



Comparison Of Fe(II) and Fe(III)-Hydralazine Complexes, A Potentiometric and Spectrophotometric Study

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ABSTRACT

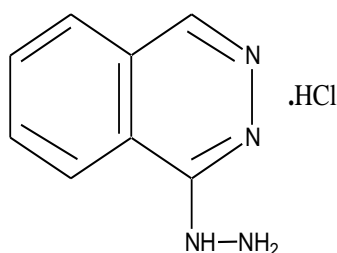
Iron in oxidation states (+2 and +3) is very essential element for human body, and its concentration significantly altered in cardio vascular disease. So the aim of the present work is to study the interaction of Fe(II) and Fe(III) with very commonly used antihypertensive drug hydralazine through potentiometric and spectrophotometric methods. The objectives of the work is to study the stoichiometry, behavior of the complexes in aqueous solution, effect of pH and behavior of this drugs towards both oxidation states of Iron. Both methods show that hydralazine forms a stable complex with both oxidation states of the metal, but the nature of complex changes with change in pH, ligand concentration and with time span. Both methods confirms 1:2 stoichiometry for Fe(II)-Hydralazine while 1:3 for Fe(III)-Hydralazine. Stabilities of both complexes were also calculated. For Fe(II)-Hydralazine complex values of $\log \beta_1$ and $\log \beta_2$ were found to be 4.99 and 7.58 respectively. For Fe(III)-Hydralazine complex $\log \beta_1$, $\log \beta_2$ and $\log \beta_3$ values were found to be 2.74, 7.39 and 11.32 respectively. At high ligand concentration hydralazine also show reducing properties. The study suggests a strong interaction of hydralazine with iron; however the nature of interaction is different with both oxidation states of iron.

Keywords: Ferrous, ferric, hydralazine, complex, potentiometric study, spectrophotometric Study

INTRODUCTION

Cardiovascular diseases are thought to be a major reason of mortality. Iron is the first and the most important trace element in cellular metabolism (Bahi et al., 2017). There is a strong relation between cardiovascular diseases and concentration of iron in the body. It has been reported that high iron levels may lead to an increased risk of cardiovascular disease (Eftekhari et al., 2013). An important and early event in the development of atherosclerosis is the initiation of lipid peroxidation by redox active iron (Stephen et al., 2001). Deposition of vascular iron is found to be closely associated with the atherosclerosis progression and LDL oxidation. Platelet and endothelial cell activation is a consequence of redox activity of iron. A potential mechanism for iron-related cardiovascular disease risk may be endothelial dysfunction. Serum ferritin is one of the strongest risk predictors of overall progression of atherosclerosis, probably due to increased oxidation of LDL cholesterol. Changes in iron stores during the follow-up period modified atherosclerosis risk; in that a lowering was beneficial and further iron accumulation exerted unfavorable effects. The increased risk of death from cardiovascular disease is a consequence of high LDL cholesterol and serum ferritin levels (Bachschmid et al., 2013).

Hydralazine is a direct acting vasodilator, with brand names of Apo-Hydral, Apresoline, Novo-Hylazin, and Apo-Hydralazine etc (Cohn et al., 2011). Its Chemical name is 1-hydrazinophthalazine monohydrochloride (Katherine, 2004). It is a white to off-white, odorless, crystalline powder. It is soluble in water, slightly soluble in alcohol, and very slightly soluble in ether. It melts at about 275 °C, with decomposition, and has a molecular weight of 196.84. It has pKa value 7.3 (Thomas and David, 2012). Hydralazine has the following structure.



Number of side effects has been observed in the use of some antihypertensive drugs. Many of these symptoms are typical of trace elements deficiencies, and it seems probable that these drugs complex metal ions in vivo. So the aim of this study is to confirm these kinds of interactions of one selected antihypertensive drug Hydralazine with two common oxidation states of iron. The objective of the study is to estimate the composition and structure of the complexes formed, and to try and correlate, at least partially, the biological action with the complexation processes that alter the homeostasis of metals such as Iron. Hydralazine may have an effective interaction with iron, so it is likely to be explained through the stability of the complexes and to analyze if this interaction may restrict the activity of this drug or not. As a result deficiency of iron may appear in living body. This interaction depends upon the nature of the donor atom present in the drug structure.

MATERIALS AND METHODS

All the chemicals used were of analytical grade (from Merck and Sigma), and employed without further purification. CO₂ free distilled deionized water was used for all experiments. Iron solution was standardized by spectrophotometric method using 1, 10-phenanthroline as coloring reagent ($\epsilon = 1.011 \times 10^4$) at $\lambda = 510\text{nm}$ (Murthy et al., 2002 and Perveen and Naqui, 2004). The acid concentration was determined by titrating against standard NaOH (Jaffery, 1989). Orion pH-meter, model SA-720, having a resolution of ± 0.001 pH unit, was used for all pH measurements. Shimadzu spectrophotometer, model UV-160A, was used to record spectra in the ultraviolet and visible region. Quartz cell with a 1-cm path length was used. Potentiometric titration was carried out in a laboratory made double walled glass cell. The temperature was controlled by circulating water, through a thermostat. The capacity of this cell was 100mL. The rubber stopper of the cell contains four holes, one for micropipette, one for purging inert gas (Nitrogen 99.999% purity), one for oxygen removal and one for the glass electrode. The solution was completely deaerated by passing N₂ gas for 15 minutes in a sealed flask and was protected with atmosphere. During experiment regular stirring was maintained by means of magnetic stirrer. The pH was measured with a combination glass electrode attached to pH meter having a resolution of ± 0.001 pH units. In each titration, the ligand solution was added first, and then the metal ion solution was added second, followed by the addition of enough water to make desirable volume. This sequence of ligand addition followed by metal addition ensured minimum metal ion hydrolysis at the start of the titration. Before each titration, the analyte solution mixture was allowed to stand for 20-30 minutes for complete equilibration.

RESULTS

To study the protonation of the ligand and complexation properties potentiometric titration was used. Initially titration was performed with hydralazine hydrochloride and the pK_a of ligand was found to be 7.50 (Figure 1). Same titrations were performed with Fe(II) and Fe(III)-Hydralazine complexes (Figure 1). Potentiometric titrations of Fe(II)-Hydralazine was also performed with varying metal to ligand (M:L) ratio (Figure 2). In equimolar solution of ligand and metal, three prominent twists near pH 5.0, 6.0 and 10.0 were observed. While when ligand was doubled in concentration, two prominent curves were found one at pH 6.0 and other at pH 9.5. In 3rd and 4th set there again two twists near pH 6.0 and 9.0 were observed. In 5th and 6th set only one depression at pH 5.5 was found. To compare the interaction of Fe(II) and Fe(III) with hydralazine, potentiometric titration was performed with complexes of both of them, keeping all the conditions same (please see experimental). These complexes were prepared in 1:1, 1:6 and 1:10 metal to ligand ratio and titrated against standard base (Figure 3, 4 and 5). pH of Fe(III)-Hydralazine complex was found to be lower than the pH of Fe(II)-Hydralazine complex in all ratio i.e. in 1:1, 1:3 and 1:6. For spectrophotometric study complexes were prepared in different M:L ratios ranging from 1:1 to 1:8 in distilled deionized water and scanned from 400 to 700 nm on double beam spectrophotometer, (Shimadzu UV-160). The absorbance of complexes was found to be increasing with increasing M:L ratio. To study the effect of pH, solution having 1:7 metal to ligand (M:L) ratio in buffers of pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and in distilled deionized water was selected. The spectra showed for both complexes that there was slight change in the λ_{max} at different pH values but the absorbance was found to be increasing with increasing pH. For the stoichiometry of complexes Mole ratio and Jobs plot methods were performed. The stoichiometry of Fe(II)-Hydralazine was studied at pH 5.0, 8.0 and in distilled deionized water at selected λ_{max} . Stoichiometry was also studied by Job's Plot method at pH 5, pH 8 and in distilled deionized water. Stoichiometry for Fe(III)-Hydralazine was employed at pH 3.0 by Mole ratio and Job's Plot method.

