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Critical Behavior of Iron(III) with a Typical Catecholate Siderophore

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Siderophores are ligands that serve as iron scavengers or iron chelators, which hinder the absorption of iron in the body; tannic acid is one of them. Since a tannic acid molecule has 8 gallic acid groups, it can bind 1-4 Fe(III) ions, depending upon ligand concentration. pH 3, 4, 5, 8 and 10 were selected for this study. The λmax and M:L molar ratio of the complexes varies with pH, as well as with ligand concentration. For low ligand concentration at pH 3, 4 and 5, the maximum absorbance of the complex was found at 650 nm, while, at pH 8 and 10, 500 nm was the λmax. For high ligand concentration, pH 550 nm was the λmax. Stability constant values of iron(III) tannic acid complexes were measured spectrophotometrically using the mole ratio method, as well as the slope ratio method, potentiometrically. The experimental results of pH titrations were treated by program "Best". The β values obtained by these methods were compared. Stability constant values were found to be 10^3, 10^8 and 10^{16} for ML, M_2L and M_4L, respectively.

INTRODUCTION

Two major biological functions that are performed by iron are oxygen transport and electron transfer in an electron transport chain. It is involved in transport and storage via hemoglobin and myoglobin in vertebrates. In an electron transfer system, it plays an important role in cytochromes and various iron-sulfur proteins. Also, important catalytic oxidation involves iron containing biocompounds [1,2].

Iron is stored in iron storage proteins, such as ferritin and hemosiderin. It exists in the body in both ferrous and ferric forms. Its maximum absorption takes place in the body in the form of ferrous. In excess, especially in the ferric form, it becomes toxic, because of deposition in the tissues as insoluble hydroxides and phosphates at physiological and higher pH, unless it is bound to iron storage protein [3,4].

These siderophores may hinder the absorption of iron in the body [5-7]. Chemically, these are of two types, hydroxamate and catecholate. The catecholate type comes under the heading of catechols and its derivatives form stable complexes with iron [8,9] and vanadium [10]. These have a very high affinity to Fe(III), so, they form highly stable complexes with Fe(III), having stability constants of 10^{30}-10^{50} [11,12].

Tannic acid belongs to the catecholic category, such as enterobactine. The stability constant for a Fe(III)-enterobectine complex is 10^{52} [13]. Common tannic acid belongs to the hydrolysable category, which decomposes in water [14,15]. Generally, its quantity found in tea is 15-20%, but, it depends on many factors, such as age and seasonal variations, extent of processing and the region to which the tea belongs. The origin, abundance, degree of purity and the factors on which the abundance of the tannic acid depends, are discussed in detail [16,17]. The structural characteristics, composition and chemical nature of the tannic acid has also been studied [18-20]. Tannic acid is a large molecule containing 8 groups of 1,2,3-trihydroxybenzoic acid (gallic acid). It can chelate more than one metal at a time (simultaneously). The stoichiometry and stability of the complexes may be pH dependent [21].

It was suggested that tannins are responsible for the interference of absorption of iron [22-24]. Tannins are the major constituents of our food stuffs and form highly stable complexes with Fe(III) [25-27]. Tannic acid, in any form of beverage, either hot or cold, consumed by the human body, may result in a deficiency of iron, because Fe(III) is considered to be chelated with tannic acid easily, even at physiological pH [28].

