A COMPARATIVE STUDY FOR CHELATION OF IRON(II) AND IRON(III) WITH LEVODOPA - AN ANTIPARKINSONIAN DRUG MOLECULE

Syed Zafar Abbas Zaidi[a] and Nasreen Fatima[a]

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In the present study interaction of Fe(II) and Fe(III) with antiparkinsonian drug molecule, Levodopa (LD), is investigated using potentiometry and spectrophotometry. Identical spectra of both of Fe(II) and Fe(III) complexes of the drug provide an evidence that similar stoichiometry was followed. Molar absorptivities of the complexes, found to be more than 100 M^{-1} cm^{-1}, showed charge transfer spectra. Addition of an antioxidant decolorized the initial intensive color of the Fe(III) complex, which was evidence of high oxidation state of iron. Catecholic ligands, being strong reductants, chelate a metal ion in high oxidation state and show LMCT bands. These observations lead to conclude that iron has high oxidation state, regardless of initial source of metal.

*Corresponding Authors
Tel: 03153237699 and 03002659510
E-Mail: mehar_zafar@yahoo.com
[a] Department of Chemistry, University of Karachi, B-12
Eastern square, Federal – B area, block-1, Karachi, Pakistan

INTRODUCTION

Dopamine analogs and iron have an unquestionable and significant role in brain function.[1-5] Iron storing protein, ferritin[6-7] is known to be present in axons of neuronal cells. Dopamine is an important neurotransmitter and its deficiency is considered to be the basic factor responsible for behavioral changes in neuro-disorders like Parkinson's disease. On the other hand iron is also considered to be one of the symptom of Parkinson disease as its accumulation is observed in Parkinsonian brain.[8] However, it has been reported that iron chelators have an ability to reduce the symptoms of Parkinson.[1-2]

Levodopa (-)-3-(3,4-dihydroxyphenyl)alanine[3-5] is a precursor of dopamine and norepinephrine[9] in the biosynthetic pathways for these neurotransmitters and these derivatives regulate a variety of physiological functions in the human body.[10] It may act as chelator for iron and is present in the different medications used for the Parkinson's disease. It has two binding sites, a catecholic and an alanine site (Fig.1). In the present study chelation ability of Levodopa towards iron has been explored.

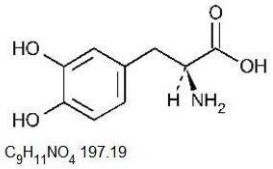

Figure 1. Structure of (-)-3-(3,4-dihydroxyphenyl)alanine (Levodopa)

The most important factor of the bioavailability of iron depends upon food sources that may restrain its absorption in the human body.[11-13] Iron exists in two forms in a living system. These are its reduced form Fe(II) and the oxidized one Fe(III). Fe(II) is the bioessential oxidation state of this metal.

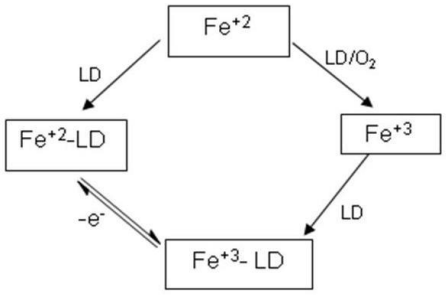

Figure 2. Possible tedox reactions in the Fe-Levodopa system

The present literature lacks in the studies related to the interaction of iron and dopamine or its analogues. Therefore we selected to investigate the chelation capabilities of different oxidation states of iron with Levodopa. Spectral characteristics of some similar complexes and their stoichiometry have already been reported.[14-15] Using these data the kinetic aspects of these complexes have also been looked into.

EXPERIMENTAL

Reagents of analytical grade were used for all the reactions. Iron salts ($FeCl_3.6H_2O$ and $Fe(NH_4)_2(SO_4)_2.6H_2O$) were of Merck and Levodopa was obtained from Wild Wind. For the preparation of the stock and sample solutions, CO_2 free distilled deionised water was used.

Potentiometric titrations

Experiment, for both cases, was performed in triplicate, having the constant ligand/metal mole ratio, i.e. 5:1, at 25 ± 1 °C. 0.05 mmol metal solution was mixed with 0.25 mmol of LD solution. The volume of reaction mixture was made up to 50.0 ml with deionised distilled water, taken in a double walled titration cell kept on a magnetic stirrer. The rubber stopper on the cell had holes for the addition of standard base, thermometer and for a glass electrode. Aliquots of standard NaOH (0.1 M) were added with the help of a micropipette.

