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# Mixed ligand complex formation of Copper metal ion with some amino acids and drug Efavirenz in ethanol-water medium

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## Abstract

The stability constant of the mixed ligand complexes of Copper (II) ion with drug Efavirenzas primary ligand and eight amino acids glycine, DL-alanine, L-glutamic acid, DL-isoleucine, DL-methionine, DL-β-phenyl alanine, DL-serine and DL-valine as secondary ligands were determined potentiometric technique in 20% (v/v) ethanol-water medium at 27 °C and at an ionic strength of 0.1 M $NaClO_4$. The formation of complex species has been evaluated by SCOGS computer program and discussed in terms of various relative stability parameters.

**Keywords:** Stability constant, Efavirenzdrug, amino acids, mixed ligand complexes

## 1. Introduction

The stability of metal complexes with medicinal drugs plays a major role in the biological and chemical activity. Amino acids are essential constituents of all living cells and contain one or more amino and carboxylic groups and have good coordination sites for the metal complexation. Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) and is used as a part of highly active anti-retroviral therapy (HAART) for the treatment of human immune deficiency virus (HIV-1). Both nucleoside and non-nucleoside RTIs inhibit the same target. The reverse transcriptase enzyme transcribes viral RNA into DNA. Unlike nucleoside RTIs, which bind at the enzyme's active site, NNRTIs bind within a pocket, termed the NNRTI pocket.Efavirenz is not effective against HIV-2, as the pocket of the HIV-2 reverse transcriptase has a different structure, which confers intrinsic resistance to the NNRTI class. It is never used alone and is always given in combination with other drugs. It is a white to slightly pink crystalline powder and it is soluble in various organic solvents but practically insoluble in water. It is chemically (4*S*)-6-chloro - (cyclopropylethynyl)-1, 4-dihydro-4- (trifluoromethyl) - 2H-3, 1-benzoxazin-2-one.Efavirenz activity is mediated predominantly by non-competitive inhibition of HIV-1 RT.

In continuation of earlier work with complexation of medicinal drug[1-8], we study ternary complexes of Copper metal ion with medicinal drug Efavirenzas primary ligand and eight amino acids as secondary ligands in 20% (v/v) ethanol-water medium at 27 °C and at an ionic strength of 0.1 M $NaClO_4$.

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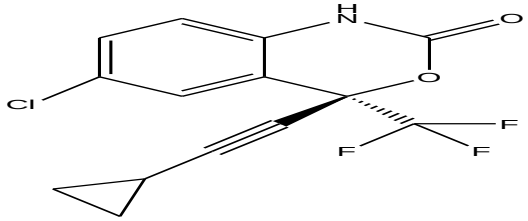

**Figure 1: Efavirenz(molecular formula $C_{14}H_9ClF_3NO_2$)**

## 2. Materials and Solution

The ligand Efavirenz is soluble in water. NaOH, $NaClO_4$, $HClO_4$ & metal salts were of AR grade. The solutions used in the potentiometric titration were prepared in double distilled water. The NaOH solution was standardized against oxalic acid solution (0.1M) and standard alkali solution was again used for standardization of $HClO_4$.The metal salt solutions were also standardized using EDTA titration. All the measurements were made at 27 °C in 20% ethanol-water mixture at 0.1M $NaClO_4$ strength. The thermostat was used to maintain the temperature constant. The pH measurements were made using a digital pH meter model Elico L1-120 in Conjunction with a glass and reference Calomel electrode. The pH-meter was adjusted with buffer of pH 4.00,7.00 and 9.18.

**Potentiometric procedure:** For evaluating the protonation constant of the ligand & the formation constant of the complexes in 20% ethanol-water mixture with different metal ions we prepared the following six sets of solutions.

(i) HClO4 (A)
(ii) HClO4+Drug (A+ L)
(iii) HClO4+Drug+ Metal (A+ L+ M)
(iv) HClO4+Amino acid (A+ R)
(v)HClO4+Amino acid + Metal(A+R+M)
(vi) HClO4+Drug+Amino acid+Metal(A+L+R+ M)

The above mentioned sets prepared by keeping M: L: R ratio, the concentration of perchloric acid & sodium perchlorate (0.1M) were kept constant for all sets. The volume of every mixture was made upto 50 ml with double distilled water. The test solutions were magnetically stirred, NaOH was added stepwise and pH reading was recorded. Graphs were obtained by plotting pH vs volume of NaOH added. These data were used to determine the pKa of ligands and logK values of metal complexes of primary and secondary ligands. The equilibrium constants of ternary complexes were calculated by using SCOGS program. The total concentrations of metal ions, free metals, free ligands and various possible species that are formed during complexation were obtained as computer output of program.

