# An Investigation on the Equilibria between Arginine and Iron(II) and Iron(III)

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ABSTRACT: Arginine plays several important roles in cellular metabolism; it is able to generate NO, which is a vasodilator. Arginine is able to complex cations in aqueous solutions because it is an amino acid. This paper studies the behavior of arginine as a ligand in the complex formation with iron(II) and iron(III) in 1.00 mol kg^{-1} NaClO$_4$ as ionic medium and at 25 °C. In the case of iron(II) the experimental data obtained by means of electromotive force measurements of galvanic cell with a glass electrode can be explained by assuming the formation of only a complex (1:1), and its stability constant is determined. Experimental data for the system iron(III)−arginine, obtained from electromotive force measurements carried out by employing glass and Pt redox electrodes, are explained by assuming the presence of mononuclear, polynuclear and mixed complexes with participation of OH$^-$. The stability constants of the assumed species are obtained and a hypothetical mechanism for the formation of the predominant complex is proposed.
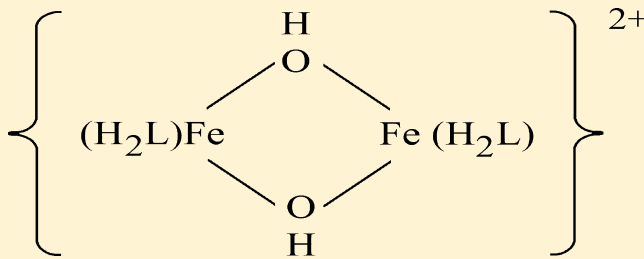
1. INTRODUCTION

Arginine is an amino acid developing important activities in the cellular metabolism by participating to the urea cycle and when it is transformed in citrulline by means of the NO synthases (NOS) is able to generate NO which behaves as vasodilator.[1,2] L-Arginine, 2(S)-amine-5-guanidilpentanoic acid, also identified as α-amine-δ-guanidinvalerianic acid, in the deprotonated form (L = C$_6$H$_{13}$N$_4$O$_2^-$) has one carboxylic, one amino, and a guanidine group. The complex formation of several amino acids (cysteine, L-cystine, DL-serine, D-serine, L-serine, taurine, aspartate and glutamate) with different cations (calcium(II), magnesium(II), cobalt(II), nickel(II), silver(I), cadmium(II), and zinc(II)) was a subject of research in our laboratory.[3−7] Few investigations are reported in the literature on the properties of arginine[8−10] as a ligand, in particular toward poisonous cations, and on the protonation constants relative to the species HL, H$_2$L, and H$_3$L.

Recently, all three protonation constants of arginine have been accurately determined at 25 °C both in 1.00 mol kg^{-1} NaCl and in 1.00 mol kg^{-1} NaClO$_4$, as ionic media, by means of electromotive force (emf) measurements by using a hydrogen electrode. In the same paper, silver and glass electrodes were employed to study the behavior of L-arginine as a ligand toward silver(I).[11] The knowledge of the protolytic equilibria of arginine and its complex formation with silver(I) at 25 °C and 1.00 mol kg^{-1} NaCl and 1.00 mol kg^{-1} NaClO$_4$, as ionic media, was very useful to investigate the behavior of arginine toward calcium(II) and magnesium(II) in the same experimental conditions.[12] The behavior of arginine as a ligand toward iron(II) and (III) was investigated very little probably for difficulties connected with the properties of iron(II) and iron(III). Albert,[13] by studying the system iron(II)−arginine, proposed the formation of the complex FeHL (log β_1 = 3.20).

The most representative paper investigating the system iron(III)−arginine was performed by Perrin.[14] The investigation was carried out at 20 °C and in NaClO$_4$ 1 mol kg^{-1}, in the range 1.62 ≤ −log c_H ≤ 2.62, where c_H represents the free hydrogen ion concentration. To explain the experimental data, the formation of the complex FeHL (log k = 8.7) alone, was assumed.

