Abstract

Complexation of iron (III) with citrate (Cit) and tartarate (Tar) anions was investigated in perturbed aqueous solutions using potentiometry, conductometry, UV/Vis. and infrared (FT-IR) spectrophotometric methods. Experiments were performed at 25 °C in 0.1M sodium chloride solutions as the electrolyte. Stepwise equilibrium formation constants and the number of moles of ligand bound to Fe (III) were obtained from potentiometric titration data. Also UV/Vis. spectrophotometric results were used to determine the pH range of complex formation.

Electrical conductivity of the Fe (III)/glucose (Glu) system showed that Glu is not bound to the iron. Potentiometric results could be used to estimate intrinsic association constants of the complexes. In the case of Fe (III)-Cit complex, two moles of Cit were bound to one mole of Fe (III) ion as determined by pH-titration and molar conductivity methods. Hill and Scatchard analysis of the Fe (III)-Cit. binding data indicated one set of binding sites, with positive cooperativity observed for the system. In the case of Fe-Tar, a complex in form of Fe (Tar)3 was formed over the pH range of 3 to 6. Hill and Scatchard analysis of the Fe (III)-Tar. data indicated that there are two unidentical and dependent sets of binding sites, and interaction between binding sites was of positive cooperativity type. FT-IR data of both complexes showed that complexation occurs by binding of carboxyl oxygen to the Fe (III) ion.

Keywords: Complexation, citrate, tartarate, pH-titration, Spectrophotometry, iron

1. Introduction

Metal ion complex formation is among the prominent interactions in nature and metal complexation is of widespread interest. Equilibrium formation constants of the complexes formed in aqueous solutions are of extreme importance for a broad variety of technologies in biology and medicine. Furthermore, perturbing of aqueous solutions is one of the useful methods for converting weak chelating agents by metal ions into more stable complex species. Glu and ethanol have the same effects on complex stability and solubility (up to pH = 14) by decreasing the dielectric constant of the solution.

The primary importance of soluble metal chelates in soil arises from their capacity to increase the solubility of added or indigenous metal cations. As a consequence, the mobility of the metal is increased by both diffusive and connective flows. Increased solubility and mobility of chelated metals have major effects on many aspects of soil chemistry and soil fertility. The movement and availability of metals to plant roots may be greatly enhanced and this remain the major objective of application of micronutrient metal chelates in soils. The solubility of iron is dependent on soil pH. Lindsay (1984) showed that in soil with pH > 5, iron deficiency has been observed in most Fe-deficient plants. Iron deficiency, being responsible for low yield and poor plant quality in some parts of the world, results in economic loss for farmers. Inorganic (mainly FeSO4) and organic (mainly Fe-chelates) sources of Fe are used, as fertilizer. Most of the agricultural soils in IRAN are calcareous with high pH. Therefore, Fe-deficiency in these soils is serious.

Fe-EDDHA is the most effective chelate for compensation of iron deficiency in plants growing in calcareous and other highly alkaline soils.\(^9\) The stability of other Fe-chelates in soil solution is lower than that of Fe-EDDHA. The stability of Fe-cit complex, in equilibrium with H\(^+\), Ca\(^{2+}\), Mg\(^{2+}\) and Al\(^{3+}\) in soil solution is limited. Cit is an effective chelating agent for Fe\(^{3+}\) even under acid soil condition.\(^8\)\(^,\)\(^10\)\(^,\)\(^12\)

In xylem, iron is transferred from roots to leaves in the form of iron-Cit. Therefore, this kind of Fe-chelate is not harmful for plants growth. Tar is another simple organic acid, which is produced by microorganism and plants in the Fe-deficient condition in soil and is able to chelate iron and other micronutrients.\(^12\)\(^–\)\(^14\) A wide array of low-molecular-weight aliphatic acids have been reported in soil, of which the following play a key role in the formation of complexes with divalent and trivalent cations: citric, oxalic, succinic, lactic, fumaric and tartaric. Among these, citric and oxalic acids are of special importance by virtue of their wide distribution in the pedosphere since they form highly stable complexes with trace elements.\(^15\) The concentration of organic acids in the soil solution is about 1·10\(^{–3}\) to 4·10\(^{–3}\) M. Substantially higher concentrations can be found in the rhizosphere, in aqueous extracts of forest litter, and possibly in soils amended with organic wastes. Excretion products of roots include a variety of organic acids, many of which (e.g., citric, oxalic, and tartaric) are capable of forming stable complexes with metal ions.\(^16\) The present work was conducted to:

