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FORMATION OF IRON GALLIC ACID COMPLEXES AT DIFFERENT pH AND DETERMINATION OF THEIR STABILITY CONSTANTS

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The stability constants of ferric complexes with 3, 4, 5, trihydroxy benzoic acid have been determined. This simple ligand was used as model of the catechol containing iron transport compounds (siderophores) found in micro-organism having very high stability constant values with ferric ion some time up to 10^{45}. The metal to ligand molar ratio is totally pH dependent. The stepwise formation constant Kn of the iron gallate reported in this paper are defined as [MLn]/[MLn-1][L]. Where [L] is the concentration of deprotonated catechol ligand. The species observed during iron gallate formation were ML, ML_2 and ML_3. The log K values calculated for these from their spectrum are $\log K_1$=14, $\log K_2$=8.5 and $\log K_3$=5. It shows that over all formation constant is nearly 10^{28}.

Key words: Iron, Gallic acid, Stability constant.

Introduction

Role of iron in the biochemical process is complicated as it involves an intricate chain of reactions. Iron also becomes toxic when in excess because of the tendency of this metal to separate in tissues as insoluble hydroxide and phosphate at physiological and higher pH unless bound to iron transfer protein or to iron storage-proteins. The increased iron input (20-25mg/day) exceeds the capacity of transferrin and ferritin, resulting in separation of insoluble iron in critical tissues. In principle, this ultimately fatal condition can be treated by administration of an iron chelating agent which would promote remobilization and excretion of the deposited iron [1].

The basic requirement of an iron chelating agent is a high and selective affinity to bind iron under physiological conditions. The tripositive ferric ion is a hard acid and consequently is bound most strongly by hard bases. The most effective of these are oxyanions, such as hydroxide, phenoxide, carboxylate, hydroxamate and phosphonate [2]. Coordination number is usually six, although some seven co-ordinate complexes are also known. Most favourable geometry is an octahedral arrangement of donor atoms, permitting the maximum possible distance between their formal or partial negative charges. Charge neutralization is an important factor and is optimum when the total charge of the six donor atoms is -3, as for the bidentate hydroxamate and tropolonate ligands [3].

Gallic acid is a good iron chelator and form highly coloured complexes with different metal to chelator ratio (1 – 3) at different pH [4]. As many as 12 possible combinations for this complex have been suggested [5]. This complexation starts from pH 3 and continues to pH 9. Below this (pH 3) strongly acid condition helps gallic acid to reduce Fe(III) to Fe (II) [6].

This iron gallic acid binding results in the reduction of iron bioavailability. On the other hand the profound insolubility of ferric hydroxide and the low equilibrium concentration of ferric ion in biological environment are overcome by enormous stability and ion selectivity of the complex [7].

In view of the strong affinity of the gallic acid for Fe(III), we have undertaken a systematic study of complexation of Fe(III), by gallic acid using spectrophotometric and potentiometric procedures. The measurement of binding constants will probably show the effect of this ligand and other polyphenolic ligands on iron availability as well as their potential use in the treatment of excessive iron in living system.

Another point of interest in this system is that it may be viewed as a model of catechols type siderophores [8].

The affinity of a ligand for iron (III) may be defined quantitatively in term of the thermodynamic constants of the equilibria involved between the aquo metal ion and ligand L. In some cases where ligand is an acid H^+ competes for L with the metal [9].

If the ligand is poly protoic acid [10] like gallic acid then the fraction of undissociated ligand α_o for gallic acid is

$$\alpha_o = \frac{[H^+]^4}{[H^+]^4 + [H^+]^3K_1 + [H^+]^2 K_1.K_2 + [H^+]K_1.K_2.K_3 + K_1.K_2.K_3.K_4}$$

and

$$\alpha_n = \frac{[A]^{-n}}{CA} \quad \text{where } \alpha_o+\alpha_1+\alpha_2+\alpha_3+.....+\alpha_n=1$$

A solution at any pH may have M, ML, ML_2 and ML_3. At some pH any one of these species may be negligible.

