Pak. J. Chem. 2(2): 91-98, 2012 Full Paper
ISSN (Print): 2220-2625
ISSN (Online): 2222-307X DOI: 10.15228/2012.v02.i02.p07

Complexation, Stability and Stoichiometry of Iron (III) with Salbutamol (Active ingredient of Asthma drug Ventolin®)

*N. Fatima
Department of Chemistry, University of Karachi, Karachi - Pakistan
Email: *dr_nfatima1@yahoo.com

ABSTRACT
Asthma is a wide speared disease all over the world. In this disease breathing is not smooth due to contraction of bronchitis. Ventolin is an effective drug for this purpose and is widely used. Its active ingredient is Salbutamol Sulphate, which is bronchodilator. Due to oxygen and nitrogen donor sites of Salbutamol (Fig I), it has been assumed that it may interact with metals present in the biological system, and therefore disturb the metals metabolism and imbalance the equilibrium. On the other hand two third of the body's iron is found in hemoglobin which is an oxygen storage protein. Therefore may iron is chelated by the Salbutamol ion in the stomach. The chelation of iron by salbutamol, stability of the complex and Stoichiometry at acidic pH of the said complex is investigated implied Spectrophotometric technique. Salbutamol formed highly colored complex with iron having maximum absorbance at 550nm. A 1:3 complex formation in buffered and non buffered solutions at 30°C was found using mole ratio and slope ratio methods. Molar extinction coefficients were determined by calibration curve method and was found very high in non buffered solution comparative to buffered solutions. Stability constant of ML_1 is found 6.3635, ML_2 is 11.919 and ML_3 is 16.858 in non buffered solution. Closer lnβ values were found in buffer of pH 3.0 and 3.5.

Key word: Asthma, Salbutamol, Iron, complexation, stoichiometry, stability, spectrphotometry

1. INTRODUCTION
Asthma affects 6 million children in the United States and 16 million people's total[1]. There are two factors which provoke asthma, triggers and inducers**.** Triggers irritate the airways and result in bronchoconstriction. The Common triggers include everyday stimuli such as: cold air, dust, strong fumes, exercise, inhaled irritants, emotional upsets and smoke[2-3]. The last one is the strongest trigger. Common inducers are Allergens and Respiratory viral infection[4-6]. In contrast to triggers, inducers cause both airway inflammation and airway hyper responsiveness and hence are recognized as causes of asthma[7-9].

These symptoms can easily be relieved by a bronchodialator such as ventolin® (containing salbutamol sulphate). salbutamol form the initial therapy of chronic as well as acute asthma[10].

Myoglobin an iron containing protein functions in the transport and short-term storage of oxygen in muscle cells, helping to match the supply of oxygen to the demand of working muscles[11]. Since Salbutamol is used as bronchodialator for asthmatic patients. There is strong possibility that Fe(III) may react with Salbutamol, could enhance its effectiveness.

In the present study chelation of Fe(III) by salbutamol is investigated. Chelation, stoichiometry, stability and molar extinction coefficients were determined by spectrophotometric technique in non buffered as well as buffer of pH 3.0 and 3.5.

Evaluation of chelation strength of Fe(III) by the drug molecule at stomach pH will provide important guideline to understand the maintenance of drug's efficacy as well as accomplishment of iron.

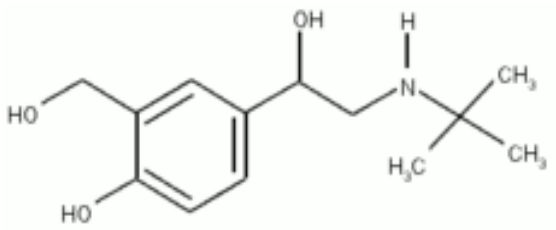

Fig-1: – (Dimethylethyl) amino]methyl]-4-hydroxy-1,3-benzenedimethanol

2. RESULTS AND DISCUSSION
A complex solution of known concentration was scanned on Thermogenesys 6 Spectrophotometer, absorbance maxima was found at 550.nm in buffer solution of pH 3.0, 3.5 and in non buffered solutions as well.

2.1 *Molar extinction coefficients*
Molar extinction coefficients of the complex at different working conditions were found by calibration curve method (Fig-2). In this method a tenfold excess of ligand is added to the metal solution. Different dilutions of the complex solutions are then prepared and the absorbances were recorded at the selected wavelength (550.0nm). Graph between

*Corresponding Author Received 13th June 2012, Accepted 17th June 2012

concentration of complex and the respective absorbance were plotted (Fig-2). Based on the implementation of the Beer's law, A = ɛbC, the straight line obtained passed through the origin and the slope of the line represented the molar extinction coefficient, ɛ (Fig-2). The value is almost same in pH 3.0 and 3.5 buffered solutions while about 10 times higher in non buffered solution (Table-I).