- Investigate the effects of perturbed aqueous solutions by Glu and Et on the stabilities of Fe (III)/Cit and Fe (III)/Tar complexes
- Estimate the stoichiometry of complex (mole ratio of ligand to metal ion) and functional groups involved in the complex formation

### 2. Experimental

#### 2.1. Materials

Iron (III) chloride, sodium chloride, Glu, sodium hydrogen Tar, Et, Tri-sodium Cit, sodium hydroxide, and hydrogen chloride were obtained from MERCK Company and used without further purification. All the solutions were prepared in deionised water obtained from a Barstead NANO pure (Electrical Resistance = 18.3 MΩ).

#### 2.2. Methods

Acid-base potentiometric titrations were carried out using a pH-meter model F-12 HORIBA equipped with a combined glass electrode and temperature controlling means. The electrode was calibrated with standard buffer solutions (phthalate and borax buffers pHs: 4.01 and 10.00, respectively) before and after each series of measurements based on the standard scale of the U. S. National Bureau of Standards.\(^17\)\(^,\)\(^18\)

Titrations were performed in a 15 mL commercial double-walled glass vessel. Temperature was kept constant inside the cell at 25 ± 0.1 °C by a MLW water circulating heating system.

Stock solutions were 10\(^{-3}\) M iron (III) chloride, 10\(^{-1}\) M sodium chloride, 10\(^{-3}\) M trisodium Cit, 4·10\(^{-2}\) M Glu, Tar 10\(^{-2}\) M sodium hydrogen, 0.5 N hydrogen chloride, 0.5 N standardized sodium hydroxide (prepared freshly) and 4·10\(^{-2}\) M Et.

The ionic strength of the solutions is maintained at constant level (0.1 mol dm\(^{-3}\)) by using the desired concentration of NaCl solution as supporting electrolyte. During the course of titrations, a stream of oxygen-free nitrogen was passed through the reaction cell to eliminate the adverse effect of the atmospheric carbon dioxide.\(^18\)\(^–\)\(^20\)

All solutions were prepared in 10\(^{-1}\) M NaCl as the electrolyte solution. The pH-metric titration curve is a plot of pH against titrant volume (0.5 N NaOH) for the ligand or its corresponding complex solution.

The iron/Cit and/or iron/Tar complex solutions, which are perturbed by Glu, consisted of 10\(^{-3}\) M Fe\(^{3+}\) chloride, 10\(^{-2}\) M Cit and 4·10\(^{-2}\) M Glu and/or Et as potentiality, the conductometric titrations were carried out using a temperature controlled conductivity meter model DS-14 Horiba. In the first experiment, specific conductivity of the iron/ligand complex with a concentration of 10\(^{-3}\) M Fe (III), 10\(^{-2}\) M ligand and 4·10\(^{-2}\) M Glu was plotted against titrant volume (0.5 N NaOH). In the second run, a blank experiment was carried out using ferric chloride (10\(^{-3}\) M stock solution) Glu solution (stock solution 4·10\(^{-2}\) M) and the related comparative conductometric titration profile was plotted. Likewise, in the third experiment a solution of Fe (III) in the presence of Glu (at the same conditions as above) were titrated with the citrate solution.

Different spectrophotometric titrations were carried out at 336 nm using a temperature controlled Shimadzu UV/Vis. spectrophotometer model 2101 PC.

Complex formation as a function of pH was examined for Fe (III)/ligand with a mole ratio of 1 : 1 at pH range of 1 to 9. Absorbance measurements were followed at λ\(_{\text{max}}\) = 380 nm. Also complex formation and completion were measured spectrophotometrically by following the process at 380 nm for titration of Fe (III) solution with the ligand (citrate or tartarate) solution. Absorbance changes at 336 nm (isobestic point) were recorded for different molar ratios of Fe (III)/ligand according to the molar ratio method for equilibrium constant estimation. The concentrations of ligand and metal ion were 10\(^{-2}\) and 10\(^{-3}\) M, respectively. A BOMEM FT-IR spectrophotometer model Arid-Zone MB-100, was used to obtain the FT-IR spectra and identification of the functional groups of the ligands responsible for complex formation. Samples were prepared and used in KBr tablets.