Observing the spectra of complex at each pH, ε (epsilon) values can be calculated. The fraction of ML, ML_2 and ML_3 at each pH can be estimated by using the equations [11,12].

If ε_m is the molar absorptivity of solution i.e. ε_m=A/Cm, ε_0 is for the metal, ε_1 for ML, ε_2 for ML_2 and ε_3 for ML_3 then.

$$\varepsilon_m = \varepsilon_0.\gamma_0 + \varepsilon_1.\gamma_1 + \varepsilon_2.\gamma_2 + \varepsilon_3.\gamma_3$$

If it is assumed that metal has no absorbance in visible region then, $\varepsilon_0.\gamma_0 = 0$ and at any pH any one or more than one of these terms may be equal to zero and the concentration of the ligand may be calculated as

$$\therefore [L] = \frac{(CA - [ML] - 2[ML_2] - 3[ML_3]) \times K_1.K_2.K_3.[H]}{[H]^4 + [H]^3K_1 + [H]^2K_1K_2 + [H]K_1K_2K_3 + K_1K_2K_3K_4}$$

These equations and their graphical representations are based on McBryde calculation and McBryde graph [13, 14].

Potentiometric study of the complex . Potentiometric method was used for the determination of formation constant of the successive complexes formed between metal ion and a ligand molecule or ion. In this connection Bjerrum calculations are very useful, which depend on finding corresponding values of $\bar{n}$ (the degree of complex formation or the ratio of complex bound ligand to the metal ion) and [L], the molar concentration of the free ligands. These are related to the formation constant of the several complexes by Bjerrum formation functions [15,16].

$$\bar{n} = \frac{\sum_{1}^{N} n\beta_n [L]^n}{1 + \sum_{1}^{N} \beta_n [L]^n}$$

The dissociation constants of gallic acid are already known [17,18]. ($K_1 = 6\times10^{-5}$, $K_2 = 3\times10^{-9}$, $K_3 = 4\times10^{-12}$, $K_4 = 1.5\times10^{-13}$)

$$\bar{n}A = \frac{K_1.K_2.[H^+] + 2K_1[H^+]^2 + 3[H^+]^3}{1 + K_1[L] + K_1.K_2[L]^2 + K_1.K_2.K_3[L]^3}$$

$[HGA] = \alpha_3 \times \frac{CH}{\bar{n}A}$ (where the calculation for α_3 has already been discussed). If [HGA] = [L]

$$\bar{n} = \frac{K_1[L] + 2K_1K_2[L]^2 + 3K_1K_2K_3[L]^3}{1 + K_1[L] + K_1K_2[L]^2 + K_1K_2K_3[L]^3}$$

There are so many methods to calculate stepwise formation constants and overall formation constants from the Bjerrum graph ($\bar{n}$ vs pL)[19].

They are highly accurate for the complexes when maximum $\bar{n}$ value is equal to 2, but there is no simple method for those complexes having n equal or greater than three, like this complex. If all the successive constants differ greatly in magnitude the formation curve has n distinct steps and $Kn^{-1} >> Kn$ [20].

When $\bar{n} = n^{-1/2}$ then approximately equal amount of ML_n and ML_{n-1} will be present in solution and the contribution of ML_{n-2} and ML_{n+1} may be neglected. According to Bjerrum, log Kn = $pL(n^{-1/2})$ if $K_1/K_2 >$ or = 2.5 [21].

Experimental

Molar absorptivities of complex at different pH. In the pH range 3-11 (with the difference of 0.5 pH units) solution were prepared . Their spectra were recorded after 1 hr on Shimadzu Model 160 in the range of 350-700nm. A second spectra was recorded after 24 hr. for each solution. From the absorbance at absorption maxima ε values were determined.