Table-1: Molar extinction coefficients of the complex at different working conditions

S. No	Working Media	Molar Extinction Coefficients (ε) $(M^{-1}cm^{-1})$
1	Buffer pH 3.0	103.07
2	Buffer pH 3.5	102.54
3	Non buffered Media	1201.32

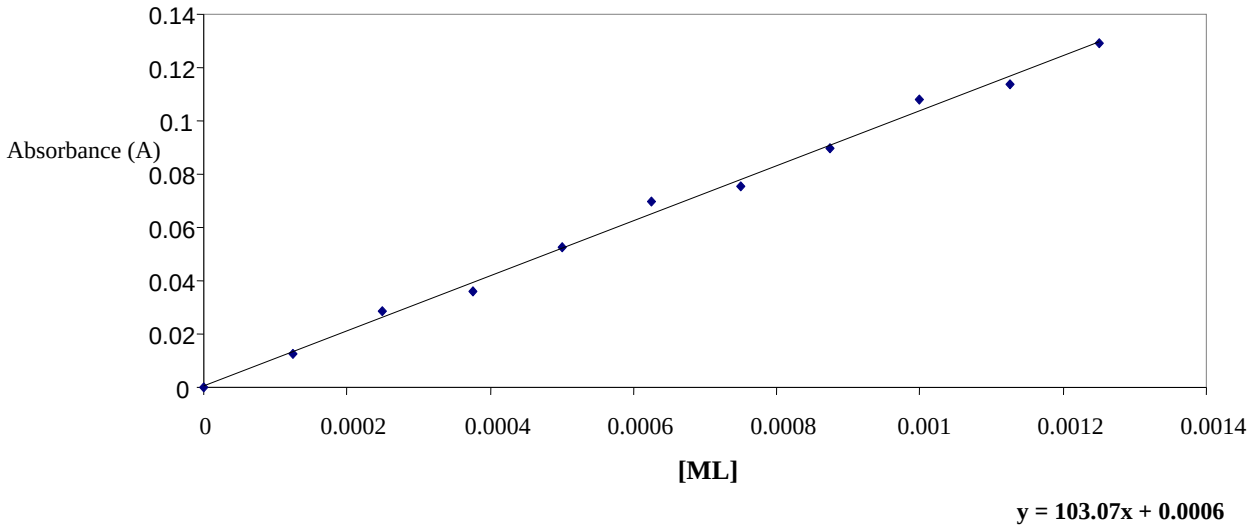

Fig-2: Calibration Curve for the Evaluation of Molar Extinction Coeff icient of Iron(III)-Salbutamol complex in buffer of pH 3.0; Temperature = 30±2°C, λmax =550 nm.

2.2 *Stoichiometry*

The sample solutions prepared were comparative to each other through the Ligand/Metal ratio and their absorbances were analyzed. Absorbance was gradually increases with the rise of ligand concentration (Fig-3) while level off at higher concentration. Tangents drawn to this curve gave an idea about the mole ratio between Fe (III) and Salbutamol which is found 1:3 regardless of solvent media (Fig-4,5).

$$M(H_2O)_6^{3+} + 3L^{-1/-2} \longrightarrow [ML_3]^{n-} + 6H_2O$$

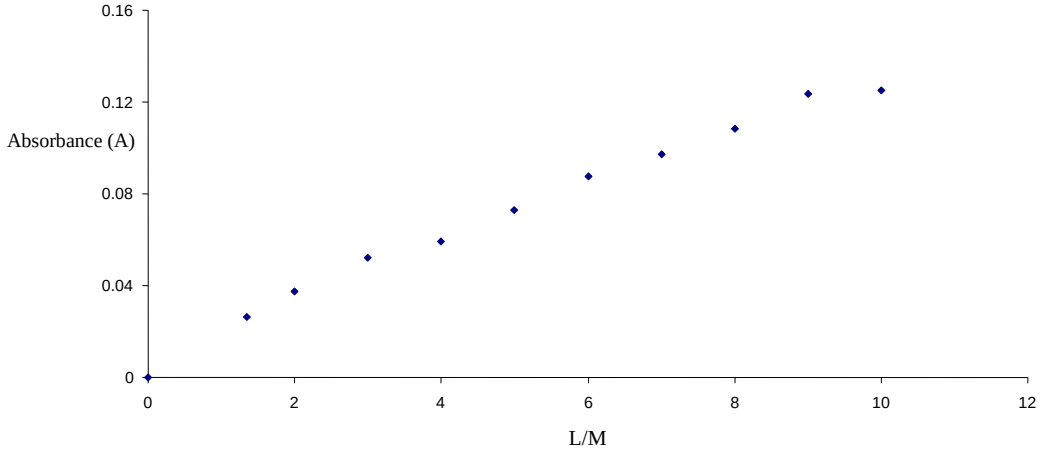

Fig-3: Mole ratio plot for Iron(III)-Salbutamol complex in buffer of pH 3.0; Concentration of Fe(III) = 1.25 mM. Temperature = 30±2°C, λmax =550 nm.