The pH changes, recorded on a JENWAY 370, were then plotted against added volume of standard NaOH solution. The equilibrium constants for each specie (ML_1, ML_2 and ML_3)**,** were calculated by circle fitting method. Furthermore, the *pK* values were calculated through the pH titration curves. The pK_1, pK_2 and pK_3 were also calculated by using the graphical method (Table 1) to correlate with the reported Levodopa ((-)-3-(3,4-dihydroxyphenyl)-L-alanine) *pK* values.[17]

Table 1. pK values evaluated by potentiometric titration method.

Complex	pK_1	pK_2	pK_3
Fe(II)-LD	*Unidentified*	7.21	9.94
Fe(III)-LD	6.42	6.60	13.81

Unidentified = the case in which humps are so close that it cannot be distinguish

Absorbance maxima

Absorbance maxima of the complex, was explored by mixing 0.005 mmol of the salt solution, with enough excess of levodopa solution. It was prepared in deionized distilled water. Following that the solution was subjected to scanning in UV-visible region on GENESYS 6 (Thermo Electron Corporation) and that λ_{max} were established to be as 430 and 730 nm, for both cases i.e. Fe(II) and Fe(III) complexes. The metal and ligand solutions have no absorbance at these wavelengths. All further work was carried out on both λ_{max}.[14-15]

Molar extinction coefficients (Serial Dilution)

Solutions of different dilutions were prepared in the de-ionized distilled water. Absorbance was recorded for all diluted solutions at selected wavelengths i.e. 430 and 730 nm. Plot of absorbance for different dilutions against metal concentration, provided the slope for determining molar extinction coefficient.

Mole ratio

Accurate amounts of the metal salts, Fe(III) and Fe(II) and the ligand were taken to prepare respective solutions in deionized distilled water. Different aliquots of ligand solution were added in 0.005 mmol metal solution and volume was kept constant for all. The absorbance was recorded at 730 nm, while temperature was maintained at 25±1 °C.[15]

Slope ratio

For this method the working solution was such that the sequence of the complex solution was split in two halves. In one half volume of 5×10^{-4} M Fe(II) was kept constant, while varying the volume of 5×10^{-3} M Levodopa, and in the other half of the samples sequence, LD was kept constant, with variable concentration of metal. The complex samples were scanned at the respective λ_{max} and the recorded absorbance was plotted verses concentration of varying specie. Similar process was followed for Fe(III) and slope of each straight line was evaluated. The ratio of the slopes helped to establish the stoichiometry of the respective complexes.

Job's plot

The solutions were prepared by mixing metal and ligand solution by continuous increase of one ingredient with the similar decrease of second ingredient. Absorbance of all samples was recorded at the λ_{max} and by plotting graph between metal composition and respective absorbances, stoichiometry was determined.

Kinetics

For the determination of rate of reaction, different concentrations of Fe(II) and Fe(III) complex solutions were observed at different time intervals. Kinetics of Fe(III)-LD complex was followed on RX20000 Rapid kinetics accessories Stop flow apparatus of Applied Photo Physics. k_{obs} was then evaluated from the slope of plot drawn between $\ln|A_t\text{-}A_\infty|$ and time.

RESULTS AND DISCUSSION

Potentiometric titrations

Potentiometric curve of Levodopa is shown in Figure 3. The plot of pH change on addition of standard NaOH to Fe(II) complex solution shows two prominent curves near pH 4.5 and 7.5. Depression in titration curve of the complex as compared to the ligand was very prominent, which confirmed the complex formation (Fig.4). These results substantiate the varying stoichiometry with the change of pH. Against that for the case of Fe(III), depression and twists in titration curves were found at pH 3, 4 and 11 (Fig.5).