High doses of iron supplements, taken together

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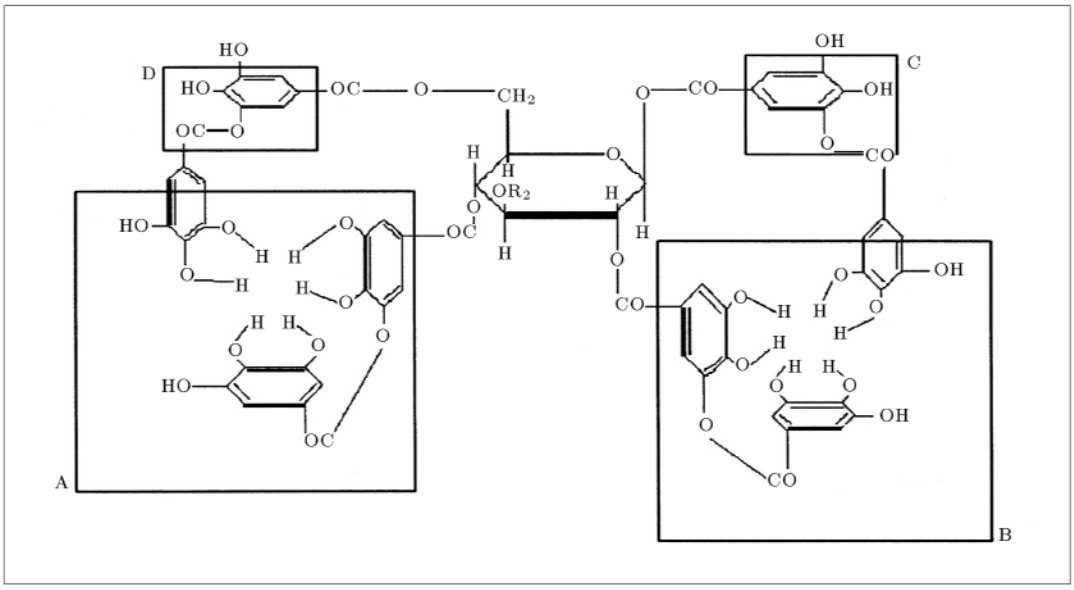

Figure 1. Chelated structure of tannic acid showing maximum protons and coordination sites.

with zinc supplements, on an empty stomach, can inhibit the absorption of zinc. Iron-fortified foods have no effect on zinc absorption, when consumed together in a single meal, whereas calcium has been found to decrease the absorption of iron. The basic requirement of an iron chelating agent is a high and selective affinity to bind iron avidly under physiological conditions [29,30].

The computer program, BEST, is also used to compute stability constants using potentiometric data. This program is used for potentiometric calculations and the variable measured in it is the pH. It is, therefore, considered logical to carry out the calculations with an algorithm, which calculates pH directly and minimizes the sum of the weighted squares of pH residuals. This is the feature that distinguishes BEST from most of the programs available for the calculation of equilibrium constants [31].

RESULTS AND DISCUSSION

Tannic acid can donate a maximum of 16 and a minimum of 2 protons from its four sites A, B, C and D (Figure 1). The complexation of any metal with a protonated ligand depends upon the deprotonation of the ligand. At very low pH, most of the ligands remain protonated and only a few binding sites may be available for a metal ion, whereas, with the increase of pH, the deprotonation increases, providing more binding sites for metal.

Potentiometric titration, therefore, seems to be the most suitable method to find the pH at which complexation occurs. A remarkable change in the potentiometric curve was observed between pH ranges 3-10, showing maximum complexation in this region. According to this, the pH selected for the detailed study were 3, 4, 5, 8 and 10 (Figure 2).

As these complexes are highly colored, spectrophotometric studies were also found to be very suitable. Two sets were prepared, having low and high ligand concentration. For low ligand concentration, the λmax observed were 600 nm for pH 3, 4 and 5, whereas 500 nm for pH 8 and pH 10 (Figure 3). For high ligand concentration peaks were found 550 nm for all considered pH (Figure 4). Stoichiometry of the complexes was determined by the mole ratio method, using two sets of experiments. In the first case, metal was kept constant with the increase of ligand. Absorbances were noted at 550 nm at different pH. The absorbance values were then plotted against the

