# Prediction of Chemical Speciation of Essential Metal ion Complexes of L-Tyrosine in Ethylene glycol and Dimethylformamide media

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## *ABSTRACT*

*Chemical speciation of complexes of Co(II), Ni(II) and Cu(II) with L-tyrosine has been studied spectrophotometrically. The complexations were carried out at different pH ranges at the wavelengths of respective complexes. The stoichiometries of the complexes were determined using Job's continuous variation method and the value was found to be 1:2 metals to ligand ratio. Stability constant values were calculated using the continuous variation method. The complexes were found to be stable over the pH range used as there was slight change in the color intensity and absorbance values.*

***Keywords**:* Cobalt(II), Nickel(II), Copper(II), Spectrophotometry, Stability constants, Toxicity.

## INTRODUCTION

L-Tyrosine is synthesized in the body from phenylalanine and it is a direct precursor of adrenaline and thyroid hormones. Metabolic transformations of tyrosine require the presence of folic acid, niacin, vitamin C and copper. Its metabolic products include melanin, estrogen and encephalin. Tyrosine is used along with tryptophan to aid in the treatment of cocaine abuse and may also be useful in the control of anxiety or depression [1-4].

The supplementation of feed with L-tyrosine is efficacious in cases where high requirements for tyrosine as a melanin precursor occur. This has been demonstrated in cats for intensively coloring the coat. L-Tyrosine may also have the potential to intensify the pigmentation of the coat/plumage of other species, but limited evidence is available [5].

L-Tyrosine is usually absorbed in the proximal small intestine. Any L-tyrosine reaching the large intestine is decarboxylated to tyramine, a biogenic amine, in the human gut. Inadequate degradation (detoxification) of formed tyramine by the gut monoamine oxidases can lead to tyramine entering the systemic circulation. Tyramine acts as a vasopressor [6-7]; it is known to be a cause of migraine headaches in humans [8]. Very recently, for a single oral administration in healthy individuals, a no observed adverse effect level (NOAEL) of 200 mg tyramine/person has

been proposed, based on a literature survey [9]. In contrast, individuals with reduced monoamine oxidase activity may suffer hypertension after ingestion of < 5 mg tyramine [10]. Intellectual deficit problems seem to occur when human neonates receive abnormally high quantities of L-tyrosine [11].

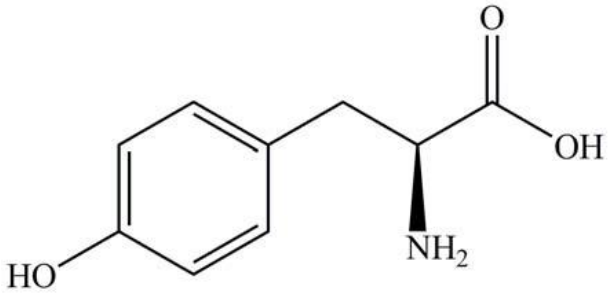


**Fig. 1:** Molecular structure of L-tyrosine

| **Description** | | | |
|---|---|---|---|
| Composition, description | Chemical formula | Purity criteria (if appropriate) | Method of analysis (if appropriate) |
| L-Tyrosine | $C_9H_{11}NO_3$ | L-Tyrosine content not less than 95.0 % on the additive | dosage in feedingstuffs: HPLC, Commission regulation (EC) No 152/2009 |

Cobalt(II), nickel(II) and copper(II) are associated with several enzymes [12-13] and any variation in their concentration leads to metabolic disorders [14]. Hence speciation study of essential meta lion complexes is useful to understand the role played by the active site cavities in biological molecules and the bonding behavior of protein residues with the metal ions. In biological fluids, metal ions exist in non-exchangeable form as in metallo-proteins or loosely bound to some bioligands as in metal-activated proteins. The loosely bound metal ions are in equilibrium with simple metal ions present in the bio-fluids. These simultaneous equilibria involving a variety of metal ions and ligands are important in bio-fluids [15].