**Table 1:Proton-ligand stability constant and metal-ligand stability constant of Efavirenzdrug & amino acids with Copper(II) at 0.1M ionic strength in 20% (v/v) ethanol-water medium.**

| Ligands | Proton-ligand stability constant | | Metal-ligand stability constant | | |
|---|---|---|---|---|---|
| | $pK_1$ | $pK_2$ | $logK_1$ | $logK_2$ | $log\beta$ |
| Efavirenz | ……. | 10.7206 | 7.0282 | 6.8544 | 13.8826 |
| DL-Alanine | 2.5336 | 9.8082 | 8.8208 | 6.9970 | 9.8178 |
| Glycine | 2.5660 | 9.7850 | 8.9278 | 7.6977 | 16.6255 |
| Glutamic acid | 2.2732 | 4.4116 | 4.0564 | 3.4113 | 7.4677 |
| DL-Isoleucine | 2.5141 | 9.7599 | 9.3618 | 7.7585 | 17.1203 |
| DL-Methionine | 2.0793 | 9.3410 | 8.5045 | 7.1715 | 15.676 |
| β-Phenyl alanine | 2.2552 | 9.0546 | 8.1655 | 7.1612 | 15.3267 |
| DL-Serine | 2.1152 | 9.1066 | 8.3817 | 7.1235 | 15.5052 |
| DL-Valine | 2.5923 | 9.6759 | 9.0102 | 7.5889 | 16.5991 |

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**Table 2: Parameters based on some relationship between formations of mixed ligand complexes of Copper (II) with Efavirenzdrug and amino acids**

| Amino Acid | $\beta_{111}$ | $\beta_{20}$ | $\beta_{02}$ | $K_L$ | $K_R$ | $K_r$ | $\Delta\log K$ |
|---|---|---|---|---|---|---|---|
| DL-Alanine | 13.8083 | 13.8826 | 15.2778 | 6.7801 | 5.5275 | 0.9470 | -1.5007 |
| Glycine | 15.4550 | 13.8826 | 16.6255 | 8.4268 | 6.5272 | 1.0131 | -0.5010 |
| Glutamic acid | 11.0834 | 13.8826 | 7.4677 | 4.0552 | 7.027 | 1.0382 | -0.0012 |
| DL-Isoleucine | 14.6395 | 13.8826 | 17.1203 | 7.6113 | 5.2777 | 0.9443 | -1.7505 |
| DL-Methionine | 13.7823 | 13.8826 | 15.676 | 6.7541 | 5.2778 | 0.9325 | -1.7504 |
| β-Phenyl alanine | 13.6931 | 13.8826 | 15.3267 | 6.6649 | 5.5276 | 0.9375 | -1.5006 |
| DL-Serine | 14.9081 | 13.8826 | 15.5052 | 7.8799 | 6.5264 | 1.0145 | -0.5018 |
| DL-Valine | 15.5356 | 13.8826 | 16.5991 | 8.5074 | 6.5254 | 1.0193 | -0.5028 |

## 3. Result and Discussion:

*I.Binary complex*: The proton ligand stability constants (pKa) of drug and amino acids were calculated by point wise and half integral method. The metal ligand stability constant logK of Cu (II) transition metal complexes with Efavirenzdrug were calculated by using Calvin Bjerrum titration techniques as adopted by Irving and Rossotti.Titration curves were obtained for different sets. During titration no precipate was formed indicating that there is no tendency to form hydroxo complexes.The stability constants of the formed complexes were investigated in the pH range of 4-6.The mean value, the average number of protons associated with the ligand $\bar{n}_A$, at different pH values were calculated.The pKa values were determined from $\bar{n}_A$. Similarly $\bar{n}$ i.e metal ligand formation number, which can be defined as average number of ligand molecules co-ordinated to the metal ions,were also obtained using Irving &Rossotti method. The $\bar{n}$ values obtained between 0.2 to 0.8 indicates 1:1 complexation and when $\bar{n}$ lies in between 1.2 to 1.8 indicate 1:2 complexation.The values of proton ligand stability constants pKa and metal ligand stability constant logKare represented in Table 1.Since we got $\bar{n}_A$between 0.2 to 0.8 and 1.2 to 1.8 indicating 1:1 and 1:2 complex formation.The order of $\log K_1 > \log K_2$is commonly observed.The reason is statistical effect,statistically coordination of a second molecule is difficult when compare to the first due to availability of less number of coordinating sites on the metal ion for the second ligand.The standard deviation for various metal ligand system is 0.036. Irving and Rossotti have proposed a relation between the stability of the complexes and basicity of the ligand by equation

$$logK = apK + b$$

The relation graph shows a straight line and the value of slope should be unity for a series of closely related ligand.

