Chemical Speciation of the Fe(III)-piroxicam and Fe(III)-tenoxicam Systems in Aqueous Solution

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Abstract. A chemical speciation study of the antiinflamatory drugs piroxicam and tenoxicam was carried out with the Fe(III) ion, under pseudophysiological conditions (T = 37ºC and I = 0.15 M). The equilibrium constants for the formation of the Fe(III)-oxicam species were obtained from UV/VIS spectrophotometric data assisted by SQUAD program. The hydrolysis constants of Fe(III) were also refined under the same experimental conditions. For tenoxicam, the constants refined correspond to the species $Fe(H_2tenox)_2^{5+}$, $Fe(Htenox)_2^{3+}$, $Fe(OH)_2(Htenox)_2^+$, $Fe(OH)_2(tenox)_2^-$, FeH_2tenox^{4+}, $FeHtenox^{3+}$, $Fe(OH)_2Htenox^+$ and $Fe(OH)_2tenox$, an analogous model was established to refine the piroxicam constants. The molar absorptivity coefficients for all the species were also calculated.

Keywords: Fe(III), tenoxicam, piroxicam, SQUAD, chemical speciation.

Resumen. Se realizó un estudio de especiación de los fármacos antiinflamatorios piroxicam y tenoxicam con el ion metálico Fe(III), bajo condiciones pseudofisiológicas (T = 37ºC y I = 0.15 M). Las constantes de formación de las especies Fe(III)-oxicam fueron obtenidas a partir de datos de espectrofotometría UV/VIS, utilizando para ello el programa computacional SQUAD. Se determinaron también las constantes de hidrólisis de Fe(III) en agua bajo las mismas condiciones experimentales. Para tenoxicam se refinaron las constantes de las especies $Fe(H_2tenox)_2^{5+}$, $Fe(Htenox)_2^{3+}$, $Fe(OH)_2(Htenox)_2^+$, $Fe(OH)_2(tenox)_2^-$, FeH_2tenox^{4+}, $FeHtenox^{3+}$, $Fe(OH)_2Htenox^+$, $Fe(OH)_2tenox$; un modelo análogo fue refinado para piroxicam. Para todas estas especies, se calcularon sus coeficientes de absortividad molar.

Palabras clave: Fe(III), tenoxicam, piroxicam, SQUAD, especiación química.

Introduction

Iron is an essential element for life processes, a number of enzymes and proteins make use of iron for their performance [1]. Around 70% of the iron in the human body is part of hemoglobin or myoglobin and 1% in form of hemoproteins and hemoenzymes. The rest of iron form part of several enzymes or proteins. Iron may be free in blood or stored in ferritin protein. It has been reported that proteins other than ferritin, such as transferrin, play a key role in the intracellular iron transporting. In the torrent blood, iron must be in the form of Fe(III) to bind apotransferrin, its transporting protein, in this way it can reach bones and liver, where it forms hemoglobin and other hemoproteins [2]. The development of new Fe(III) chelating agents is of great importance, because of their potential applications in medicine, in antioxidant therapies and antineoplastic agents [3-6].

Nowadays the rheumatoid arthritis is one of the most common conditions, manifested by muscle and articulations inflammation [7-9]. The treatment includes intake of non steroidal antiinflamatory drugs (NSAIDs) of the oxicam family, being piroxicam and tenoxicam the widest used (Scheme 1a and 1b). It has been documented that complexation with metal ions improved the oxicams antiinflamatory effects [10-15].