## MATERIALS AND METHODS

All the reagents used in this work were of analytical grade purity and were used without further purification. All weighing were carried out using Shimadzu TX223L analytical balance, while spectrophotometric measurements were obtained on UV-Visible Spectrophotometer. 0.1M $CoCl_2.6H_2O$, $NiCl_2.6H_2O$ and$CuCl_2.2H_2O$ were prepared in a $100cm^3$volumetric flask each. Similarly, 0.1M solution of L-tyrosine was prepared in $100cm^3$ volumetric flask. The stoichiometric ratio of M(II) to ligand in the complexes were determined by Job's continuous

variation method. Series of volumes (1cm$^3$) of the 0.1M of M(II) solutions were pipetted into four 50cm$^3$ volumetric flasks and aliquot (2 cm$^3$) of the 0.1M ligand were added, keeping the mole fraction of the solution constant. The absorbances of each of the solution was taken at the wavelength of maximum absorbance of the complex which was initially determined by varying the wavelengths from 200-400 – 800nm.

## UV–vis measurements

The optical densities of some metal-complexes were measured by UV–vis spectrophotometry at specific pH values. The UV-visible spectra were taken using a Shimadzu SP65 UV Visible spectrophotometerin 200-800 nm range using a 1.0 cm quartz cell pathlength at a controlled temperature of 25±0.1 ˚C with a Cole–Parmer bath.

## RESULT AND DISCUSSION

The results of species and Step-wise protonation constants of M(II)- L-Tyrosine in DMF and EG-water mixtures along with some important statistical parameters are given in Table 1&2.

**Table 1: Stability constants of L-tyrosine complexes with Co(II), Ni(II) and Cu(II) in DMF-water mixtures.**

| | **Log $K_{MLH}$** | |
|---|---|---|
| % V/V | 111 | 110 |
| **Co(II)** | | |
| 0.0 | 8.65 | 13.00 |
| 10 | 8.55 | 12.90 |
| 20 | 8.50 | 12.75 |
| 30 | 8.88 | 15.72 |
| 40 | 7.80 | 10.50 |
| 50 | 7.70 | 10.35 |
| 60 | 7.60 | 10.25 |
| **Ni(II)** | | |
| 0.0 | 8.65 | 13.00 |
| 10 | 8.18 | 14.53 |
| 20 | 10.97 | 15.31 |
| 30 | 8.61 | 13.69 |
| 40 | 9.23 | 12.77 |
| 50 | 11.10 | 15.48 |
| 60 | 17.74 | 21.44 |
| **Cu(II)** | | |

| | | |
|---|---|---|
| 0.0 | 8.65 | 13.00 |
| 10 | 7.30 | 14.18 |
| 20 | 9.79 | 17.54 |
| 30 | 9.88 | 19.73 |
| 40 | 10.25 | 18.24 |
| 50 | 11.01 | 15.68 |
| 60 | 17.39 | 20.78 |

**Table 2: Stability constants of L-tyrosine complexes with Co(II), Ni(II) and Cu(II) in EG-water mixtures.**

| | **Log $K_{MLH}$** | |
|---|---|---|
| % V/V | 111 | 110 |
| **Co(II)** | | |
| 0.0 | 8.65 | 13.00 |
| 10 | 12.43 | 16.43 |
| 20 | 10.11 | 14.24 |
| 30 | 11.42 | 15.87 |
| 40 | 9.43 | 13.82 |
| 50 | 12.99 | 13.96 |
| 60 | 7.80 | 14.82 |
| **Ni(II)** | | |
| 0.0 | 8.65 | 13.00 |
| 10 | 14.88 | 18.46 |
| 20 | 6.08 | 13.86 |
| 30 | 15.43 | 14.52 |
| 40 | 9.25 | 17.74 |
| 50 | 10.01 | 15.63 |
| 60 | 9.26 | 14.65 |
| **Cu(II)** | | |
| 0.0 | 8.65 | 13.00 |
| 10 | 9.26 | 15.71 |
| 20 | 6.69 | 13.00 |
| 30 | 12.60 | 16.56 |
| 40 | 9.79 | 14.25 |
| 50 | 9.19 | 15.65 |
| 60 | 8.04 | 14.85 |