The little knowledge of the equilibria taking place between iron(III) and arginine and the disagreement between proposed species and constants, induces a study of the system iron(III)−arginine in a wide range of reagent concentrations to verify if polynuclear and/or complexes with the participation of hydrogen ions are present. The method of the constant ionic medium, proposed by Biedermann and Sillèn,[15] was adopted. This method, differently to the method of ionic strength, foresees in the solution the presence of a salt considered inert and at high concentration with respect to the reagents. Biedermann and Sillèn[15] proved experimentally that, even with substituting up to 15% of the ions of the ionic medium with those of the reagents, the activity coefficients of the reagents remain constant despite the variation of the reagent concentration. The adoption of this method allows the substitution of activities by concentrations. The ionic medium in this work was 1.00 mol kg^{-1} NaClO$_4$.

To perform a complete investigation on the behavior of arginine toward iron(III), it was decided to carry out electromotive force measurements (emf) by a redox (Fe^{3+}/Fe^{2+}) electrode, together with the glass electrode. To obtain from the redox potential, the concentration of iron(III) at

equilibrium, it was necessary to know preliminary equilibria involving iron(II) with arginine. For this reason this paper consists of two parts. In the first part the equilibria between arginine and iron(II) and in the second those between iron(III) and arginine are studied.

2. EXPERIMENTAL SECTION

2.1. Method of Investigation. *2.1.1. Method of Investigation: Iron(II)–Arginine.* The investigation of the equilibria between iron(II) and arginine was performed by supposing the absence of the mixed complexes with participation of ion hydrogen and of polynuclear species in iron(II). This hypothesis will be proved *a posteriori*.

Similarly to the equilibria between arginine and other bivalent cations,[12] it could be reasonably supposed that the following equilibria can be written.

$$Fe^{2+} + wHL \leftrightarrow Fe(HL)_w \tag{1}$$

In a constant ionic medium, equilibrium 1 is connected with a constant, defined as follows:

$$c_{Fe(HL)w} = \beta_w c_{Fe2+} c_{HL}{}^w \tag{2}$$

To find the prevailing values of w and of the constant β_w, the emf of the following cell was measured:

$$RE/solution \ S/GE \tag{I}$$

In I RE is a reference electrode, below described, GE is a glass electrode, and the solution S had the following general composition: C_H M in H^+; $C_{Fe(II)}$ M in Fe(II); $(1 - C_H - 2C_{Fe(II)})$ M in Na^+ and 1.00 M ClO_4^- (M denotes mol kg^{-1}). With "C_x", the total concentration of the species x is indicated, while "c_x" indicates its free concentration at equilibrium.

In a constant ionic medium, at 25 °C and in mV units, the emf of the cell (I) can be expressed as follows:

$$E_I = E_I^o + 59.16 \log c_H + E_j \tag{3}$$

E_I^o is a constant, E_j is the liquid junction potential due to the hydrogen ion concentration.[15] In the selected experimental ionic medium, according to Biedermann and Sillèn, it was found $j = -60$ mV mol^{-1} Both were determined in the first part of each titration, in the absence of ligand, when $C_H = c_H$ and $C_{Fe(II)} = c_{Fe(II)}$. After the determination of E_I^o and E_j, the ligand, in the selected ionic medium, was introduced in the solution S. Its concentration was gradually increased, by keeping constant C_H and $C_{Fe(II)}$. In correspondence c_H decreased. Measurements at different C_H and $C_{Fe(II)}$ were carried out. The knowledge of analytical concentrations of the reagents and the free concentration of hydrogen ions obtained by the emf of the cell (I) constitute the basis of the elaboration to obtain the values of w and β_w.

