# Study of Complex Formation of Fe(III) with Tannic Acid

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**Summary:** Free ferric ion is not available in living system either due to the formation of $Fe(OH)_3$ or by chelation with some biological ligands like tannins, gallic acid and other siderophores. Tannins are the major constituents of our beverages and food. Since tannic acid molecule has 8 gallic acid groups, it can bind 1-4 Fe(III) ion depending upon ligand concentration. They form highly stable colored complexes with Fe(III) at pH range 3-10 so the pH 3, 4, 5, 8 and 10 are 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, 5 the maximum absorbance of complex was found at 600nm while at pH 8 and 10, 500nm was the λmax. For high ligand concentration at all considering pH 550nm was the $\lambda_{max}$.

## Introduction

Iron is essential trace element for all living organisms. Two major biological functions which performed by this metal are oxygen transport and electron transfer in electron transport chain. It is involved in transport and storage by hemoglobin and myoglobin in vertebrates. In 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 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 deposite in the tissues as insoluble hydroxides and phosphates at physiological and higher pH unless it bound to iron storage protein such as ferritin and hemosiderin. The ligands which serve as iron scavangers or iron chelators are called siderophores. These siderophores may hinder the absorption of iron in the living body [3-5]. Chemically these are of two types, hydroxamate catecholate type comes under the heading of catechols and its derivatives form stable complexes with iron [6,7] and vanadium [8]. These have very high affinity to the Fe(III), so they form highly stable complexes with Fe(III) having stability constants $10^{30}$-$10^{50}$ [9,10].

Tannic acid belongs to catecholic category like enterobactine. The stability constant for Fe(III)-enterobectine complex is $10^{52}$ [11]. Tannic acid are the major constituents of our beverages and food stuffs [12]. Common tannic acid belongs to the hydrolysable category which, decompose in water [13]. Generally its quantity found in tea is the 15-20%, but it depends on many factors such as age variations, seasonal variations, extent of processing and the region of the tea belongs to. The origin, abundance, degree of purity and the factors on which the abundance of the tannic acid depends, are discussed in detail [14,15]. The structural characteristics, composition and chemical nature of the tannic acid has also been studied, it contains 8 gallic acid functionalities/molecules [16-18].

It was suggested that tannins are responsible for the interference of absorption of iron [19,20]. Tannins are the major constituents of our food stuffs and form highly stable complexes with Fe(III) [21-23]. One of the most important reasons to cause anemia is due to the fact that, maximum absorption of iron in the biological environment takes place. in the form of ferrous, but we intake a large amount of iron by the food stuffs in the form of ferric. Therefore, tannic acid in any form of beverages either hot or cold, consumed by the human bodies resulted in deficiency of iron. Because Fe(III) is chelated with tannic acid easily even at physiological pH [24].

Tannic acid is a large molecule containing 8 groups of 1,2,3-trihydroxybenzoic acid (gallic acid).It can chelate more than one metals at a time (simultaneously). The stoichiometry and stability of the complexes may be pH dependent. The study of complexation of this multidentate ligand with Fe(III) seems to be valuable and interesting.

## Results And Discussion

Tannic acid is a large molecule having 8 units of 1,2,3-trihydroxybenzoic acid (gallic acid) functionalities. It can donate maximum 16 and

minimum 2 protons from its four sites A, B, C and D. (Figure-1). The complexation of any metal with deprotonated ligand depends upon the deprotonation of ligand, which is dependent on pH and its pK value. At very low pH the most of the ligands remain protonated and only few binding sites might be available for a metal ion. Whereas with the increase of pH the deprotonation increases providing more binding sites for metal.