Regarding the Fe(III) interaction with different oxicams, from elemental analysis of prepared solids, it has been proposed that the complexes with isoxicam, tenoxicam and piroxicam

[16-17] are formed in the 1:3 metal:oxicam stoichiometry; in all the cases, the coordination sphere would involve the enolate oxygen atom and the carbonilic oxygen of the amide group. However, the crystal structure confirming this stoichiometry has not yet been reported. In solution, our group has investi-
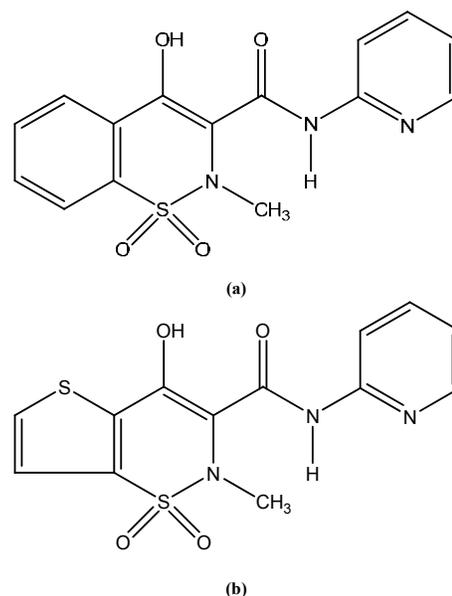
Scheme 1. Chemical structure for a) piroxicam and b) tenoxicam.

gated the complexation of Fe(III) with piroxicam and tenoxicam in methanol and acetone; these studies point out that the stoichiometry of the formed species is strongly dependent on the solvent. For instance, in methanol we found mononuclear complexes metal-oxicam in 1:1 and 1:2 stoichiometry. In contrast, in acetone the polynuclear metal-oxicam species are favored, with stoichiometries 2:1, 2:2 and 2:3 [18].

Being Fe(III) one of the most abundant ions, with around 3 mg of circulating iron in blood plasma (transferrin-bound iron and non-transferrin-bound iron), and having a main role in chronic diseases, it is of vital importance the knowledge of its interactions with intake drugs and the physicochemical characterization of the species formed in aqueous solution under pseudophysiological conditions. In this work we studied the interaction of Fe(III) with piroxicam and tenoxicam at T = 37°C and a ionic strength of 0.15 M in aqueous solution, in order to obtain the stoichiometry of the predominant species and the corresponding formation constants.

Results and discussion

1. Hydrolysis Constants of Fe(III)

So far, the study of Fe(III) in solution is controversial, mainly because of the complexity of the established equilibria due to the kinetics effects, and the number of the polynuclear-hydroxo species reported. While in acid solutions, the $Fe_2(OH)_2^{4+}$ dimmer and two ($FeOH^{2+}$ and $Fe(OH)_2^+$) mononuclear species are present, in neutral or basic solutions the $Fe(OH)_3$ and $Fe(OH)_4^-$ species are predominant. At high enough concentrations, the $Fe_3(OH)_4^{5+}$ species has been reported in solution [19]. Although the formation constants, as well as the absorption spectra, of some of these complexes have been investigated, the study under pseudophysiological conditions (37°C and I = 0.15 M) is desirable.

In this direction, an hydrolysis study of Fe(III) was undertaken prior to investigate the interaction with tenoxicam and piroxicam. The recorded spectra were introduced to the SQUAD program [20] to refine the equilibrium constants. Table 1 shows the hydrolysis constants calculated by employing a reported model [19] together with the Fe(III) concentrations used in this study. Table 1 also shows the values of σ and U, the standard deviation of the data set and the sum of quadratic residues, respectively. Figure 1 displays the molar absorptivity coefficients of the different species; it was found that the absorption maxima for Fe^{3+} and $FeOH^{2+}$ correspond to those previously reported [19] as well as the isosbestic point at 275 nm. It is worth noting that for pH < 2.0 it is possible for the complexes $FeCl^{2+}$ and $FeCl_2^+$ to be formed [21], therefore the reported stability constants, all along this study, should be considered as for the Fe(III) generalized species in this pH region. At a ionic strength of 0.1 M we have estimated that up to 50% of the Fe(III) can form chloride complexes, but at our ionic strength of 0.15M, the participation of chloride for forming complexes with Fe(III) may be lesser.

Table 1: Hydrolysis constants of Fe(III) under pseudophysiological conditions.