2.1.2. Method of Investigation: Iron(III)–Arginine. The study of complex formation between iron(III) and arginine is performed without preliminary hypothesis. By considering the species as independent reagents, the following equilibrium can be formulated:

$$qFe + pH + rL \leftrightarrow Fe_qH_pL_r \tag{4}$$
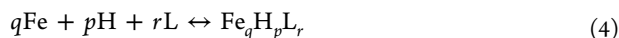
In eq 4, charges are omitted and $q \geq 1$. If $q > 1$, polynuclear complexes in iron(III) are formed, $p \neq 0$, indicates the formation of complexes mixed with participation of protons and $r \geq 1$.

The equilibrium 4 is defined by the constant:

$$\beta_{q,p,r} c_{Fe3+}{}^q c_H{}^p c_L{}^r = c_{FeqHpLr}$$

The aim of this research is to determine the prevailing values of q, p, and r and the relative constants.

For this purpose the emf of the following cells was measured:

$$RE/solution \ S1/GE \tag{II}$$

$$RE/solution \ S1/Pt \tag{III}$$

In II and III RE and GE indicate reference and glass electrode, while Pt is a platinum electrode able to measure the ratio $R = c_{Fe3+}/c_{Fe2+}$.

The solution S1 had the following general composition: C_H M in H^+; $C_{Fe(II)}$ M in Fe(II); $C_{Fe(III)}$ M in Fe(III) ; $(1 - C_H - 2C_{Fe(II)} - 3C_{Fe(III)})$ M in Na^+ and 1.00 M ClO_4^- (M denotes mol Kg^{-1}).

In a constant ionic medium, at 25 °C and in mV units, the emf of the cells can be written as

$$E_{II} = E_{II}^o + 59.16 \log c_H + E_j \tag{5}$$

$$E_{III} = E_{III}^o + 59.16 \log R + E_j \tag{6}$$

where E_{II}^o and E_{III}^o, two constants, and, E_j, the liquid junction potential[15] are determined in the first part of each measurement, in the absence of ligand; that is, $C_H = c_H$ and $C_{Fe(III)} = C_{Fe(II)} = c_{Fe3+} = c_{Fe2+}$ and of course $R = 1$.

In the second part of the measurement, the ligand in the ionic medium was added to the solution S1 and its concentration was gradually increased, by keeping C_H, $C_{Fe(III)}$ and $C_{Fe(II)}$ constant. In this way also $-\log c_H$ increased gradually. For each experimental point the analytical concentration C_H, $C_{Fe(III)}$, $C_{Fe(II)}$, and C_L were known and the emf measurements provided $-\log c_H$ and $\log R$. The elaboration of the experimental data will be carried out in the suitable section.

2.2. Material and Analysis. A L-arginine p.a. product (Sigma) was twice crystallized from water, dried at 120 °C until constant weight. The absence of impurities was proven by thermogravimetric and polarographic analysis.

Stock solutions of $Fe(ClO_4)_3$ and $Fe(ClO_4)_2$ were prepared and analyzed as previously described.[16] $NaClO_4$, $HClO_4$, NaOH, were prepared and analyzed as previously described,[17,18] as well.

2.3. Details on Experimental Apparatus. All measurements were carried out in a thermostat room at 25.0 °C and in a thermostat at 25.00 ± 0.05 °C.

The emf measurements of cell (I) and (II) were carried out with a Metrohm, model 654 pH-meter, equipped with a glass electrode of the same firm. The emf measurements of cell (III) were carried out with a Keithley model 2000. Foils of bright Pt, boiled in concentrated HNO_3, washed with bidistilled water, and finally burned in an ethanol flame were used in cell (III).

The salt bridge was similar to that described by Forsling et al.[19] and the RE = Ag, $AgCl/0.01$ M Cl^-, 0.99 M ClO_4^-, 1.00 M Na^+ was prepared according to Brown[20] (M denotes mol kg^{-1}). The agreement of data obtained from direct and back emf measurements allows the assumption that measurements were performed in solution at real equilibrium. Values of E_I and E_{II} reach constant values a few minutes after each addition, whereas it was necessary to wait (45– 60) min to have constant values of E_{III}. Such values remain constant for several hours.