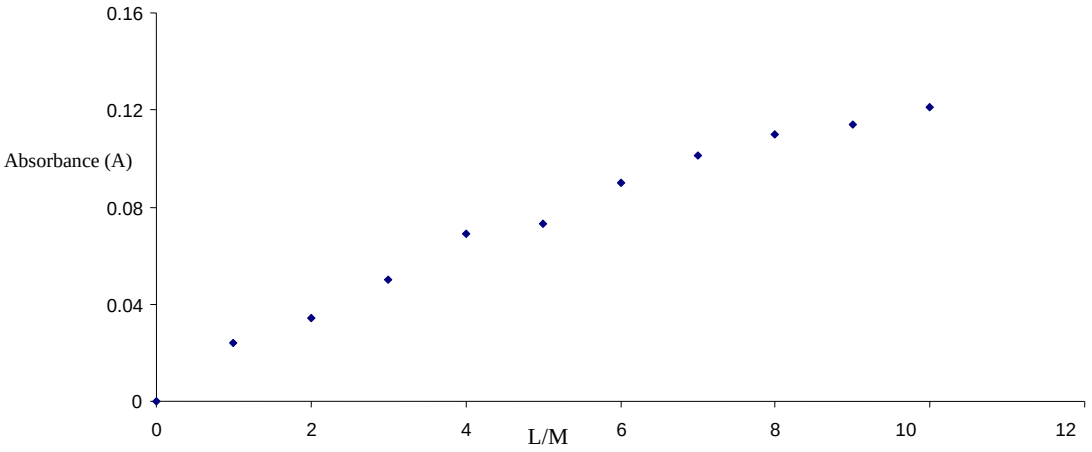

Fig-4: Mole ratio plot for Iron(III)-Salbutamol complex in buffer of pH 3.5; Concentration of Fe(III) = 1.25 mM. Temperature = 30±2°C, λmax =550 nm**.**

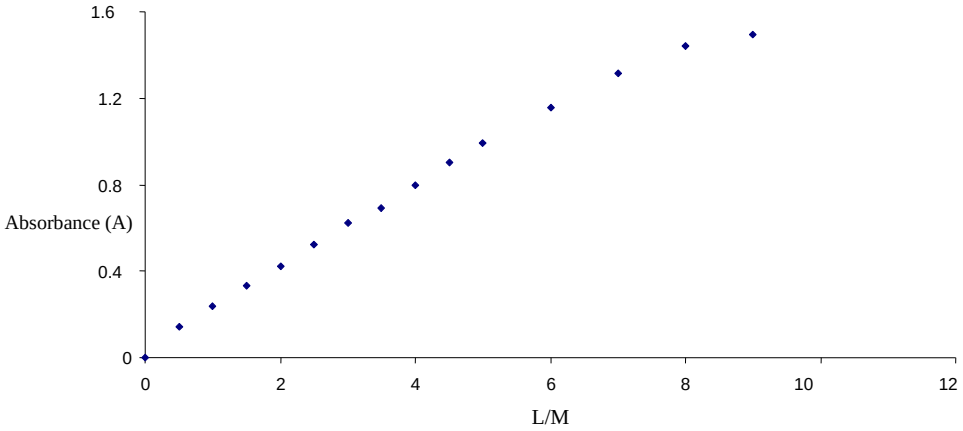

Fig-5: Mole ratio plot for Iron(III)-Salbutamol complex in aqueous media; Concentration of Fe(III) = 1.25 mM. Temperature = 30±2°C, λmax =550 nm.

2.3 *Slope Ratio Method*

Stability of complexes depends strongly upon nature of ligand regardless of many other factors. In case of pH 3.0, computational studies of the spectrphotometric data (of Fig-2) was carried to determine stability constants (β)[13-14]. Same studies were done in working conditions of pH 3.5 buffer and in non-buffered solutions.

Since 1:1, 1:2 and 1:3, M:L species were formed during the spectrphotometric titration. Therefore,

$$\beta_1 = \frac{[ML]}{[M]_r\,[L]_r} \qquad \text{----------Equation (1)}$$

Similarly

$$\beta_2 = \frac{[ML_2]}{[M]_r\,[L]_r^2} \qquad \text{----------Equation (2)}$$

and

$$\beta_3 = \frac{[ML_3]}{[M]_r\,[L]_r^3} \qquad \text{----------Equation (3)}$$

Taking ln on both sides of the equation (1)

$$\ln \beta_1 = \ln ([ML]/ [M]_r\,[L_1]_r)$$

$$\ln \beta_1 = \ln ([ML]/ [M]_r) - \ln [L_1]_r)$$

$$\ln ([ML]/ [M]_r) = \ln [L_1]_r + \ln \beta_1 \quad \text{----------Equation (4)}$$

According to the equation of straight line,

$$y = m x + c$$

By plotting a graph between 'x' and 'y', a straight line was obtained. The slope of the line is denoted by 'm' whereas 'c' represents the intercept.

Applying straight line equation on equation (4), a linear graph is observed between $\ln[L_1]_r$ and $\ln ([ML]/ [M]_r)$ (Fig-6,9,12). Therefore the intercept of the line represents 'lnβ' and the slope gives the mole ratio of metal and ligand[13-14]. Using equation 2 and 3 in the same manner $\ln\beta_2$ and $\ln\beta_3$ of ML_2 and ML_3 were determined (Fig-7,8,10,11,13,14).

