid

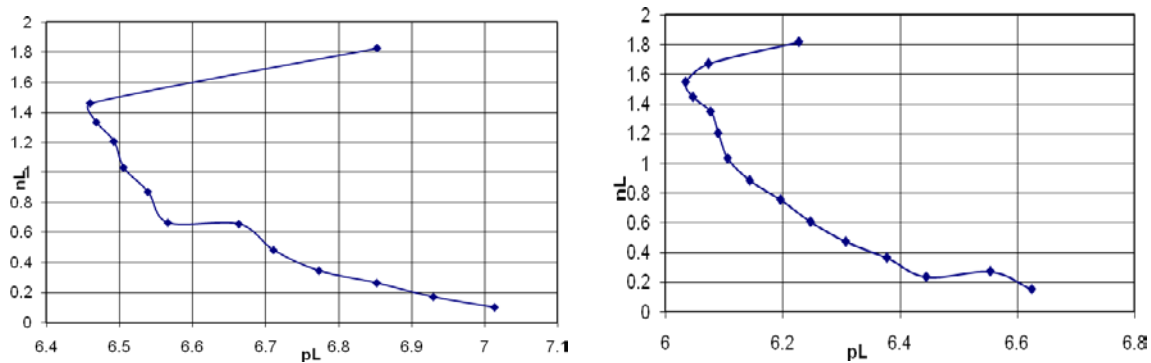
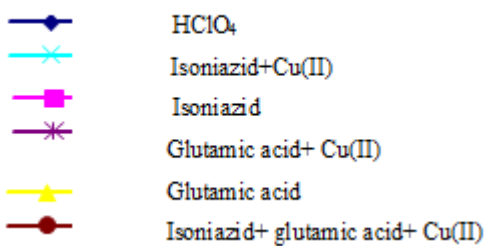


Figure 8. $\bar{n}_L = f(pL)$ curves of mixed ligand complexes

a. Cu(II)-Isoniazid-L-glutamic Acid

$$\bar{n}_L = 0.5 \quad \log K = 6.70$$

b. Zn(II)-Isoniazid-L-glutamic Acid

$$\bar{n}_L = 0.5 \quad \log K = 6.30$$

RESULTS

Serum level of zinc and copper has important in patients with adult pulmonary tuberculosis. Because while serum level of zinc is limited in this patient, copper level increases.

The stable complex of Cu(II)-isoniazid has been chemically or structurally characterized before (Bogden and et al,1978). It was shown to cause nearly immediate cell lysis of streptomycin-resistant strain of mycobacteria(Sakar et al. 1976) and to chelate with transition metals enhance antituberculostatic drug's biological activity. The relation between mycobacteria tuberculosis and amino acids wasn't statistically significant. That is why it is important to know how the stability constants of this complexes can be determined.

The complexes formation of mixed ligand were examined at different pH by spectrophotometric method. Absorbtion spectra of isoniazid was shown in figure 2. The maximum absorbance was observed at 262.5 nm. The maximum absorbance of complexes were recorded at: isoniazid-Cu(II)= 256.0 nm; isoniazid-Zn(II)= 262.5 nm.

Table 1: Formation Constants of binary-ligand complexes (I=0.11; t=25±0.1°C)

Ligand-metal	logK ₁	logK ₂	Logβ
Cu(II) - L-glutamic acid	8.39± 0.01	7.92± 0.01	16.31± 0.01
Zn(II) - L-glutamic acid	5.76± 0.01	4.97± 0.01	10.73± 0.01
Cu(II) - L-asparagine	7.87± 0.01	5.74± 0.01	13.61± 0.01
Zn(II) - L-asparagine	7.41± 0.01	5.56± 0.01	12.97± 0.01
Cu(II) - Isoniazid	12.29± 0.01	-	12.29± 0.01
Zn(II) - Isoniazid	11.07± 0.01	-	11.07± 0.01

The formation of ternary complexes were identified quantitatively by the pH of precipitation of ML, MY, MLY titration curves which indicates the higher value of pH of precipitation of ternary system than corresponding binary system(Irving et al.1953). Titration curves of solutions (M-INH-GLUT; M-INH-ASP) are shown in Figure 4 and Figure 7. Besides potentiometric results were examined for ternary complexes and shown in table 2.

Table 2: Formation Constants of ternary - ligand complexes (I=0.11, t=25.0 ± 0.1°C)

Ternary complex	logK ₁	logK ₂	Logβ
Cu(II) - L-asparagine - Isoniazid	7.29 ± 0.01	-	7.29 ± 0.01
Zn(II) - L-asparagine - Isoniazid	4.09± 0.01	-	4.09± 0.01
Cu(II) - L-glutamic acid - Isoniazid	6.70± 0.01	-	6.70± 0.01
Zn(II) - L-glutamic acid - Isoniazid	6.30± 0.01	-	6.30± 0.01

It is observed from Table 2 that the order of stability constants, copper > zinc, is the same for metal(II)-isoniazid binary and metal(II)-isoniazid-L-asparagine or metal(II)-isoniazid-L-glutamic acid mixed complexes, but corresponding stability constant values are lower in mixed complexes.