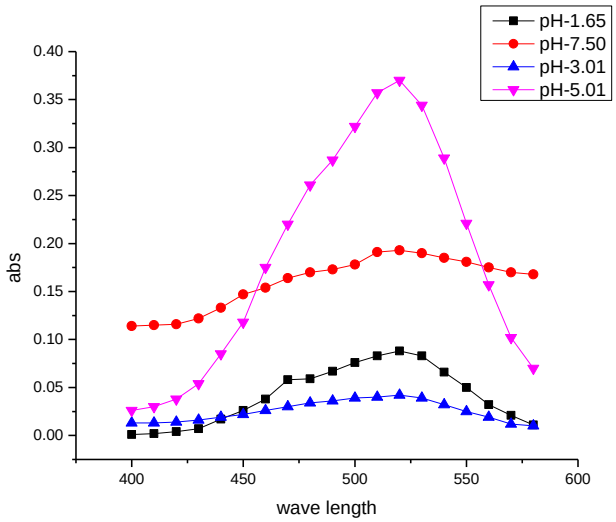


Fig.2. Absorption spectra of Co(Tyro)

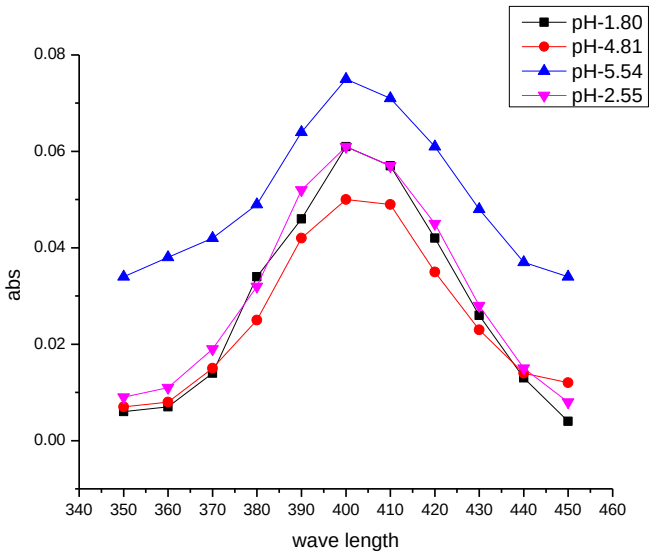


Fig.3. Absorption spectra of Ni(Tyro)

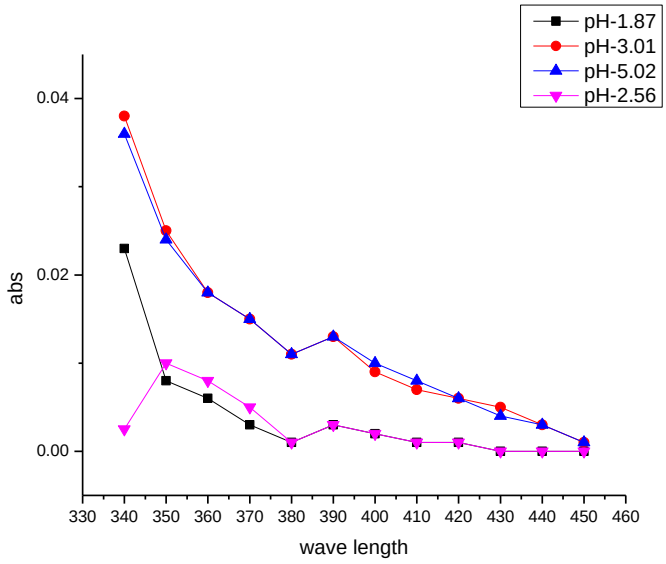


Fig.4. Absorption spectra of Cu(Tyro),1:2 metals to ligand ratio

## Job's Method of Continuous Variation

The stoichiometric composition of the coordination compound is determined using Job's continuous variation method. The method consists of mixing equimolar solutions of metal and ligand in varying proportions in such a manner that the total concentration of the metal and the ligand is constant in the resulting mixtures. The absorbance of a series of mixtures is measured at a suitable wavelength. If x mole/liter of L are added to (1 - x) mole/liter of M and if C1, C2 andC3 are the equilibrium concentrations in moles/liter of M, L and $ML_n$, respectively, the following relations hold good

C1={(l-x)-C3}

C2={x-nC3}

$C3= C1.C_2^n$

The number of L's(n) attached to metal ion can easily be determined from the position of the maximum or minimum in the graph. If the complex species are coloured and the metal ion and the ligand are colourless, the plots of absorbance versus composition will give a maximum at the composition of the complex. Job's method is ordinarily applicable to systems in which only one complex is present. Gould and Vosburgh have, however, shown that even if a second equilibrium exists in addition to the main reaction, Job's condition is obeyed. The method is applicable to systems which obey Beer's Law.