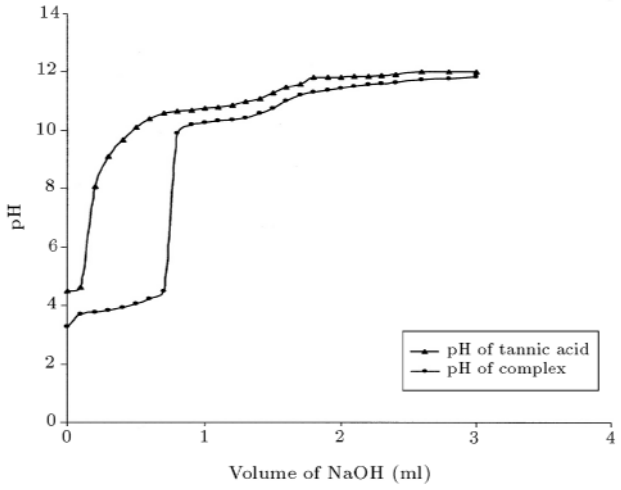

Figure 2. Plot for potentiometric titration.

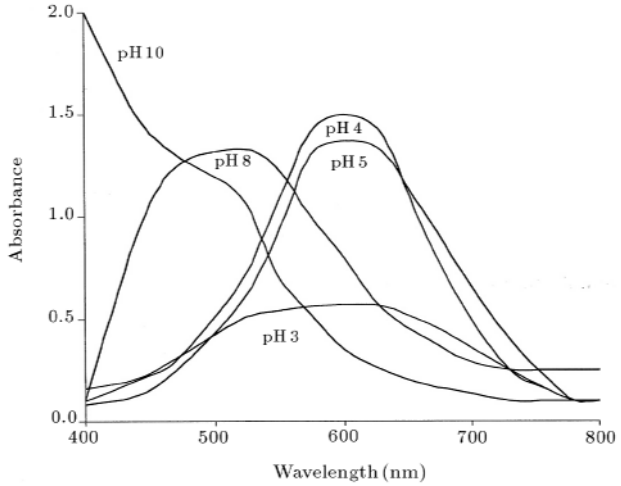

Figure 3. Absorption peaks at variable pH for low ligand concentration.

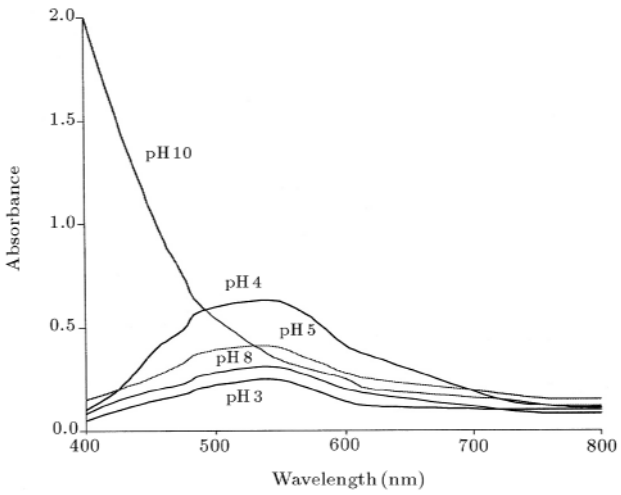

Figure 4. Absorption peaks at variable pH for high ligand concentration.

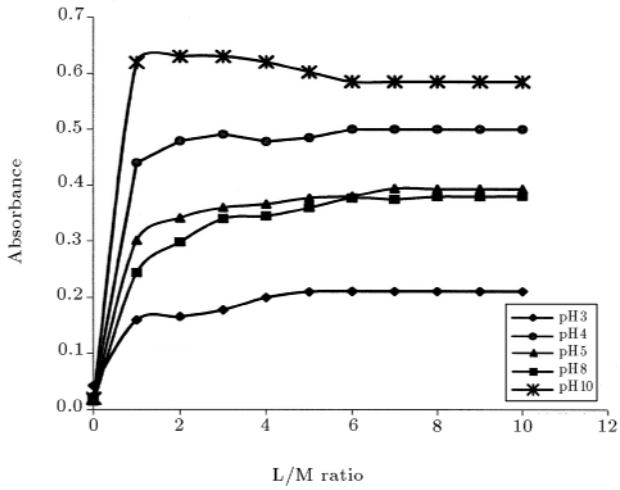

Figure 5. Mole ratio method at variable pH (metal constant).