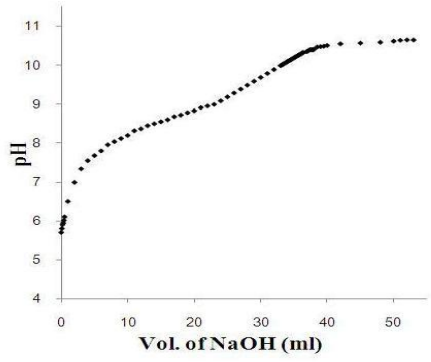

Figure 3. Potentiometric titration plot of Levodopa with NaOH

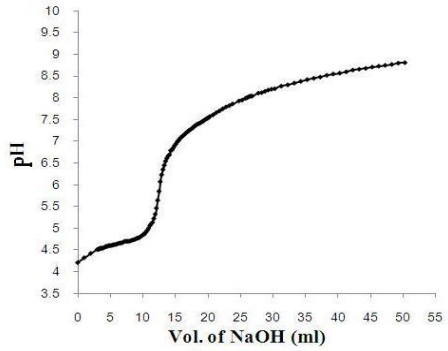

Figure 4. Potentiometric titration plot of Fe(II)-Levodopa with NaOH

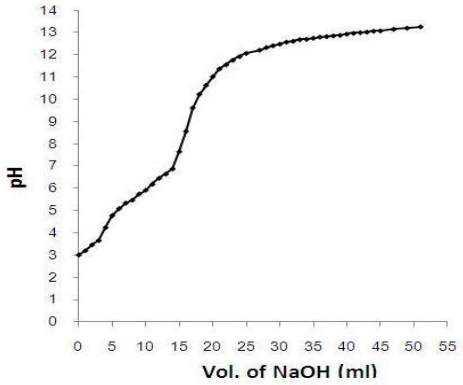

Figure 5. Potentiometric titration plot of Fe(II)-Levodopa with NaOH

Absorbance maxima

U.V. visible spectroscopy was used to investigate the spectral characteristics, complexation of Fe(II) and Fe(III) with Levodopa. For this purpose iron complexes of LD in the volumetric ratio of 1:5 were scanned spectrophotometricaly in the range of 300 to 800 nm.

A green colored complex of Fe(II) and levodopa was formed at 25+1 °C within 3 to 4 minutes with continuous increase of absorbance while in the case of Fe(III) the color appears abruptly within seconds and fades sharply. Absorbances were recorded at both the selected wavelengths. Observed spectras revealed that Fe(II) and Fe(III) complexes of LD show an absorbance maxima in the visible region (Fig.6).

In order to confirm the hypothesis that green colored complex with Levodopa is formed in +3 oxidation state of iron, complex of Fe(II)-LD was treated with a reducing agent (ascorbic acid). This resulted into immediate color loss, indicating that Fe(II) was first converted to Fe(III) by the aerial oxidation and that Levodopa worked as catalyst for this reaction. Distinct peaks of Fe(II) and Fe(III) complexes of LD were observed at 430 and 730 nm.

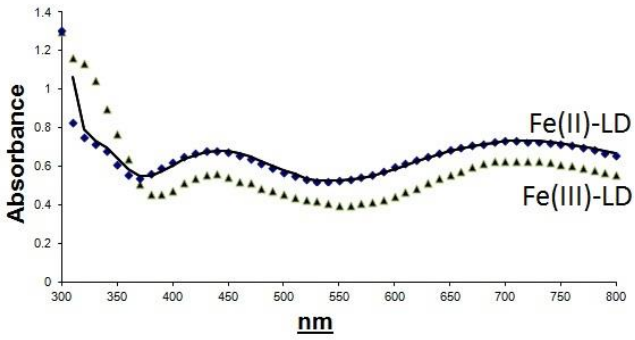

Figure 6. UV-VIS spectra of Fe(II)-LD and Fe(III)-LD complexes

Molar extinction coefficients (serial dilution)

Molar absorptivity of Fe(II)-LD complex was investigated using serial dilution method (Table 2). Absorption maxima in a non buffered aqueous medium where identified to be at 430 nm and 730 nm. At 430 nm molar absorptivity was found to be 237.03 M^{-1} cm^{-1}. The trend line at 730 nm is comparatively higher than that of 430 nm and provides a molar absorptivity of about 302.6 M^{-1} cm^{-1} (Fig.7).

Table 2. Molar extinction coefficients by serial dilution method.