Potentiometric titrations. The titration was carried out in a double walled glass cell. Temperature was controlled by circulating thermostated water through the jacket (capacity of cell was 75ml.). The solution was completely sealed from the atmosphere. The rubber stopper on the cell had holes for microburett for the addition of standard base, for purging inert gas and for glass electrode. pH was measured with a combination glass electrode attached to an Orion SA model 720 pH meter having a resolution of ± 0.001 pH units.

pH Titration of iron gallic acid complex: 50ml of deionized and CO_2 free water was taken in the above mentioned cell. 0.2 m moles of gallic acid and 0.05 m moles of ferric nitrate were dissolved in this water. Purified nitrogen gas was purged through the solution for half an hour. The temperature was controlled at 30° by means of circulating water from the water bath. The solution was stirred on a magnetic stirrer.

1M sodium hydroxide solution was prepared and standardized by 1M standards HCl solution. To the gently stirred acid solution of the ligand, prepared as described above, standard base was added in sufficiently small increments (0.05ml) to provide 50 or more experimental points for each run. Equilibrium conditions, determined by a constant meter reading falling within an interval of less than ± 0.002 pH unit was obtained for each experimental point before proceeding with the next step.

For most system protonation and deprotonation of ligand and complexation is rapid and complete in the time required for mixing.

pH Titration of gallic acid . A similar titration was done at 30° with gallic acid only. The ferric ion was replaced by another tripositive metal (Bi) which was inert towards gallic acid.

Results and Discussion

In the titration between complex and NaOH, changes in curve were at different pH, showing depression in pH values as opposed to the titration of gallic acid only. From pH 2.8 to 3.8 the difference in between two curves was approxi-

mately 1 or nearly one. Around pH 4 it was about 2 and above this pH, it was nearly 3. This comparison strongly indicates the release of hydrogen ions during complexation. (Table 1, Fig. 1).

$$Fe + H_3GA \longrightarrow Fe(HGA) + 2H^+$$
$$Fe + 2H_3GA \longrightarrow Fe(HGA)_2 + 4H^+$$
$$Fe + 3H_3GA \longrightarrow Fe(HGA)_3 + 6H^+$$

at high pH where the ligand is in form of H_2GA

$$Fe + 3H_2GA \longrightarrow Fe(HGA)_3 + 3H^+$$

These results substantiate the deduction about stoichiometry, that 1:1 complex is formed at low pH, 1:2 complex is formed around pH 5 and above pH 7 the stoichiometry approaches 1:3. Bjerrum calculation was based on CH, $\bar{n}A$,$\bar{n}$ and [L], calculated by the derived formulas and equations. Concentration of ligand [L] or [HGA] at any pH was calculated with help of α_3 (Table 2).

A graph between n and p (L) was plotted (Fig.2). The stability constant values computed from this graph at n 0.5, 1.5 and 2.5 for K_1, K_2 and K_3 respectively and it was found that $\log K_1 = \sim 13.2$ $\log K_2 = \sim 10.4$ $\log K_3 = \sim 6.5$. With F the help of these dissociation constant different fraction of M,ML,ML_2 and ML_3 were also calculated at different pH values and species distribution diagram was generated (Table.3, Fig.3). This species distribution diagram showed that 1:1 complex formation started from pH 3.5 to pH 6, 1:2 from 6 to 8.5 was found and formation of 1:3 was complete at pH 11.

In the spectrophotometric study solution of the complex were prepared with buffers of different pH (from 3 to 8). The spectra of these solutions were scanned from wavelength 450nm – 700 nm. Fresh solution and aged solution (after 24 hr) both were scanned to get the absolute ε values. (Table 4, Fig.4). These ε values were calculated at various pH and wavelengths.