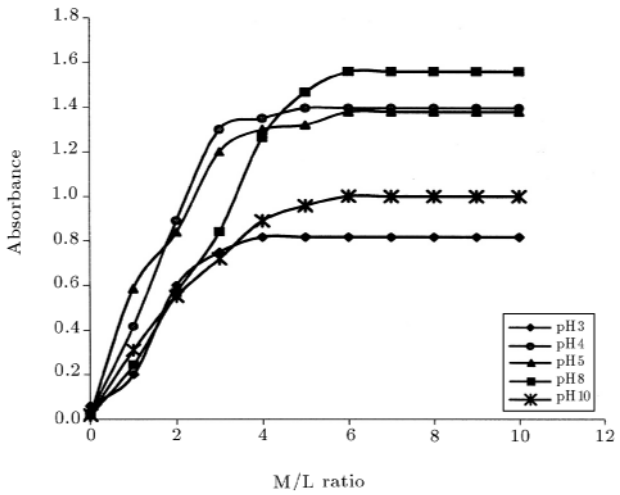

Figure 6. Mole ratio method at variable pH (ligand constant).

L/M ratio and the ML ratio was found to be 1:1 at all considered pH (Figure 5). In the second set, the ligand was kept fixed while the metal was gradually increased. The absorbance was then noted at the 600 nm and 500 nm λmax of the complex and the absorbance was plotted versus the M/L ratio. At low pH 3, 4 and 5, the ML ratio was found to be 2:1 and at pH 8 and 10 it reached 4:1 (Figure 6).

It is shown that the ligand has the capability to bind 1-4 metal ions, due to several binding sites A, B, C and D (Figure 1), where A and B are preferable, as compared to C and D sites. At low pH, since the ligand is in protonated form and only one proton from each trihydroxy benzene ring is removed, A and B positions form only two bonds with each metal (act as bidentate) and the remaining coordination sites of the metals might be satisfied with water molecules. Whereas, at high pH values, it is completely free from protons and positions A and B act as hexadentate, positions C and D may act as bidentate for third and fourth metals.

For high ligand concentration, when ligand is in excess, generally, the stoichiometry of the complexes varies from 1:1-1:3. Due to a larger size of ligand, there may be a steric hindrance for 1:3, so, each metal is coordinated as the maximum by two ligands. As the opposite site of the ligand is also available, therefore, simultaneously, two metals may be chelated by one ligand at all considered pH values. The most suitable geometry for these complexes under these conditions is cyclic, having 3 metals chelated with 3 ligands and so on, therefore, the empirical formula of the complex remains the same as 1:1.

Stability constant (K) values were determined spectrophotometrically, as well as potentiometrically, for each specie (i.e., ML, M_2L and M_4L). Before the determination of K_{eq} values, ε (molar extinction

Table 1. ε values for different species at specific λmax.

Sps.	Wavelength (λ) (nm)	Molar Absorptivity (ε) lit. mol ^{1}.cm ^{1}
ML	550	7000
M_2L	650	14000
M_4L	500	16000

coefficient) values were also calculated (Table 1). The concentration of the complex of each particular ratio was determined by using its ε value. The concentration of free metal was calculated from the concentration of the complex, while the concentration of the free ligand was calculated by using the following expression:

$$p[L]_{free} = pK_a \quad \log[HL]_{init} \quad [NaOH].$$

Then K_{eq} values were calculated, which were found to be in the ranges of 10^3, 10^8 and 10^{16} for ML, M_2L and M_4L, respectively.

The equation for the equilibrium constant was further modified in logarithm form, as follows:

$$\log K = \log[M_nL]/[L] \quad n \log[M],$$

$$\log[M_nL]/[L] = \log K \quad n \log[M].$$

According to this, the plot of $\log[M_nL]/[L]$ versus log [M] may give a straight line. The slope of that line "n" should indicate the amount of metal chelated. Intercept should give the value of K for the species (Figures 7 to 10) and (Tables 2 and 3). The values of the stability constants obtained were, again, comparable to those which were obtained and calculated by different methods (Table 4). However, the slope ratio plots do not seem to be linear because of their low R^2 values. This affects the accuracy of pK values obtained for the complexes.

