American Journal of Analytical Chemistry, 2015, 6, 551-558
Published Online May 2015 in SciRes. http://www.scirp.org/journal/ajac
http://dx.doi.org/10.4236/ajac.2015.66053

Investigation of Iron Complex Formation of Anti-Hypertensive Drug: Methyldopa

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Received 7 April 2015; accepted 18 May 2015; published 20 May 2015

Abstract

The drug administered for any disease may play an unwanted function in biological system. They may have multiple counter effects, one of which is their interaction to bioactive metals. Iron is most common bio essential metal and is reported to interact with antihypertensive drug methyldopa. In the present study, above said complex is analyzed by UV-Visible spectrophotometry. Formation constant of the complex is calculated by using mole ratio method and single point statistical method which is in the range of 10^{10}, values are also calculated which are independent of pH like formation constant. Absorbance maxima were found to be dependent on pH. At lower pH complex shows two broad bands centered at 430 nm and 730 nm. With the rise in pH later peak shifts toward lower wavelength, so 615 nm is selected for further studies. Molar extinction coefficient of the complex is explored by serial dilution method. At all wavelengths it increases with increase in pH. Mole ratio and slope ratio methods are used for exploring stoichiometry. Metal to ligand combining ratio in the complex is 1:2 at pH 4.0 and pH 4.5 while 1:3 at pH 5.0 and pH 5.5.

Keywords

Iron, Methyldopa, Stoichiometry, Formation Constant, UV-Spectrophotometery

1. Introduction

Iron, is one of the most abundant biological metal, existing in two oxidation states. In its lower oxidation state, it is more soluble and more biologically available. Iron supplements are among the most recurrently recommended medicines [1]. Patients are often treated with several drugs and some time they consume more than one phar-

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How to cite this paper: Fiaz, T., Fatima, N., Zaidi, S.Z.A., Abbas, T. and Kazimi, M.R. (2015) Investigation of Iron Complex Formation of Anti-Hypertensive Drug: Methyldopa. *American Journal of Analytical Chemistry*, **6**, 551-558.
http://dx.doi.org/10.4236/ajac.2015.66053

maceuticals. When patients ingest two or more drugs simultaneously, there is a risk of drug-drug interaction [2]. As Iron has strong affinity toward Nitrogen and Oxygen donor ligand that is why variety of drugs can form chelates with Iron. Reduction in the absorption of many drugs like, penicillamine [2], levodopa [3], carbidopa, ciprofloxacin and methyldopa [3]-[7], is caused due to Iron. The major mechanism by which iron interacts with these drugs is the formation of iron-drug complexes [8]. Methyldopa (MD) is one of the catecholic molecules which are liable to interact with Iron. It is chemically known as 1-methyl-3, 4-dihydroxyphenylalanine, it is a catecholamine widely used anti-hypertensive drug with structure illustrated in **Figure 1**. The MD is a centrally acting alpha$_2$-adrenoreceptor agonist, which reduces sympathetic symptoms and results in decrease in blood pressure [9].

Different analytical methods and techniques have been employed for the analysis of catechol derivatives in pharmaceuticals or in biological samples. These procedures include titrimetry, Fluorimetric determination, kinetic studies, amperometry, gas chromatography, high-performance liquid chromatography (HPLC), chemiluminescence and voltammetric analysis [10]-[24]. These methods are not simple and involve procedures with severe control of the experimental conditionsor otherwise are associated with expensive or delicate instruments. Stoichiometry and other spectral characteristics of Levodopa and other similar complexes have already been reported but still there is lack of data reporting formation constant of said complexes [25]-[28].

In the present study using a simple spectrophotometric technique Formation constant of the Methyldopa complex of Iron (II) is explored in the pH range of 4.0 to 5.5. Two different methods of calculation are used and results found are in agreement with each other, Stoichiometry of the complex is also reviewed by using Mole ratio and Slope ratio method.