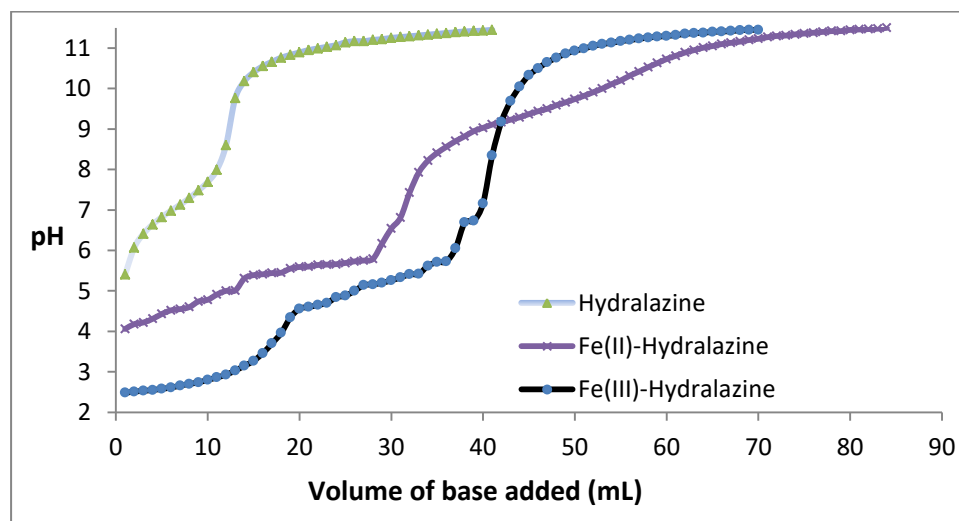


Figure 1: Potentiometric titration of Hydralazine, Fe(II)-Hydralazine and Fe(III)-Hydralazine.

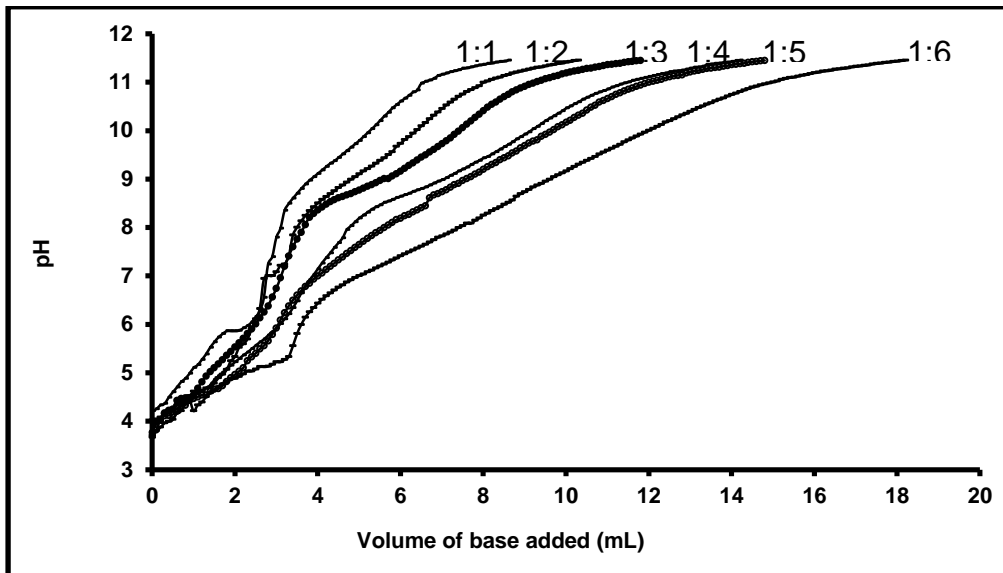


Figure 2: Potentiometric titration of Fe(II)-Hydralazine in different M:L ratio.

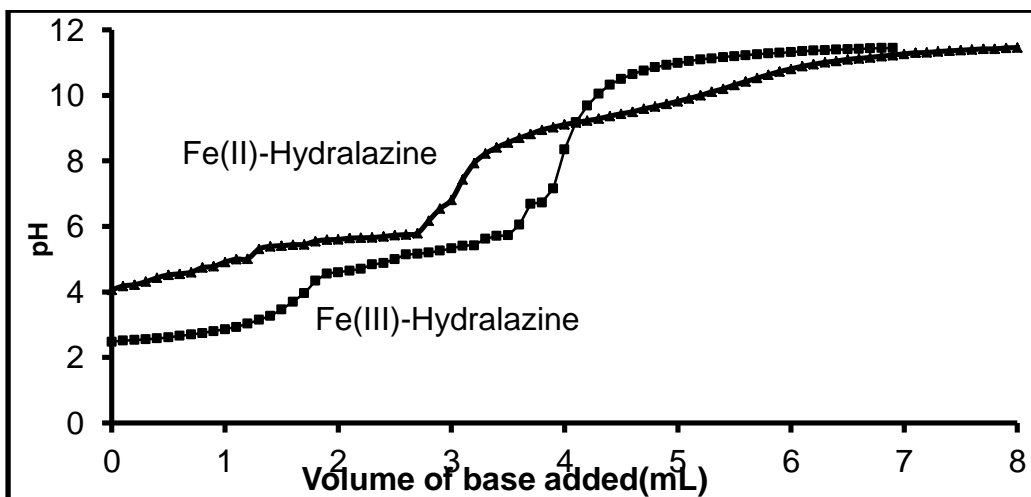


Figure 3: Comparison between Fe(II) and Fe(III)-Hydralazine complexes(1:1).

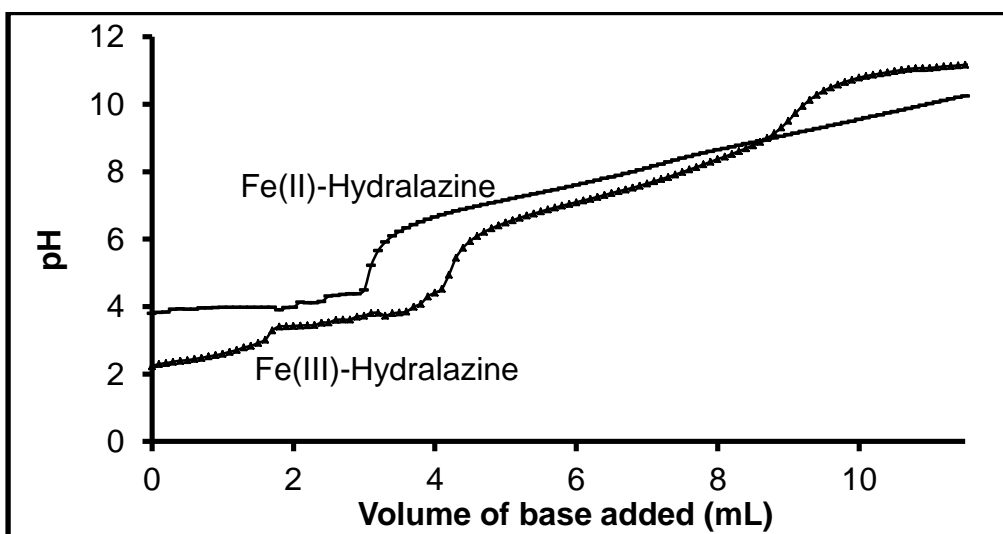


Figure 4: Comparison between Fe(II) and Fe(III)-Hydralazine complexes (1:6).