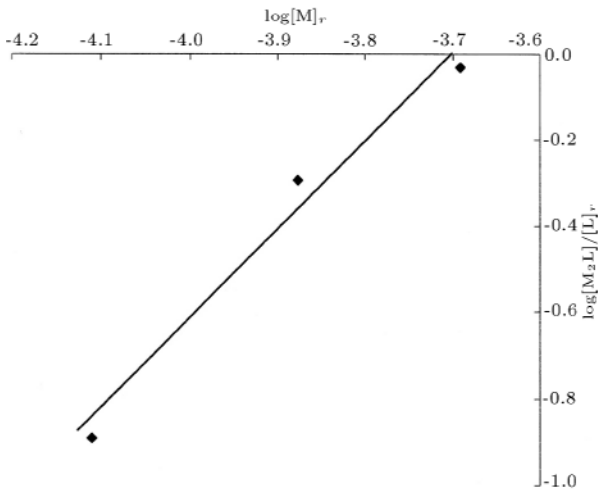

Figure 7. Plot for slope ratio method at pH 3.

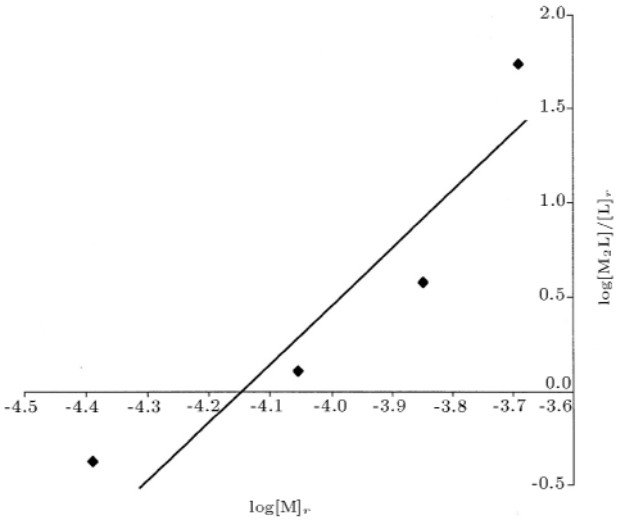

Figure 8. Plot for slope ratio method at pH 4.

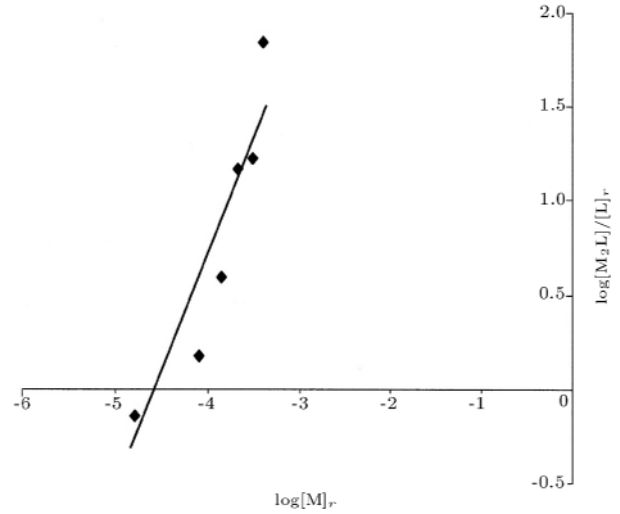

Figure 9. Plot for slope ratio method at pH 5.