***II. Mixed ligand complexes***: The formation of 1:1:1 mixed ligand complex were identified by the pH of precipitation of ML, MR and MLR titration curves. These curves indicate the higher value of pH of precipitation of ternary system than corresponding binary systems. The relative stabilities of mixed ligand complexes were quantitatively

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expressed in terms of ΔlogK, Kr, $K_L$ and $K_R$ values which are defined by equations:

$$\Delta logK = log\beta_{111} - (logK_{10} + logK_{01}) \quad (1)$$

$$K_r = \frac{\beta_{111}^2}{(\beta_{20}\beta_{02})} \quad (2)$$

$$K_L = \frac{\beta_{111}}{logK_{10}} \quad (3)$$

$$K_R = \frac{\beta_{111}}{logK_{01}} \quad (4)$$

Where $\beta_{111}$ is the equilibrium constant of ternary system. $\beta_{20}$ is the overall stability constant of primary complexes. $\beta_{02}$ is the overall stability constant of secondary complexs.

The equilibrium constants of ternary systems of Cu (II) transition metal ion and relative stability parameters are shows in Table 2. The ternary complexes of copper metal ions with Valine(15.53)show higher values of stability whereas Glutamic acid(11.08) ternary complexes show low values of stability. This may be attributed to the aliphatic nature of secondary ligand, steric effect and chelation formation.The order of stability of equilibrium constants of ternary systems of Cu(II) transition metal ion with respect of secondary ligand is Efavirenz: valine>gly.>serine >isoleu.>ala.>methionine >β-phenyl ala.>glut. Acid The comparison of $\beta_{111}$with $\beta_{20}$ and $\beta_{02}$ of these systems reveals the preferential formation of ternary complexes over binary complexes. The low positive values of $K_L$ and $K_R$ indicates less stability of ternary complexes with respect to binary complexes of primary as well as secondary ligands. The Kr value is positive but less, which indicates lower stability of ternary complexes. This may be attributed to the interactions outside the coordinated sphere such as formation of hydrogen bonding between coordinated ligands, charge neutralization, chelate effect and electrostatic interactions between non coordinated charge groups of ligands. The negative values of ΔlogK have been found in all systems, which show the formation of ternary complex but less stable and destabilized nature of complexes which has been reported in N and O coordination of amino acids. The negative value of ΔlogK may be due to the higher stability of its binary complexes, reduced number of coordination sites, steric hindrance, electronic consideration, difference in bond type, geometrical structure etc.

Thompson and Lorass pointed out that more negative ΔlogK value of ternary complexes is due to the electrostatic repulsion between the negative charge on the ligand and amino acids. Steric hindrance consideration is the most important factor because in the present studies of ternary complex, primary ligand coordinates with the metal ion in the lower pH range and form 1:1 and 1:2 complex. In solution, ternary complex forms as the titration curve run below the Cu (II)-drug titration curve, it is evident that the entry of the secondary ligand amino acids faces steric hindrance due to bigger size of the Cu (II)-drug complex as compared to aquo ion, which tries to restrict the entry of the secondary ligand in the coordination sphere of the Cu (II) metal ion and thus reduces the stability of ternary complexes.

***III. Species distribution curves:*** Accordingto the result given by SCOGS computer programme, the concentration of different species distributed are as follows:

$$C_1 = HL \rightleftharpoons H + L \quad (1)$$
$$C_2 = HR \rightleftharpoons H + R \quad (2)$$
$$C_3 = M + L \rightleftharpoons ML \quad (3)$$
$$C_4 = M + R \rightleftharpoons MR \quad (4)$$
$$C_5 = M + L + R \rightleftharpoons MLR \quad (5)$$

The species distribution curves of Cu(II)LR systems were obtained by plotting percentage concentration of various possible species formed during complexation vs pH of solution as shown in figure 2. In Cu(II)LR ternary systems, primary as well as secondary ligands forms 1:1 and 1:2 binary complexes. The species distribution curves of free metal (M), free ligands L and R indicates that there is a slowly decrease in concentration of free metal ions with increase in pH whereas increase in concentration of ligands with pH and indicates higher percentage concentration of FL than FR. The species distribution diagram of Cu

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(II)LR system shows the formation of mixed ligand complexes. The concentration for the formation of drug (L) and HR represented by $C_1$ and $C_2$ show continuous decrease with increasing pH. The concentration of $C_5$ species continuously increases, confirm the formation of ternary complexes Cu (II)LR.