2. Experimental

2.1. Materials

Analytical grade reagents were used throughout the study. $Fe(NH_4)_2(SO_4)_2{\cdot}6(H_2O)$ was obtained from Merck and Methyldopa was obtained from Wild Wind, CO_2 free distilled deionized water was used for the preparation of buffer and complex solutions.

2.2. Absorbance Maxima

Absorbance maxima of the complex, was investigated by treating 0.5 mM of Fe(II) solution with adequate excess of Methyldopa solution prepared in Acetate buffer of desired pH. The pH of the complex was recorded by JENWAY370 pH meter and SCHIMADZU model number UV-160A was used for scanning complexes in visible region. Spectrum of the complex indicated presence of a broad peak centered at 615 nm and another peak at 430 nm, shifting of the peak with rise in pH was also observed that is why wavelength of 615 nm was selected for further study as illustrated in **Figure 2**.

2.3. Molar Extinction Coefficients and Serial Dilution

Solutions of different dilutions were prepared in Acetate buffer of desired pH. Absorbance was recorded for all diluted solutions at selected wavelengths *i.e*. 615 nm. **Table 1** demonstrates a plot of absorbance for different dilutions against metal concentration provided the slope for determining molarextinction coefficient [28].

2.4. Mole Ratio

Accurate amounts of Iron (II) salt was used to prepare stock solution of metal in deionized distilled water. Stock solution of Methyldopa was prepared in acetate buffer of required pH. Different aliquots of ligand solution were

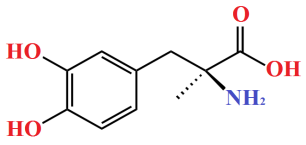

Figure 1. Methyldopa structure chemically known as 1-methyl-3, 4-dihydroxyphenylalanine.

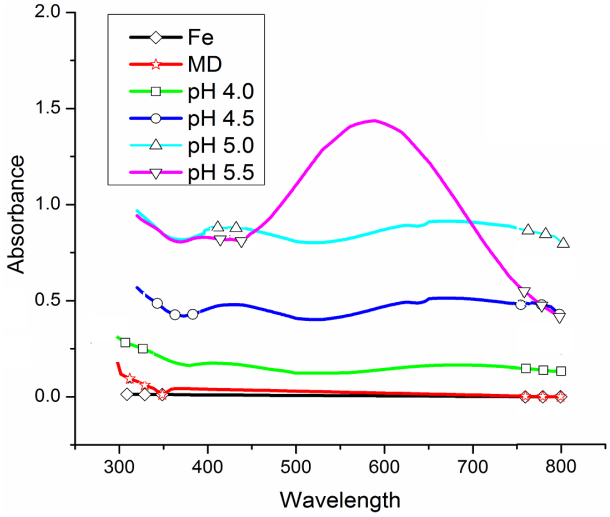

Figure 2. UV-VIS spectra of Fe(II)-MD complex at variable pH.

Table 1. Molar absorptivity of Fe(II)-MD complex in different pH; [Fe(II)] = 0.5 mM; T = 25˚C ± 1˚C.

Molar absorptivity of Fe (II)-MD complex			
pH	Wavelength		
	430 nm	**615 nm**	**730 nm**
4.0	1410	1126	1066
4.5	2030	2016	1740
5.0	2460	2848	1770
5.5	2642	3498	1912

added in 0.5 mM metal solution in order to get various ligand metal ratios ranging from 0.5:1 to 9:1. The final volume was maintained with respective buffers in all cases. The absorbance was recorded at 615 nm and temperature maintained at 25˚C ± 1˚C is illustrated in **Figure 3** [29].