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3. Results and Discussion

Potentiometric titration is a simple and rapid method to study the systems in which the equilibrium state is attained within a few minutes. It is essential that the total concentration of the starting solution to be titrated and the titrant solution must be different, and thus the total concentrations of the components of solution under study vary in accordance with the dilution rules during the stepwise addition of the titrant. Hence, the change in the equilibrium concentration could be followed by means of the appropriate electrode system.

As potential oxygen donor ligands, Cit and Tar ions (acid or salt forms) are interesting and have gained special attention not only because of the structural chemistry of their coordination modes, but also because of their importance in plant biochemistry. The roots in soil trap the cations like Fe^{3+} and can secrete these materials. This indicates their ability to chelate metal ions and form stable complexes.7,18,21

The pH-titration method is one of the most reliable methods, which can be used whenever the complexation is accompanied by a change in pH. The chelation of the iron ion by Cit, for example, may be represented by multiple equilibria as:

\[ \text{HL}^2^- + \text{Fe}^{3+} \rightleftharpoons \text{FeL} + \text{H}^+ \] (I)

\[ \text{FeL} + \text{HL}^2^- \rightleftharpoons \text{FeL}^2^- + \text{H}^+ \] (II)

Since two protons are formed in the reactions of (I) and (II), the addition of Cit to the metal ion solution should result in a decrease in pH. Titration curve (plot of pH versus volume of the base used) can be obtained by titration of separate mixtures of Cit solution and the corresponding complex solution by a standard NaOH solution. A typical potentiometric titration curve is shown in Figure 1.

The curve for metal-Cit complex is well below that of the Cit ligand profile alone, and the decrease in pH shows that complexation occurs well and becomes complete at high pH values. The two successive or stepwise equilibria between the iron ion metal (M) and citrate or the ligand (L) for the complex of ML\(_2\) type may be written in general as:

\[ \text{M} + \text{L} \rightleftharpoons \text{ML} \quad K_1 = [\text{ML}]/[\text{M}][\text{L}] \] (1)

\[ \text{ML} + \text{L} \rightleftharpoons \text{ML}_2 \quad K_2 = [\text{ML}_2]/[\text{ML}][\text{L}] \] (2)

and the overall reaction (I and II) is:

\[ \text{M} + 2\text{L} \rightleftharpoons \text{ML}_2 \quad \beta = K_1K_2 = [\text{ML}_2]/[\text{M}][\text{L}]^2 \] (3)

Bjerrum called \( K_1 \) and \( K_2 \) the formation constants, while the equilibrium constant \( \beta \) for the overall reaction is known as the stability constant.

A quantity like \( n \) may now be defined as the average number of ligand molecules bound to the metal ion, which may be written as:

\[ \bar{n} = (\text{total conc. of ligand bound}) / (\text{total conc. of metal ion}) \] (4)

or

\[ \bar{n} = ([\text{ML}] + 2[\text{ML}_2])/(\text{M} + [\text{ML}] + [\text{ML}_2]) \] (5)

While \( \bar{n} \) has a definite value for each species of complex, it may have any value between 0 and the largest number of ligand molecules bound. The numerator of equation (5) gives the total concentration of bound ligand species. The denominator gives the total concentration of all forms of the metal (free and complex species). For the special case in which \( \bar{n} = 1 \), equation (5) becomes:

\[ [\text{ML}] + 2[\text{ML}_2] = [\text{M}] + [\text{ML}] + [\text{ML}_2] \] (6)

\[ [\text{ML}_2] = [\text{M}] \]

Employing the result in equation (3) and (5), we obtain the following relation:

\[ \beta = K_1K_2 = 1/[L]^2_{\text{free}} \]

or

\[ \log \beta = -2 \log [L]_{\text{free}} \]

and finally:

\[ \text{pL}_{\text{free}} = 1/2 \log \beta \text{ at } \bar{n} = 1 \] (7)

In which \( \text{pL}_{\text{free}} \) represents \(-\log[L]_{\text{free}}\). Bjerrum has also shown that, to a first approximation,
\[ pL_{free} = \log K_1 \quad \text{at } n = 1/2 \]  
(8)

\[ pL_{free} = \log K_2 \quad \text{at } n = 3/2 \]  
(9)

It should now be possible to obtain the individual complex formation constants \( K_1 \) and \( K_2 \) and the overall stability constant \( \beta \) if one knows the two values \( n \) and \( pL_{free} \).

