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pH Effect on Stoichiometry and Stability of Ferrous Complexes of (-)-3-(3,4-dihydroxyphenyl)-L-alanine

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ABSTRACT
Living organisms contain many bio-molecules that are comprised of oxygen and nitrogen donor atoms and therefore have high affinity for metal ions, such as iron, present in the body. The stability constants of these chelates are very high in general. One of such molecules is (-)-3-(3,4-dihydroxyphenyl)-L-alanine which is present in brain to control the normal function of neurons. In case of neurodisorder disease, Parkinson's, this molecule is orally administered in the form of tablets. The pH of stomach is acidic and blood is about neutral, therefore in the present work, the chelation, stoichiometry and stability of iron complexes of (-)-3-(3,4-dihydroxyphenyl)-L-alanine is studied at acidic to neutral pH. Reaction of this molecule in vitro showed interesting results. Stoichiometry of the complex is found 1:3 and it remains pH independent (in buffered & non buffered solutions).While stability of the complex increases with the rise of pH as revealed by molar extinction coefficient values.

Keywords: Iron, Levodopa, stoichiometry, pH effect, molar extinction coefficient.

1. INTRODUCTION
Metals by themselves and in proper balance to one another have important biochemical and nutritional functions[1-2]. The bioavailability of these minerals is also a very important factor, depending on the food source and also upon the pH of stomach[3-4].

Iron plays significant role for normal brain and nerve function through its involvement in the synthesis of neurotransmitters[5]. Severe iron deficiency anemia may result in many diseases[6].On the other hand dopamine is considered basic factor, responsible of behavioral changes in neurodisorder disease[7-9]. Iron and dopamine both play significant role in normal function of brain. Also the excess iron is found in brain in Parkinsonian patients but still the relation of iron and dopamine is ambiguous[10-11].

Dopa of dihydroxyphenyl alanine is a precursor of dopamine and norepinephrine. It is levorotatory stereoisomer of dopa[12] while d-enantiomer leads to clinical side effects[13]. Levodopa is (-)-3-(3,4-dihydroxyphenyl)-L-alanine, (Fig. 1) is a derivative of Amino acid (alanine) containing catechol moiety. Catechol has high affinity for metal ions specifically iron, therefore Levodopa is expected to chelate iron strongly[14-17].

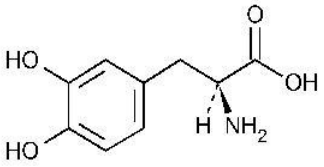

Fig-1: (-)-3-(3,4-dihydroxyphenyl)-L-alanine Commonly known as Levodopa

In the beginning part of the project, the complex formation of (-)-3-(3,4-dihydroxyphenyl)-L-alanine with iron was carried. Fe^{2+} forms intense color complex with (-)-3-(3,4-dihydroxyphenyl)-L-alanine having absorbance maxima in visible region. Spectral characteristics of the said complex, stoichiometry and effect of pH on complex formation are explored. Current research is carried to investigate the stability of complex at low pH, which in turn is helpful to understand the chelation of iron with administered Levodopa in stomach.

2. EXPERIMENTAL
A. R. grade reagents were used for all the reactions. CO_2 free distilled deionized water was used to prepare the solution.

2.1 *Absorbance maxima:*
To explore wavelength of the maximum absorbance the complex of Fe^{2+} (-)-3-(3,4-dihydroxyphenyl)-L-alanine, 0.005mmol of Fe^{2+} salt solution was mixed with enough excess of levodopa solution (prepared in deionized distilled water). The stock solution was subjected to scanning in UV-visible region on GENESYS 6 (Thermo Electron Corporation). Using the resultant spectra, suitable wavelengths were selected for further study. The complex solution showed absorbance maxima at two wavelengths; 430nm and 730nm. The metal and ligand solutions have no absorbance at these wavelengths (Fig. 2). All further work was carried out on both wavelengths.