2.5. Slope Ratio

The slope ratio method was used to find the Stoichiometry of the complex. Two series of solutions were prepared. In first half constant volume of 0.5 mM Fe(II) was treated with variable volumes of 5 mM Methyldopa, where as in the other half of the analysis, MD was kept constant versus variable volumes of Fe(II) solution. Resulting complexes were scanned at selected wavelength of 615 nm and the recorded absorbance was plotted versus concentration of varying specie. Stoichiometry of the complex was interpreted by calculating ratio of slope of two straight lines [30]. **Figure 4** is a plot of slope ratio at pH 5.5. The same method was used at pH 4.0, 4.5 and 5.0. All experiments were performed in triplicate in order to get consistent results.

3. Results and Discussion

3.1. Molar Extinction Coefficient

The results show that Methyldopa-Fe have two distinct peaks at low pH, which appear at 430 and 730 nm. The absorbance increases at these wavelengths with the rise of pH. However, the peak at 730 shifts to lower wavelength, as pH is increased. The molar extinction coefficient values were evaluated by serial dilution of complex (standard curve method). The values found are indicated in **Table 1** and are found to be very high, increasing with the pH. The high value indicates charge transfer band either LMCT or MLCT.

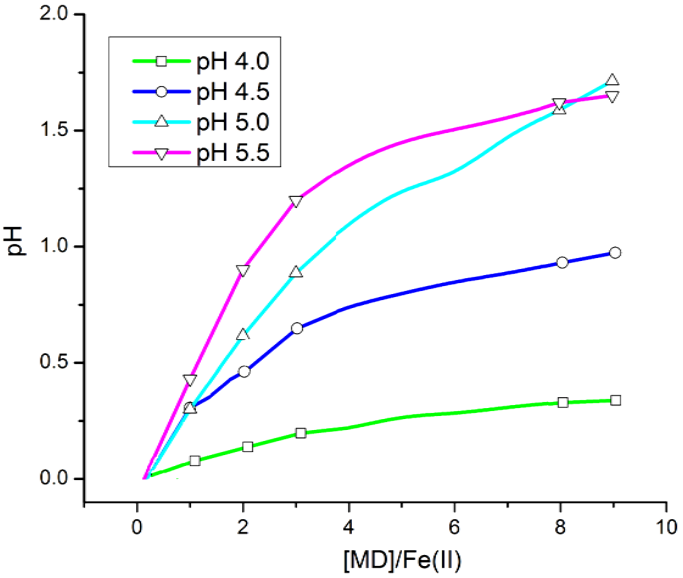

Figure 3. Stoichiometry of Fe(II)-MD complex by mole ratio method in Acetate bufferof variable pH; [Fe(II)] = 0.5 mM; T = 25˚C ± 1˚C; Selected Wavelengh = 615 nm.

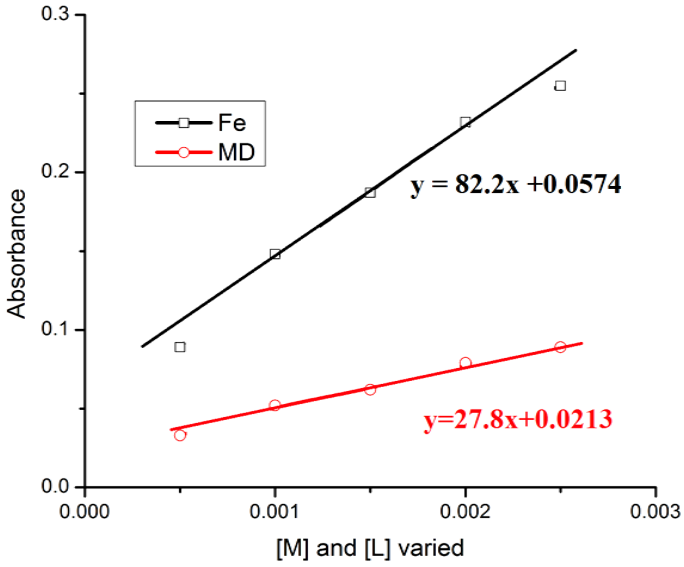

Figure 4. Plots of slope ratio method of Fe(II)-MD, in Acetate Buffer of PH 5.5; Absorbance vs. concentration of variable reagent, T = 25˚C ± 1˚C; Selected Wavelength = 615 nm.