The values of $E_I - E_I^o$ and $E_{II} - E_{II}^o$ were reproducible within ± 0.2 mV, whereas those of $E_{III} - E_{III}^o$ were reproducible within ± 0.1 mV.

Nitrogen (99.995 %) from a cylinder, further purified by passing through 10 % NaOH, 10 % H_2SO_4 and the ionic medium, was bubbled through S and S1 to eliminate oxygen and CO_2 from the solutions.

3. RESULTS

3.1. Results of Investigation on Iron(II)–Arginine.
Experimental data were obtained for $C_{Fe(II)}$ = (0.010, 0.025 and 0.050) mol kg^{-1} at different C_H and arginine concentrations.

By introducing the $-\log c_H$, obtained from the emf of cell (I), in the material balance of the analytical excess of hydrogen ion, the free concentration of the ligand could be calculated, as follows:

$$C_H = c_H + k_1 c_H c_L + 2k_1 k_2 c_H^2 c_L + 3k_1 k_2 k_3 c_H^3 c_L \tag{7}$$

In eq 7, as above explained, the absence of polynuclear and mixed complexes with the participation of hydrogen ions is assumed. Furthermore, on the basis of the studies of Hedström,[21] hydrolytic species of iron(II) are not considered.

In Figure 1 the distribution of arginine as a function of $-\log c_H$ is plotted. It can be observed that, in the investigated $-\log c_H$
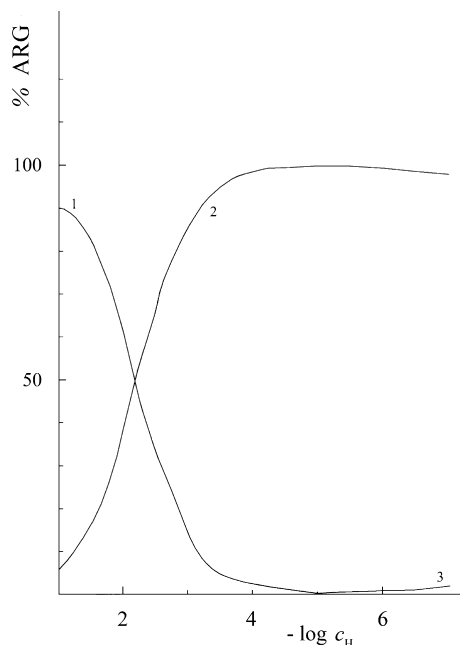

Figure 1. Arginine distribution as a function of hydrogen ion concentration. The curves 1, 2, and 3 are relative to H_3L, H_2L, and HL, respectively.

range to study the system Fe(II)–arginine, the ligand is present prevalently in the protonated form. It is assumed that it acts toward iron(II) in the form HL. Its free concentration, below indicated as c'_L, can be calculated from eq 7.

The formation function **n** (i.e., the average number of ligand per iron(II), central group) could be calculated from the material balance of arginine divided to $C_{Fe(II)}$, as follows:

$$\mathbf{n} = (C_L - c_{L'} - 2k_2 c_H c_{L'} - 3k_2 k_3 c_H^2 c_{L'})/C_{Fe(II)} = \Sigma w \beta_w$$

$$c_{L'}{}^w/(1 + \Sigma \beta_w c_{L'}{}^w) \tag{8}$$

In eq 8, according to Hedström,[21] the hydrolysis of iron(II) could be neglected and the hypothesis was formulated that

