Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2013, 4(3):54-58

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Determination of the stability constants of mixed ligand complexes of bio-molecules and amino acids with Ni(II) by potentiometric titration method

R. P. Phase[1], A. G. Shankarwar[2*], S. G. Shankarwar[3] and T. K. Chondhekar[3]

[1]Department of Chemistry, L. B. S. Sr. College, Partur(M. S.), India
[2]Department of Chemistry, S. B. E. S. College of Science, Aurangabad(M. S.), India
[3]Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad(M. S.), India

---

ABSTRACT

A potentiometric titration technique has been used to determine the stability constants for the various complexes of Ni(II) with bio-molecules(drugs) (L) as primary ligand and amino acids (R) as secondary ligand. Ternary complexes of amino acids are formed in a stepwise mechanism, whereby (L) binds to Ni(II),followed by interaction with ligand (R), form ternary complexes through a simultaneous mechanism. The formation constants of the complexes were determined at 30°C and ionic strength 0.1M $NaClO_4$. The relative stabilities of the ternary complexes are compared with those of the corresponding binary complexes in terms of Δlog K, K_R, K_L and K_r values. The concentration distribution of the complexes is evaluated.

Keywords: Biomolecules, binary complex, ternary complex, stability constant, SCOGS.

INTRODUCTION

Metal complexes are widely used in various fields such as biological processes, pharmaceuticals, analytical processes, separations techniques, etc. Metal complexes play a vital role in nature, they have been extensively used in clinical applications as enzyme inhibitors [1], anti-bacterial [2,3], antiviral [4-6] and as anti-cancerous [7-9]. Different kinds of metals have been employed in these complexes including platinum, gold, vanadium, iron, molybdenum, cobalt, tin, gallium, copper and many others [8]. Metal complexes of adenine (A) have been shown to possess anticancer activity [9-13].

Recently, there has been considerable interest in the study of binary and ternary complexes by pH-metric method [14-16] using biomolecules (drugs). Drugs have various functional groups present in its structure, which can bind to metal ions present in human body [17]. Metal complexes of drugs are found to be more potent than parent drugs [18]. Chemistry of drugs attracts many researchers because of its applications in medicinal chemistry. Interesting results have been reported earlier on complex formation reactions of drug-amino acid-metal ion, mixed ligand complexes [19-23].

Ethambutol hydrochloride (L_7) chemically known as (+) 2, 2′ -(ethane-1,2 diyldiimino)dibutan-1-ol is an anti-tubercular drug [24]. Aspartame (*N*-(l-α-aspartyl)-l-phenylalanine-1-methyl ester) (L_4) is a dipeptide of aspartic acid and phenylalanine, used as an artificial, non-saccharide sweetener in the pharmaceutical and food industry. Literature survey revels that, over the last decade there has been tremendous work done on the study of metal complexes [25,26]. The studies in metal-ligand complexes in solution of a number of metal ions with carboxylic acids, oximes, phenols etc. would be interesting which throw light on the mode of storage and transport of metal ions in biological kingdom. With the view to understand the bio-inorganic chemistry of metal ions, Banerjee et al. [27] have synthesised a number of mixed-ligand alkaline earth metal complexes. Irving and Williams [28] had

studied the order of stability of metal complexes of transition metal ions by comparing the ionic radius and second ionisation potential of metal ions, as it is valid for most nitrogen and oxygen donor ligands. Deosarkar [29] have studied stability constants of Al(III), Cr(III) and Fe(III) metal ion complexes with substituted sulphonic acid. Pund [30] have investigated interaction between La(III) and Nd(III) metal ions and 1-(4-hydroxy-6-methylpyrimidine)-3-substituted thiacarbamide of 0.1 M ionic strength pH metrically. Speciation of binary complexes of Ca(II), Mg(II) and Zn(II) with L-glutamic acid in DMSO-water Mixtures has been studied [31]. Recently, S.A.Lahsasni [32] have reported the Mixed-Ligand Complex Formation of Cu(II) with 1,2- Diphenylethylenediamine as Primary Ligand and Amino Acids as Secondary Ligands and A.B. Patil [33] have investigated the interaction of Mn (II), Co (II), Ni (II), Cu (II) and Zn (II) metal ions with penicillamine (PEN) has been studied by pH-metric technique at 0.1 M (KNO3) ionic strength at 302 ± 0.5 K in aqueous medium. Although research work on dipeptides (biomolecules) have been carried out by many workers [34-36] but no sigficant quantitative studies were made on ternary chelates of ethambutol hydrochloride and aspartame selected for this study in solution.