Graphs of ε vs pH and ε vs wavelength, were plotted (Fig.5). Spectral properties of the complex (molar absorptivities and λ_{max} values) at different pH are listed in (Table.4). Following equations were used for further calculation of [L] as well as for [M], [ML], [ML_2] and [ML_3].

at pH = $3\varepsilon m = \varepsilon_1(ML)/Cm$

at pH = 5 $\varepsilon m = \varepsilon_1(ML)/Cm + \varepsilon_2(ML_2)/Cm$

at pH = 7 $\varepsilon m = \varepsilon_2(ML_2)/Cm + \varepsilon_3(ML_3)/Cm$

and at pH = 8 $\varepsilon m = \varepsilon_3(ML_3)/Cm$

$[M] = Cm - ([ML] + [ML_2] + [ML_3])$

$$[L] = CA - ([ML] + 2[ML_2] + 3[ML_3]) \times \frac{K_1.K_2.K_3[H]}{[H]^4 + [H]^3K_1 + [H]^2K_1K_2 + [H]K_1K_2K_3 + K_1K_2K_3K_4}$$

TABLE 1. pH TITRATION OF GALLIC ACID AND COMPLEX WITH NaOH.

NaOH Vol. (ml)	pH GA.Sol.	pH Comp.Sol	pH diff.	[HGA]	p[HGA]	nA	n^-	m
0.0	3.4	2.9	0.5	7.3×10^{-18}	17.14	2.96	0.0	-4
0.1	3.8	3.1	0.7	5.6×10^{-17}	16.25	2.92	0.3	-3.2
0.2	4.2	3.5	0.7	8.0×10^{-15}	14.10	2.84	0.4	-2.4
0.3	4.7	3.9	0.8	9.0×10^{-14}	13.05	2.67	0.6	-1.6
0.4	5.9	4.1	0.8	4.4×10^{-13}	12.36	2.56	0.9	-0.8
0.5	7.2	4.3	2.8	1.5×10^{-12}	11.83	2.45	1.1	0.0
0.6	7.8	4.8	3.0	6.5×10^{-11}	10.18	2.20	1.3	0.8
0.7	8.2	5.1	2.9	4.4×10^{-10}	9.36	2.10	1.6	1.6
0.8	8.7	6.0	2.7	4.8×10^{-9}	8.30	2.00	2.0	2.4
0.9	9.2	6.6	2.6	8.0×10^{-8}	7.1	1.99	2.4	3.2
1.0	9.8	7.0	2.8	4.6×10^{-7}	6.3	1.97	2.5	4.0
1.1	10.0	7.2	2.8	1.2×10^{-6}	5.92	1.96	2.7	4.8
1.2	10.3	8.4	1.9	1.7×10^{-5}	4.77	1.60	2.9	5.6
1.3	10.5	9.6	0.9	1.0×10^{-4}	4.00	1.50	3.0	6.4

m = C_B-C_A/Cm., C_B = cons. of base, CA = conc. of acid and Cm = conc. of metal., [M] = 1×10^{-3}, [GA] = 4×10^{-3}, molarity of NaOH = 0.8M.

TABLE 2. DETERMINATION OF THE CONCENTRATION OF THE LIGAND AT DIFFERENT pH.

pH	$[H]^4$	$K_1[H]^3$	$K_1K_2[H]^2$	$K_1K_2K_3[H]$	α_3	[L]
3.5	10^{-14}	$10^{-14.5}$	10^{-19}	$10^{-26.5}$	10^{-14}	10^{-16}
4.0	10^{-16}	10^{-16}	10^{-20}	10^{-27}	10^{-11}	10^{-15}
4.5	10^{-18}	$10^{-17.5}$	10^{-21}	$10^{-27.5}$	10^{-10}	10^{-14}
5.0	10^{-20}	10^{-19}	10^{-22}	10^{-28}	10^{-9}	10^{-13}
5.5	10^{-22}	$10^{-20.5}$	10^{-23}	$10^{-28.5}$	10^{-8}	10^{-12}
6.0	10^{-24}	10^{-22}	10^{-24}	10^{-29}	10^{-7}	10^{-11}
6.5	10^{-26}	$10^{-23.5}$	10^{-25}	$10^{-29.5}$	10^{-6}	10^{-10}
7.0	10^{-28}	10^{-25}	10^{-26}	10^{-30}	10^{-5}	10^{-9}
7.5	10^{-30}	$10^{-26.5}$	10^{-27}	$10^{-30.5}$	10^{-4}	10^{-8}
8.0	10^{-32}	10^{-28}	10^{-28}	10^{-31}	10^{-3}	10^{-7}
8.5	10^{-34}	$10^{-29.5}$	10^{-29}	$10^{-31.5}$	$10^{-2.5}$	$10^{-6.5}$
9.0	10^{-36}	10^{-31}	10^{-30}	10^{-32}	10^{-2}	10^{-6}
9.5	10^{-38}	$10^{-32.5}$	10^{-31}	$10^{-32.5}$	$10^{-1.5}$	$10^{-5.5}$