2.2 *Molar extinction coefficients:*
Different dilutions of the stock complex solution were then prepared in deionized distilled water. Absorbance of all diluted solutions was recorded at selected wavelengths (430 and 730nm). ε was determined as the slope of straight line

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on a graph plotted between recorded absorbance against metal concentration. The same work was repeated using buffer of pH 3.0, 4.0, 5.0 and 6.0.

2.3 *Mole ratio*

Accurate amount of Ferrous ammonium sulfate was taken to prepare metal solution in deionized distilled water, while the ligand solutions were prepared in buffer solutions of desired pH. At each pH, different aliquots of ligand solution were added in 0.005mmol metal solution and volume was kept constant for all. The absorbance was recorded at 430nm and 730nm, while temperature was maintained at 25±1°C.

3. RESULTS AND DISCUSSION

3.1 *Spectral Characteristics*

A green colored complex of Fe(II) and levodopa was formed at pH 4.0, 5.0 and 6.0 at 25±1°C except pH 3.0. Absorbances were recorded at both the selected wavelengths. Spectra in Fig. 3 show drastic change with varying pH (Fig. 3). No green color was observed in buffer of pH 3.0. Spectra in the above mentioned range has no peak.

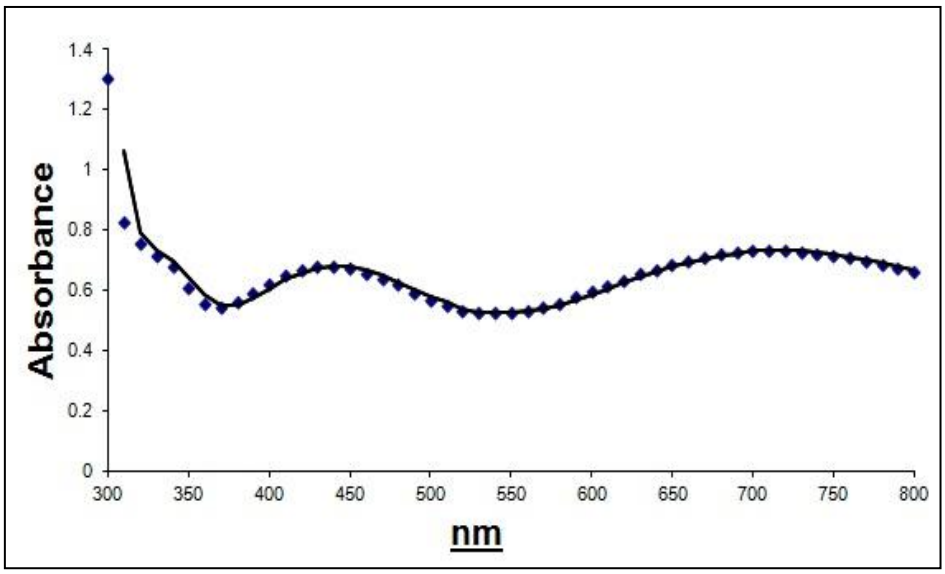

Fig-2: Spectra of Fe (II) complexed with Levodopa in non-buffered aqueous solution. Fe(II) = 0.05mmol, 10 fold ligand solution