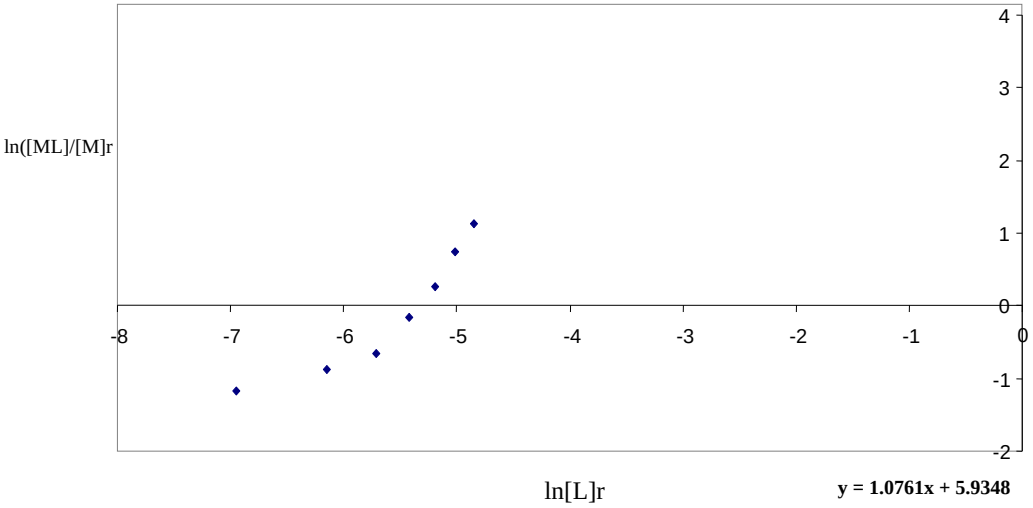

Fig-6: Plot for formation constant of ML_1 complex in buffer of pH 3.0.
Intercept = 5.9348, Slope =1.0761

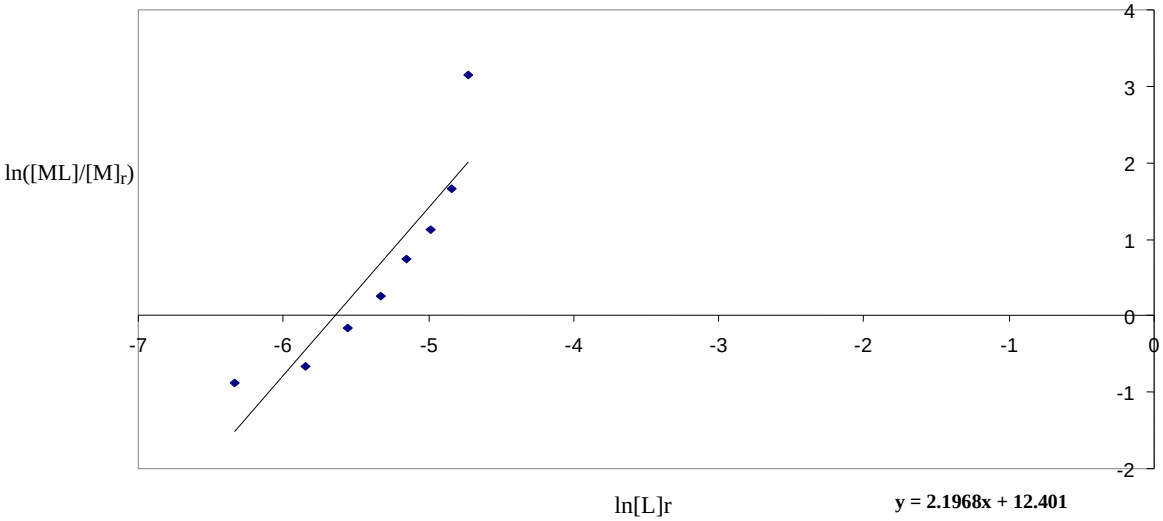

Fig-7: Plot for formation constant of ML_2 complex in buffer of pH 3.0.
Intercept = 12.401, Slope =2.1986

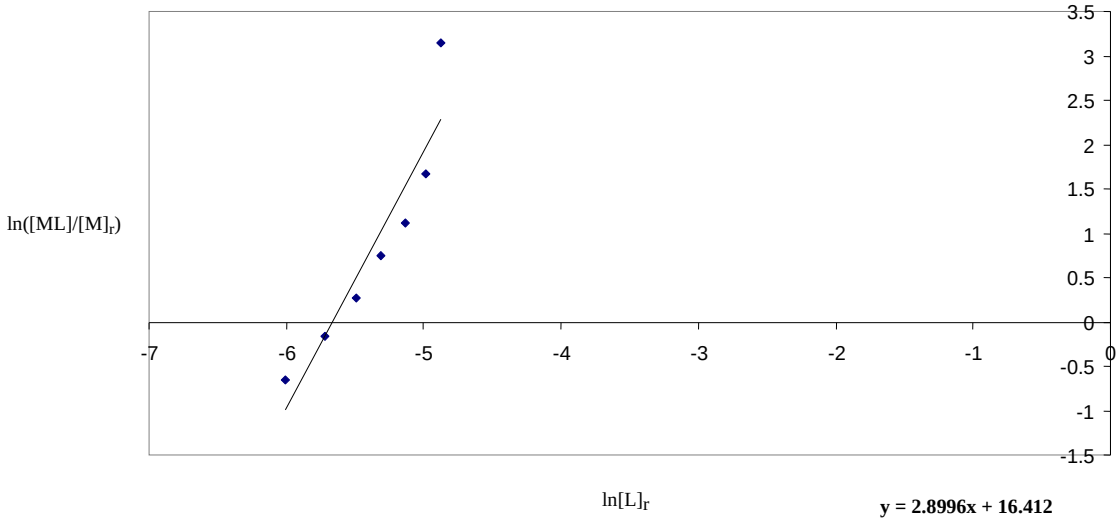

Fig-8: Slope ratio Plot of formation constant for ML_3 complex in buffer of pH 3.0.
Intercept = 16.412, Slope = 2.8996