The stoichiometric composition of the complex is found to be 1:2 (metal: ligand) in all the Co(II), Ni(II) and Cu(II) L-Dopa chelate complexes (Fig. 5).

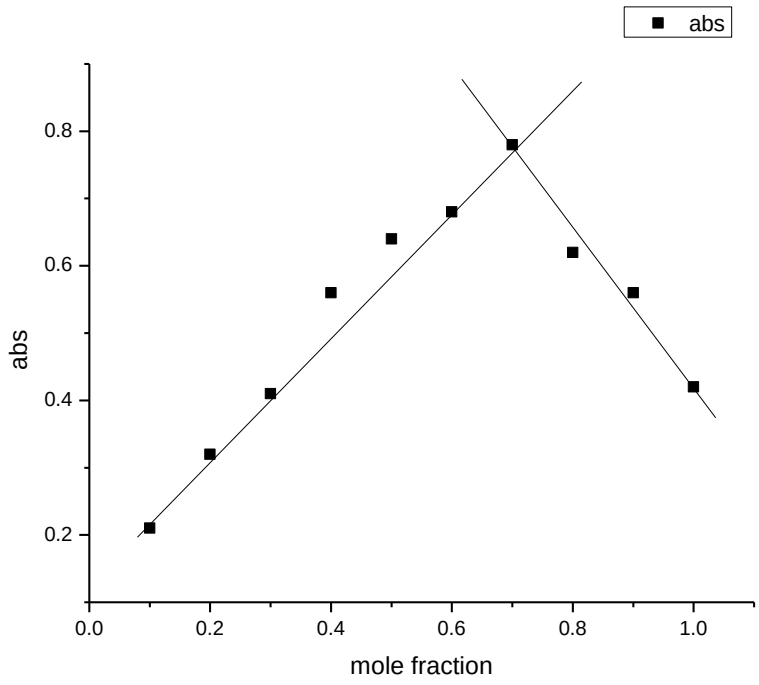


Fig. 5. Mole Ratio Determination (Job's Method) for Co(II)-L-tyro complex

## Calculation of Stability Constants

The formulae of highly colored complex ions can be determined in solution using spectrophotometric techniques. Consider the formation of coordination complex $[ML_n]^{z+}$ from a metal ion $M^{z+}$ and a ligand L (Eq. 1):

$$M^{z+} + nL^{o} \rightleftharpoons [ML_n]^{z+} \quad (1)$$

Assuming that no other species in solution is absorbing radiation of a particular frequency, the Beer- Lambert law (Eq. 2) can be used to calculate the concentration of the complex ion:

$$A_{obs} = [\varepsilon_M c_M + \varepsilon_L c_L + \varepsilon_{MLn} c_{MLn}]l \quad (2)$$

$A_{obs}$ = total absorbance of all species in solution
l= pathlength of the cell (taken as 1 cm in this experiment)
$\varepsilon_M$, $\varepsilon_L$, $\varepsilon_{MLn}$= molar extinction coefficients of the absorbing species
$c_M$, $c_L$, $c_{MLn}$ = concentration of the absorbing species

The conditional stability constants of the complexes were determined from Job's curves Fig 2. Optical density of solution prepared by taking a large excess of ligand (metal to ligand ratio) can be considered as first approximation, to be due to the complex. Knowing the optical

density, one may determine values for the extinction coefficient of the complex with the help of Beer's Law. From this value, it is possible to calculate the concentration of the complex in various mixtures and obtain the value of stability constant[43].The conditional stability constants of a complex can be determined from Job's curves. The two methods usually adopted are as follows

**Distribution Diagrams**

The percentage of metal ion in the form of various complex species is given by equation

$$PS = \frac{m(j) * \beta_{m(j)l(j)h(j)} * FL_i^{l(j)} * FH_i^{h(j)}}{\sum_{j=0}^{N} \beta_{m(j)l(j)h(j)} * FL_i^{l(j)} * FH_i^{h(j)}} * 100$$

This information is highly useful in choosing ingredient concentrations to increase or decrease the concentrations of selected species in DMF- and EG- water mixtures. The variations of species are shown in Figs.6-8 for metal-Tyro complexes in DMF- and EG-water mixtures.