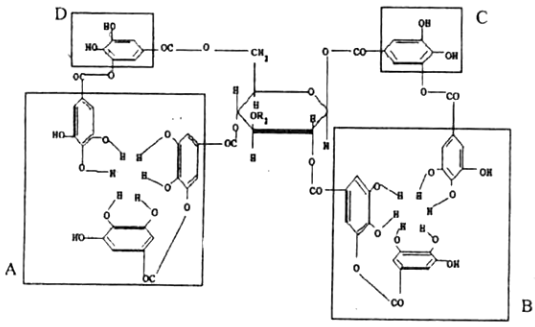


Fig. 1: Chelated structure of tannic acid showing maximum protons and coordination sites

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

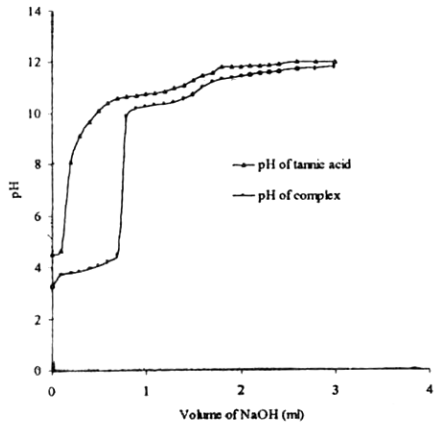


Fig. 2: Plot for Potentiometric Titration

As these complexes are highly colored therefore spectrophotometric studies was also found to be very suitable. Two sets were prepared, for low ligand concentration absorption peaks of the complexes were observed at 600nm for pH 3, 4 and 5 whereas at 500nm for pH 8 and pH 10 respectively. (Figure-3 ). For the high ligand concentration, we got the peaks at 550nm for all considered pH (Figure- 4). Stoichiometry of the complex was determined by mole ratio method using two sets of experiments. In the first case metal was kept constant with the increase of ligand. Then the absorbances were noted at the 550nm λmax of the complex. The absorbance values were then plotted against L/M ratio, by extrapolating, values the ML ratio found to be 1:1 at all considered pH. (Figure-5). In the second set ligand was kept fixed while metal was gradually increased. Then the absorbance was noted at the 600nm and 500nm λmax of the complex. Then the absorbance was plotted versus M/L ratio. At low pH 3, 4 and 5, ML ratio was found to be 2:1 and at pH 8 and 10 ML ratio was found to be 4:1 (Figure-6).

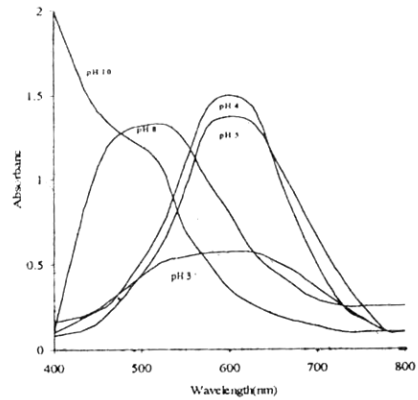


Fig. 3: Absorption Peaks at Variable pH for Low Ligand Concentration

Ligand has capability to bind 1-4 metal ions due to several binding sites A, B, C and D. Where A and B are preferable as compared to C and D sites. At low pH, since ligand is in protonated form, A and B positions form only two bonds with each metal (act as bidentate) and remaining coordination sites of the metals might be satisfied with water molecules. However at high pH values the ligand is completely free from protons therefore, position A and B act as hexadentate whereas position C and D will be act as bidentate for third and forth metal.