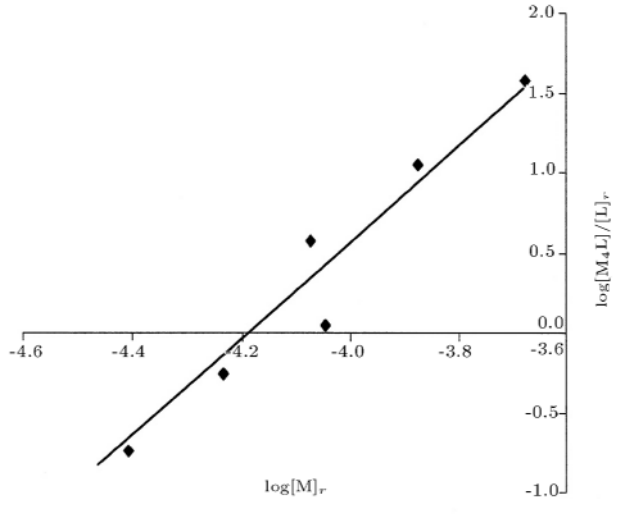

Figure 10. Plot for slope ratio method at pH 8.

Table 2. Calculation for stability constants by slope ratio method at pH 3 ($\varepsilon_{600\ nm}$ = 14000 lit.mol $^{-1}$.cm $^{-1}$).

Vol. of Metal (ml)	M/L Ratio	Abs.	$[M_2L]$	$[L]_r$	$[M]_r$	log $[M]_r$	log $[M_2L]/[L]$
1	1	0.159	1.135 e ^{5}	8.865 e ^{5}	7.730 e ^{5}	-4.110	-0.892
2	2	0.471	3.364 e ^{5}	6.636 e ^{5}	1.327 e ^{5}	-3.877	-0.295
3	3	0.674	4.814 e ^{5}	5.186 e ^{5}	2.037 e ^{5}	-3.691	-0.032
4	4	0.818	5.842 e ^{5}	4.158 e ^{5}	1.168 e ^{5}	-3.932	0.147

Concentration of metal and ligand = 0.001 M; Volume of ligand = 1 ml; $[M]_t$ = Total metal or added metal; $[L]_t$ = Total ligand or added ligand; $[M]_r$ = Remaining metal [M]t-2[M_2L]; $[L]_r$ = Remaining ligand $[L]_t$-[M_2L]; log K = log $[M_2L]/[L]_r$-2 log $[M]_r$.

Table 3. Calculation for stability constants by Slope ratio method at pH 8 ($\varepsilon_{500\ nm}$ = 16000 lit.mol $^{-1}$.cm $^{-1}$).

Vol. of Metal (ml)	M/L Ratio	Abs.	$[M_4L]$	$[L]_r$	$[M]_r$	log $[M]_r$	log $[M_4L]/[L]$
1	1	0.244	1.525 e ^{5}	8.475 e ^{5}	3.900 e ^{5}	-4.408	-0.744
2	2	0.567	3.543 e ^{5}	6.457 e ^{5}	5.830 e ^{5}	-4.234	-0.260
3	3	0.842	5.262 e ^{5}	4.738 e ^{5}	8.952 e ^{5}	-4.048	0.045
4	4	1.264	7.900 e ^{5}	2.100 e ^{5}	8.400 e ^{5}	-4.075	0.575
5	5	1.467	9.168 e ^{5}	8.320 e ^{6}	1.330 e ^{4}	-3.876	1.042
6	6	1.559	9.743 e ^{5}	2.570 e ^{6}	2.103 e ^{4}	-3.677	1.578

Concentration of metal and ligand = 0.001M; Volume of ligand = 1ml; $[M]_t$ = Total metal or added metal; $[L]_t$ = Total ligand or added ligand; $[M]_r$ = Remaining metal $[M]_t$-4[M_4L]; $[L]_r$ = Remaining ligand $[L]_t$-[M_4L]; log K = log $[M_4L]/[L]_r$-4 log $[M]_r$.

Table 4. Comparison of the stability constant values obtained by different methods.

Sps.	log β values		
	Mole Ratio Method	Slope Ratio Method	Best Program
ML	3	*	3.8
M_2L	8	8	8.9
M_4L	16	16	15

EXPERIMENTAL

Analytical grade (AR) reagents were used without any further purification. Double distilled deionized water was used in a working solution and in the preparation of all solutions of reagents and buffers. This double distillation was taken by the deionizer (i.e. Amberlite resin RA-401 from BDH chemicals), in order to make it free of cations (Check by the conductivity method).