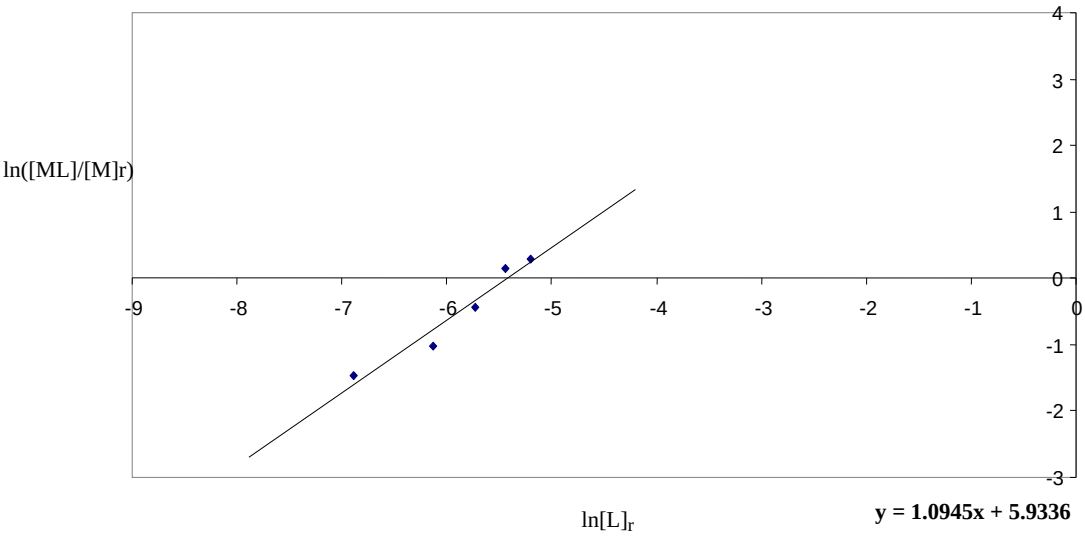

Fig-9: Plot for formation constant of ML_1 complex in buffer of pH 3.5.
Intercept = 5.9336, Slope =1.0945

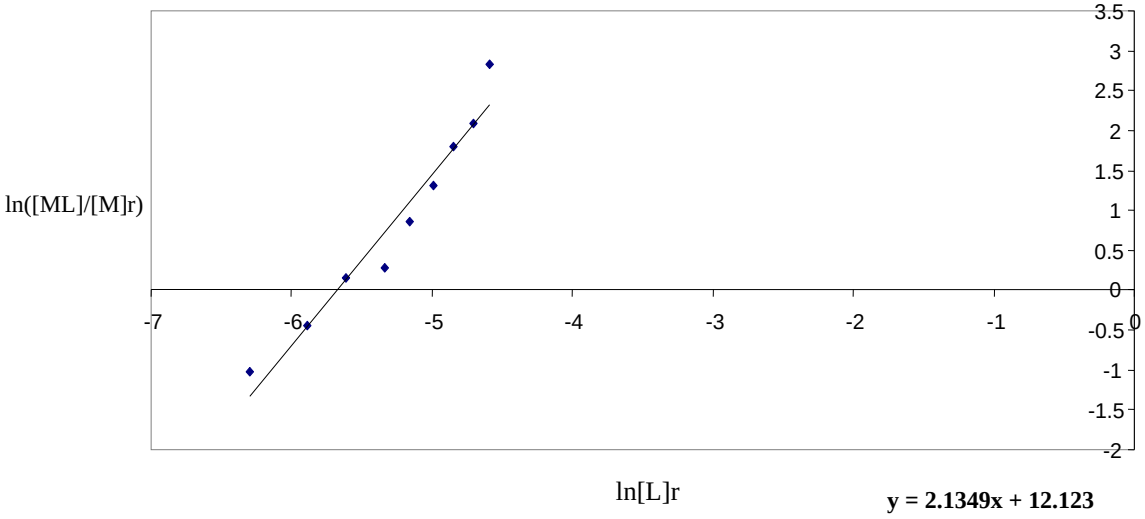

Fig-10: Plot for formation constant of ML_2 complex in buffer of pH 3.5.
Intercept = 12.123, Slope = 2.1349