Species	log (standard deviation)	σ	U
$FeOH^{2+}$	−3.2092 (0.08)	1.66×10^{-2}	2.85×10^{-1}
$Fe(OH)_2^+$	−5.5640 (0.03)		
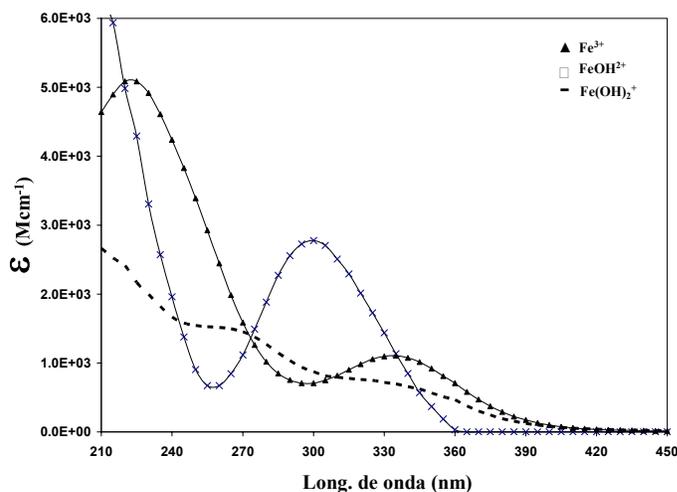
Fig. 1. Calculated molar absorptivity coefficients of the Fe(III) species in aqueous solution.

2. Fe(III)-tenoxicam speciation in aqueous solution

A selection of the absorption spectra for the Fe(III)-tenoxicam system at the 1:1 and 1:2 Fe(III):tenoxicam molar ratios are shown in Figures 2a and 2b, respectively. For the molar ratio 1:3 the spectra are very similar to those of the free oxicam. At pH > 6.0 there were not significant changes on the spectra of the systems. For the 1:1 and 1:2 molar ratios, a isosbestic point appears at 245 nm which was not present in the spectra of the free tenoxicam [22] in the pH zone 0.9 < pH < 2.0. Therefore, a new equilibrium for the Fe(III)-tenoxicam exists, which might be related with the deprotonation of the complex.

When the absorbance spectrum as a function of pH at 250 nm is represented for each of the molar ratio studied (Figure 3), there is a change in the curve in the zone 2.0 < pH < 3.0, which cannot be attributed to the free oxicam (its neutral species is predominant in this pH zone). Because of the observed band is basically that of the oxicam, the changes could be related with the hydrolysis of the Fe(III)-tenoxicam complexes.