At all pH, work was carried on three wavelengths, 430, 615 and 730, selected purposely. At 430 nm the ε increases with pH. Same trend is found at all wavelengths. Considering ε on a single pH, it is interesting to note that at pH 4.0 and 4.5, the highest value is found at 430 nm, while at pH 5.0 and 5.5, ε is higher at 615 nm. This observation may correspond to the result, that, at low pH, 2 MD molecules chelate iron (II), while with the rise of pH, all six coordination sites of iron are occupied by MD, forming $Fe(MD)_3$. as suggested in **Figure 5**.

3.2. Stoichiometry

Stoichiometry is evaluated by mole ratio and further confirmed by slope ratio method. It has been found that at low pH $Fe(H_2O)_2(MD)_2$ forms while it converts to $Fe(MD)_3$ due to de-protonation of ligand at higher pH as

showed in **Table 2**. Since the pH have a significant effect on complex formation, indicate that, the chelation of metal take place through catecholic side.

Results obtained by Slope ratio method are in good agreement.

3.3. β and Formation Constant Evaluation

β value of ML_1, ML_2 and ML_3 species formed gradually in the solution of varying stoichiometric ratio were calculated by using moleratio data applying single point statistical method where β is the ratio of complex concentration to the product of remaining concentration of metal and ligand at each data point. Overall formation constant of the complex was calculated by using same method; direct moleratiois also used to calculate K_f in this method formation constant is ratio of equilibrium concentrations of products and reactants [30].

The overall formation constant for $Fe(MD)_3$ is found very high about 10^{10}. The values of K_f remains unaffected by pH when determined in **Table 3** and **Table 4** at 615 nm. Step wise formation constant at each pH is also similar *i.e.* no significant change is observed with pH. K_f obtained by the two methods graphical and statistical handling of mole ratio data is consistent.

4. Conclusions

Acidic pH was selected for study, No spectral evidence of complexation was observed at pH below 4 even in the