With this shortcoming and our continuation interest [37] herein, we reports the pH metric study of the formation and stability of bivalent metal ion ternary chelates, MLR where M=Ni(II), L_4= Aspartame and L_7= Ethambutol hydrochloride and R_3=Leucine, R_6 = phenylalanine in 20% (v/v) ethanol-water mixture at 30 ± 0.1^0C and 0.1M (NaClO4) ionic strength.

MATERIALS AND METHODS

Drug sample of ethambutol hydrochloride and aspartame in pure form were obtained from pharma industries. Ethanol was purified as described in literature [38]. Double distilled water was used for the preparation of ethanol-water mixture and stock solution of aspartame and ethambutol hydrochloride. All chemicals NaOH, $NaClO_4$, $HClO_4$ and metal salts were of Analar grade. The solutions used in the potentiometric titrations were prepared in double distilled water. The NaOH solution was standardized against oxalic acid solution (0.1 M) and then standard alkali solution was used for standardization of $HClO_4$. The metal salt solutions were also standardized using EDTA titrations (Vogel, 1978). Experimental procedure by potentiometric titration technique, involves the titration of carbonate free solution of

(1) Free HClO4 (A),
(2) Free HClO4 + Ligand-Drug,
(3) Free HClO4 + Ligand-Drug + Metal ion,
(4) Free HClO4 + Ligand-Amino acid,
(5) Free HClO4 + Ligand-Amino acid + Metal Ion,
(6) Free HClO4 + Ligand-Drug + Ligand-Amino acid + Metal Ion,

Against standard solution of sodium hydroxide, with drug ethambutol hydrochloride or aspartame and amino acids. The total volume of solution was kept 50 ml by the adding distilled water. Titrations were carried out using a digital pH meter (Elico model (LI-127) in conjunction with combined glass electrode. The ionic strength of solutions was maintained constant i.e. 0.1 M by adding appropriate amount of 1M sodium perchlorate solution. Titrations were carried out in 20% (v/v) ethanol-water medium at 30°C in an inert atmosphere by bubbling oxygen free nitrogen gas through an assembly containing the electrode to expel out CO_2. The proton-ligand and metal-ligand binary formation constants were determined by Irving-Rossotti method [39]. The formation constants and various statistical parameters of ternary complexes were evaluated by using computer program SCOGS [40,41].

RESULTS AND DISCUSSION

(a) Binary metal complexes

Proton ligand constant of Primary ligand L_4,L_7 and secondary ligand R_3,R_6 have been determined by Irving-Rossotti technique .Their metal-ligand formation constants were also determined for the comparison with those of the ternary system. For this we have given emphasis on studies of binary systems under identical condition with those for ternary systems. The values are presented in Table 1. Primary ligand and secondary ligand both forms 1:1and 1:2 complexes with Ni (II) ions.

