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

I. Abdul Tawab Khan and Z.T. Maqsood

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 $\lambda_{\text{max}}$ 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 $\lambda_{\text{max}}$. For high ligand concentration, pH 550 nm was the $\lambda_{\text{max}}$. 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 $\beta$ values obtained by these methods were compared. Stability constant values were found to be $10^2$, $10^8$ and $10^{16}$ for ML, M$_2$L and M$_4$L, 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 transport 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)-enterobactine 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...
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.](image-url)
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 $\lambda_{\text{max}}$ 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$_2$L and M$_3$L). Before the determination of $K_{\text{eq}}$ values, $\varepsilon$ (molar extinction
Table 1. $\varepsilon$ values for different species at specific $\lambda_{\text{max}}$.

<table>
<thead>
<tr>
<th>Sps.</th>
<th>Wavelength ($\lambda$) (nm)</th>
<th>Molar Absorptivity ($\varepsilon$) lit. mol$^{-1}$.cm$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML</td>
<td>550</td>
<td>1000</td>
</tr>
<tr>
<td>M$_2$L</td>
<td>650</td>
<td>14000</td>
</tr>
<tr>
<td>M$_4$L</td>
<td>500</td>
<td>16000</td>
</tr>
</tbody>
</table>

Coefficient values were also calculated (Table 1). The concentration of the complex of each particular ratio was determined by using its $\varepsilon$ 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:

$$p[L]_{\text{free}} = pK_\alpha - \log[H\!\!L]_{\text{init}} - [\text{NaOH}].$$

Then $K_{\text{eq}}$ values were calculated, which were found to be in the ranges of $10^3$, $10^8$ and $10^{16}$ for ML, M$_2$L and M$_4$L, respectively.

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

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

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

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.
Table 2. Calculation for stability constants by slope ratio method at pH 3 ($\varepsilon_{1100 \text{ nm}} = 14000 \text{ lit/mol}^{-1} \text{cm}^{-1}$).

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

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 ($\varepsilon_{500 \text{ nm}} = 16000 \text{ lit/mol}^{-1} \text{cm}^{-1}$).

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

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 $[M]_t-[M_4L]$; $[L]_r$ = 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.

<table>
<thead>
<tr>
<th>Sps.</th>
<th>log $\beta$ values</th>
<th>Mole Ratio</th>
<th>Slope Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Method</td>
<td>Method</td>
</tr>
<tr>
<td>ML</td>
<td>3</td>
<td>*</td>
<td>3.8</td>
</tr>
<tr>
<td>$M_2L$</td>
<td>8</td>
<td>8</td>
<td>8.9</td>
</tr>
<tr>
<td>$M_4L$</td>
<td>16</td>
<td>16</td>
<td>15</td>
</tr>
</tbody>
</table>

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$9H$_2$O 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 micropipette 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 $\lambda_{max}$. 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