polynuclear and mixed complexes with participation of protons are not present in appreciable concentration. The formation function, **n**, versus $-\log c_{L'}$ is plotted in Figure 2. It can be
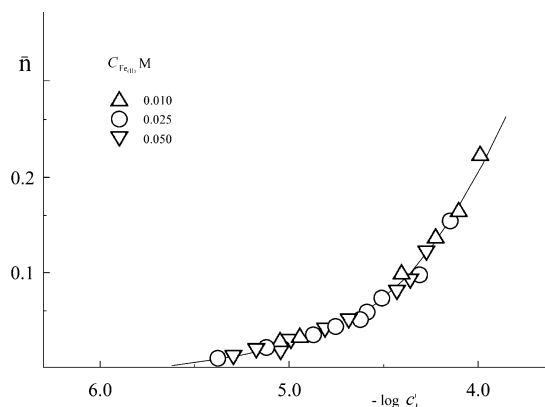

Figure 2. Formation function of iron(II) arginine complexes. The curve is calculated by using the value of the constant proposed in the text. M denotes mol kg^{-1}.

observed that all the experimental points fall on the same curve, then **n** is not a function of the total concentration of iron(II). The hypothesis previously formulated is verified. Furthermore, as the trend of the points of Figure 2 shows a slope ≤ 1, it could be deduced that w assumes the value 1 and eq 8 becomes

$$\mathbf{n} = \beta_1 c_{L'}/(1 + \beta_1 c_{L'})$$

which, by adopting the graphical method proposed by Sillèn,[22] can be normalized in the following:

$$N = u/(1 + u)$$

where the position $u = \beta_1 c_{L'}$ is proposed.

By superimposing the normalized curve on the experimental points and moving the two plots parallel to abscissa, in the position of best fit, the value of $-\log c_{L'}$ corresponding to log u = 0 was obtained. On the basis of the assumed mathematical position, it corresponds to log β_1 = 3.40 ± 0.05. The limit of error corresponds to the maximum shift between normalized curve and experimental points for an agreement still acceptable.

The agreement between points and curve supports the validity and the accuracy of the procedure.

3.2. Results of Investigation on Iron(III)–Arginine.
Solutions with C_H = (0.050; 0.070; 0.100 and 0.140) mol kg^{-1} and $C_{Fe(III)}$ = $C_{Fe(II)}$ = (1.00; 0.50; 0.25; 0.125; 0.0625; 0.0313) $\times 10^{-3}$ mol kg^{-1} were investigated in the range $1.7 \leq -\log c_H \leq 3.3$.

From the value of the constant of Fe(II)–arginine above determined, it could be calculated that the presence of an iron(II) complex with arginine can be neglected in the $-\log c_H$ range studied in this section. Without loss of accuracy, it can be assumed that $C_{Fe(II)} = c_{Fe(II)}$, so that $c_{Fe(III)}$ was easily calculate from R.

From the measured values of c_H and c_{Fe}, on the basis of the study of Hedström,[23] the iron(III) hydrolysis could not be neglected.

The iron(III) material balance without preliminary hypothesis can be written as follows:

$$C_{Fe(III)} = c_{Fe(III)} + \Sigma\Sigma q\beta_{q,p,r}c_{Fe(III)}{}^{q}c_{H}{}^{p}c_{L}{}^{r} + x_{1,1}c_{Fe(III)}c_{H}{}^{-1}$$
$$+ 2x_{2,2}c_{Fe(III)}{}^{2}c_{H}{}^{-2} + x_{1,2}c_{Fe(III)}c_{H}{}^{-2}$$

where $x_{1,1}$, $x_{2,2}$, and $x_{1,2}$ are the values proposed by Hedström.[23]

The hydrolysis corrected value

$$C_{Fe} = C_{Fe(III)} - (x_{1,1}c_{Fe(III)}c_{H}{}^{-1} + 2x_{2,2}c_{Fe(III)}{}^{2}c_{H}{}^{-2}$$
$$+ x_{1,2}c_{Fe(III)}c_{H}{}^{-2})$$

was used to define the following function:

$$\eta = \log(C_{Fe}/c_{Fe}) = \log(1 + \Sigma\Sigma q\beta_{q,p,r}c_{Fe(III)}{}^{q-1}c_{H}{}^{p}c_{L}{}^{r}) \tag{9}$$