TABLE 3. FRACTION OF THE COMPLEX SPECIES AT DIFFERENT pH.

pH	[M]/Cm	$[ML_1]$/Cm	$[ML_2]$/Cm	$[ML_3]$/Cm
2.8	0.999	7.3×10^{-4}	4.0×10^{-9}	–
3.1	0.995	5.0×10^{-3}	2.0×10^{-7}	–
3.5	0.935	6.5×10^{-2}	3.4×10^{-5}	–
3.9	0.540	0.450	3.0×10^{-3}	–
4.1	0.200	0.800	2.0×10^{-2}	–
4.3	3.2×10^{-3}	0.470	0.520	2.0×10^{-5}
4.8	2.6×10^{-4}	0.170	0.800	1.3×10^{-4}
5.1	6.8×10^{-8}	3.0×10^{-3}	0.980	1.0×10^{-2}
6.0	5.0×10^{-10}	2.5×10^{-4}	0.890	0.100
6.6	1.7×10^{-10}	1.4×10^{-4}	0.820	0.170
7.0	1.0×10^{-12}	1.0×10^{-5}	0.460	0.530
7.2	1.0×10^{-13}	1.0×10^{-6}	0.24	0.750
8.4	–	–	2.3×10^{-3}	0.998

TABLE 4. MOLAR ABSORPTIVITY OF Fe(GA)n AT DIFFERENT pH AND DIFFERENT WAVELENGTH.

	410	420	430	440	450	460	470	480	490	500	510	520
3.5	2800	2400	2000	1800	1640	1600	1520	1480	1420	1420	1280	1240
4.0	3000	2800	2480	2240	2000	1920	1800	1720	1720	1680	1640	1640
4.5	3400	3200	2800	2600	2400	2320	2320	2280	2280	2240	2240	2240
5.0	3920	3520	3360	3200	3040	3000	2300	2800	2800	2920	2960	3000
5.5	4000	4000	4000	3800	3600	3600	3640	3680	3720	3800	3840	3880
6.0	6000	3920	3880	3840	3800	3760	3800	3840	3880	3920	3960	3960
6.5	4400	4400	3920	3760	3600	3200	3000	3200	3400	3600	3800	3920
7.0	4400	4400	3920	3600	3200	3200	3360	3440	3520	3600	3640	3680
7.5	4400	4000	3846	3520	3200	3000	3200	3440	3360	3600	3560	3520
8.0	5600	5200	4800	4400	4000	4000	4000	4000	4000	4000	3920	3846
8.5	6000	5200	4800	4400	4000	4000	4400	4400	4800	4800	4800	4800
9.0	6400	6000	5600	5200	4800	4800	4800	5200	5200	5200	5200	4800
9.5	6400	6000	5600	5200	5200	5200	5200	5200	5200	5200	5200	4800
10.0	6400	6000	5800	5600	5400	5360	5200	5200	5200	5200	4800	4800
10.5	6200	6000	5800	5600	5400	5360	5200	5200	5200	5200	4800	4800
	530	540	550	560	570	580	590	600	610	620	630	640
3.5	1240	1200	1200	1200	1160	1160	1120	1080	1080	1040	1000	1000
4.0	1600	1600	1600	1560	1560	1560	1520	1520	1480	1440	1360	1280
4.5	2240	2240	2240	2200	2160	2080	2040	2000	1920	1840	1760	1680
5.0	3000	3000	3000	2960	2920	2880	2840	2800	2720	2640	2560	2480
5.5	3920	3920	3960	3920	3840	3760	3680	3600	3520	3400	3320	3200
6.0	4400	4400	4000	3960	3840	3760	3600	3600	3520	3400	3320	3200
6.5	4400	4400	4000	3920	3840	3760	3600	3560	3520	3446	3360	3280
7.0	3720	3760	3800	3760	3720	3680	3640	3520	3440	3280	3120	3000
7.5	3480	3440	3420	3360	3320	3280	3240	3200	3120	3040	2960	2880
8.0	3760	3680	3600	3400	3200	3000	2800	3000	2800	2600	2400	2200
8.5	4400	4000	3600	3400	3280	3120	2920	2800	2400	2200	2000	1800
9.0	4800	4400	4000	3800	3600	3200	2800	2600	2400	2200	1800	1400
9.5	4800	4400	4000	3800	3600	3200	2800	2400	2200	2000	1800	1600
10.0	4800	4500	4000	3800	3600	3200	2800	2400	2200	2000	1800	1400
10.5	4800	4560	4000	3800	3600	3200	2800	2400	2200	2000	1800	1400