Spectrum data corresponding to the worked molar ratios were introduced to the SQUAD, program and different models for the chemical equilibria refined. For each molar ratio 24 spectra were introduced working in the wavelength range from 230 to 450 nm with a total of 44 points for each spectrum. Besides fixing the equilibrium constant values (β, equation 1) of the tenoxicam acid-base species [20] and their corresponding molar absorptivities [22], to optimize the refining process, the hydrolysis constants of Fe(III) and their molar absorptivities were also introduced to SQUAD. For the model refinement,
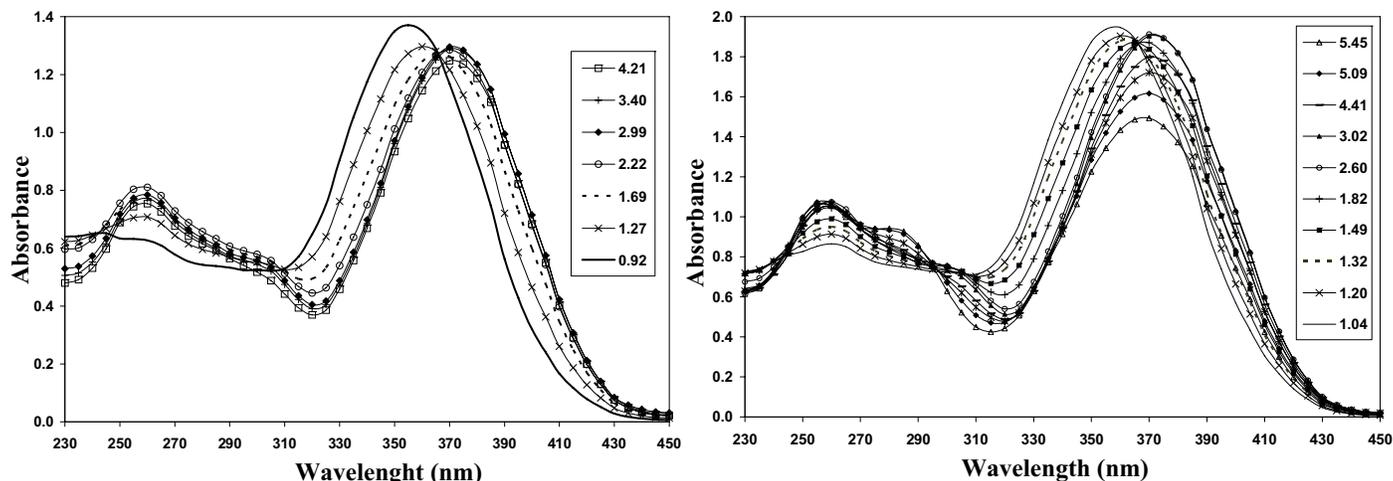
Fig. 2. Absorption spectra of the Fe(III)-tenoxicam system as a function of the pH under pseudophysiological conditions, [Fe(III)] = 6.0×10^{-5} M, a) 1:1 and b) 1:2 molar ratio.
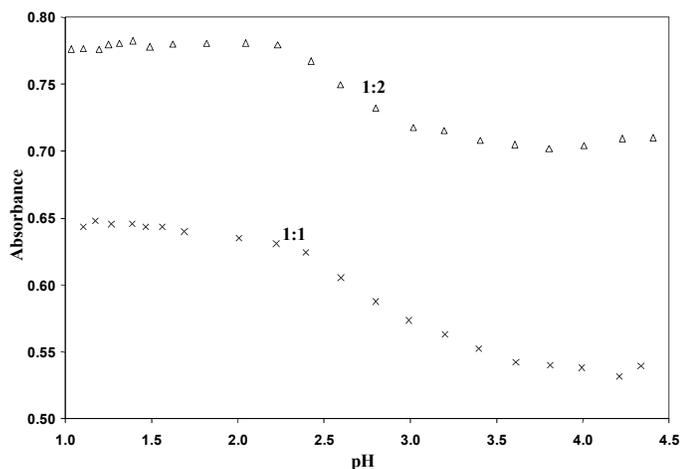
Fig. 3. Absorbance as a pH function of the Fe(III)-tenoxicam system at 250 nm, curves are for the 1:1 and 1:2 molar ratios at [Fe(III)] = 6.0×10^{-5} M.
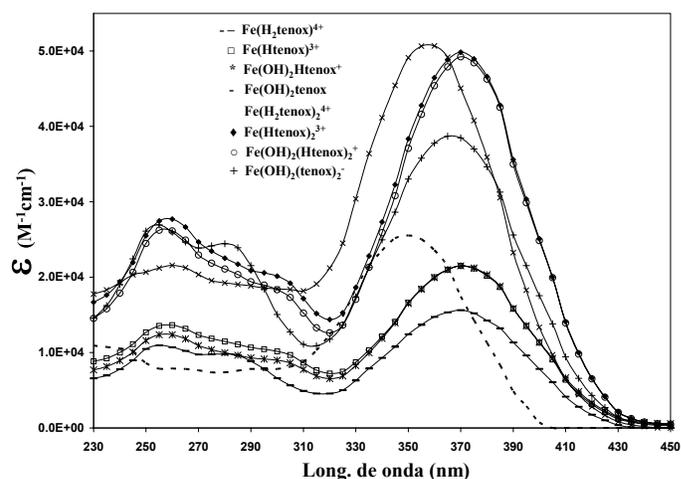
Fig. 4. Molar absorptivities of the species of the Fe(III)-tenoxicam system.

a combination of the experiment results with the 1:1 and 1:2 Fe(III)-Oxicam ratios were employed. First, we worked the results of the 1:1 ratio and those of the 1:2 ratios in independent SQUAD runs, then the output of these runs were used for modeling the global system.