In Figure 3 an example of experimental data is plotted in the form η versus $-\log c_{H}$. Points obtained at the same C_{H}, but at
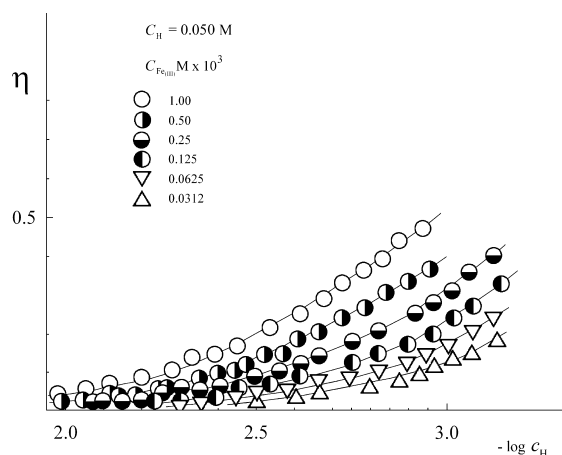

Figure 3. Dependence of the function η versus $-\log c_{H}$ as an example for C_{H} = 0.050 mol kg^{-1}. The curves are calculated by means of the constants of Table 1.

different C_{Fe} fall on different curves, in particular as η is an increasing function of C_{Fe}, polynuclear complexes are present and $q \geq 1$.

To explain the experimental data it is necessary to study the dependence of η on the concentration of the ligand.

For this purpose, it is convenient to remember the distribution of arginine as a function of $-\log c_{H}$, shown in Figure 1. It is evident that, in the investigated $-\log c_{H}$ range, the ligand is present in the form H_2L. In the following its free concentration will be indicated as c_{L}''.

The dependence of η on $-\log c_{L}''$ is shown in Figure 4, where, as an example, data obtained at the same C_{Fe}, but different analytical excess of hydrogen ions, C_{H}, are plotted.

It can be observed that η is a decreasing function of C_{H}, then species with a loss of protons are formed. As η is a function of three variables, it is not possible to study its dependence contemporary on the three variables by graphical methods. By applying the normalized curves method proposed by Sillèn,[22] conditional constants are found by keeping constant in a first step two variables and studying the dependence on the third. In successive steps the dependence on the other variables was studied in order to obtain the species present and their stability constants, as described previously.[24,25]

However, independently of the graphic elaboration, the primary experimental and analytical data are elaborated by means of a PC graphical program[26] and a PC program, BSTA.[27] Both from graphical method and PC elaboration, the same results were obtained. The assumption of the presence of the species $Fe(H_2L)_2$, $FeH_{-1}(H_2L)_2$, $Fe_2(H_2L)_2$, and $Fe_2H_{-2}(H_2L)_2$ with stability constants reported in Table 1 explained well the experimental data.

The constants of Table 1 are used to calculate the theoretical curves drawn on the experimental points in the relative plots. The error limits correspond to the maximum possible shift between points and curves for a still acceptable agreement. The good agreement between points and curves supports the validity of the procedure and the species assumed with the relative constants.
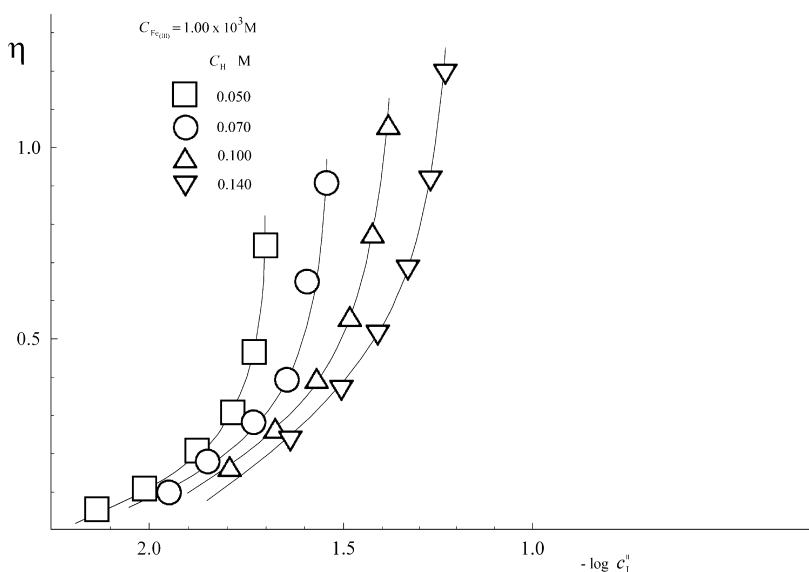