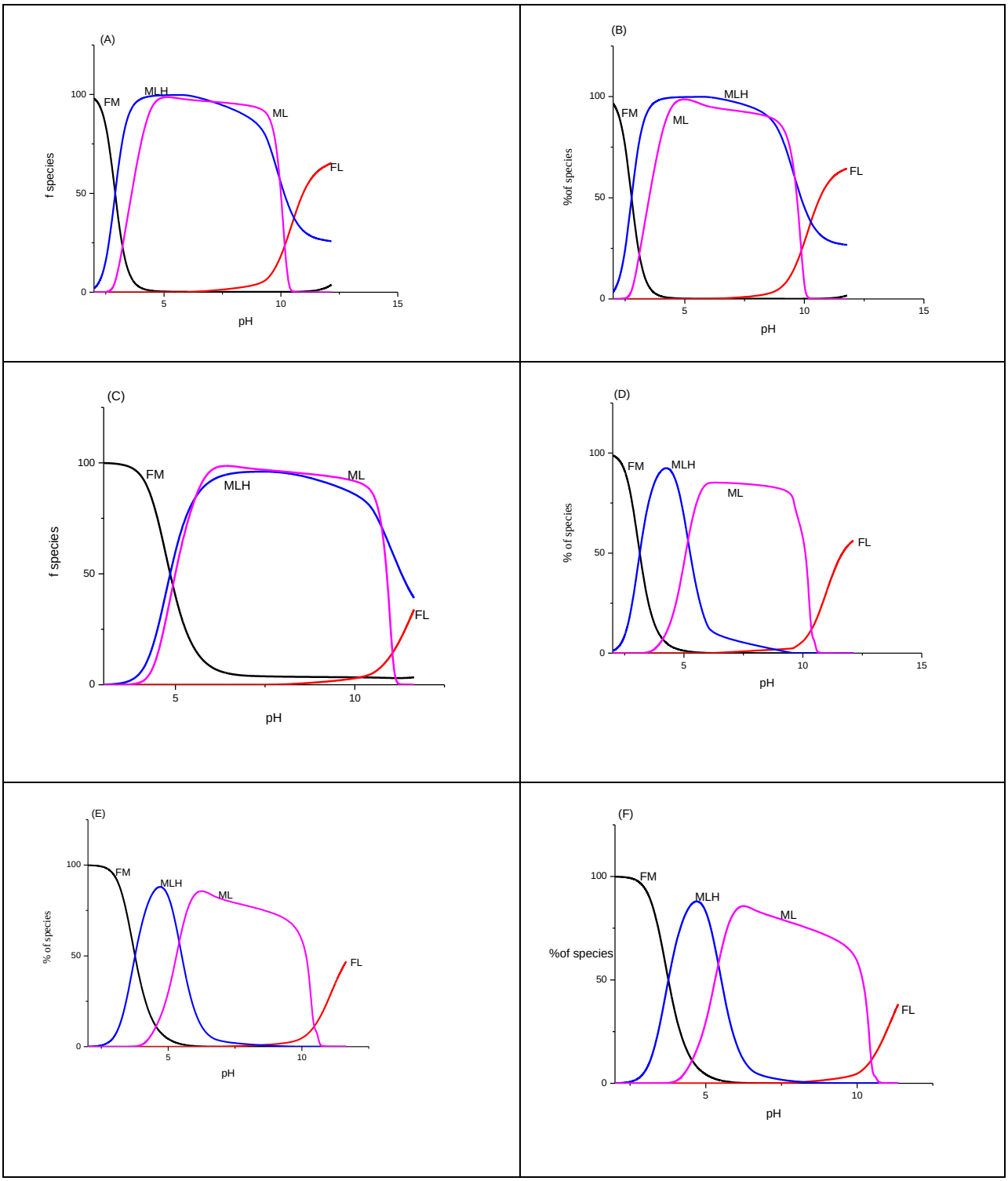


**Fig.6:** Distribution diagrams of Co(II)-L-tyrosine in (A) 10.0 (B) 30.0 (C) 50.0 (% v/v) DMF-water mixtures (D) 20.0 (E) 40.0 (F) 60.0 (% v/v) EG-water mixtures.