Table 2 compiles the best models obtained for each of the molar ratio metal:oxicam, as well as σ and U. The reported equilibrium constant is the associated with the generalized equilibrium:

$$pM^{3+} + qL^- + rH^+ \Leftrightarrow (M_pL_qH_r)^{(p3-q+r)}$$

$$\beta_{pqr} = \frac{\left[M_pL_qH_r^{(p3-q+r)}\right]}{\left[M^{3+}\right]^p\left[L^-\right]^q\left[H^+\right]^r} \qquad (1)$$
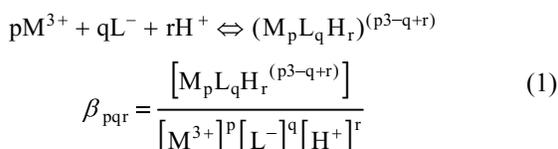
From Table 2 it can be realized that six acid-base equilibria for the Fe(III)-tenoxicam system exist:

$$[Fe(H_2tenox)_2]^{5+} \Leftrightarrow [Fe(Htenox)_2]^{3+} + 2H^+$$
$$pKa = 1.345 \qquad (2)$$

$$[Fe(Htenox)_2]^{3+} + 2H_2O \Leftrightarrow [Fe(OH)_2(Htenox)_2]^+ + 2H^+$$
$$pKa = 2.920 \qquad (3)$$

$$[Fe(OH)_2(Htenox)_2]^+ \Leftrightarrow [Fe(OH)_2(tenox)_2]^- + 2H^+$$
$$pKa = 4.905 \qquad (4)$$

$$[Fe(H_2tenox)]^{4+} \Leftrightarrow [Fe(Htenox)]^{3+} + H^+$$
$$pKa = 0.910 \qquad (5)$$

$$[Fe(Htenox)]^{3+} + 2H_2O \Leftrightarrow [Fe(OH)_2(Htenox)]^+ + 2H^+ \\ pKa = 2.950 \quad (6)$$

$$[Fe(OH)_2(Htenox)]^+ \Leftrightarrow [Fe(OH)_2(tenox)] + H^+ \\ pKa = 4.790 \quad (7)$$
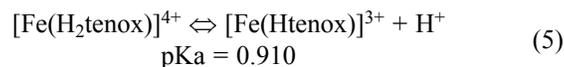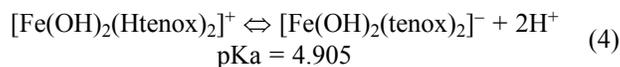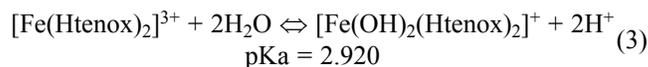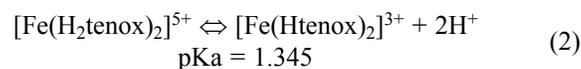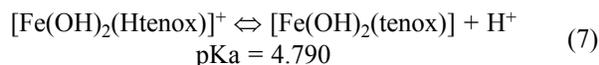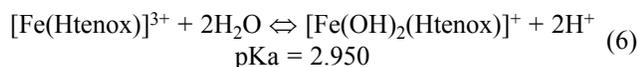
The molar absorptivities for the species calculated by SQUAD are depicted in Figure 4.

Once the values of the equilibrium constants were obtained, the species distribution diagram were constructed (Figure 5) by employing the MEDUSA program [23].

3. Fe(III)-piroxicam speciation in aqueous solution

Figure 6 shows the selected spectra for the Fe(III)-piroxicam system, at the 1:1 and 1:2 Fe(III):piroxicam molar ratios. Similar to the Fe(III)-tenoxicam system, in this case the molar ratio 1:3 resembles the free piroxicam spectrum, observing evident changes for the 1:1 and 1:2 molar ratios. At pH > 6.0 there were not significant changes on the spectra of the systems, as it was found for the Fe(III)-tenoxicam system.