Table 1: Proton-ligand and metal-ligand stability constants in binary system

Ligands	pK_1	pK_2	$logK_1$	$logK_2$
Aspartame (L_4)	3.6134	8.3301	6.7324	4.9044
Ethambutol hydrochloride (L_7)	-	6.3248	6.5672	5.8982
Leucine (R_3)	2.1333	10.4844	5.5575	3.5438
Phenyl alanine (R_6)	2.2157	9.2296	5.4034	4.0929

(b) Ternary metal complexes

The stability of mixed ligand complexes is mainly governed by the characteristics of the approaching secondary ligand. The stability therefore depends mainly on the ring size which affects the overall basicity of the secondary ligand. It can be inferred that the stability of complex depends more on the length and spatial configuration of chelate-ring than on the acidity of the complexing agent. At the pH of secondary ligand combination, the formation of mixed ligand can be represented by equilibria (1) and (2)

$M_{(aq)} + L \rightarrow ML$ (1)

$ML + R \rightarrow MLR$ (2)

Only 1:1:1 ternary complex have been used in this study to ensure the exclusive formation of the simplest ternary complex MLR. By considering the proton-ligand and metal-ligand constants of ligands, the species that exist in complexation equilibria have been plotted in figures 1,2,3,4 as a function of pH. The parameters K_L, K_R, K_r and $\Delta \log K$ are generally used to indicate the relative stability of ternary complexes as compared to the binary complexes.

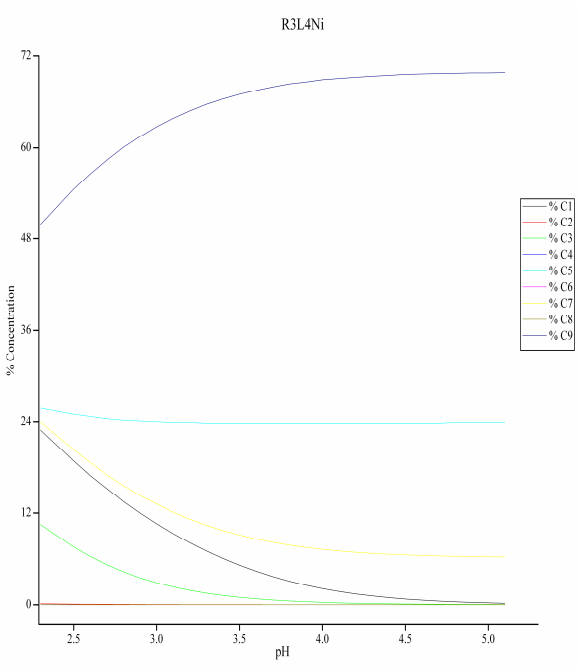

Figure 1

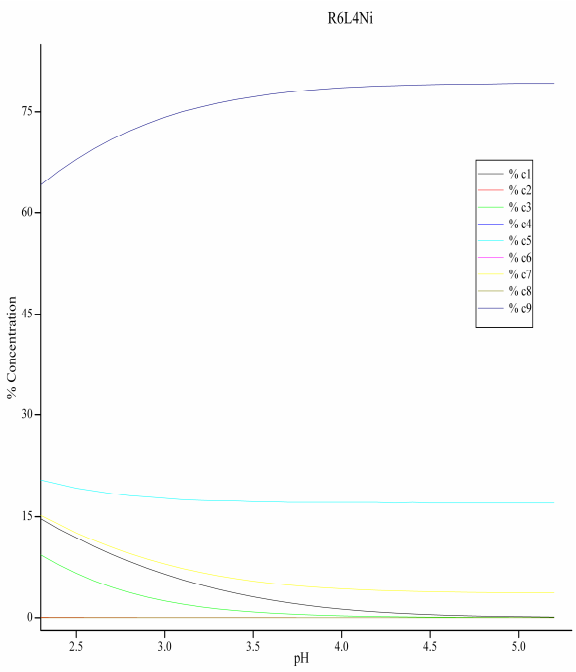

Figure 2

In all the ternary systems, distinct inflections were observed in the titration curves, indicating the formation of chelates. Formation of ternary complexes was further confirmed from the non-superimposible nature of theoretical composite curves on the experimental curve in the region of ternary complex formation. The species distribution curves, as a function of pH were generated using the computer program SCOGS, also supports the formation of ternary chelates. Similarly the percentage curves of the species FM, FL and FR are shows that the initial concentration of free metal is decrease with increasing pH. This indicates that all of the metal is in bound state in the form of binary and ternary complexes. The free ligand concentration FL and FR show slight increase during the process with increasing pH. This may be attributed to the dissociation of slight excess ligands present in the system.