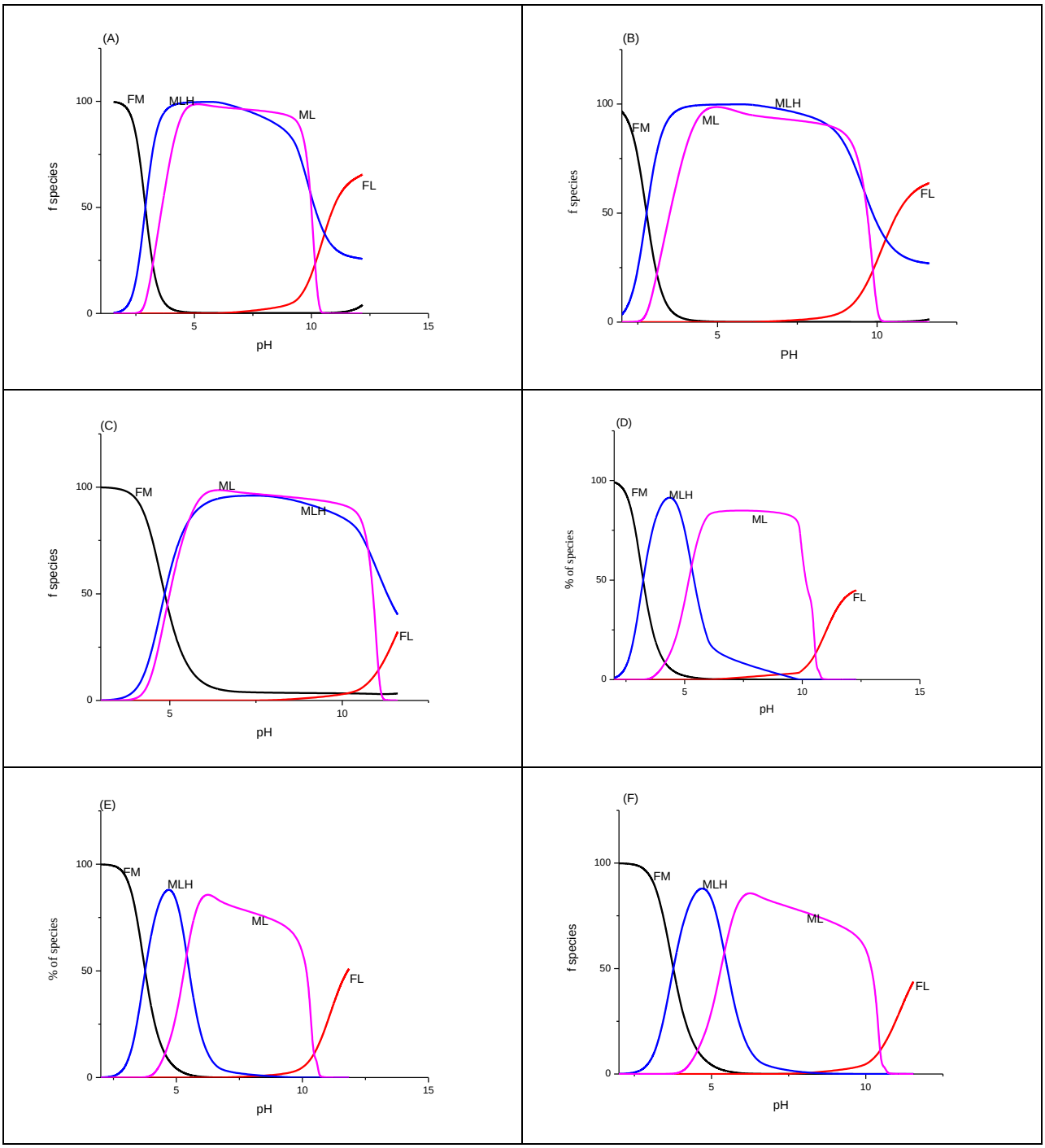


**Fig.7:** Distribution diagrams of Ni(II)-L-tyrosine in (A) 10.0 (B) 30.0 (C) 50.0 (% v/v) DMF-water mixtures (D) 20.0 (E) 40.0 (F) 60.0 (% v/v) EG-water mixtures.