For piroxicam the isosbestic point appears at 240 nm, in the pH region 1.0 < pH < 2.3. The maximum of 250 nm, which for the free oxicam increases with pH, in the Fe(III)-piroxicam system shows a hypochromic effect. A diminishing of the maximum at 300 nm is also observed.

Figure 7 displays the absorbance as a function of pH at 250 nm for this system, in contrast with the absorbance of the free piroxicam, the absorbances of the 1:1 and 1:2 molar ratios present changes in the observed pH region. These changes cannot be associated with the acid-base behavior of the free ligand, because in this zone the predominant free oxicam species is the neutral form.

Employing the same methodology described for the Fe(III)-tenoxicam system, the chemical model for Fe(III)-piroxicam was established. Table 3 resumes the obtained results and includes the corresponding σ and U statistical parameters of the data.

The molar absorptivities for the species present in the Fe(III)-piroxicam system calculated by using SQUAD are depicted in Figure 8.

Figure 9 shows the corresponding Fe(III) species distribution diagram, elaborated with MEDUSA, calculated using SQUAD data.

Finally it is worth to mention that there is no evidence of insoluble species presence in the basic pH region; this fact indicates that the $Fe(OH)_{3(s)}$ species formation is not favored, because the interaction of the ion with the oxicam is stronger.

Conclusions

In this work the speciation of the antiinflamatory drugs tenoxicam and piroxicam with Fe(III) was studied under pseudo-physiological conditions (T = 37 ± 0.5°C and I = 0.15 M). From spectrophotometric data, processed using SQUAD, it

was possible to refine the formation constants for the complexes $Fe(H_2tenox)_2^{5+}$, $Fe(Htenox)_2^{3+}$, $Fe(OH)_2(Htenox)_2^+$, $Fe(OH)_2(tenox)_2^-$, FeH_2tenox^{4+}, $FeHtenox^{3+}$, $Fe(OH)_2Htenox^+$, $Fe(OH)_2tenox$. A similar set of species and chemical models were employed for the Fe(III)-piroxicam system. The absorptivity coefficients for all the species were also computed. It is interesting to note the number of formed species and to point out that the stoichiometries found in this study are different from those reported in chemical literature.

Experimental

Reagents and equipment

Tenoxicam and piroxicam drugs of analytical grade were obtained from Sigma and anhydrous $FeCl_3$ from Merck. Prior to used, carbonate free NaOH solutions, Baker, were standardized with potassium phthalate acid, Merck. Analytical grade HCl and NaCl, Baker, were also employed. All the solutions were prepared using deionized water.

Spectrophotometry data were obtained with a Perkin-Elmer Lambda 18 spectrophotometer, using quartz cells of 1 cm of optical path length. pH measurements were done with a Mettler Toledo MA235 pH/ion analyzer by means of a Ag/AgCl combined cell. Titration volumes were added from a digital burette II of Merck. All the experiments were carried out under controlled temperature conditions (37 ± 0.5°C), employing a thermal bath MGW Lauda C 12 T1, and under N_2 atmosphere.

Speciation Study Methods

In order to obtain the stability constants of the Fe(III) hydroxo-complexes, a 5.0×10^{-4} M aqueous solution of Fe(III) was prepared, its ionic strength was then adjusted to I = 0.15M with NaCl. Titration with a 0.01M solution of NaOH was performed spectrophotometrically, and finished when precipitation of $Fe(OH)_3$ was observed. Reproducibility was verified repeating the titration procedure three times. The pH interval in these set of experiments was 1.0 < pH < 3.0. Spectra were recorded each 0.1 pH units in the 210-450 nm wave length range. The spectra data were corrected by dilution effects before treatment.

For the purpose of obtaining the stability constants for the complexes formed by Fe(III) and tenoxicam or piroxicam, the molar ratio method using 1:1, 1:2 and 1:3 metal:oxicam ratios as a function of pH was applied. In this case the worked pH interval was 1.0 < pH < 10.0. As in the study of hydrolysis, titrations were repeated three times. The concentration of Fe(III) was set at 6.0×10^{-5} M, while the oxicam was adjusted to fit the proper molar ratio. The spectra data were recorded each 0.1 pH units in the 230-450 nm wave length range, adjusting the system pH by adding 0.01 M solution of HCl. The experiment was repeated three times to verify its reproducibility and spectra were corrected to consider dilution.