Complex	Wavelength (nm)	ε (M^{-1} cm^{-1})
Fe(II)-LD	430 nm	237
Fe(II)-LD	730 nm	302.6

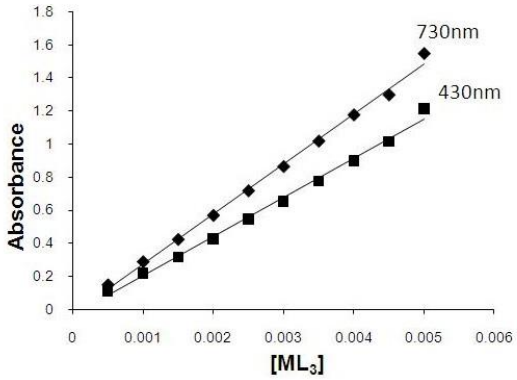

Figure 7. Molar absorptivity by serial dilution method in non-buffered medium; [Fe(II)-LD_3]=5.0x10^{-4} M,=25+/-1 °C; λ_{max}=430 and 730 nm.

Mole ratio

The plots of absorbances against the mole ratio of Fe(II) and Fe(III) complex with LD guide us to suggest a mole ratio of 1:3 for Fe(II)-LD as well as for the complex of Fe(III)-LD (Fig.8-10). The complexation of Fe(III) with Levodopa is faster than that of Fe(II) complexation. The study was therefore verified through a stop flow apparatus and the same mole ratio was found. Molar absorptivity (Table 3) and formation constant (Table 5) were also evaluated by mole ratio method.

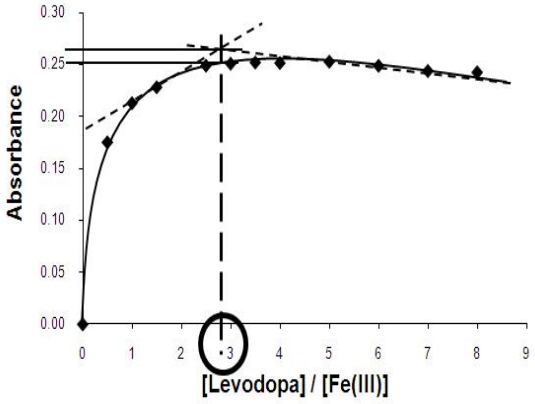

Figure 8. Stoichiometry by mole ratio method of Fe(III)-LD in non-buffered medium; [Fe(III)]=5.0x10^{-4} M; T=25+/-1 °C; λ_{max}=430 nm

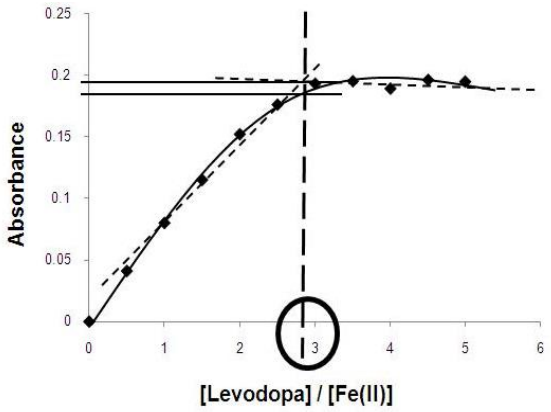

Figure 9. Stoichiometry by mole ratio method of Fe(II)-LD in non-buffered medium; [Fe(II)]=5.0x10^{-4} M; T=25+/-1 ºC; λ_{max}=430 nm

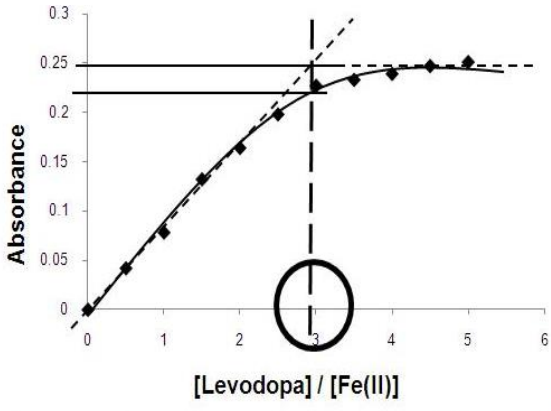

Figure 10. Stoichiometry by mole ratio method of Fe(II)-LD in non-buffered medium; [Fe(II)]=5.0x10^{-4} M; T=25+/-1 °C; λ_{max}=730 nm

Table 3. Molar extinction coefficients by mole ratio method.