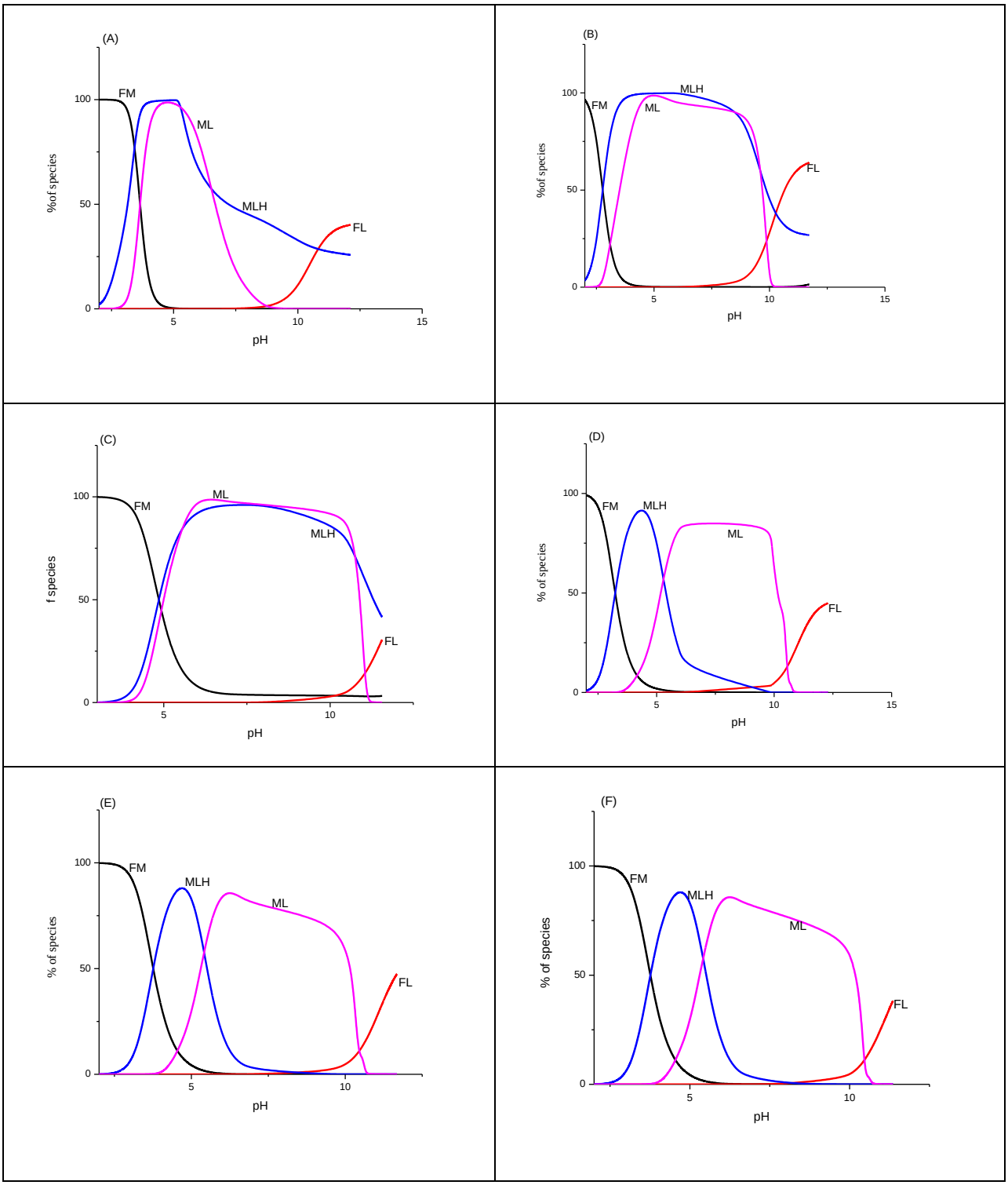


**Fig.8:** Distribution diagrams of Cu(II)-L-tyrosine in (A) 10.0 (B) 30.0 (C) 50.0 (% v/v) DMF-water mixtures (D) 20.0 (E) 40.0 (F) 60.0 (% v/v) EG-water mixtures.