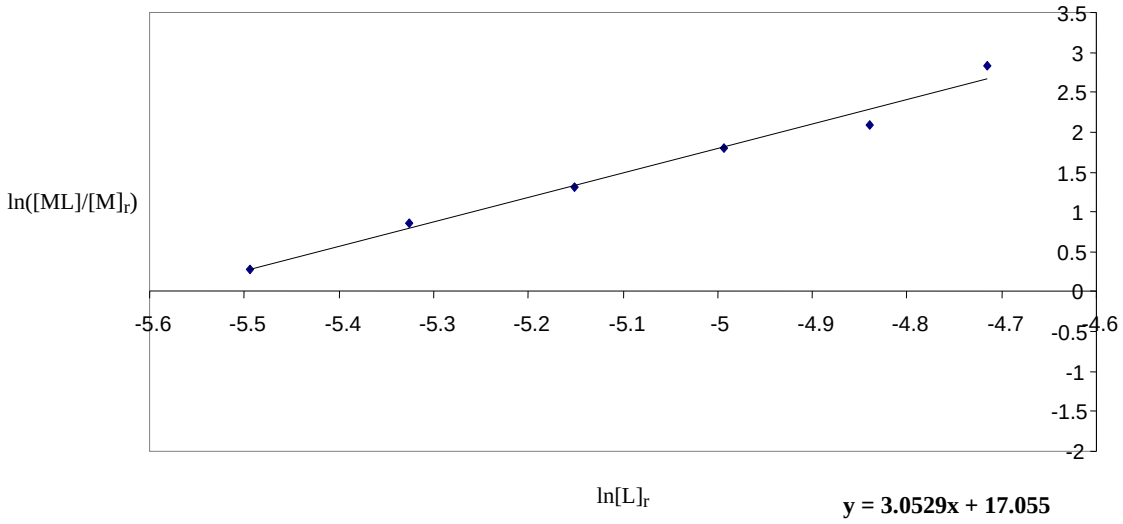

Fig-11: Plot for formation constant of ML_3 complex in buffer of pH 3.5.
Intercept = 17.055, Slope = 3.0529

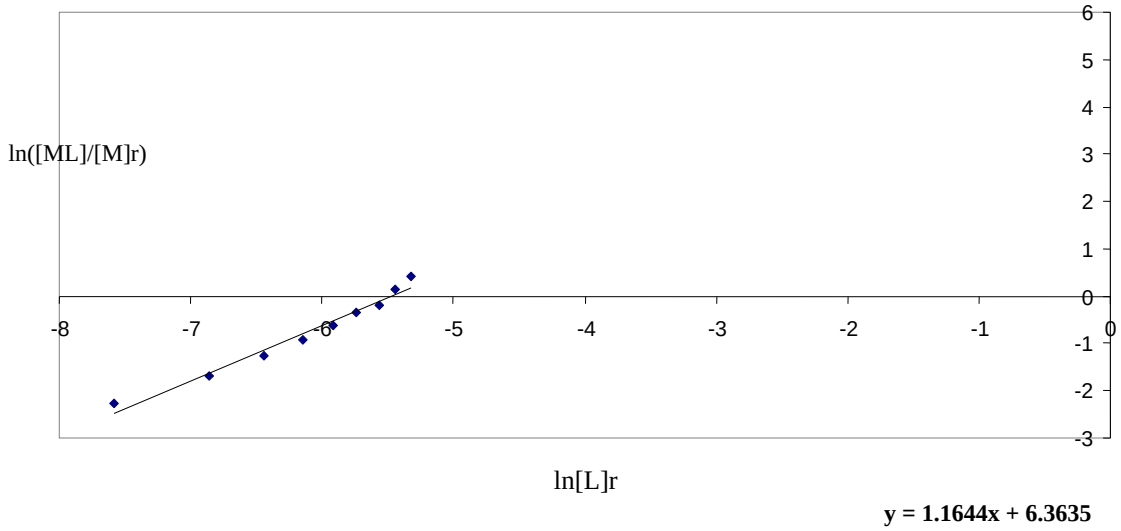

Fig-12: Plot for formation constant of ML_1 complex in aqueous media.
Intercept = 6.3625, Slope = 1.1644