In this study the conditional formation constants were calculated and these constants were found to be in agreement with the formation constants of ternary systems. This result affords us to find the stability constants of mixed complexes. In this calculation, the pK values of ligands and the formation constants of complexes which they formed with metals are used as data. The conditional formation constants, namely the stability constants of mixed complex can also be calculated. The difference between the formation constants of mixed and binary systems is a parameter which characterizes the formation behaviour of mixed ligand complexes (Irving et al., 1953; Itoh et al,1974; . Bajpai et al., 1993).

$$\Delta \log K = \log K_{MLY} - \log K_{MY}$$

The difference is an equilibrium constant of the following equation.



If $\Delta \log K$ is negative, then equilibrium favours the left hand side.

The conditional formation constant equals the " β_1 values" of the mixed complex. The formation constants of mixed complex found in this work are in agreement with the calculated conditional formation constants of $\beta_1 = K_1 \cdot K_2$ mixed complex.

Cu(II)-INH-ASP, Cu(II)-INH-GLUT, Zn(II)-INH-ASP, Zn(II)-INH-GLUT systems are also in agreement with our observations.

The concentration of various species formed in the complex were found by means of the calculated formation constants and were plotted as a function of pH. The species distribution curve shows that ternary complex species were formed at 6.00 pH(80% and 90 %) (Figure 9 and Figure 10).

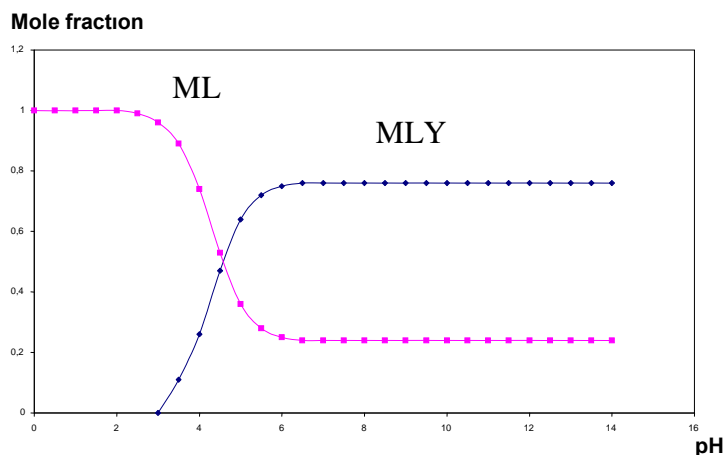


Figure 9. Species distribution curves of the Cu (II)-INH-Aminoacids

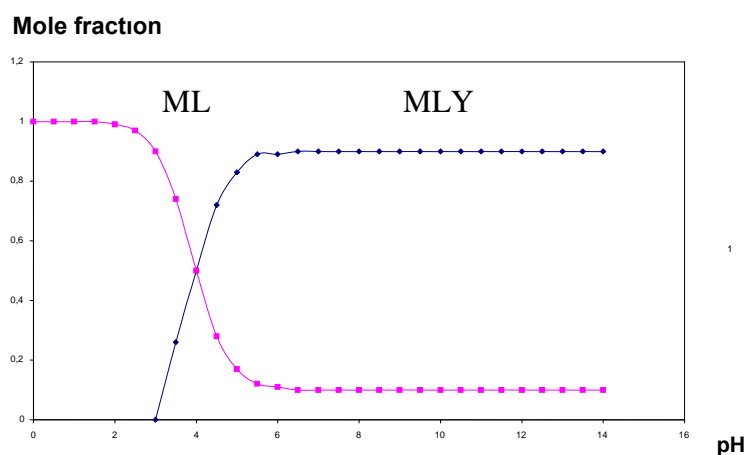


Figure 10. Species distribution curves of the Zn (II)-INH-Aminoacids

DISCUSSION

As a result, in this study conditions for ternary complexes with isoniazid have been discussed and it has been shown that the presence of complex formation. Two methods, UV spectrophotometry and potentiometry were successfully applied to formation of isoniazid complex systems and their stability constants were also determined. Comparing results are shown us to form ternary complexes of isoniazid-metal with L-asparagine and L-glutamic acid can increase antitumor activity, provide Zn(II) and Cu(II) balances and prevent hepatic toxicity as well as other side effect. In the light of these information, Zn-L-asparagine, Zn-L-glutamic acid tablet forms can be searched for tuberculosis patients by pharmacologists. The present technique is also very helpful in finding whether a complex system is formed or not; if formed its stability constants can also be determined. In this way, formation complexes greatly help in understanding the binding efficiency of the ligand as well as some of its possible side effects if used as a drug in metal overloading diseases.