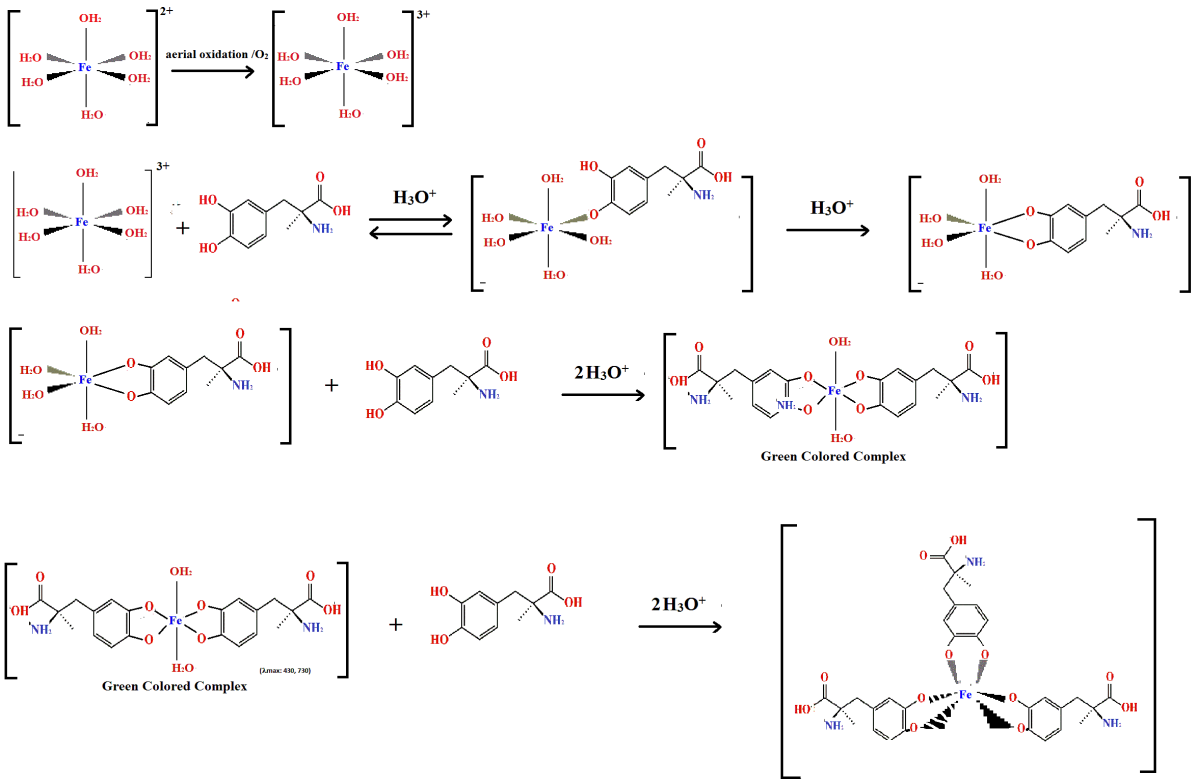

Figure 5. Suggested reaction mechanism between Methyldopa and iron.

Table 2. Stoichiometry of Fe(II)-MD Complex at 615 nm olar Absorptivity of Fe(II)-MD Complex in different pH; [Fe(II)] = 0.5 mM; T = 25˚C ± 1˚C.

	Stoichiometry			
pH	**4.0**	**4.5**	**5.0**	**5.5**
Sloperatio method	2:1	2:1	3:1	3:1
Moleratio method	2:1	2:1	3:1	3:1

Table 3. Overall formation constant K_f of Fe(II)-MD Complex.

Formation constant				
pH	**4.0**	**4.5**	**5.0**	**5.5**
Statistical method	3.4E+05	1.6E+06	1.4E+09	9.2E+09
Graphical method	6.2E+05	2.8E+06	3.6E+09	1.2E+11

Table 4. Stepwise formation constants of Fe(II)-MD Complex.

β values by statistical method				
pH	**4.0**	**4.5**	**5.0**	**5.5**
β_1	2.57	3.34	2.83	3.38
β_2	5.54	6.23	5.98	6.15
β_3	___	___	9.16	9.2

presence of catecholic ligand, which is otherwise reported to catalyze this oxidation [27]. This observation also supports that Iron is present in its higher oxidation state in investigated complex which is not possible at a very low pH. Spectra and mole ratio curves showed formation of ML_2 complex at 4.0 to 4.5 pH, while an evidence of ML_3 type complexation was found at higher pH. The shifting of peak also indicates variation in nature of complex. The results were verified by slope ratio method. Effect of pH shows that chelation depends on de-protonation of MD. It indicates that chelation is through catecholic moiety which has strong affinity for Iron (III). Therefore, the high values of ε are indication of LMCT charge transfer bands, which is characteristic of catecholic ligands. These findings are consistent with the results of $Fe^{2+/3+}$ LD system [27].

The K_f values of the complex was found very high, and remained constant regardless of pH. The complex of iron formed with MD is very strong.

Strong complexation at pH 4.0 and above while no complex formation up to pH 3.5 reveals that this drug can effectively be taken orally as the pH of stomach (1 to 3.5) does not affect its availability in presence of Fe.

According to Campbell *et al*., iron supplements reduce the bioavailability of many drugs including methyldopa due to chelate formation, however no complexation observed at pH lower than 4.0, indicate that chelation and therefore reduction of bioavailability did not occur in stomach.

Acknowledgements

Authors are grateful to Dr. Muhammad Baqar Ali (MBBS) of Claims Med Inc. for fruitful discussion about methyldopa and iron supplements. He facilitated in concluding that this drug can effectively be taken orally as the pH of stomach does not affect its availability in presence of Feat pH 4.0 up to pH 3.5.

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