Complex	Wavelength	ε (M^{-1} cm^{-1})
Fe(III)-LD	730 nm	788
Fe(II)-LD	430 nm	380
Fe(II)-LD	730 nm	498

Slope ratio

The stoichiometric results from slope ratio method were found 1:3 (Table 4), in good agreement with mole ratio method (Fig.11-12).

Table 4. Stoichiometry by slope ratio method of Fe(III)-LD and Fe(II)-LD in non-buffered medium.

Metal Complex	Wavelength (nm)	Stoichiometry from slope ratio (L/M)
Fe(III)-LD	730	3.1
Fe(II)-LD	730	2.9

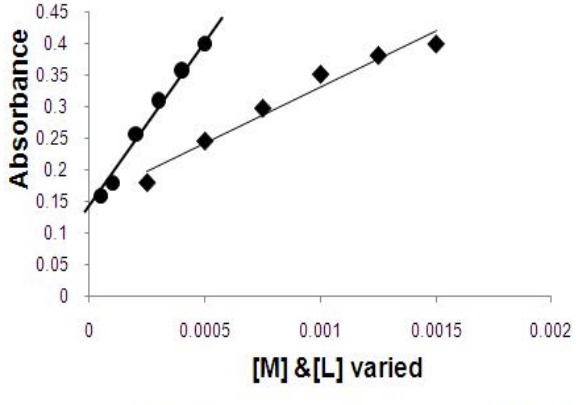

Figure 11. Plots of slope ratio method of Fe(III)-LD, in non-buffered medium; Absorbance vs. concentration of variable reagent, T=25+/-1 °C; λ_{max}=730 nm; Variable:♦ [LD]; ● [Fe(III)]

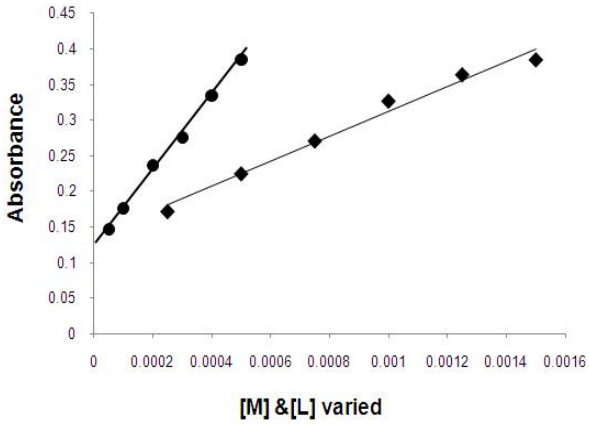

Figure 12. Plots of slope ratio method of Fe(II)-LD, in non-buffered medium; Absorbance vs. concentration of variable reagent, T=25+/-1 °C; λ_{max}=730 nm; Variable:♦ [LD]; ● [Fe(II)]

Job's plot

Job's plot method also verified the stoichiometry ratio i.e. 1:3 in both the cases of Fe(II) and Fe(III) complexation with Levodopa (Fig.13). Overall formation constant of Fe(II)-LD at 430 and 730 nm were also evaluated (Table 5).

Table 5. Overall Formation constants by mole ratio and Job's plot method.

Metal complex	Wavelength (nm)	log K_f values	
		mole ratio	Job's method
Fe(III)-LD	730 nm	9.75	-----
Fe(II)-LD	430 nm	11.95	9.61
Fe(II)-LD	730 nm	11.45	10.31

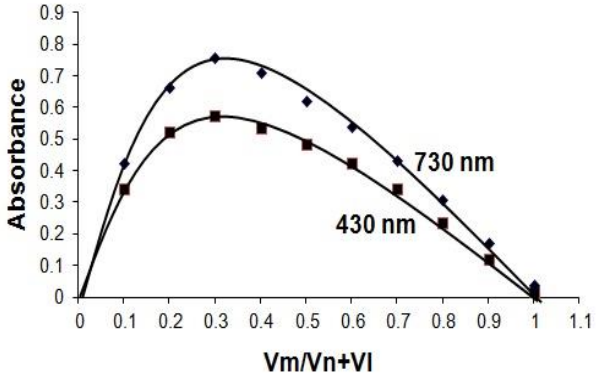

Figure 13. Stoichiometry by Job's method of Fe(II)-LD, in non-buffered medium; [Fe(II)]=5.0x10^{-4} M; T=25+/-1 °C; λ_{max}=430 and 730 nm

The observations obtained by all of the three methods given above were consistent and suggested 1:3 stoichiometry for the complexes of Fe(II) and Fe(III) with Levodopa.