Equation (4) shows that the concentration of bound ligand must be determined before \( n \) can be evaluated. The horizontal distances represented by the lines in Figure 1 between the titration curve for Cit alone and for Cit-iron complex, give the amount of alkali consumed in the reactions at specified pH value:

\[
\begin{align*}
HL^2^- + Fe^{3+} + OH^- & \leftrightarrow FeL + H_2O \quad (10) \\
FeL + HL^2^- + OH^- & \leftrightarrow FeL_3^{2-} + H_2O \quad (11)
\end{align*}
\]

This quantity of alkali is exactly equal to the concentration of the protons released or the ligand bound. According to equation (4), dividing the [L]_{bound} by the total concentration of the metal ion, gives the value of \( n \) at each pH. The concentration of the acid species at defined pH is taken as the difference between the initial concentration \([HL]_{init}\) of Cit and the concentration of alkali added ([NaOH]).

\[
[L]_{free} = K_a([HL]_{init} - [NaOH])/(H_3O^+) \quad (12)
\]

or

\[
pL_{free} = pK_a - \log([HL]_{init} - [NaOH]) \quad (13)
\]

Where \([L]_{free}\) is the free concentration of the Cit ligand. For example, from Figure 1, the horizontal distance at pH = 6.87 for 10.5 mL sample is 1.5 \( \mu \text{L} \) NaOH, or \( 0.714 \cdot 10^{-4} \) M. Hence, the total concentration of Fe (III) is \( 4.76 \cdot 10^{-4} \) M, and \( n \) is obtained from equation (4):

\[
\bar{n} = 0.714 \cdot 10^{-4}/4.76 \cdot 10^{-4} = 0.15
\]

Then from equation (13) we have:

\[
pL_{free} = 6.4 - 6.87 - \log[(4.76 \cdot 10^{-3}) - (0.714 \cdot 10^{-4})] = 1.85
\]

Likewise at pH = 8.5, the horizontal distance gives:

\[
[NaOH] = 9.61 \cdot 10^{-4} \text{ mol/L}
\]

and

\[
\bar{n} = 9.61 \cdot 10^{-4}/4.76 \cdot 10^{-4} = 2.02
\]

\[
pL_{free} = 6.4 - 8.5 - \log[(4.76 \cdot 10^{-3}) - (9.61 \cdot 10^{-4})] = 0.32
\]

The values of \( \bar{n} \) and \( pL_{free} \) at various pH values are then plotted as shown in Figure 2.

A curve such as that shown in Figure 2 is known as the formation curve. It reaches a limit at \( \bar{n} = 2 \), illustrating the maximum number of Cit molecules bound to one atom of iron as 2. From this curve at \( \bar{n} = 0.5 \), \( \bar{n} = 3/2 \), and \( \bar{n} = 1.0 \), the approximate values for \( \log K_1 \), \( \log K_2 \) and \( \log \beta \) could be obtained, respectively. Parameters of \( \log K_1 \), \( \log K_2 \), and \( \log \beta \) values for Fe-Cit complex were obtained 1.7, 1.03 and 2.73, respectively.

The conductivity measurement curve for citrate ligand and for Fe^{3+}/Cit is shown in Figure 3.

By increasing the amounts of NaOH, the electrical conductivity of the complex decreases and at point of 20 \( \mu \text{L} \) NaOH, it reaches to the minimum. Then with increasing NaOH increases electrical conductivity. In the case of Cit alone, the electrical conductivity increased after addition of the NaOH solution, while for the curve of Fe-cit complex this is observed at the point at which complexation
becomes complete. The electrical conductivity for glucose in the presence of Fe (III) is also shown in Figure 4.

As the figure depicts, with increasing concentration of NaOH the electrical conductivity of the above mixture decreases, which means, there is no complexation between iron and Glu but Glu and other materials like Et are able to prevent the precipitation of iron in the form of Iron (III) hydroxide via decreasing the dielectric constant of the aqueous solution. The molar conductivity of the complex solution as a function of added citrate is shown in Figure 5.

With increasing the amount of ligand, the molar conductivity of the complex solution decreases and then becomes constant. There are two points in the curve at ratios \( \bar{n} = 1 \) and \( \bar{n} = 2 \), (when the complexation is completed). The molar conductivity could be written in terms of species conductivity as:

\[
\Lambda_M = 1000 \frac{\kappa}{M} \quad (14)
\]

Where \( \kappa \) is specific conductivity (in mS/cm) and \( M \) is the molar concentration of the ligand.