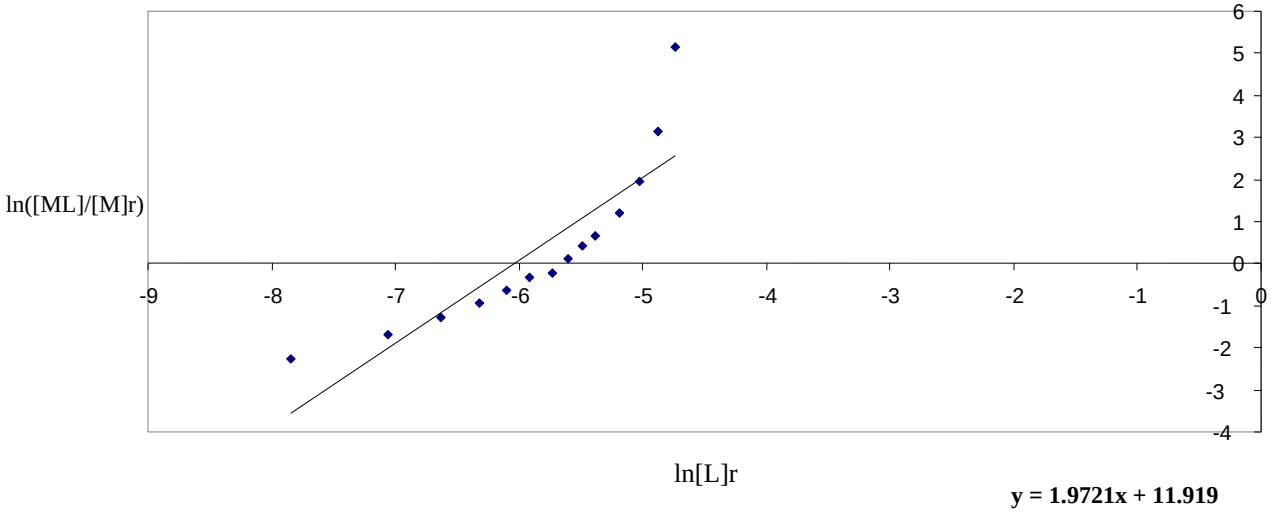

Fig-13: Plot for formation constant of ML_2 complex in aqueous media.
Intercept = 11.919, Slope = 1.9721

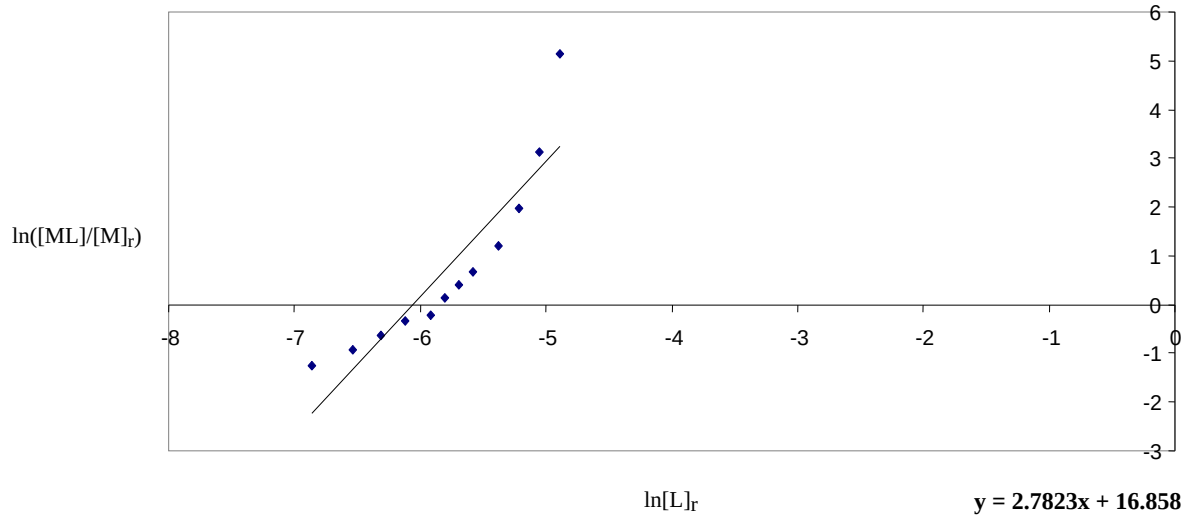

Fig-14: Plot for formation constant of ML_3 complex in aqueous media.
Intercept = 16.858, Slope = 2.7823

By the help of this method, ln β of respective complexes at different pH conditions were explored and summarized in Table-2.

Table-2: Stability Constants of Fe(III)-Salbutamol Comlexes

S. No	Solvent media	lnβ		
		ML_1	ML_2	ML_3
1	Buffer of pH 3.0	5.9348	12.401	16.412
2	Buffer of pH 3.5	5.9336	12.123	17.055
3	Non-buffered Solution	6.3635	11.919	16.858

3. EXPERIMENTAL

All reagents used were of A. R. grade. Throughout the experiments, temperature was maintained at 30°C $\pm$ 2°C. Known amount of Ferric sulfate was weighed carefully on an analytical balance SHIMADZU Model No. AUW 220, and dissolved in de-ionized distilled water with a small volume of concentrated hydrochloric acid for acidification. The final volume was then made up to 250.0ml.

This Fe (III) solution was standardized using hydroquinone and 1,10-orthphenanthroline solutions after addition of Na-$NaCH_3COO$ buffer[12].

Stock solution of Salbutamol sulphate for known concentration were prepared in de-ionized distilled water by dissolving calculated amount of AR grade reagent. Different dilutions of the stock solutions were then made according to the requirement of the experiments. For each and every set fresh solution were prepared to avoid the expected oxidation of ligand molecules by air. Same method is used to prepare stock solution in buffer of pH 3.0 and

3.5. The required buffers of pH 3.0 and 3.5 were prepared by adding formic acid in standard NaOH, and the pH of buffers were maintained by using Jenway model No. 370 having a resolution of $\pm$ 0.01.

The prepared solutions (in each media i.e. pH 3.0, pH 3.5 and in de-ionized distilled water) of metal salt, ligand and their complex were scanned between 300nm and 800 nm on the UV-Visible spectrophotometer Thermo Genesys 6 in order to find out the Absorbance maxima(λ_{max}).