ACKNOWLEDGEMENT

This work was part of the Project supported by the Marmara University, Scientific Research Commission (Project number 2004/097/081004), to whom the authors wish to Express their thanks

REFERENCES

- [1] Ammar RA, Mutir EMA. *Fluid Phase Equilib.* 301:51, 2011.
- [2] Bajpai S, Limaye SN, Saxena MC. Formation Constants and Extrastabilization of Mixed Complexes of Co(II), Ni(II), Cu(II), Zn(II), and Cd(II) with α, α'' -dipyridil-o-phenanthroline and Amino Acids. *Acad Sci.Lett.* 16: 237-241, 1993.
- [3] Bernheim F. Metabolism of mycobacteria, In. H. Birk houser and H.Bloch. Advances in tuberculosis research. vol.4. S. Karger AG., Basel, Switzerland, pp.1-38, 1951.
- [4] Bogden JD, Lintz DI, Joselow MM, Charles J and Salaki JS. Effect of pulmonary tuberculosis on blood concentrations of copper and zinc. *Am J Clin Pathol.* 67(3): 251-6, 1977.
- [5] Bogden JD, Lintz DI, Joselow MM, Charles J and Salaki JS. Copper/Zinc ratios in whole blood, plasma and erythrocytes in pulmonary tuberculosis. *Health Lab Sci.* 15(1): 38-43, 1978.
- [6] Bowles A and. Segal W Kinetics of utilization of organic compounds in the growth of mycobacterium tuberculosis. *J. Bacteriol.* 90: 157-163, 1965.
- [7] Brill S, *Transition Metal Biochemistry*, Springer-Verlag, Berlin-Heidelberg-New York .pp 186, 1977 .
- [8] Epinosa A and Mansilla MIA, Valenzuela PA Munoz Comparative study of partial least squares and a modification of hybrid linearanalysis calibration in the simultaneous spectrophotometric determination of rifampicin, pyrazinamide and isoniazid. *Anal.Chim.Acta.* 427: 129-136, 2001.
- [9] Francis O. Segarra MD Pulmonary Tuberculosis Treated with High doses of isoniazid plus glutamic acid. *Chest Journal*, 37: 382-389, 1960.
- [10] Hanson JC, Structure of a Copper- Isoniazid Complex. *Proceedings of the Biochemical Society.* 24:1369-1371, 1981.
- [11] Haropriya M, Rani G. *Bioresour technol.* 96:1387, 2005.
- [12] Lapa RAS, Lima JLFC. Fluorimetric determination of isoniazid by oxidation with Ce(IV) in a multicommuted flow system. *Anal.Chim.Acta.* 419: 17-23, 2000.

- [13] Long ER Utilization of nitrogen for growth. In Chemistry and Chemotherapy of Tuberculosis, 3rd. Ed pp. 80-85,1958 .
- [14] Lyon RH, Hall WH, Martinez CC. *J.Bacteriol.* 117(1) 151-156, 1974 .
- [15] Rieber M, Bemski G. *Biochem.Biophys.* 131: 655-658, 1969.
- [16] Sadeg N, Pertata H. Rapid specific and sensitive method for isoniazid determination in serum. *J.Chromatogr.B.* 675: 113-117,1996.Safavi A, Karimi MA, Hormozi NMR Sensitive indirect spectrophotometric determination of isoniazid. *Spectrochim. Acta.* part A 60. 765-769, 2004 .
- [17] Sakar B and Kruck TPA In: Peisach J, Aisen P and Blumberg WE, Editors, The Biochemistry of Copper, Academic Press, New York, pp. 183, 1966.)
- [18] Seifart HI, Gent WL, Parkin DP. High Performance liquid chromatographic determination of isoniazid , acetylisoniazid and hydrazine in biological fluids. *J.Chromatogr.B.* 674: 269-275, 1995 .
- [19] Sorkin E, Roth W Erlenmayer H _Über die Beeinflussung tuberkulostatischer Wirkungen durch Cu(II). Metallionen und biologische Wirkung. *Helv.Chim.Acta*, 35:,1736-1741,1952.
- [20] Magare BK and Millind B. Ubale, Solution behaviour of copper complexes with an antibacterial drugs and amino acids. *Der Chemica Sinica.* 2(2) : 158-164, 2011.
- [21] Karaderi S, Bilgic D. Zinc(II) and cadmium(II) binary complexes with creatinine and their mixed-ligand complexes with L-asparagine or L-glutamic acid: Potentiometric studies. *Main Group Metal Chemistry.* 29(3): 144-155, 2006.
- [22] Karaderi S., Bilgic D, Dolen E, Pekin M. Determination of stability constants of mixed ligand complexes of Cu(II) with creatinine and ethylenediamine tetraacetic acid or l-glutamic acid: Potentiometric and spectrophotometric methods. *Reviews in Inorganic Chemistry.* 27(6): 459-472 , 2007.
- [23] Irving, HM and Rossotti HS. Methods for computing successive stability constants from experimental formation curves, *J.Chem. Soc.*, 3397-3405, 1953, The calculation of formation curves of metal complexes from pH titration curves in mixed solvent 2904-2910, 1954.
- [24] Itoh T, Kaneko T, Izumi Y, Chibata I, Itoh I.Synthetic Production and Utilisation of Amino Acids. Halsted Press, New York, Ch. 6., 1974.
- [25] You TY, Niu L, Gui JY. Detection of hydrazine, methylhydrazine and isoniazid by capillary electrophoresis with a 4 pyrididyl hydroquinoneself assembled microdisk platinumium electrode .*J. Pharma.Biomed.Anal.*19: 231-237,1999 .