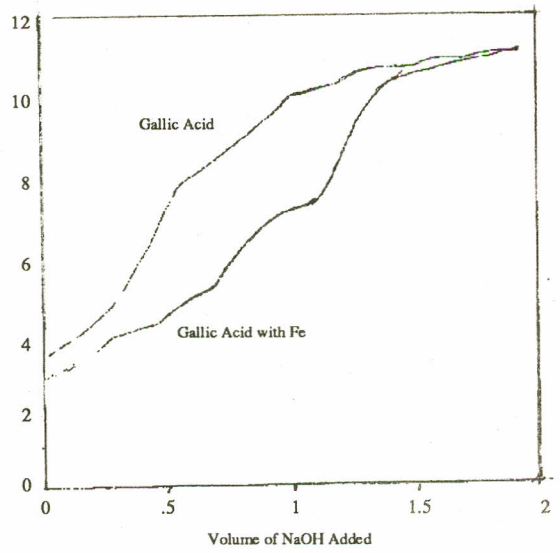

Fig. 1. pH Titration of ligand and complex.

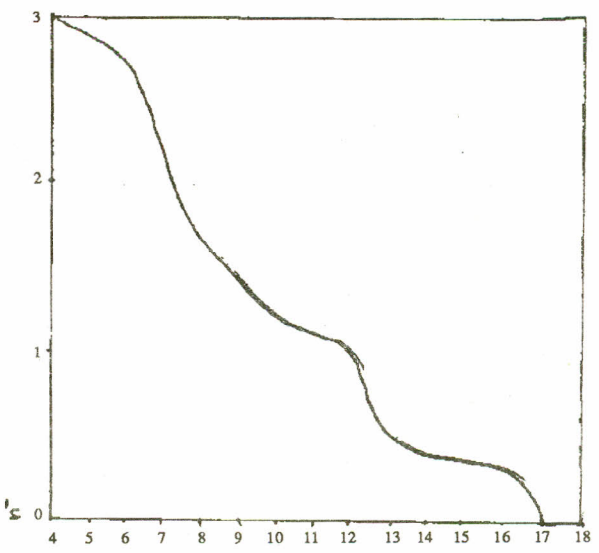

Fig. 2. Bjerrum graph.