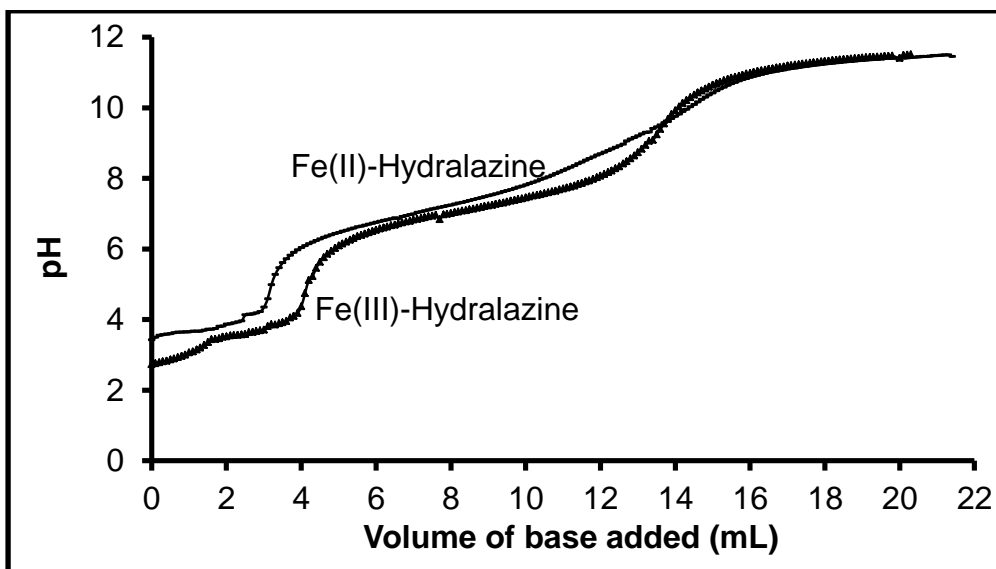


Figure 5: Comparison between Fe(II) and Fe(III)-Hydralazine complexes (1:10).

DISCUSSION

Potentiometric study

The potentiometric titration curves were used to study the protonation of the ligand and complexation properties. As a reference titration was initially performed with hydralazine hydrochloride, which showed one break with pK_a 7.50 (Machado et al., 2002). Same titrations were performed with Fe(II) and Fe(III)-Hydralazine complexes (Figure 1). The pH of both (Fe(II)-and Fe(III)-hydralazine complexes) was found to be less than the ligand which may be due to the release of H^+ ion as a result of complex formation or by acidic pH of metal. In both cases a prominent depression in titration curves of the complexes as compared to the ligand is suggestive of stable complex formation between metal and ligand (Skoog et al., 2004). Potentiometric titrations were also performed with varying metal to ligand (M:L) ratio. The titration curves showed that complex formation between iron (II) and (III) and Hydralazine is possible at wide pH range, but at different pH different species might occur. The shapes of the potentiometric titration curves were used to study the steps of complexation. The features of the curve showed the average number of ligand bound to metal and gave indications about species likely to account for experimental data and a rough estimation of their stabilities (Figure 1).

For Fe(II)-Hydralazine complex in equimolar solution of ligand and metal, three prominent twists near pH 5.0, 6.0 and 10.0 were observed. While when ligand was doubled in concentration, two prominent curves were found one at pH 6.0 and other at pH 9.5, which may be due to the formation of two types of species that is ML and ML_2 respectively. In 3rd and 4th set there again two twists near pH 6.0 and 9.0 were observed. In 5th and 6th set only one depression at pH 5.5 was found. This is noticeable in all titration curves that with increasing ratio of ligand the curves were becoming steeper, showing an increase in the buffering action (Figure 2). For Fe(III)-Hydralazine complex, the titration curve showed three breaks near pH 3.0, 5.0 and 10.0. It showed the formation of three types of species (Figure 1). To compare the interaction of Fe(II) and Fe(III) with hydralazine, potentiometric titration was performed with complexes of both of them, keeping all the conditions same (please see experimental). These complexes were prepared in 1:1, 1:6 and 1:10 metal to ligand ratio and titrated against standard base. pH of Fe(III)-Hydralazine complex was found to be lower than the pH of Fe(II)-Hydralazine complex. It might be due to the fact that aqueous solution of Fe(III) salt has pH lower than Fe(II) salt (Figure 3).

As mentioned earlier that, the Fe(II)-Hydralazine complex showed the formation of three types of species. The ML species was forming between the pH ranges 4 to 5, and then the complex stabilizes itself and the second species, most probably ML_2 present from pH 6 to 8. Third species might be ML_3 seemed to be formed in very low concentration, or having very low stability. Hence, the dominating species are ML and ML_2 . Fe(III)-Hydralazine complex also showed the formation of three types of species, but the dominating species are ML and ML_3 . The first species i.e. ML formed between pH 2 to 4. The second species might be ML_2 forms between pH 4 to 5. The third species ML_3 showing high stability forms between pH 5 to 10 (Figure 3). Fe(II)-Hydralazine favors more tendency to form 1:2 complex. While in Fe(III)-Hydralazine ML_3 stoichiometry shows high stability. This behavior can also be explained in terms of charges of ferrous and ferric. Due to more positive charge (+3) of ferric, it has more attraction towards the lone pairs of ligand as compared to ferrous. So, it binds three ligand molecules more strongly than Fe(II), which can bind two ligand more strongly due to its less positive charge (+2). When potentiometric titration of Fe(II) and Fe(III) hydralazine was compared in high M:L ratios, it was observed that as the ligand concentration was increasing, both curves were becoming similar. When M:L ratio was 1:10, both curves were overlapped. This observation suggests that with high concentration of ligand, hydralazine may reduce iron from +3 to +2 state, and Fe(III)-Hydralazine complex converted to Fe(II)-Hydralazine complex (Figure 4 and 5).

Spectrophotometric study

For spectrophotometric study complexes were prepared in different M:L ratios ranging from 1:1 to 1:8 in distilled deionized water and scanned from 400 to 700 nm on double beam spectrophotometer, (Shimadzu UV-160). The absorbance of complexes was found to be increasing with increasing M:L ratio. The value of λ_{max} was selected from these spectra (Table 1). The value of molar absorptivity constant (ϵ) was calculated for both complexes, which was found to be $13,000 \text{ LM}^{-1}\text{cm}^{-1}$ and $1700 \text{ LM}^{-1}\text{cm}^{-1}$ for Fe(II)-Hydralazine and Fe(III)-Hydralazine complexes (non-buffered aqueous medium) respectively. To study the effect of pH, solution having 1:7 metal to ligand (M:L) ratio in buffers of pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and in distilled deionized water was selected. The spectra showed for both complexes that there was slight change in the λ_{max} at different pH values but the absorbance was found to be increasing with increasing pH. At low pH, due to the excess of H^+ ions the donor nitrogen atoms of the ligand were less accessible than at high pH. In acidic pH i.e. at pH 4.0, the complex with 1:7 metal to ligand ratio had absorbance less than 0.2 at its λ_{max} , while at pH 5.0 it was more than six times greater and in basic pH it became ten times of that. At pH 9.0 a sharp peak was not obtained (Figure 6, Table 1). Table 1 shows that at pH 3.0 both complexes Fe(II)-Hydralazine and Fe(III)-Hydralazine, behave separately, but at pH 5.0 and above, both complexes have almost same λ_{max} values (Table 1). At pH 5.0 and above, the spectra of Fe(II) and Fe(III)-Hydralazine complexes are almost same, but after pH 5.0 the absorbance of Fe(II)-hydralazine complex is more than Fe(III)-hydralazine.