In case of $R_3L_4Ni(II)$ system. Fig.1.shows that, the initial concentration of MLR is about 50 percent at pH 2.3 and then increases sharply up to pH 3.7 where it becomes 68 percent and then the rate of formation becomes slow till it reaches finally to about 70 percent. The percentage of species H_2L and MR represented by C_1 and C_7 is approximately same initially and then sharply decreases as the pH increases. It will be seen that their concentrations reaches to minimum at pH 3.7 where the formation of mixed ligand complex reaches to maximum i.e. equal to 68%. After this pH, the rate of formation becomes slow and tends to constant value. It may be concluded that the initial higher concentration of species H_2L sharply decreased to give HL and then its rapid dissociation in to H and L. This free ligand L then interacts with MR to give formation of MLR after pH 2.3. The mechanism of formation of ternary complex by different equilibria discussed in previous Ni(II) system is totally applicable to $R_6L_4Ni(II)$ system and the stability constant of this complex is 11.39 which is smaller than $R_3L_4Ni(II)$ system.

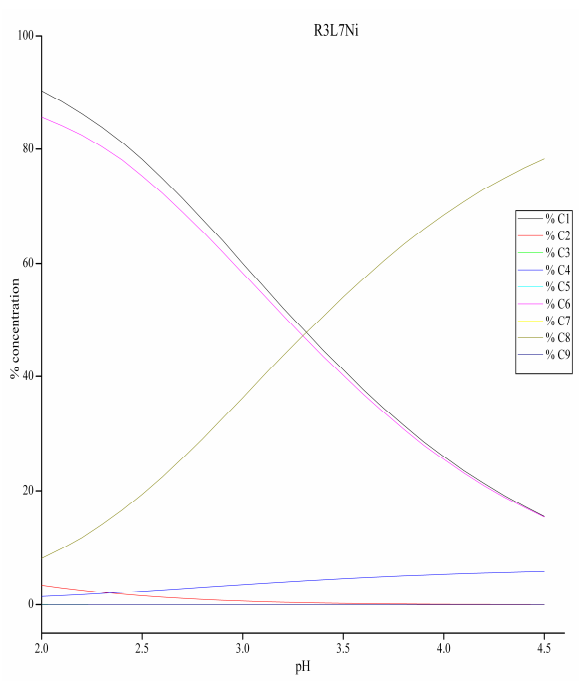

Figure 3

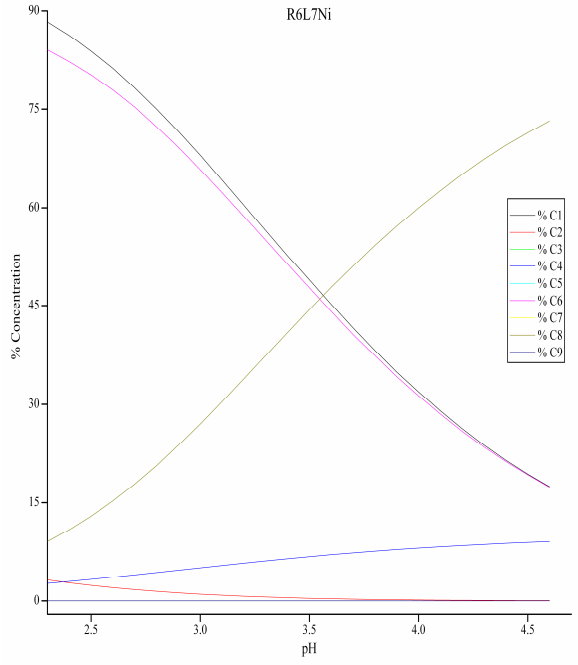

Figure 4

In case of $R_3L_7Ni(II)$ system. Fig.3.shows that, The initial percentage concentration of ternary complex MLR is 8.17 at pH 2.0 and increases slowly until it reaches to maximum value i.e. about 78 at pH 4.5. The percentage of HL and MR represented by C_1 and C_6 are highest at the initial stage i.e. 90 and 86 respectively. The concentration of these species decreases with increasing pH and reaches to minimum at pH 4.5. The decreasing trend of these species indicates that they are utilized in the formation of ternary complex. This is supported by the increasing concentration of ternary complex in the same pH range.