For pH titration, CO_2 free water was prepared by boiling redistilled and deionized water for 10 minutes and then cooling it in airtight flasks. A 0.050 M solution of Potassium Hydrogen Phthalate, which has the pH value of 4.01 at 25°C, was used to calibrate the pH meter.

pH titration was undertaken using a pH meter (ORION Research Analog pH meter/model SA 920A). All solutions were equimolar and standard solutions of NaOH and $Fe(NO_3)_3 9H_2O$ were used. In the first set of titration, 10 ml of water and 50 ml (0.0010 M) of tannic acid were taken in a beaker, then, aliquotes of standard 0.10 M NaOH were added with the help of a micro-pipette with continuous stirring by a magnetic stirrer and the pH variation was measured after each addition. In the second set of titration, the same procedure was repeated for the complex of Fe(III)-tannic acid. After selecting the pH, the absorption peaks of Fe(III)-tannic acid complex at pH 3, 4, 5, 8 and 10 were determined by scanning with a 400-800 nm Shimadzu model-160 (UV-VIS) spectrophotometer. For the spectrophotometric study, two sets were prepared at each pH. In the first case (taking high ligand concentration), the metal was kept constant while the ligand was gradually increased. Absorbances were recorded on Spectronic 21 (Bausch and Lomb) on the observed λmax. The same procedure was repeated for the second case (taking low ligand concentration), in which the ligand remained constant while the metal was increased.

REFERENCES

1. Hay, R.W. "Bio-inorganic Chemistry", *Ellis Horwood Series in Bio-inorganic Chemistry*, Halsted Press, John Willey & Sons, **(1)**, pp 102-7, NY, USA (1987).