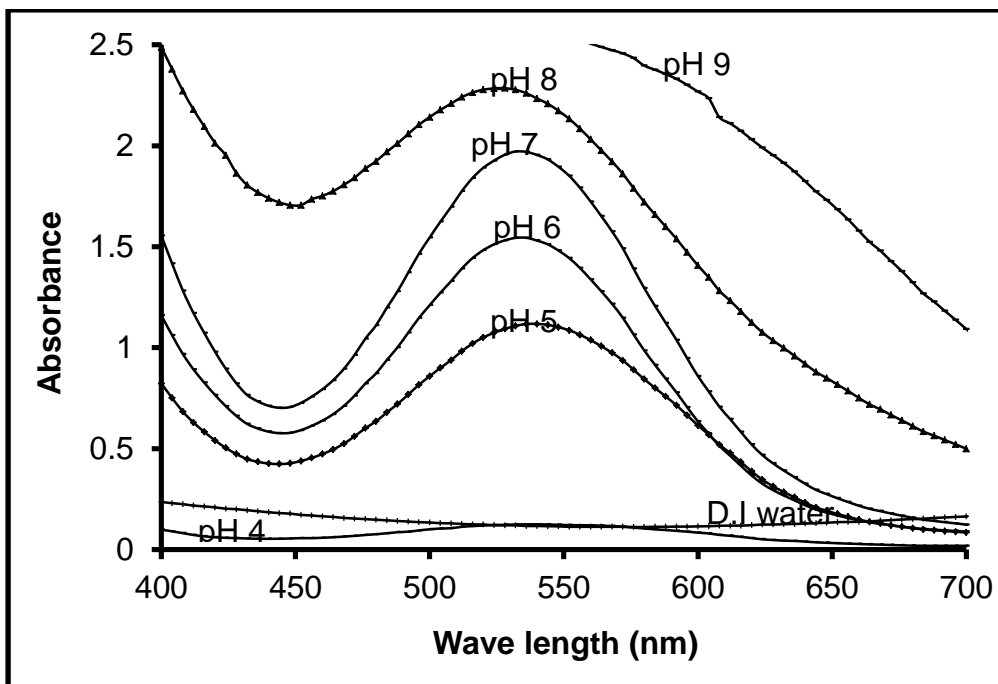


Figure 6: Effect of pH on Fe(II)-Hydralazine complex (1:7).

Table 1: Wave lengths selected at different pH values for Fe(II) and Fe(III)- Hydralazine complexes.

pH	λ_{max} selected for Fe(II)-Hydralazine (nm)	λ_{max} selected for Fe(III)-Hydralazine (nm)
3.0	----	575
4.0	536	556
5.0	540	540
6.0	536	540
7.0	532	536
8.0	410	410
9.0	410	410
Distilled deionised water	540	540

Fe(II)-hydralazine and Fe(III)-hydralazine complexes were compared in buffer of pH 3.0. It was observed that initially at pH 3.0 Fe(II)-hydralazine formed in very small amount as compared to Fe(III)-hydralazine (Figure 7). But in high concentration of ligand, the formation of complex is faster (Figure 8). It was also observed that in highly acidic medium Fe(III)-hydralazine complex formed instantaneously, but Fe(II)-hydralazine complex did not form even after couple of hours (Figure 9). The spectra of both complexes were recorded at different time intervals at pH 3.0 and it was found that Fe(II)-hydralazine formed very slowly and absorbance increased gradually and slowly. Fe(III)-hydralazine complex formed instantaneously at this pH, but after 24 hours its absorbance decreased (Figure 10 and 11).

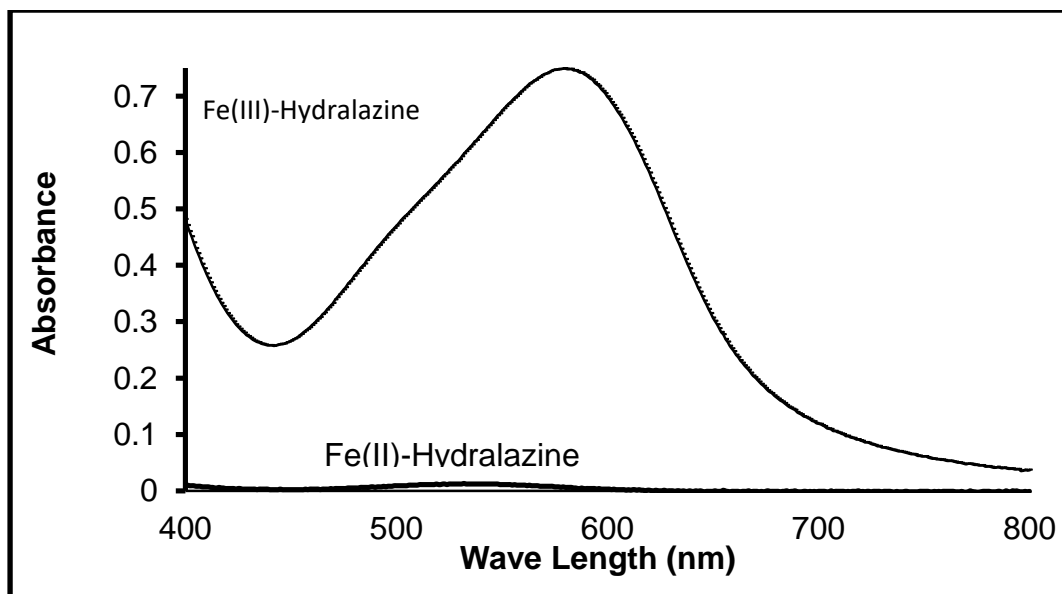


Figure 7: Spectra of Fe(II)-Hydralazine and Fe(III)-Hydralazine at pH 3.0 after 30 minutes.

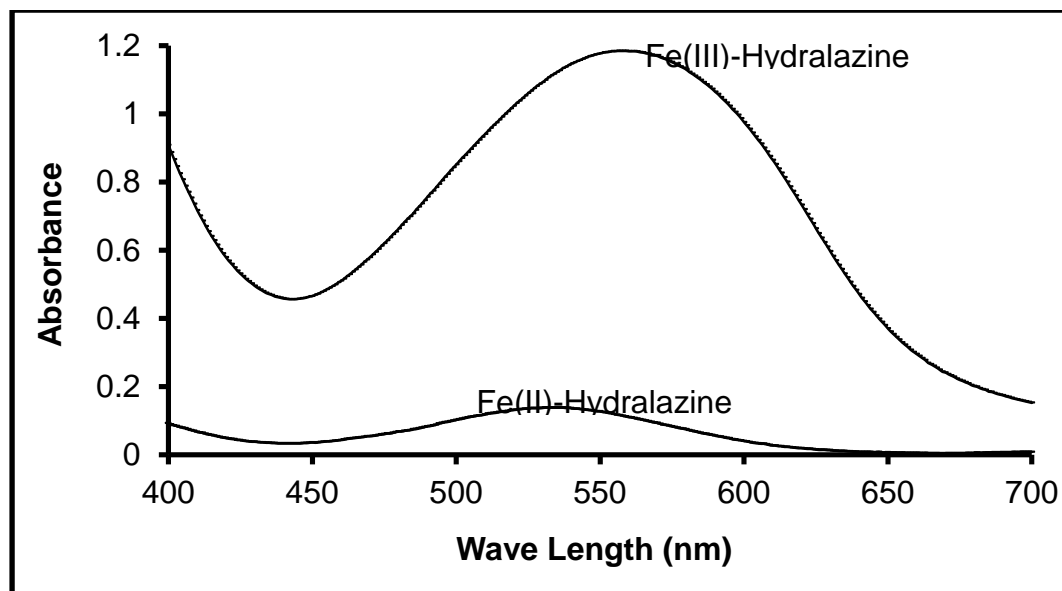


Figure 8: Spectra of Fe(II)-Hydralazine and Fe(III)-Hydralazine with high ligand concentration.