Figure 4. Dependence of the function η (defined in the text) on the free concentration of the ligand, in the form $c_{L}'' = H_2L$, as an example. Experimental points at the same iron(III) concentration, but different C_{H}, fall on different curves. The curves are calculated by using the constants of Table 1. M denotes mol kg^{-1}.

Table 1. Species Assumed to Explain the Experimental Data Obtained Studying the System Iron(III)−Arginine and Relative Stability Constants

proposed species	constants
$Fe(H_2L)_2$	$\log \beta_{1,0,2} = 2.20 \pm 0.10$
$FeH_{-1}(H_2L)_2$	$\log \beta_{1,-1,2} = -0.60 \pm 0.08$
$Fe_2(H_2L)_2$	$\log \beta_{2,0,2} = 6.25 \pm 0.10$
$Fe_2H_{-2}(H_2L)_2$	$\log \beta_{2,-2,2} = 0.55 \pm 0.10$

3.3. Discussion. The behavior of arginine as a ligand toward iron(II) and iron(III) was investigated by varying widely the concentration of the reagents.
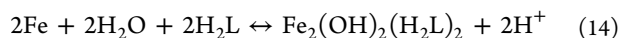
The species assumed to explain the experimental data for iron(II) and the relative stability constant agrees with the result proposed by Albert,[13] by taking into account the different experimental conditions.

Perrin[14] studied the system iron(III) concentration in a very narrow reagent concentration range, so that it should be very hard to find evidence for polynuclear species. Although his investigation was carried out in the range more acid than that in this paper, the species HL was assumed as a ligand.

Some values of the constants of Table 1 could seem very low [for the complex $FeH_{-1}(H_2L)_2$ a negative value is proposed], but if the equilibria taking place with loss of protons are considered in the following form, omitting charges,

$$Fe + H_2O + 2H_2L \leftrightarrow Fe(OH)(H_2L)_2 + H^+ \qquad (13)$$

and

$$2Fe + 2H_2O + 2H_2L \leftrightarrow Fe_2(OH)_2(H_2L)_2 + 2H^+ \qquad (14)$$

the constants relative to eq 13 and eq 14 can be expressed, respectively:

$$\beta_{1,-1,2} = c_{Fe(OH)(H2L)2} c_H c_{Fe}^{-1} c_{H2L}^{-2}$$

and

$$\beta_{2,-2,2} = c_{Fe2(OH)2(H2L)2} c_H^{2} c_{Fe}^{-2} c_{H2L}^{-2}$$

By introducing in both equations K_w, the following can be obtained:

$$\beta_{1,-1,2}^* = \beta_{1,-1,2} K_w^{-1} = c_{Fe(OH)(H2L)2} c_{Fe}^{-1} c_{OH}^{-1} c_{H2L}^{-2}$$

$$\text{with} \quad \log \beta_{1,-1,2}^* = 13.78 - 0.06 = 13.72$$

and

$$\beta_{2,-2,2}^{**} = \beta_{2,-2,2} K_w^{-2} = c_{Fe2(OH)2(H2L)2} c_{(OH)2}^{-2} c_{Fe}^{-2} c_{H2L}^{-2}$$

$$\text{with} \quad \log \beta_{2,-2,2}^{**} = 27.56 + 0.55 = 28.11$$

The value of $\log K_w = 13.78$ was obtained previously[11] in the same experimental conditions. The high values for the constants can explain the little percentage of hydrolyzed iron(III) found in this investigation.