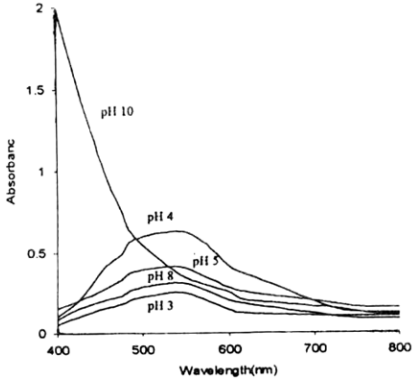


Fig. 4: Absorption Peaks at Variable pH for High Ligand Concentration

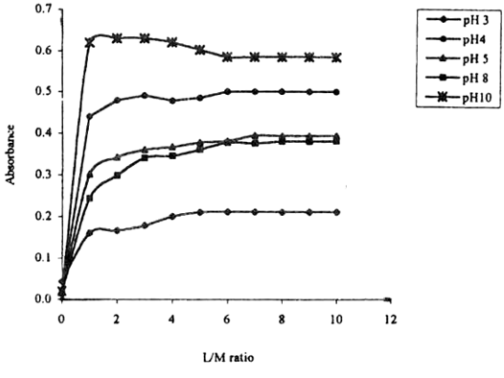


Fig. 5: Mole Ratio Method at Variable pH (metal constant).

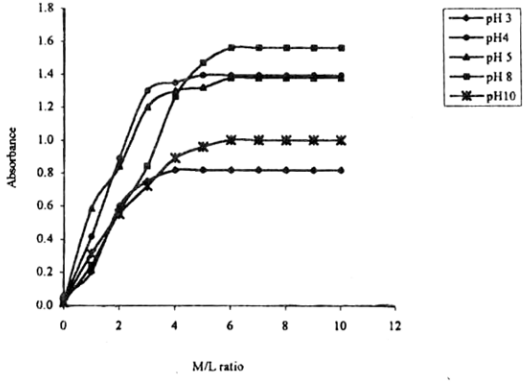


Fig. 6: Mole Ratio Method at Variable pH (ligand constant).

The above results show that the concentration of ligand and metal also effects the stoichiometry of the complex. For this purpose two sets were studied, one in which ligand was in excess over the metal and in second metal was in excess over the ligand. In the case of low ligand concentration when pH was very low i.e. 3, only a few coordination sites were available therefore 2 metal ions may chelate with 1 ligand due to less ligand concentration through site A and B, while remaining coordination sites of metal are satisfied with water molecules. When pH is considered, it is found that at medium, high and extremely high pH, co-ordinating sites of ligand increase due to increase in deprotonation so, 2 metal ions may chelate with 1 ligand molecule through its six sites. At extremely high pH all 12 –OH groups deprotonate and metal can bind through all of its binding sites so four metals may chelate with one ligand molecule through its sites A, B, C and D, while remaining coordination sites of metal are satisfied with water molecules.

For high ligand concentration, when ligand is in excess, generally the stoichiometry of the complexes vary from 1:1-1:3, but due to larger size of ligand there may be a steric hindrance for 1:3. So each metal is coordinated 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 in these conditions is cyclic having 3 metals chelated with 3 ligands and so on therefore, the empirical formula of the complex remains same as 1:1.

## Experimental

Analytical grade (AR) reagents were used without any further purification. Double distilled deionized water was used in working solution and preparation of all solution 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).

pH Titration was done using 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 first set of titration 10ml water and 50ml (0.0010M) tannic acid was taken in a beaker, then aliquotes of standard 0.10M NaOH was added with the help of

micro-pipette with continuous stirring on a magnetic stirrer and the pH variation was measured after each addition. In the second set of titration 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 using by scanning from 400-800nm 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) metal was kept constant while ligand was gradually increased. Absorbances were recorded on Spectronic 21 (Bausch and Lomb) on the observed $\lambda_{max}$. Same procedure was repeated for the second case (taking low ligand concentration) in which ligand remained constant while metal was increased.

## Conclusions

Since mostly the iron taken by food is in Fe(III) form but forms highly stable complex with tannic acid present in beverages, so becomes non available for body. However, the peoples intake large amount of iron but experiences the deficiency of iron/blood. An antioxidant is devised to be taken with food so that it can reduce Fe(III) into the absorbable specie i.e. Fe(II).

The $\lambda_{max}$ and mole ratio varied with the variation of pH which confirms the pH dependence of the complexation. Results obtained from potentiometry were also supported by spectrophotometry. After these studies, it is clarified that the beverages containing tannic acid should not be used in more quantity in our daily routine and must not be taken just after the meal.

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