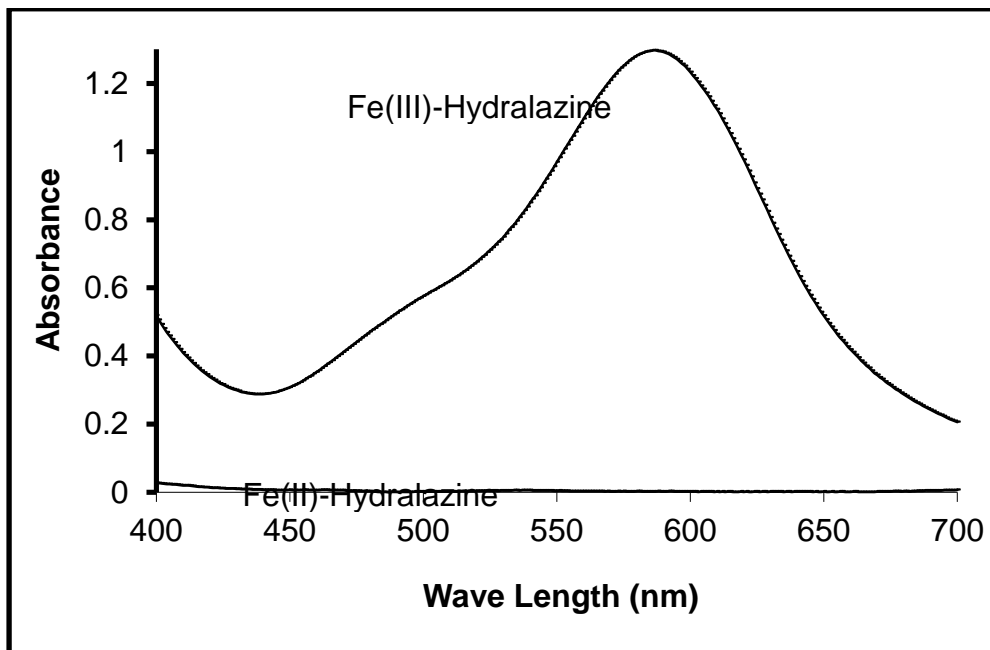


Figure 9: Spectra of Fe(II)-Hydralazine and Fe(III)-Hydralazine in highly acidic medium after 6 hours.

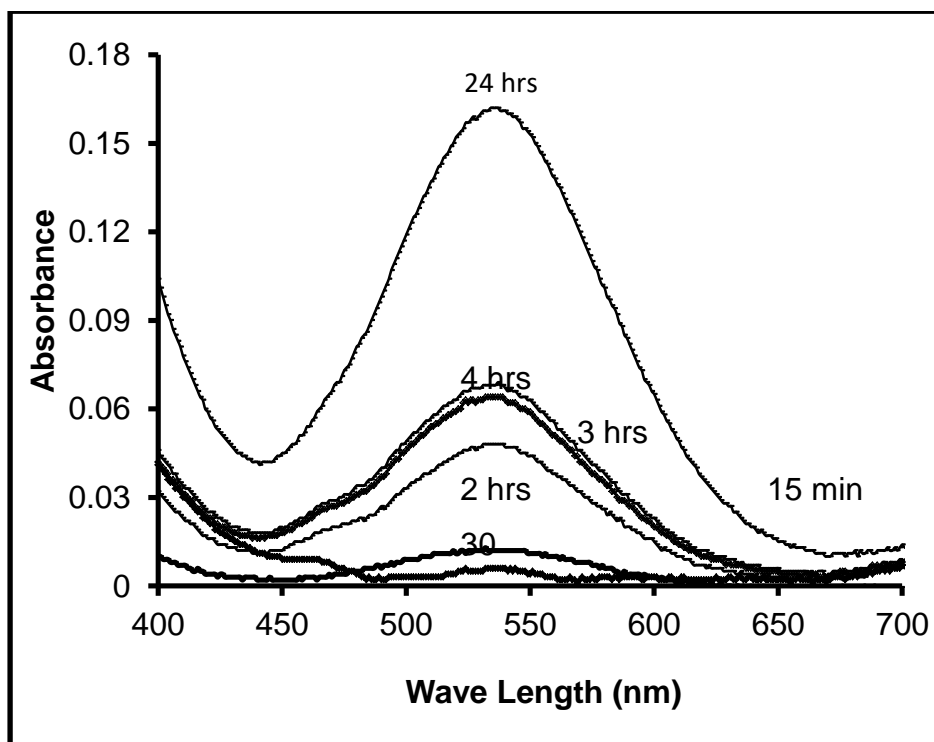


Figure 10: Spectra of Fe(II)-Hydralazine at pH 3.0 at different time intervals.

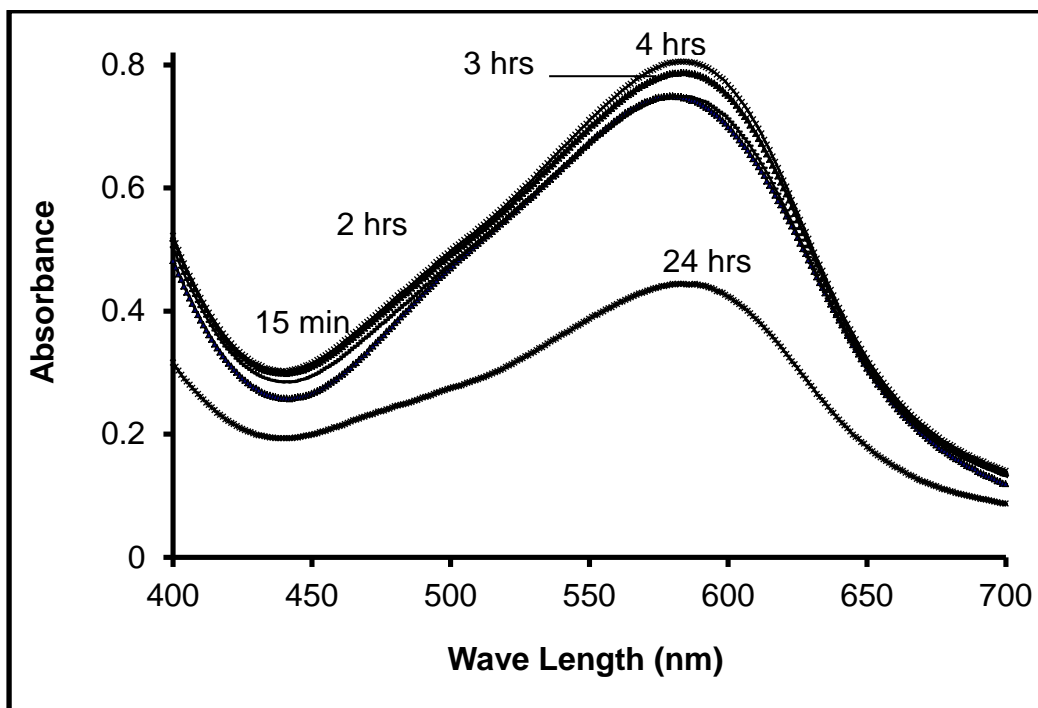


Figure 11: Spectra of Fe(III)-Hydralazine at pH 3.0 at different time intervals.