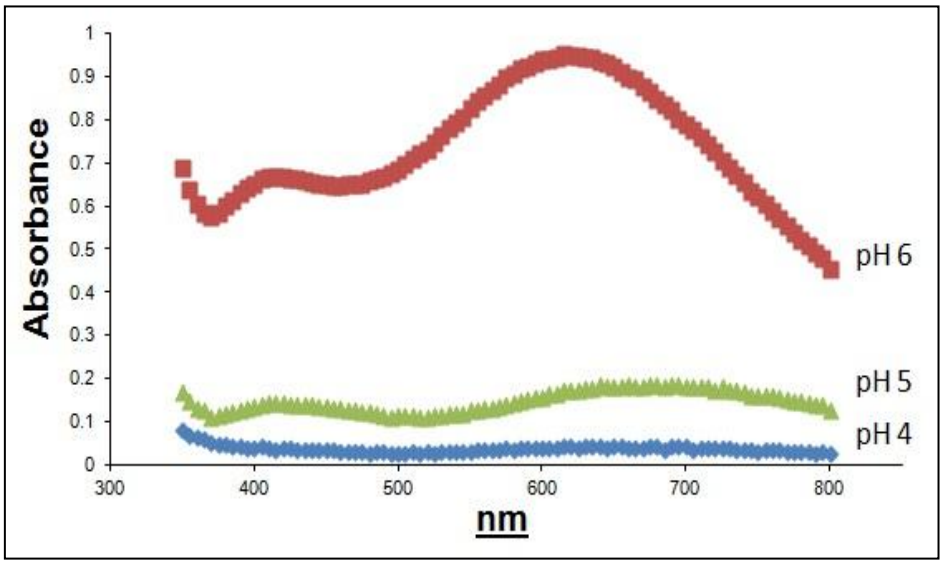

Fig-3: Spectra of Fe (II) complexed with Levodopa, pH 4.0, 5.0 and 6.0 buffer solutions. Fe (II) = 0.005 mmol, 10 fold ligand solution

3.2 *Molar Absorptivity*

Fe(II) and levodopa formed a complex at pH buffer 4.0, 5.0 and 6.0 at 25+1°C and absorbance was recorded at all the selected wavelengths and molar absorptivities were evaluated (Figure 4-5,Table-1). Two peaks at 430 and 730nm were found in aqueous non buffered media as well as in buffer of pH 4.0, 5.0 and 6.0. The value of on these selected wavelengths increases simultaneously with pH. However in pH 6.0 buffered solutions, peak shift is observed

and the maximum value of □ is found at 615nm, that is equal to 2500M-1cm-1. In all cases □ at 430nm is found a little higher than the □ at 730nm.

Table-1: Molar Absorptivity of Fe(II)-Levodopa Complex at different pH at all Selected wavelengths

pH	Molar Absobtivity ($M^{-1}cm^{-1}$)		
	430nm.	730nm.	615nm.
Non buffered	237	302.6	----
4.0	783.1	633.7	----
5.0	866.8	796.2	----
6.0	1834	1735	2528

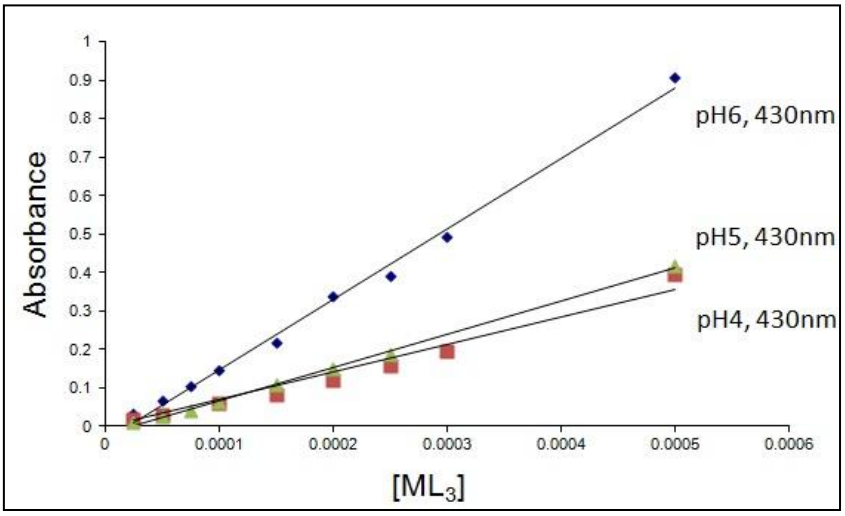

Fig-4: Molar Absorptivity of Fe (II)-Levodopa Complex, pH 4.0, 5.0 & 6.0, λ = 430nm.