A complex solution which contain excess ligand than Fe(III), showed significant absorbance at selected wavelength. This solution was used for calibration curve method. As the ligand was in excess in the case, it is assumed that all the Fe(III) formed complex. Therefore the concentration of the complex is equal to the concentration of the metal ion taken initially in th-e sample.

Different volumes of this complex from 1.0 ml to 10.0 ml were taken and diluted with de-ionized distilled water up to a final volume of 10.0ml and their absorbance were recorded. Same procedure was used to find out the molar extinction coefficient in buffer of pH 3.0 and 3.5.

A fixed volume (1.0 ml from stock) of Fe (III) salt solution was taken in different volumetric flasks of 10.0 ml. Different volumes of the already prepared equimolar solution of Salbutamol sulphate was added to the volumetric flasks and their total volume made up with deionized distilled water. An intense violet color Iron (III) – Salbutamol complex was observed instantly.

Absorbances versus ligand to metal ratio were obtained. A graph of which helps to evaluate stoichiometry of the complex. Computational method was applied to implement the slope ratio method that explores the stability

constants of the complex at experimental conditions[13-14]. Same procedure was used for the analysis at pH 3.0 and 3.5. For which the final volume of complex solutions were maintained upto a certain value with respective buffers. Each experiment in the analysis was repeated at least thrice to obtain accuracy and minimize errors.

4. CONCLUSION

Salbutamol chelates iron as a bidentate ligand. Three molecules of Salbutamol sulphate bind an Iron satisfying all six coordination sites and form an octahedral complex.

In a buffer of pH 3.0, pH 3.5 and in non-b uffered media, the analysis showed that the mole ratio between iron and Salbutamol sulphate comes out to be 1:3, regardless of the pH conditions in which the analysis was carried out. The results of pH 3.0 and pH 3.5 are very much similar whereas the molar extinction coefficient in aqueous media is very high as compared to the other two.

The stability constants of ML_1, ML_2 and ML_3 are considerably high although the trend observed was similar in all three solvent systems.

The results showed that at acidic pH (in stomach), salbutamol is able to make strong complex with Ferric ion and therefore may create iron deficiency in asthmatic patients. Since literature reported anemia in asthmatic patients, the present study is consistent with the result.

Still the binding sites of the investigated drug molecule has question mark. Complex formation and stability constants and stoichiometry affected by increasing pH should be examine and explored.

5. REFERENCES

1. Masoli, M., Denise, F., Shaun, H., Richard, B. "Global Burden of Asthma(TM)" (PDF). Retrieved on 2008-08-08, **(2004)**.
2. Lugogo, N., Que, L. G., Fertel, D., Kraft, M., Asthma. In: Mason, R. J., Broaddus, V. C., Martin, T. R., Murray Nadel's Textbook of Respiratory Medicine. 5th ed. Philadelphia, Pa: Saunders Elsevier; **(2010)**, Chap 38, http://dx.doi.org/10.1016/B978-1-4160-4710-0.00038-9.
3. Brozek, J. L., Bousquet, J., Baena-Cagnani, C. E., Bonini, S., Canonica, G. W., Casale, T. B., J. Allergy Clin. Immunol. **(2010)**, 126(3), 466-76, http://dx.doi.org/10.1016/j.jaci.2010.06.047.
4. Zhao, J., Takamura, M., Yamaoka, A., Odajima, Y., Iikura, Y., Pediatr Allergy Immunol. **(2002)**, 13(1), 47–50, http://dx.doi.org/10.1034/j.1399-3038.2002.00051.x.
5. Lilly, C. M., J. Allergy Clin. Immunol. **(2005)**, 115, 526–31, http://dx.doi.org/10.1016/j.jaci.2005.01.028.
6. Chen, E., Miller, G. E., Brain Behav Immun. **(2007)**, 21(8), 993-9, http://dx.doi.org/10.1016/j.bbi.2007.03.009.
7. Gold, D. R., Wright, R., Annu Rev Public Health. **(2005)**, 26, 89–113, http://dx.doi.org/10.1146/annurev.publhealth.26.021304.144528.
8. Chen, E., Miller, G. E., Brain Behav. Immun. **(2007)**, 21(8), 993-9, http://dx.doi.org/10.1016/j.bbi.2007.03.009.
9. Fanta, C. H., N Engl J Med. **(2009)**, 360, 1002-1014, http://dx.doi.org/10.1056/NEJMra0804579.
10. Rodrigo, G. J., Rodrigo, C., Hall, J. B., Chest. **(2004)**, 125(3), 1081–102, http://dx.doi.org/10.1378/chest.125.3.1081.
11. Andrews, N. C., Engl J Med, **(1999)**, 341:1986-1995.
12. Vogel, A Textbook of Quantitative Inorganic Analysis, 3rd Ed., p. 294, 310 & 787.
13. Fatima, N., Maqsood, Z. T., J. Saudi Chem. Soc., **(2005)**, 9(3), 519-528.
14. Ali, K., Fatima, N., Maqsood, Z. T., Kazmi, S. A., J. Iran. Chem. Soc., **(2004)** 1(1), 65-70.