Kinetics

In the kinetic study, rate constant values were plotted against concentration of Levodopa. A direct relationship was found in the case of Fe(III)-LD complex, and that k_{obs} increases with the rise in the ligand concentration (Fig.14). Similar trend was observed in case of Fe(II)-LD complex at lower concentrations of the ligand. However at higher concentration the k_{obs} of complex become independent of ligand concentration (Fig.15). This demonstrates that Fe(II)-LD Kinetics goes through two pathways and it follows two step mechanism i.e. A ligand dependent along with a ligand independent pathway (Fig.2).

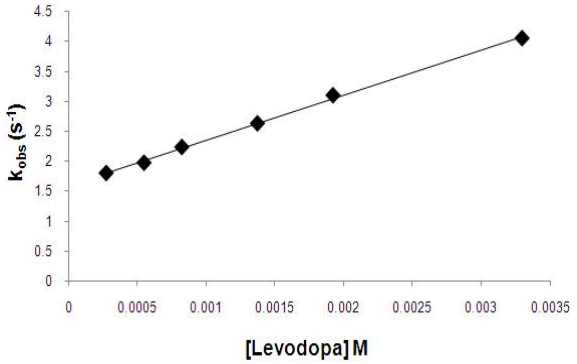

Figure 14. Graph of observed rate constant dependence of Fe(III)-LD complex in non-buffered medium; [Fe(III)]=5.0x10^{-4} M; T=25+/-1 °C; λ_{max}=730 nm

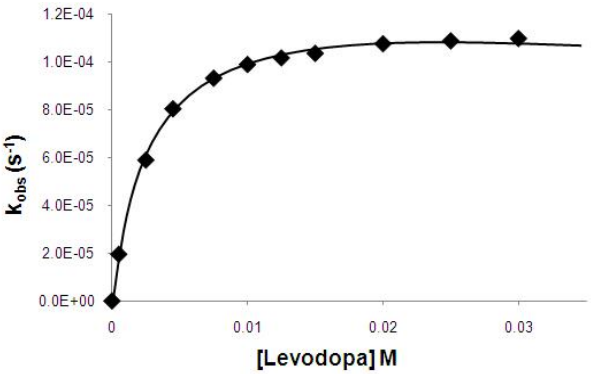

Figure 15. Graph of observed rate constant dependence of Fe(II)-LD complex in non-buffered medium; [Fe(III)]=5.0x10^{-4} M; T=25+/-1 °C; λ_{max}=730 nm

CONCLUSION

Interaction of antiparkinsonian generic drug i.e. Levodopa, has been studied with +2 and +3 oxidation states of iron. Spectral analysis revealed that both the states of iron, form green color complex with Levodopa with the same spectral characteristics, whilst rate of reaction for the formation of Fe(III)-LD is higher as compared to Fe(II)-LD complex. Green colored complex with Levodopa is formed by +3 state of iron. This observation is substantiated by treating green complex solution with a reducing agent such as ascorbic asid , which resulted in disappearance of color.

LMCT bands were observed and molar absorptivities of the complexes were evaluated, which is comparatively high at 730 nm than 430 nm. *pK* were evaluated for Fe(II) and Fe(III) complex with LD by Potentiometric plots. At selected λ_{max}, different methods explored ML_3 complex formation. Calculated log K_f showed comparative results of Fe(II)-LD complex.

Kinetic results for the complex formation tendencies of the two systems (Fig.13-14) revealed that formation of Fe(III)-LD complex is a fast single phase reaction while Fe(II)-LD complexation is relatively slow and two phase reaction. Observation leads us to conclude that Fe(II) is immediately converted to Fe^{+3} in the presence of Levodopa and it is +3 state of iron which is complexing with Levodopa. Figure 2 displays the suggested mechanism for this complex formation.

Variation in pH is instrumental in bringing such changes which influence the functioning of human body and its various organs. Hence kinetic behaviour of iron in its two oxidation states towards its complex formation with Levodopa may be controlled by the pH of the system. Therefore further study in this direction is desirable.

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