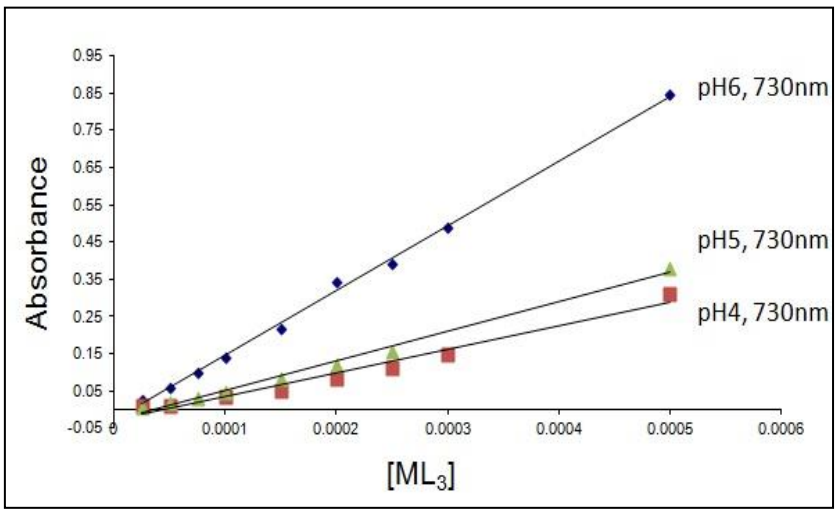

Fig-5: Molar Absorptivity of Fe (II)-Levodopa Complex, pH 4.0, 5.0 & 6.0, λ = 730nm.

3.3 *Stoichiometry*

Applying mole ratio method, In each set, different aliquots of ligand solution were added to 0.005mmol Fe (II) solution in order to get 0.5-10 times L:M mole ratio. Gradual increase was observed in absorbance with the increase of ligand to metal mole ratio (Fig 6). At high concentration of ligand the absorbance is independent of Ligand concentration. Tangent drawn on the curve in Figure 6-9 gives a value of ~3 showing a 1:3 metal to ligand ratio in the complex.

$$[Fe(H_2O)_6]^{2+} + H_2LD \longrightarrow [Fe(H_2O)_4LD] + 2H_3O^+$$

$$[Fe(H_2O)_4LD] + H_2LD \longrightarrow [Fe(H_2O)_2(LD)_2]^{2-} + 2H_3O^+$$

$$[Fe(H_2O)_2(LD)_2]^{2-} + H_2LD \longrightarrow [Fe(LD)_3]^{4-} + 2H_3O^+$$

Stoichiometry of Fe (II) and Levodopa Complex was further studied on pH 3.0, 4.0, 5.0 and 6.0. Consistency was found in the results as shown in the Figure 7-9 (Table-2). At all the considered pH ML_3 complex is formed regardless of the solution pH.

Table-2: pH effect on the Stoichiometry of Complex

pH	Stoichiometry $Fe^{2+}(LD)_3$
Aqueous (non buffered)	1:3
4.0	1:3
5.0	1:3
6.0	1:3