These observations suggest that the rate of formation of Fe(II)-hydralazine is very slow at pH 3.0. At pH 4.0 it increases a bit, that's why we got λ_{max} in between 540 and 575, i.e. 556 nm. At pH 5.0 and above the rate of formation of Fe(II)-hydralazine was higher. So after pH 3.0 hydralazine reduced iron from +3 to +2 state instantaneously after formation of complex and the final complex is Fe(II)-Hydralazine complex. In case of Fe(III)-Hydralazine some ligand was used to reduce iron, while the remaining made complex with Fe(II), that's why the absorbance of Fe(III)-Hydralazine was lesser than Fe(II)-Hydralazine.

Stoichiometry of iron- hydralazine complexes

Mole ratio and Jobs plot methods were performed to study stoichiometry of complexes. The stoichiometry of Fe(II)-Hydralazine was studied at pH 5.0, 8.0 and in distilled deionized water at selected λ_{max} . Stoichiometry was also studied by Job's Plot method at pH 5, pH 8 and in distilled deionized water. The metal to ligand ratio (M:L) for Fe(II)-Hydralazine was found to be 1:2 (Figure 12 and 14). Stoichiometry for Fe(III)-Hydralazine was employed at pH 3.0 by Mole ratio and Job's Plot method. The stoichiometry for Fe(III)-Hydralazine was found to be 1:3. The absorbance readings were taken at 575 nm, which was the selected λ_{max} of Fe(III)-Hydralazine complex at this pH. According to the mole ratio method the stoichiometry was found to be 1:3 (ML₃) (Figure 13 and 15).

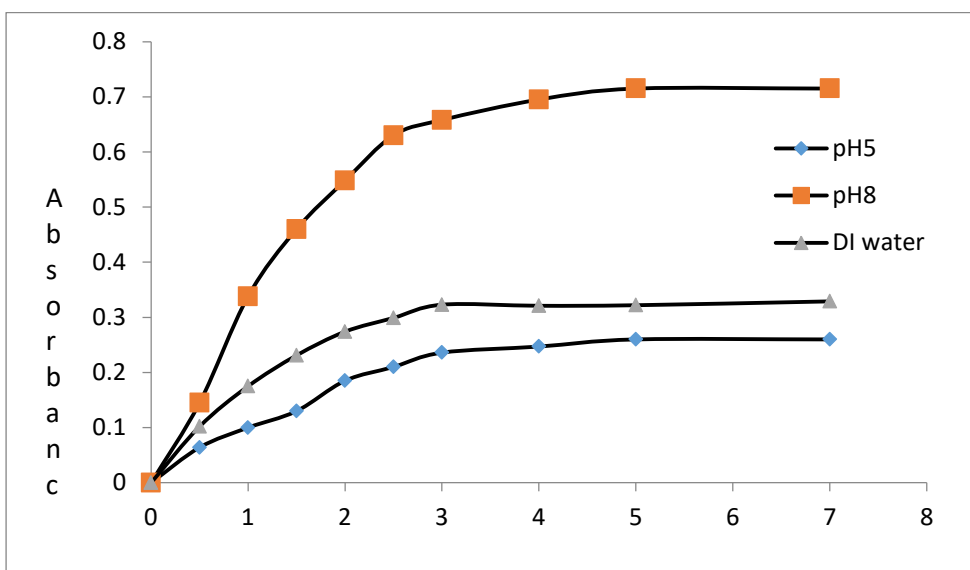


Figure 12: Mole Ratio Plots of Fe(II)-Hydralazine at pH 5, 8 and Deionized Water.

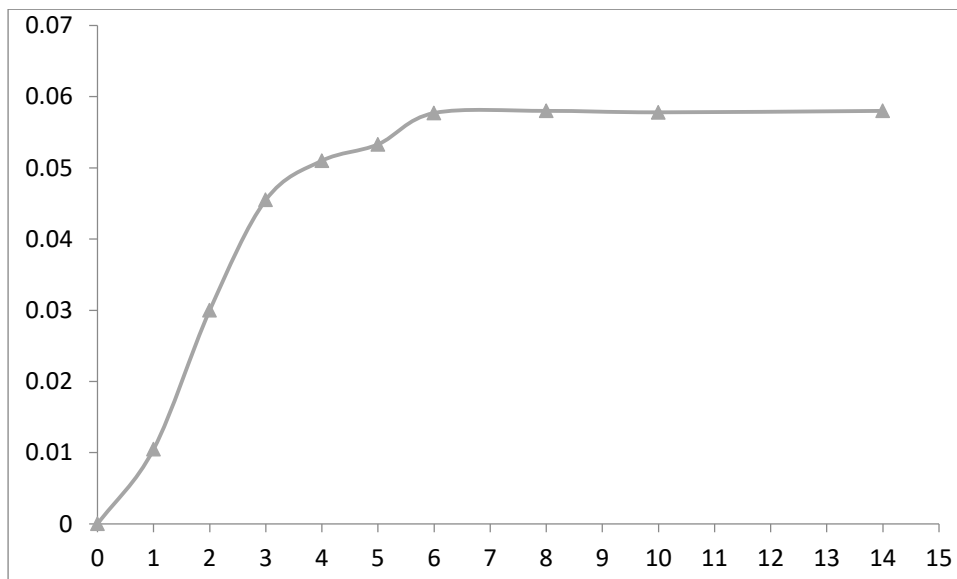


Figure 13: Mole Ratio Plots of Fe(III)-Hydralazine at pH 3.

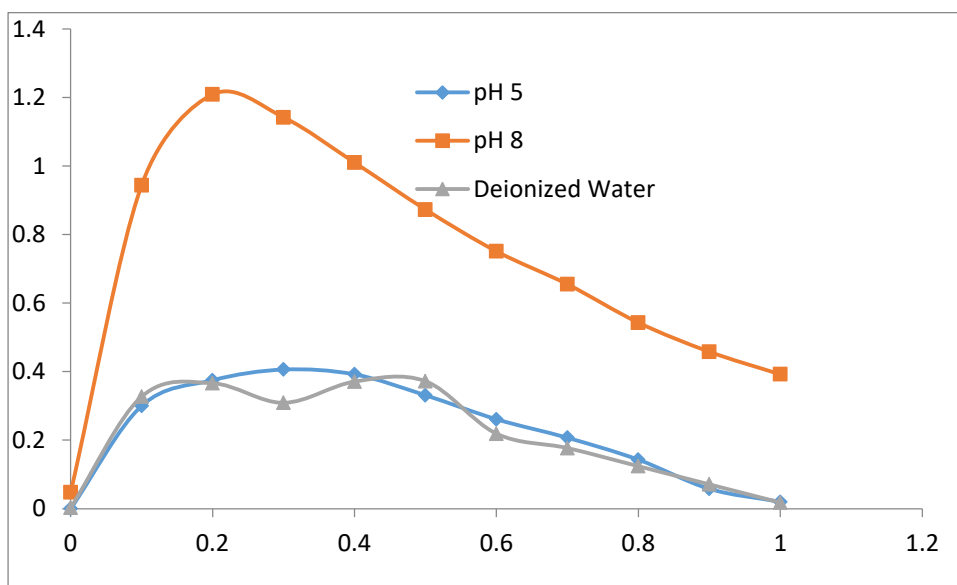


Figure 14: Job's Plot Fe(II)-Hydralazine.