4. CONCLUSION

The assumed species with the relative stability constants explain well the experimental data. The formation of a complex FeHL was expected for iron(II), while it was necessary to assume the presence of many species, even polynuclear and with loss of protons complexes to explain the data obtained in the investigation on iron(III).

Only the conclusion of the study of Perrin[14] can be partially compared with the results here obtained. However, Perrin, by studying the system iron(III) arginine, formulated *a priori* the hypothesis of the formation of 1:1 complex, limited his investigation to a very narrow concentration range of iron(III) and to the range $1.6 \leq -\log c_H \leq 2.6$. It would be hard to find polynuclear species in iron(III) without significant variation of the cation concentration.

A comment on the results obtained in this investigation can be formulated by observing the composition of the proposed complexes.

The contemporary presence of OH bound to iron(III) and arginine in the form H_2L can be well explained because iron(III) hydrolysis[23] begins at very low values of $-\log c_H$ and, in this $-\log c_H$ range, the ligand is present in the form H_2L, as it is supported by the values of the protonation constants.

The comparison between the composition of the here proposed species and those proposed by Hedström[23] to explain the iron(III) hydrolysis shows some analogy. In both cases the ratios 1:1 and 2:2 between iron(III) and OH^- are present.

Although emf measurements are not able to provide information on the structure of the formed species, but taking into account literature suggestions, some hypothesis on the mechanism of formation of the assumed complexes can be proposed. Gaines et al.[28] prepared an iron−phenanthroline solid compound with properties corresponding to the "diol" structure. Bjerrum,[29] on the basis of a comparison between hydrolysis and amino complexes data, deduced that a structure of type "ol" (like $Fe_2(OH)_2^{4+}$) is generally acceptable. Hedström[23] to explain the prevailing binuclear species assumed, to explain his data on hydrolysis of iron(III), proposed a "diol" structure similar to the following structure V:

(V)
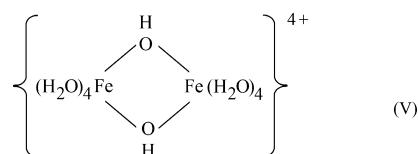
More recently, in a research on the complex formation between iron(III) and glycine,[30] the existence of a "diol" structure similar to structure V, with glycine instead of water molecules, is proposed to explain the assumed species.

To formulate a hypothesis of a formation mechanism of the here proposed species, it seems important to stress that in the structure proposed by Gaines et al.,[30] Bjerrum,[29] and Hedström,[23] the "diol" configuration involves a binuclear complex. In conclusion the hypothesis of the "diol" mechanism similar to that above-described in structure VI seems probable. In structure VI the water molecules are omitted for simplicity.

(VI)
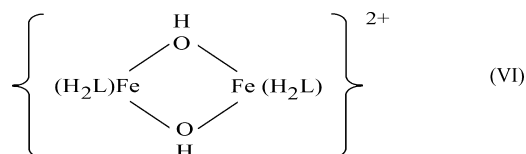
The values of the stability constants of Table 1, when compared with the hydrolysis Hedström[23] data and the experimental evidence found in our investigation, show that by increasing $-\log c_H$ beyond 3.5, also the hydrolyzed iron(III) percentage increases and a precipitate is formed. To obtain a soluble species involving arginine and iron(III) at physiological

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hydrogen ion concentrations, the formation of more complex species with the participation of other ligands has to be supposed.

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Notes

The authors declare no competing financial interest.

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