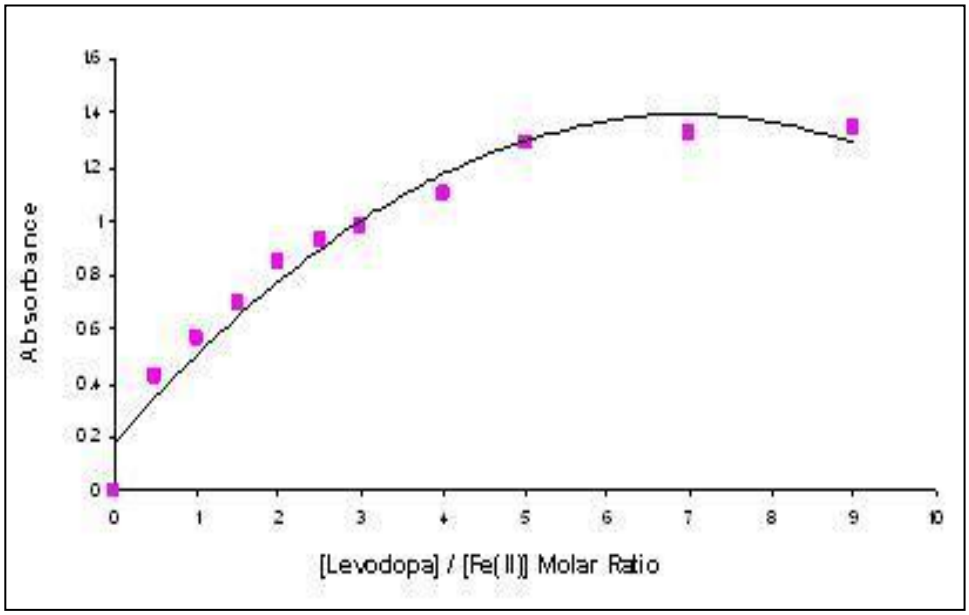

Fig-6: Stoichiometry of Fe (II)-Levodopa Complex in non-buffered aqueous solution. Fe (II) = 0.005mmol, λ = 730nm.

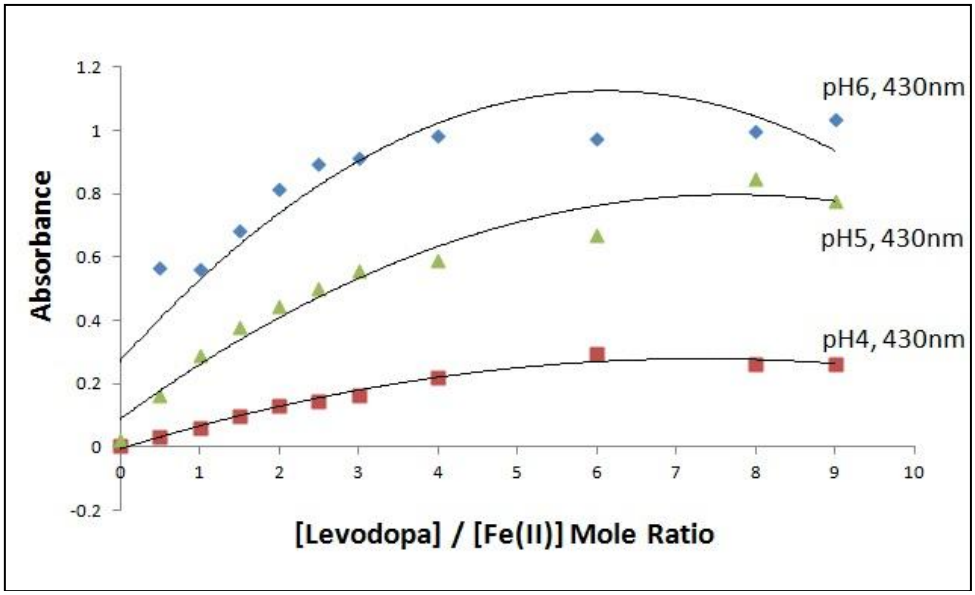

Fig-7: Stoichiometry of Fe (II)-Levodopa Complex, pH 4.0, 5.0 and 6.0. Fe (II) = 0.005mmol, λ = 430nm.