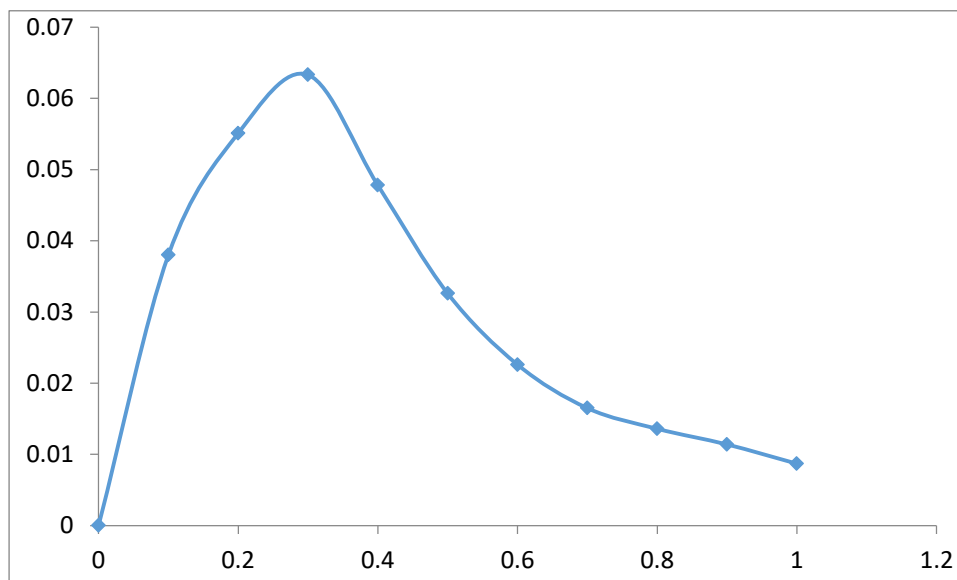


Figure 15: Job's Plot Fe(III)-Hydralazine.

Stability of complexes

Stabilities of both complexes were calculated using potentiometric method and verified by computer program "BEST". In this method, theoretically calculated stability constant values were refined till the least σ_{fit} value. An input data file "FOR004.DAT" was written and with the help of various options of the program, the value of σ_{fit} was refined. Program "BEST" also helped to calculate the species distribution. These results were generally obtained in terms of mole fraction of species with respect to pH. Species distribution at different pH was calculated, and finally their diagram was drawn (Figure 16 and 17).

Stability was also calculated by spectrophotometric method. Using the value of molar absorptivity, the concentration of the complex formed, and then concentration of the remaining metal and remaining ligand at equilibrium were calculated. These concentrations were calculated at different ratio and at different pH.

Using the equation [Petrucci et al., 2007 and Ali et al., 2004].

$$\beta = \frac{[ML_n]}{[M]_r [L]_r^n}$$

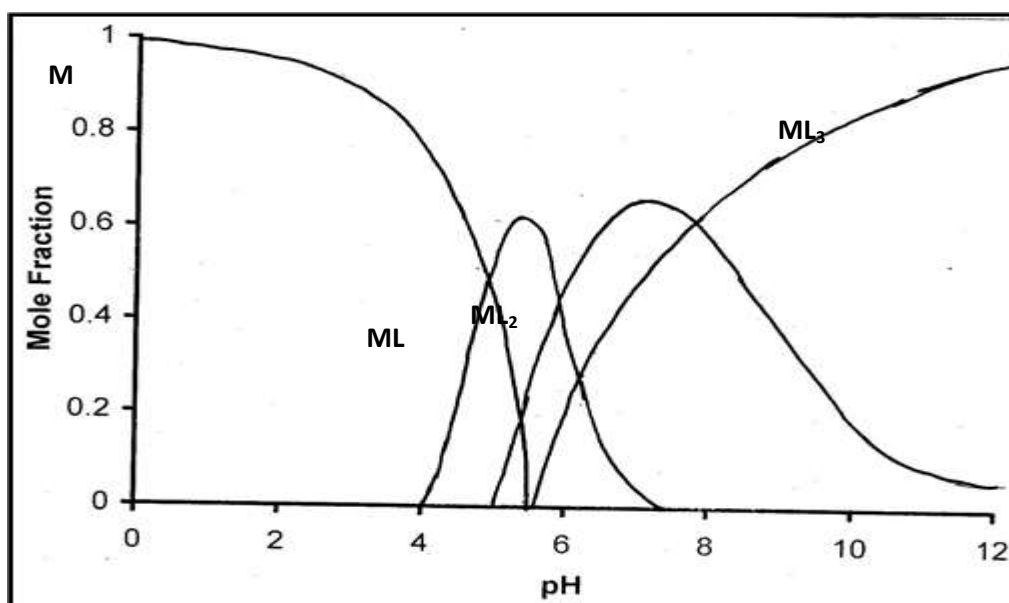


Figure 16: Species distribution curve F(II)-Hydralazine Complex.

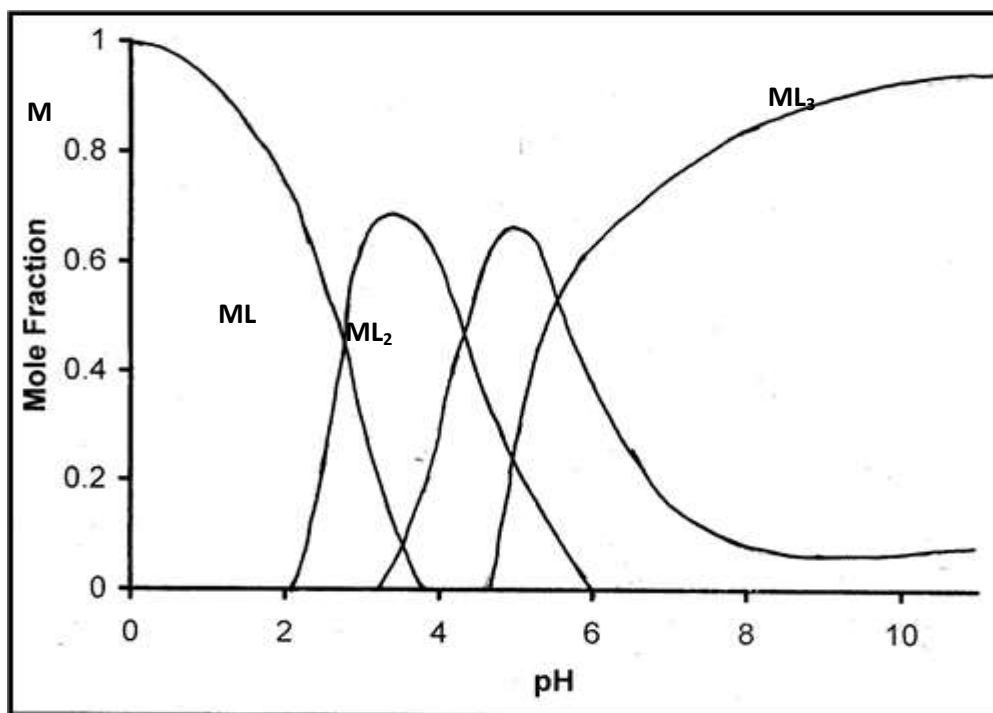


Figure 17: Species Distribution Diagram of Fe(III)-Hydralazine.