# STRUCTURES OF COMPLEXES

Structures of L-Tyrosine complexes with Co(II), Ni(II) and Cu(II) where S is either solvent or water molecules are shown in Fig.9.

**L-Tyrosine - Metal Complexes**

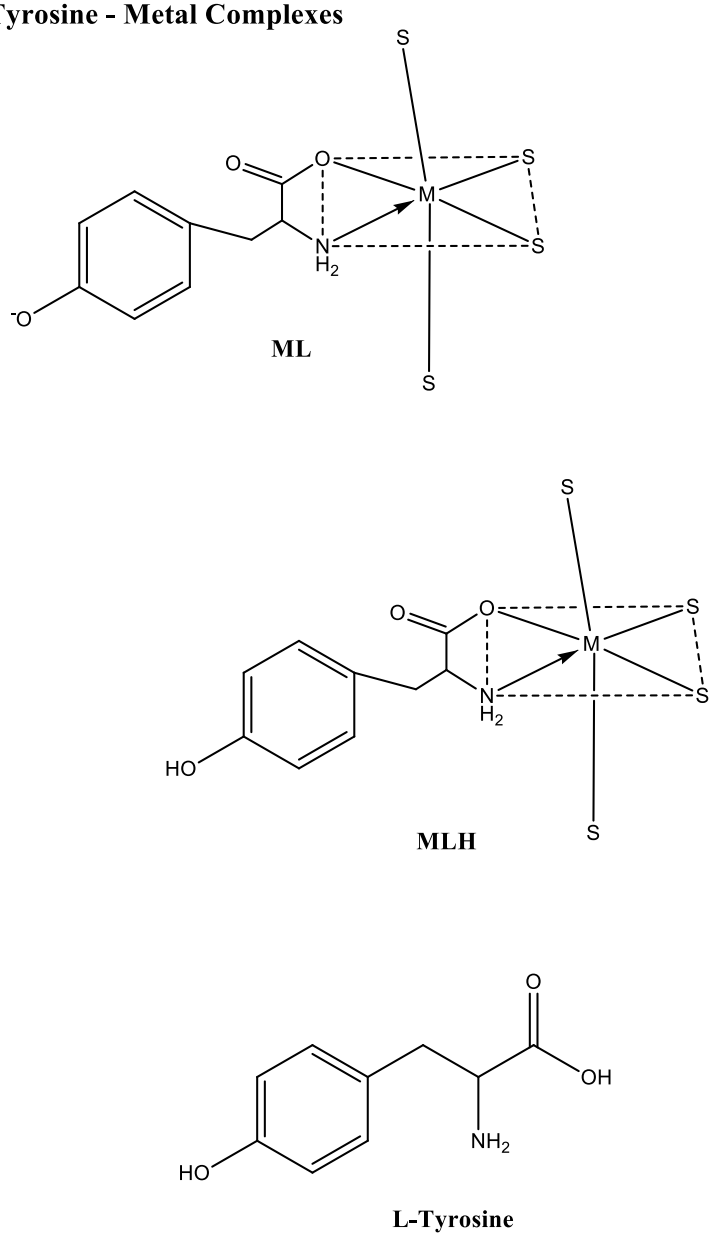


**Fig. 9. Structures of L-tyrosine complexes, where S is either solvent or water molecules.**

# CONCLUSIONS

The aim of the present study is to probe into the nature of interactions and speciation of the complexes of Co(II), Ni(II) and Cu(II) with Tyro in DMF- and EG- water mixtures. The following conclusion can be drawn based on the speciation studies performed on these systems.

1. The binary species refined for the Tyro complexes of Co(II), Ni(II) and Cu(II) are ML and MLH in both the (DMF- and EG- water) media. These models are validated by statistical treatment of the data.

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