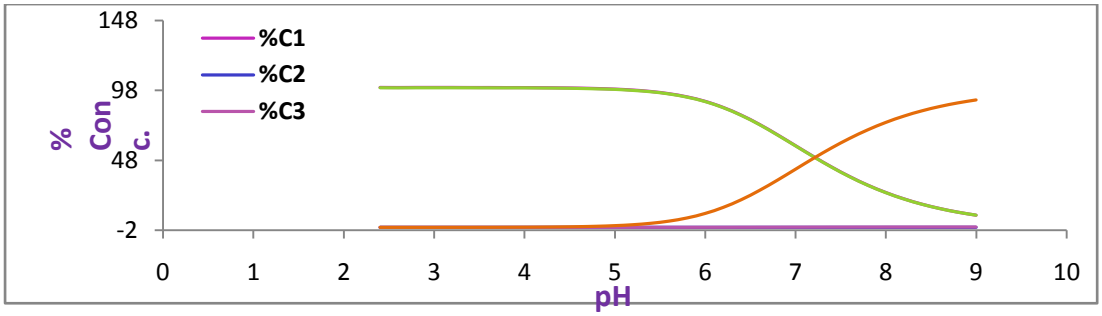


**Figure 2: Species distribution curve of Cu (II) $LR_8$ system ( pH versus % conc. of various possible species)**

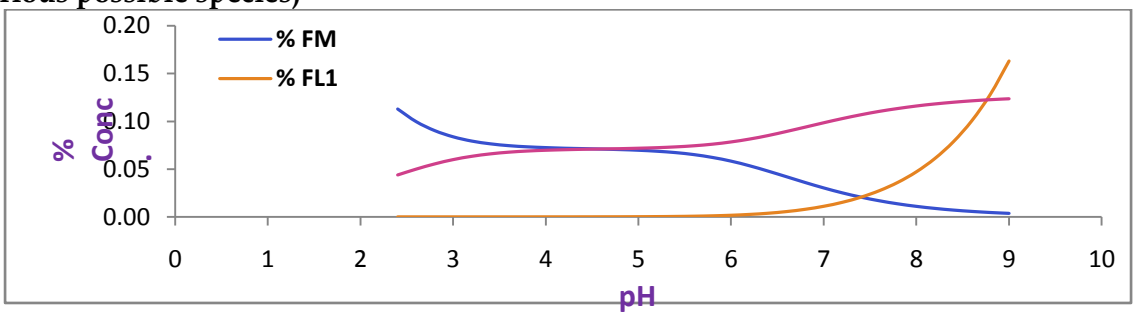


**Figure3 :Species distribution curve of Cu (II) $LR_8$ system (pH versus % conc. of free metal & free ligands)**

## 4. References

1. S.V. Thakur, MazaharFarooqui and S.D.Naikwade, ***Asian J Biochemical & Pharma Res.***,3(3): 34-43, 2013.
2. S.V. Thakur, MazaharFarooqui and S.D.Naikwade, ***IntJPharmTech Res***.,5(4): 1508-1515, 2013.
3. Shailendrasingh Thakur, M.A. Sakhare, MazaharFarooqui and S.D.Naikwade,***J Basic& Applied sci.***,10(1): 48-51, 2013.
4. Shailendrasingh Thakur, S.D. Naikwade and MazaharFarooqui, *J Medicinal Chem& Drug discovery*(Special Issue), 107-118, 2015.
5. R.L. Ware, MazaharFarooqui and S.D.Naikwade, *Int J Emerging Tech in Computational & Applied Sci*.5(2):123-128, 2013.
6. R.L.Ware, Shoeb Peerzade, S.D. Naikwade and MazaharFarooqui,*J Chemical & Pharma Res.*,5(8): 59-63, 2013.
7. ShailendrasinghThakur,S.A. Peerzade,A.J.KhanandR.L.Ware, *InternationalMultilingual Research Journal****Printing Area*** (Special Issue),47-51, Dec.2017
8. S.V. Thakur,M.A.Sakhare,S.N.Sampal and H.U.Joshi,International *Multilingual Research Journal****Printing Area*** (Special Issue),169-173, Dec.2017