The method of continuous variation, or Job’s method, is based on changes in the absorption characteristics of metal ion or ligand when a complex is formed. Optical density measurements are made for a series of solutions, which contain variable ratios of ligand to metal ion, while keeping the reactant concentration constant. The point at which absorbance reaches a maximum corresponds to the maximum number of ligands bound in the complex.22

Previously Fitch and Stevenson (1984) pointed out that stability constants obtained by the Hill plot are doubtful when binding at one site decreases binding affinity at subsequent sites, which appears to be the case for metal complexes with humic substances.4,23–25

The Scatchard plot approach has been the method of choice of most investigators. Non-linear curves have been obtained, from which stability constant for binding at two sets of binding sites (\( \log K_1 \) and \( \log K_2 \)) can be calculated.26,27

The molar ratio method is the most often used procedure for photometric titration study of complex formation processes. The essence of these methods is that the solution absorbance is measured at constant \( T_L \) (total conc. of ligand) or \( T_M \) (total conc. of metal), The total concentration of the other component being variable. If a single, stable complex is formed, the diagram shows two intersecting straight lines. The ratio of the \( T_L/T_M \) corresponding to the point of intersection gives the quotient of the stoichiometric coefficients. If a moderately stable complex is formed, then the diagram does not display a sharp break: the initially linear section gradually approaches a limiting value and becomes horizontal (as observed in the case of Fe-cit complex). When a weak complex is formed, the horizontal section of the curve cannot be attained in realistic \( T_L \) or \( T_M \) concentration ranges, particularly when it is essential that the constancy of the activity coefficients be ensured in such measurements. In the event of stepwise complex formation, if all of the complexes are sufficiently stable and the equilibrium processes do not overlap each other, the molar ratio diagram consists of several linear sections. The \( \lambda_{\text{max}} \) of the Cit complex was observed at 380 nm, and the functional groups responsible for ligation of the Cit to Fe\(^{3+} \) were determined by the FT-IR spectrophotometry method. At first, the electrical conductivity of the Cit complex decreases with increasing ligand ratio and then shows an increasing trend. In Figure 6, the absorbance of the complex solution at 336 nm (isobestic point of all complex species) is plotted against the mole ratio of \([L]/[M]\).
The initially linear section of the curve gradually approaches a limiting value and then becomes horizontal. The first and second break points of the curve can be attributed to the molar ratios of 1:1 and 2:1 of ligand to the metal ion. In order to investigate the effect of pH on the stability of the complex and obtain the optimum pH of the complex formation, the electronic spectra of the complex are obtained at different pH values.

For a weak soluble acid in water, we have:

\[ \text{HA} + \text{H}_2\text{O} \leftrightarrow \text{H}_3\text{O}^+ + \text{A}^- \quad (15) \]

\[ K_a = \frac{\left[ \text{H}_3\text{O}^+ \right] \left[ \text{A}^- \right]}{\left[ \text{HA} \right]} \quad (16) \]

If the pH and concentrations of HA and A\(^-\) are known, then \( pK_a = -\log(K_a) \) value of the acid can be estimated. When \( [\text{A}^-] = [\text{HA}] \), then \( pK_a = \text{pH} \).

The pH in this point can be shown as \( \text{pH}_{1/2} \), because this point is the midpoint of the titration curve. By plotting the Abs. (at 380 nm) as a function of pH, the \( pK_a \) can be easily calculated. The absorbance of the solution (A) is related to the molar concentration of the complex by means of the Beer-Lambert relation. Where \( \varepsilon \) is the molar absorptivity of the complex, I is the path-length of cell and C is molar concentration, we may write:

\[ [\text{A}^-] = \frac{A}{1 \varepsilon I} \quad (17) \]

Where A\(^-\) is the ligand species with the maximum absorption. When \( [\text{HA}] = [\text{A}^-] \)

Inserting equation (17) into equation (16) gives:

\[ [\text{H}_3\text{O}^+] = K_a(C/[\text{A}^-] - 1)) = K_a(C\epsilon I/A-1) \quad (18) \]

or

\[ \text{pH} = pK_a - \log(C\epsilon I/(A-1)) \quad (19) \]

Since \( C = [\text{HA}] + [\text{A}^-] \)

Then at sufficiently high pH value all the ligand must be in the form of A\(^-\) and under this condition, we have:

\[ C = [\text{A}^-] \text{ and pH} = \varepsilon pK_a = \text{Abs} \quad (20) \]

Equation (20) coincides with the midpoint of the Figure 7 in which half of the reaction of the complex formation is achieved.