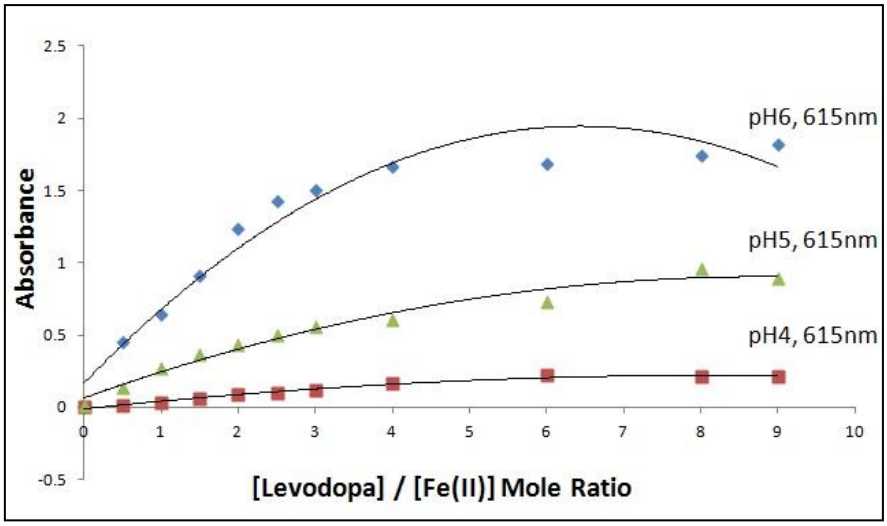

Fig-8: Stoichiometry of Fe (II)-Levodopa Complex, pH 4.0, 5.0 and 6.0. Fe (II) = 0.005mmol, λ = 615nm.

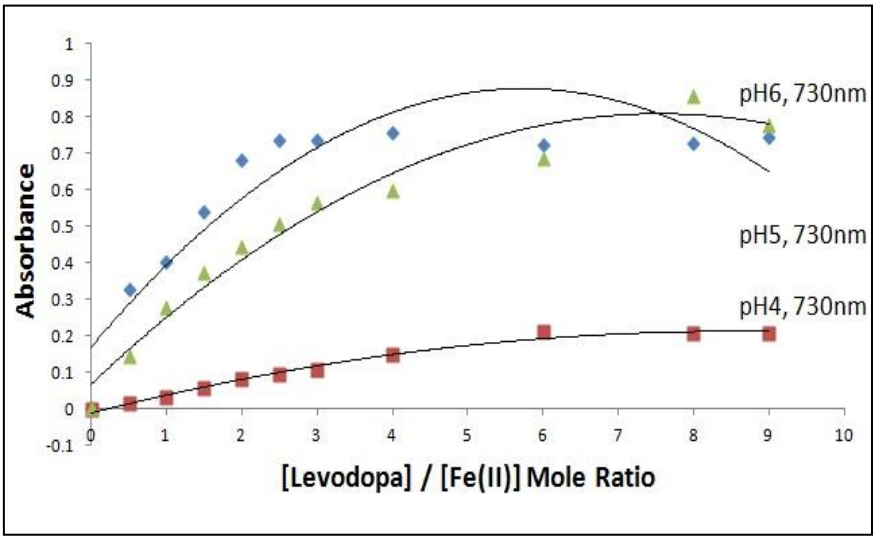

Fig-9: Stoichiometry of Fe (II)-Levodopa Complex, pH 4.0, 5.0 and 6.0. Fe (II) = 0.005 mmol. λ = 730nm.

4. CONCLUSIONS

Complex formation of Fe (II) L-dopa is studied at pH 3.0, 4.0, 5.0 and 6.0 buffered and also in non buffered media. Two distinct peaks were found in complex spectra, one at 430nmand another at 730nm. In pH 6.0 buffer, the 730nm peak sharpens while shifted to 615nm.

At the selected wavelengths, Fe (II):H_2L mole ratio plot showed an ML_3 complex formation in spite of pH; evident of stability of the investigated complex. The same result is verified by molar extinction coefficient values that increase with the increase of pH. In ML_3 complex, three levodopa molecules occupied all six positions around the metal in an octahedral molecule. Thus levodopa acts as bidentate ligand forming a strong chelate.

Levo-dopa forms very strong complex with Fe(II) at stomach pH. The result is consistent with literature. It showed that the Levodopa in drug molecule is chelated by iron and therefore may not efficiently reach to its required destination.

The question arises whether this chelation helps the L-dopa to cross blood brain barrier or not. This important point requires further investigation to be explored.

Since pH of brain is approximately 10. Work at high pH may provide imperative information about chelation of Fe(II) by this molecule in brain. In-vitro study at high pH is in progress and will soon be published.

5. ACKNOWLEDGEMENT

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