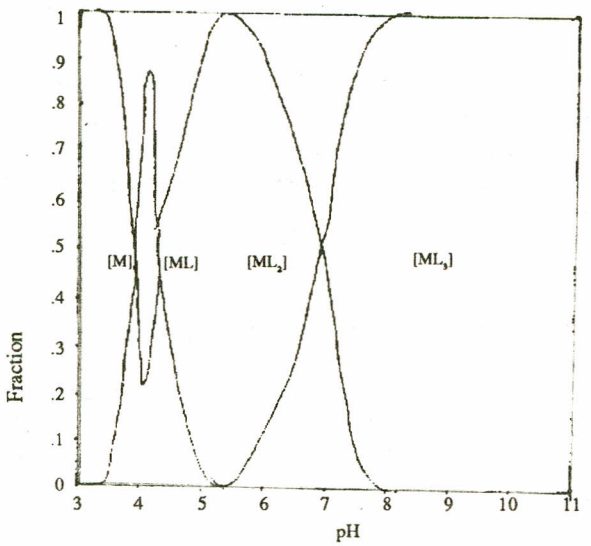

Fig. 3. Species distribution graph.

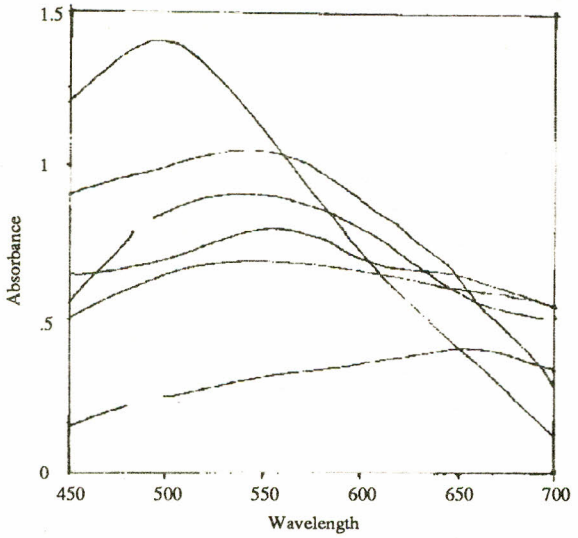

Fig. 4. Spectra of iron gallic acid complex.

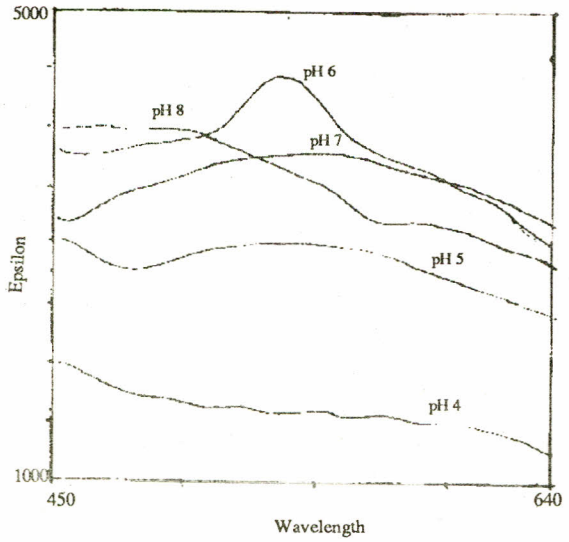

Fig. 5. Plot of molar absorptivity at different pH.

The conc. of [L] at different pH were calculated by these equations. The pH and λ_{max} were selected by the help of the graph between ε and λ_{max} which shows that wave length 620 from pH 3.5 and 4 is most suitable for ML, λ_{max}, 480 from pH (8-11) for ML_3 and 600 for ML_2 (from pH 5-7.5).

After calculalting the concentration of ML, ML_2 and ML_3 at different pH equilibrium constants were obtained by using the simple equations;

$$\text{and then } K_1 = \frac{[ML]}{[M][L]} \quad K_2 = \frac{[ML_2]}{[ML][L]} \quad \text{and } K_3 = \frac{[ML_3]}{[ML_2][L]}$$

at the stage where [M]=[ML] K_1=1/[L], where [ML]=[ML_2] K_2=1/[L] and where [ML_2]=[ML_3], K_3=1/[L]

After averaging the logK values, the following results were found; log K_1~ 14, log K_2~ 8.5, log K_3~ 5 and for protonated 1:1 complexes logK11~ 17.