From this observation it may be concluded that the higher concentration of primary ligand sharply decreases because of its rapid dissociation, resulting into the formation of free ligand L. This species then interacts with MR to give final product MLR. The decrease in percentage concentration of primary ligand is slightly more as compared to that of MR. Therefore, it may be concluded that the excess formation of free ligand L species might be utilized in the formation of ML. Since its concentration also slightly increases with increasing pH. The initial concentration of ternary complex 8.17 is due to the initial reaction between M, L and R. The mechanism of formation of ternary complex by different equilibria discussed in previous Ni(II) system is totally applicable to the $R_6L_7Ni(II)$ system and the mixed ligand stability constant of this complex is 11.22 which is smaller than Ni(II)-L_7-R_3 system.

Table 2: Stability constants in ternary complexes of Ni(II).

L	R	β_{111}	β_{02}	β_{20}	K_L	K_R	Kr	ΔlogK
L_4	L-Leucine	12.2887	9.1013	11.6368	5.5563	6.7312	3.8393	-0.0012
L_4	DL-Phenyl alanine	11.3855	9.4963	11.6368	4.6531	5.9821	1.6379	-0.7503
L_7	L-Leucine	12.1213	9.1013	12.4654	5.5541	6.5638	2.6759	-0.0034
L_7	DL-Phenyl alanine	11.2205	9.4963	12.4654	4.6533	5.8171	0.4793	-0.7501

It has been observed from Table 2 that the stability constant of ternary complexes of L_4 are found to be higher than L_7. The positive values of $K_{L,}$ K_R and K_r indicate the stability of ternary complex. But the negative $\Delta \log K$ values indicate that the ternary complexes are relatively less stable than 1:1 binary complexes of primary as well as secondary ligands.

CONCLUSION

It is observed that the ternary complex formation is less favored over corresponding binary 1:1 complexes. It is possible due to availability of less number of coordination sites for secondary ligands on primary complex ML than on free metal ion.