2. Miller, D.D. and Shriker, B.R. "Nutritional bioavailability of iron", *ACS. Sym. Ser. 203. Am. Chem. Soc.*, C. Kies, Ed., (1), pp 12-25, New York, USA (1982).
3. Sultana, F. and Kazmi, S.A. "Co-ordination of hydroxamate type siderophores with iron in the presence of biologically available trace metals", *Pak. J. Pharm. Pb. Univ. LHR.*, **9**(2), pp 23-27 (1994).
4. Gray, H.B. and Lever, A.B.P. "Physical bioinorganic chemistry series", (5), M.L. Thomas, Ed., V.C.H. Ltd., pp 103-109 (1989).
5. Berthold, F.M., Gertraud, M.M. and Kenneth, N.R. "Iron carriers & iron protiens", *Physical Bio-inorganic Series*, M.L., Thomas, Ed., (1), pp 3-10, Published by V.C.H. Ltd., UK (1988).
6. Maqsood, Z.T. and Kazmi, S.A. "Determination and comparison of stability constant, enthalpy and entropy of formation of iron (III)-gallic acid complexes", *Jour. Chem. Soc. Pak.*, **5**(1), pp 30-35 (1993).
7. Ali, K., Fatima, N. and Zahida, T.M. "Chelation of V(V) with hydroxamate ligands and their stability relation with H^+ concentration", *J. Saudi Chem. Soc.*, **3**(7), pp 355-359 (2003).
8. Syed, A.K. and Saqib, M. "Reactivity of iron(III) complexes of gallic acid", *Inorganic Chemica Acta.*, **137**(1), pp 151-54 (1987).
9. Maqsood, Z.T. and Kazmi, S.A. "Study of iron and gallic acid complexes at pH 4", *J. Research (Science)*, **2**(3), pp 17-19 (1990).
10. Fatima, N. and Syed, A.K. "Complex formation of vanadium ions with various catecholes", *J. Chem. Soc. Pak.*, **24**(1), pp 49-56 (2002).
11. McArdle, J.V., *Encyclopaedia of Chemical Technology*, M. Grayson, Ed., John Willey & Sons Inc., (13), p 764, NY, USA (1981).
12. Martell, A.E. and Smith, R.M., *Critical Stability Constants*, (1), pp 123-126, Plenum Press, NY (1977).
13. Bezkorovainy, A., *Biochemistry of Non Heme Iron*, (1), pp 306-20, E., Frieden, Ed., Plenum Press, NY (1980).
14. Finar, I.L., *Organic Chemistry*, (2), 4th Edition, p 702, English Language Book Society & Longman Group Limited, London (1975).
15. Hagerman, A.E., Zhao, Y. and Johnson, S. "ACS symposium series", *Am. Chem. Soc.*, pp 209-22, Washington DC, USA (1997).
16. Azhar, I., Ahmed S.W. and Usmanghani, K., *Tannins: Their Chemistry and Bioactivity*, (1), pp 9-11, Bureau of Composition, Compilation and Translation Press, University of Karachi, Pakistan (1997).
17. Shakir, S., Begum, A., Ali, A., Jamil, K. and Qadri, R.B. "Studies on the chemical constituents of different varieties of black. tea", *Jour. Chem. Soc. Pak.*, **19**(1), pp 34-37 (1997).
18. Koji, N., Toshio, G. and Sho, I., *Natural Product Chemistry*, (2), pp 165-66, Kodansha Ltd., Academic Press, Inc., NY, USA (1990).
19. Brown, R., Klein, A. and Hurrell, R.F., *The Bioavailability of the Trace Minerals Iron & Zinc*, D. Southgate, I. Johnson and G. Fenwick, Eds., pp 152-53, RSC Special Publication, (72), Cambridge (1988).
20. South, P.K. and Miller, D.D., *Food Chemistry*, (2), pp 167-72, Elsvier Science Ltd., USA (1998).
21. Iffat, A.T., Maqsood, Z.T., Ali, K. and Nisar, S. "Interaction of tannic acid with higher oxidation state of iron", *Jour. Chem. Soc. Pak.*, **26**(2), pp 151-155 (2004).
22. Swain, T. and Bate Smith, E.C., *Comparative Biochemistry*, (3), pp 101-105, A.M. Florkin and H.S. Mason, Eds., Academic Press, New York, USA (1962).
23. Iffat, A.T., Fatima, N. and Maqsood, Z.T. "Study of complex formation of Fe(III) with tannic acid", *J. Chem. Soc. Pak.*, **27**(2), pp 174-177 (2005).
24. White, T., *The Chemistry of Vegetable Tannins*, (1), pp 13-18, Society of Leather Trades Chemists, Croydon (1956).
25. Walker, R.J. and Williams, R., *Iron in Biochemistry & Medicine*, (1), pp 786-90, A., Jacobs, and M., Wordwood, Eds., Academic Press 45, NY, USA (1977).
26. Pittand, C.G. and Martell, A.E. "Inorganic chemistry in biology & medicine", *ACS Symposium Series*, (1), pp 210-15, NY, USA (1980).
27. Zahida, T.M. and Syed, A.K. "Formation of iron gallic acid complexes at different pH and determination of their stability constant", *J. Pak. Chem. Sci. Ind. Res.*, **6**(11), pp 113-119 (1993).
28. SHI, X., Dalal, N.S. and Jain, A.C., *Food Chemistry Toxicology*, (1), pp 16-19, Pergamon Press, New York, USA (1991).
29. Sultana, F. and Maqsood, Z.T. "Effect of other metals on iron bioavailability in presence of selective chelators", *Karachi Univ, J. of Sci.*, **23**(1), pp 59-65 (1999).
30. Harris, C.M. and Livingstone, S.E. "Chelating agents and metals chelates", p 95, F.P. Dwyer and D.P. Mellor, Eds., Academic Press, New York, USA (2001).
31. Motekaitis, R.J. and Martell, A.E. "BEST- A new program for rigorous calculation of equilibrium parameters of complex multi-component systems", *Can. J. Chem.*, (60), pp 2403-2409 (1982).