It proves the strong affinity of iron (III) towards 3, 4, 5, trihydroxy benzoic acid at different pH including the physiological pH.

The pH titration curve also indicates bonding sites. It shows that the coordinations are through catecholes not from carboxylate. The pKa of carboxylic proton is around 4 and if lone pair present on carboxylate oxygen goes to metal then the pH of the complex solution at the starting point should be much more less than the starting pH of gallic acid solution. The difference in pH between these two solutions before adding any base was found to be nearly 0.5 which is not accountable. If the carboxylate group has any interaction with iron (III) than complexation should be maximum near pH 4 but it does not happen like that. So it confirms that the main coordinators iron (III) are phenolate not carboxylate that is the reason that we can select gallic acid as a model for catechol type siderophores.

References

1. C.G. Pittand, A. E. Martell, *Chelating Agents , Inorganic Chemistry in Biology and Medicine* (A.C.S. 1980).
2. A.E. Martell and R.M. Smith, *Critical Stability Constants* (Plenum, New York, 1977), Vol. 1-4.
3. S.J. Lippard, H. Schugar and C. Walling, Inorg. Chem., **6**, 1825 (1967).
4. G.A. El-Inaney, F.Ziauddin, B.El-Shetary and Y.M. Issa, J. Chem. Soc. Pakistan, **7** (3) (1985).
5. C.A. Vander Heijden, P.A.C.M. Jansen, J.J. Strike, Fd. Chem. Toxicol., **24**, 10 (1986).
6. L.F.Longinova, V.V. Medynsteve and B.I.Khomotove, (U.S.S.R) Zh. Obsch. Khim, **42**, 739 (1972).
7. H.S.Waxman and E.B.Brown, Prog. Hematology, **6**, 338 (1969).
8. Alex Avdeef, Stephen R.Softner, Thomes, L.Bergente

and K.N.Raymond, J. Chem. Soc., **100**, 5362 s (1978).
9. R. Assa, B.G. Malstrom. P. Saltman and T. Vanngard, Biochim. Biophys. Acta, **75**, 203 (1963).
10. Adon A. Gordus, *Theory and Problem of Analytical Chemistry* (McGraw Hill, New York, 1987).
11. W.A.E.McBryde, Can. J. Chem., **43**, 1917 (1964).
12. W.A.E.McBryde and G.F. Atkinson, Can.J.Chem., **39**, 510 (1961).
13. W.A. McBryde, Can. J. Chem., **46**, 2385 (1968).
14. W.A. McBryde, J.L. Rohr, J.S. Penciner and J.A. Page, Can. J. Chem., **48**, 2574 (1970).
15. J.Bjerrum and E.J. Nielson, Acta. Chem. Scand., **2**, 297 (1948).
16. J.Bjerrum, *Metal Ammine Formation in Aqueous Solution* (P. Haase and Son, Copenhagen, 1941).
17. K.N. Sahu and A.K. Bhatacharia, J.Indian Chem. Soc., **41**, 11 (1964).
18. Sandmann, J. Beverly, Chein, H. May and A. Robert, Anal. Lett, **A2**, 149 (1985).
19. Gordon A. Carlson, James P. McReynold and Frank H. Verhock,Aug. Acta. Chem. Scan., **67**, 1334 (1945).
20. F.J. Rossoti and H.S. Rossoti, Acta. Chem. Scand., **9**, 166 (1955).
21. H. Irving and H.S. Rossoti, Acta. Chem. Scand., **10**, 72 (1956).
22. R.J. Motekaitis and A.E. Martell, Can. J. Chem., **60**, 2403 (1982).
23. P.J. Motekaitis and A.E. Martell, Can. J. Chem., **60**, 168 (1982).