In this point pH = 2.73 which is in good agreement with \( \log \beta = 2.73 \) obtained from Figure 2.

Analysis of binding data on the basis of Scatchard and Hill models lead us to the existence and type of the cooperativity in such system. The Scatchard relation is a plot of \( \bar{n}/[\text{L}]_{free} \) vs. \( \bar{n} \) in which a nonlinear pattern is observed for cooperative systems. Figure 8 shows the Scatchard diagram for the Fe-Cit complex.

The results of the binding data show the existence of one set of binding sites, with positive cooperativity in the
system. On the basis of the Hill equation, the binding parameters for such a system could be estimated as shown in Figure 9. Equation (21) represents the linear form of the Hill equation for the system described above, where \( n_H \) and \( K \) are the Hill coefficient and binding constant of the complex, respectively.

\[
\log(\bar{n}/(2 - \bar{n})) = \log K + n_H \log[L]_{\text{free}} \quad 0 < \bar{n} < 2 \quad (21)
\]

The Hill coefficient, \( n_H \), clearly shows a positive cooperative effect in this system since the obtained \( n_H \) is greater than unity. Linear regression of the data from Figure 9 according to equation (21) gives: \( n_H = 1.96 \) and \( \log K = 2.71 \).

The value of \( \log K \) is in excellent agreement with \( \log \beta = 2.73 \) obtained from Figures 2 and 7.

The results of FT-IR spectrophotometry of the ligand and the complexes are shown in Figures 10 and 11, respectively.

Comparison of the wavenumber of the Cit and the corresponding complex shows that some functional groups contributed to the chelation. Results showed that the wavenumber of 3450 cm\(^{-1}\) in both cases has not changed, which means the alcoholic hydroxyl groups did not participate in the binding of the ligand to iron (III). Complexation of iron with Cit leads to the changes of the peak position of hydroxyl groups and the width of peak decreases which indicates the binding of the carboxylic groups to the iron. In the same manner, the study of complexation of Tar ion with Fe\(^{3+}\) is also investigated. In this case, a complex in the form of Fe(Tar)_3 forms and completes (Figure 12) over the pH range of 3–6.

Hill and Scatchard analysis of the data indicate that there are two unidentical and dependent sets of binding sites and the interaction between binding sites is of positive cooperative type. In Figures 13, 14 and 15 the Scatchard and Hill plots are shown, respectively.
4. Conclusion

It is concluded that we can use many chelating agents, which are initially unstable in calcareous soils. However by perturbing the aqueous solution, they could be converted to stable chelates and therefore are usable to compensate the iron deficiency in calcareous soils.

The advantages of these chelates are:

a) They are much cheaper than the other chelating agents, such as EDDHA, EDTA, etc.
b) The preparation method is simple and does not need expensive apparatus and technology
c) Most of the weak chelating agents could be converted to the stable forms in this manner

In this research the stability constant of Fe-Tar was found to be three times more stable than that of Fe-Cit complex, but both complexes were soluble and stable in the presence of NaOH up to pH = 14. Therefore, by using organic acids like citrate, tartarate and maleate, it is possible to make a Fe-complex with high stability usable in soil systems. In a greenhouse study (data not shown) we found that the prepared complexes are able to compensate the iron deficiency in soybean plants and apple trees which were planted in high calcareous soils.

5. Acknowledgement

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6. References

Povzetek
Kompleksacijo železa(III) s citratnimi (Cit) in tartratnimi (Tar) anioni smo študirali v vodnih raztopinah z uporabo potencimetrije, konduktometrije, UV-Vis in FT-IR spektroskopije. Določili smo število vezanih ligandov na železov ion in konstante ravnotežij. Na železov ion se vežeta dva citratna in trije tartratni ioni. Hill-Scatchard analiza daje pri Fe(III)-Cit en set vezavnih mest, pri Fe(III)-Tar pa dva seta. FT-IR spektri potrjujejo vezavo ligandov na železov ion s karboksilsnimi kisikovimi atomi.