For Fe(II)-Hydralazine complex stability was calculated at pH 5.0, 8.0 and in distilled deionised water from the observations obtained from mole ratio method, at each pH values. As the mole ratio (M:L) for Fe(II)-Hydralazine was obtained 1:2 at all pH values, so the values for β_1 and β_2 were calculated. Consistency was found in the results. The approximate $\log \beta_1$ and $\log \beta_2$ values obtained for Fe(II)-Hydralazine complex were 4.99 and 7.58 respectively (Table 2).

Table 2: Stability constant values for Fe(II)-Hydralazine complex obtained from different methods.

	Spectrophotometric			Average	Potentiometric (BEST)
	pH 5.0	pH 8.0	Distilled deionized water		
$\text{Log}\beta_1$	4.92	5.39	4.38	4.99	5.60
$\text{Log}\beta_2$	7.56	7.89	7.28	7.58	7.50

For Fe(III)-Hydralazine complex stability was calculated at pH 3.0. The $\log \beta_1$, $\log \beta_2$ and $\log \beta_3$ values for Fe(III)-Hydralazine complex were found to be 2.74, 7.39 and 11.32 respectively (Table 3). The values of stabilities for Fe(II)-Hydralazine and Fe(III)-Hydralazine complexes obtained from both potentiometric and spectrophotometric methods are in good agreement with each other.

Table 3: Stability constant values obtained from different methods for Fe(III)-Hydralazine complex.

	Spectrophotometric	Potentiometric (BEST)
$\text{Log}\beta_1$	2.74	4.00
$\text{Log}\beta_2$	7.39	6.50
$\text{Log}\beta_3$	11.32	10.50



CONCLUSION

The results obtained through this study confirm a strong interaction between the drug molecule and iron in both +2 and +3 oxidation states. This interaction is irrespective of the pH, as the H^+ are not in competition with metal present in the surroundings due to particular coordination conditions required for the bonding. So it can be said that the variation in the pH in the gastrointestinal tract may not cause considerable changes on the complexation. The ratio of the concentration of metal and ligand is responsible for the stoichiometry and stability of the complex formed. It was found that Fe(II) may form trans- bis complexes. Therefore after addition of two drug molecules, further addition in the coordination sphere of metal is prohibited. On the other hand Fe(III) may be able to form 1:3 (ML_3) type of complexes. Results composed from the potentiometric study and spectrophotometric findings for the β values of the species exhibit good stability of the complexes at various pH values. Stabilities of these complexes were found to be high as $\log \beta_3$ value for Fe(III)-hydralazine was nearly 10, while $\log \beta_2$ for Fe(II)-hydralazine is nearly 7. As hydralazine is also a reducing agent [1], so it may convert trivalent metal in to divalent. The reduction properties of hydralazine were found to be pH dependent. Therefore at low pH, i.e. at pH 3.0 reduction is very slow, and drug is able to form ferri complex, while with Fe(II) chelation was found to be very slow, so at this pH stability and stoichiometry of Fe(III)-hydralazine can be easily studied. Reduction may not help in release of metal from the resultant complex as divalent metals also have good affinity towards this drug, but some drug may be released because stoichiometry may change from 1:3 to 1:2, but above pH 3.0 the complex exists only in Fe(II) form. Accordingly when concentration of ligand is high the rate of reduction increases and therefore degree of complexation decreases.

REFERENCES

1. Ali K, Fatima N, Maqsood ZT, Kazmi SA, 2004. Complexation of vanadium(IV) with hydroxamate chelators and their stability relation with pH, *J. Iran. Chem. Soc.*, 1(1), 65-70. DOI: <https://doi.org/10.1007/BF03245772>
2. Bachschmid MM, Schildknecht S, Reiko Matsui, Rebecca Zee, Dagmar Haeussler, Richard A. Cohen, David Pimental, Bernd van der Loo, 2013, Vascular aging: Chronic oxidative stress and impairment of redox signaling—consequences for vascular homeostasis and disease, *Ann. Med.* 45(1), 17–36. DOI: 10.3109/07853890.2011.645498
3. Bahi GA, Bidie AP, Meite S, Djaman AJ, 2017, Exploring the iron metabolism in multidrug resistant tuberculosis (MDR-TB) patients in treatment, *Int. J. Biol. Chem. Sci.* 11(3), 1039-1045. DOI: <https://dx.doi.org/10.4314/ijbcs.v11i3.9>
4. Cohn JN, McInnes GT, Shepherd AM, 2011, Direct-Acting Vasodilators, *J Clin Hypert.*, 13(9), 690–692, DOI: 10.1111/j.1751-7176.2011.00507.x
5. Eftekhari MH, Khosravi HM, Shidfar F, Zamani A, 2013. Relation between Body Iron Status and Cardiovascular Risk Factors in Patients with Cardiovascular Disease. *Int J Prev Med.* 4(8): 911–916. DOI:10.4103/2008-7802.182733
6. Jaffery GH, Bassett J, Mendham J, Denney RC, 1989, Vogel's Text Book of Quantitative Chemical Analysis, Longman Scientific and Technical, 5th edition, 292.
7. Imad S, Nisar S, Maqsood ZT, 2010, A study of Redox properties of Hydralazine Hydrochloride, an antihypertensive drug, *J. Saud. Chem. Soc.*, 14, 241-245, DOI:10.1016/j.jscs.2010.02.003
8. Katherine, M.H., 2004, Vasodilators In Medical Toxicology, Richard, C.D., (Ed.), Lippincott Williams & Wilkins: Philadelphia, 3rd edition, 714-715
9. Machado JD, Gomez JF, Betancor G, Comacho M, Brioso MA, Borges Ricardo, 2002, Hydralazine Reduces the Quantal Size of Secretory Events by Displacement of Catecholamines From Adrenomedullary Chromaffin Secretory Vesicles, *Circulation Res.*, (91), 830-836, DOI:10.1161/CIRCRESAHA.117.310812.
10. Murthy CP, Ali SFM, D Ashok, 2002, University Chemistry, 1, New Age International (P) Limited Publishers, India, 280.
11. Perveen, P, Naqui, II, *The Nucleus*, 41 (1-4), 63-6. 2
12. Petrucci RH, Harwood WS, Herring FG, Madura JD, 2007, Solubility and Complex Ion Equilibria: General Chemistry, Principles and Modern Applications, Pearson Education International: London, 9th edition, 762-763
13. Skoog DA, West DM, Holler FJ, Crouch SR, 2004, Potentiometry: Fundamentals of Analytical Chemistry, Thomson Books: Mexico, 623-627
14. Stephen JD, Elizabeths B, Monika H, Judson D, Russell NG, John K, Joseph AV. 2001, Iron Chelation Improves Endothelial Function in Patients With Coronary Artery Disease, *Circulation*, 103, 2799-2804. DOI:10.1161/CIRCULATIONAHA.117.030114.
15. Thomas LL, David AW, 2012, Foye's Principles of Medicinal Chemistry, Seventh Edition, Lippincott Williams & Wilkins, Philadelphia, 781