REFERENCES

[1] Louie AY, Meade TJ, *Chem . Rev.*, **1999,** 99, 2711.
[2] Patel RN, Singh N, Shukla K, Chauhan UK, Chakraborty S, Gutierrez JN, Castineiras A, *J. Inorg. Biochem*., **2004,** 98**,** 231.
[3] Fairlamb AH, Henderson GB , Cerami A, Proc.Natl.Acad.Sci.U.S.A., **1989,** 86, 2607.
[4] Balcarova Z, Kasparakova J, Zakovska A, Novakova O, Sivo MF , Natile G, Brabeck V, *Mol. Pharmacol*., **1998,** 53**,** 846.
[5] LaFemina RL, *J. Virol*., **1992,** 66**,** 7414 .
[6] Moore PS, Jones CJ, *J. Biochem*., **1995,** 307, 129.
[7] Rixe O, Ortuzar W, Alvarez M, Parker R, Reed E, Paull K, Fojo T, *Biochem. Pharmacol*., **1996,** 52, 1855.
[8] Bakhtiar R, Ochiai EI, *Gen. Pharmacol*., **1999,** 32, 525.
[9] Hammud HH, Nemer G, Sawma W, Touma J, Barnabe P, Bou-Mouglabey Y, Ghannoum A, El-Hajjar J, Usta J, *Chem. Biol. Interact*., **2008,** 173, 84.
[10] Schubert J, *Sci. Am*., **1966,** 40**,** 214.
[11] Kirschner S, Wei YK, Francis D, Bergman JG, *J. Med. Chem*., **1966,** 9, 369.
[12] a) Livingstone SE, Nolan JD, Mihkelson AE, *Inorg. Chem*., **1968,** 7, 1447; b) Livingstone SE, Nolan JD, Mihkelson AE, *Inorg. Chem*., **1970,** 9, 2545.
[13] Rosenberg B, Van Camp L, Trasko JE, Mansour VH, *Nature*, **1969,** 222**,** 385.
[14] Martell E, Smith RM, *Critical Constants-1: Amino acids*, New York, **1974**.
[15] Sigel H, *Metal Ions in Biological Systems-2*, Marcel-Dekker, Inc., New York, **1973**.
[16] Beck MT, *Chemistry of Complex Equilibria*, Van Nostrand, New York, **1970,** 174.
[17] Lehninger AL, *Physical Rev.*, **1950,** 30, 393.
[18] Richards FM, Wyckoff HM, Allewel NM, *Neurosciences*, **1969,** 901.
[19] Dogan A, *Ind. J. of chem*., **2003,** 42A, 1632.
[20] Sekhon BS, Gandhi L, *J. Ind. Chem. Soc*., **2006**, 83**,** 868.
[21] Gary BS, Dwivedi P, *J. Ind. Chem. Soc*., **2006,** 83, 229.
[22] Sharma S, Shah M, Patel N, *J. Ind. Chem. Soc*., **2006,** 83, 620.
[23] Sanna D, Agoston G, Micera G, *Polyhedron*, **2001,** 20**,** 937.
[24] Lee CS, Benet LZ, *Anal Profiles Drug Subs*, **1978,** 7, 231.
[25] Martell AE, Calvin M, *Chemistry of metal chelate compounds*, Prentie Hall, Inc. Englad, Cliffs, N.J., **1962**.
[26] Rossotti FHC, Rossotti H, *The determination of stability constants*, McGraw Hill Book Co.
[27] Banerjee AK, Rao TVR, *J. Indian Chem. Soc*., **1968,** 63, 480.
[28] Irving H, William RJP, *J. Chem. Soc.*, **1953,** 3192.
[29] Deosarkar SD, Jahagirdar HG, Narwade ML, *Acta Ciencia Indica*, **2009,** Vol.XXXVC,No.2**,** 277.
[30] Pund DA, Bhagawatkar, Tayade DT, Rathod RB, *J. Ind. Chem. Soc*., **2010,** 3(2**)**, 246.
[31] Ananda Kumar B, Raju S, Bharath Kumar Naik K, Sailaja BBV, Nageswara Rao G, *Der Pharma Chemica*, **2011,** 3(5), 155.
[32] Lahsasni SA, Ammar RA, Amin MF, Shoukry EM, *Int. J. Electrochem. Sci*., **2012 7,**7699.
[33] Patil AB, *Rasayan J. chem*., **2012,** 5(4), 490.
[34] Mukherji GN, Chakraborty PK, *J. Indian chem. Soc*., **2001,** 78, 565.
[35] Sovgo I, Petocz G, *J. Chem. Soc., Dalton Trans*, **1987,** 1717.
[36] Gergely A, Farkas E, *J. C. S. Dalton*, **1981,** 747.
[37] Shankarwar SG, Shankarwar AG, Phase RP, Chondhekar TK, *Int. J. chem. Sci*., **2008,** 6(1), 385.
[38] Vogel AI, *"A Text Book of Practical Organic Chemistry"*, Pergamon Green and Co.Ltd.London, **1956**.
[39] Irving HM, Rossotti HS, *J. chem. Soc*., **1954,** 2904.
[40] Sayce IG, *Talanta*, **1988,** 15, 1397.
[41] Sayce IG, *Talanta*, **1972,** 19, 831.