Table 2: Fe(III)-tenoxicam refined equilibrium constants, calculated from experimental data under pseudophysiological conditions.

Species	log β (standard deviation)	σ	U
Fe(H$_2$tenox)$^{4+}$	10.95 (0.20)	2.88×10^{-3}	5.55×10^{-3}
Fe(Htenox)$^{3+}$	10.04 (0.18)		
**Fe(OH)$_2$Htenox$^+$	4.50 (0.17)		
Fe(OH)$_2$tenox	−0.29 (0.19)		
Fe(H$_2$tenox)$_2^{5+}$	22.18 (0.01)	7.27×10^{-3}	4.75×10^{-2}
Fe(Htenox)$_2^{3+}$	19.5*		
***Fe(OH)$_2$(Htenox)$_2^+$	13.66 (0.04)		
Fe(OH)$_2$(tenox)$_2^-$	3.85 (0.06)		

* It was fixed during the refining process.
** Fe(OH)$_2$tenox$^+$ is formally equal to Fe(OH)tenox$^+$.
*** Fe(OH)$_2$(Htenox)$_2^+$ is formally equal to Fe(tenox)$_2^+$.
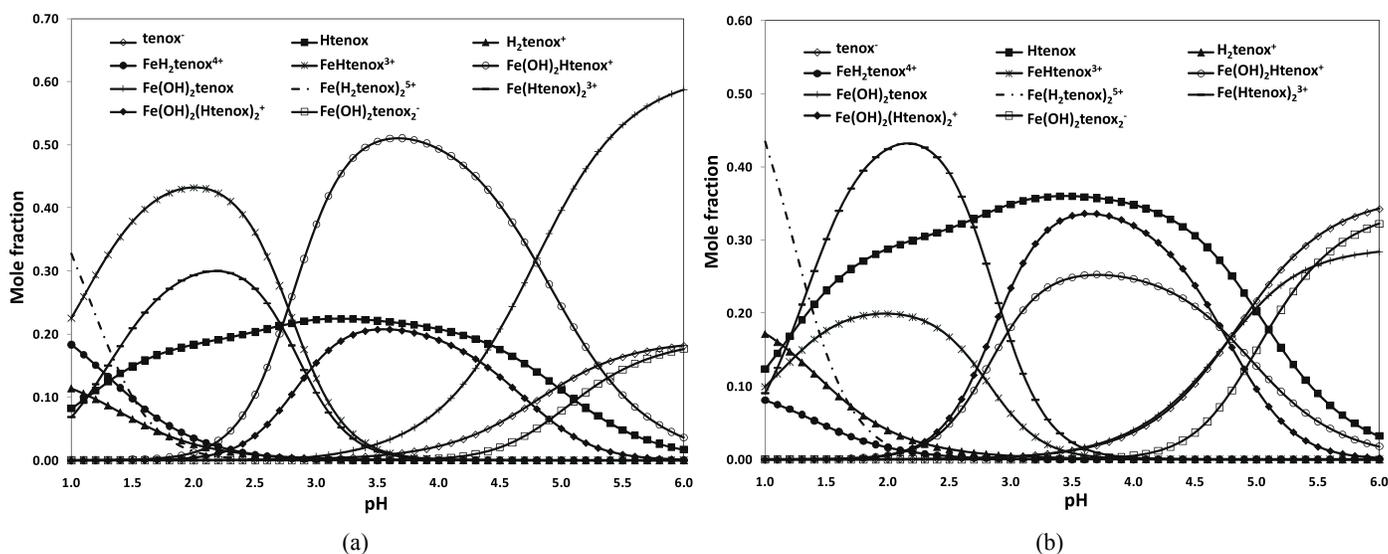
Fig. 5. Tenoxicam species distribution diagrams a) 1:1 and b) 1:2 molar ratio at [Fe(III)] = 6.0×10^{-5} M.
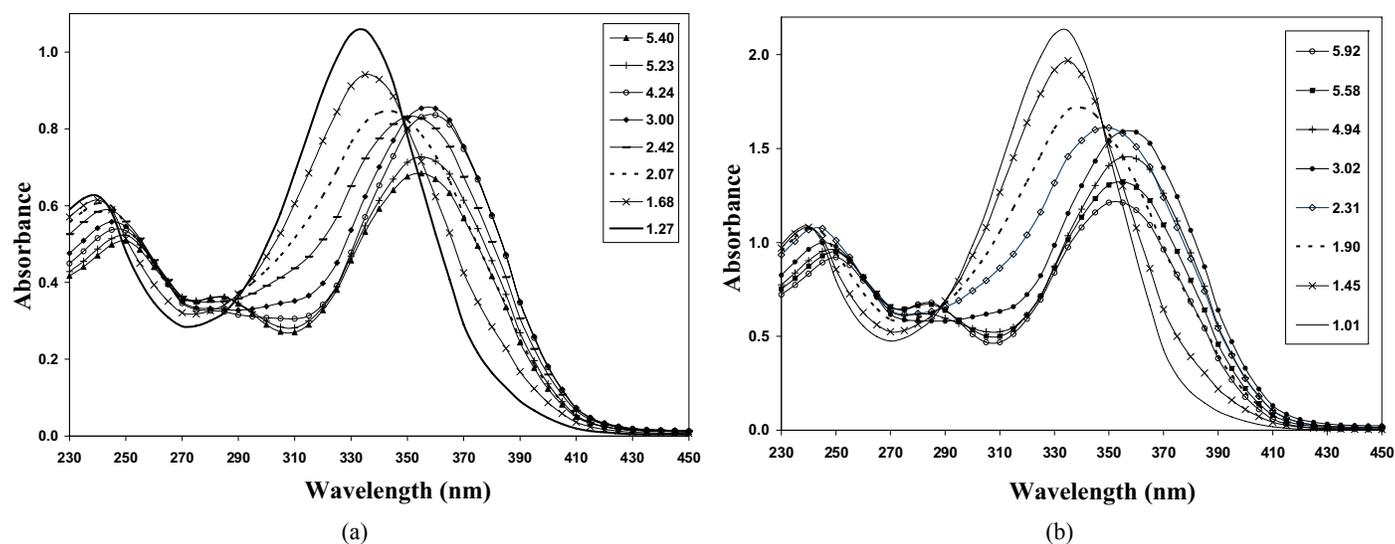
Fig. 6. Absorption spectra of the Fe(III)-piroxicam system as a function of the pH under pseudophysiological conditions, [Fe(III)] = 6.0×10^{-5} M, a) 1:1 and b) 1:2 molar ratio.
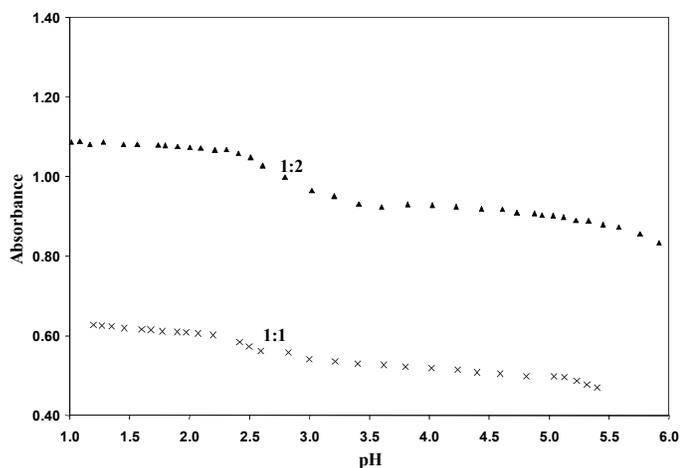
Fig. 7. Absorbance as a pH function of the Fe(III)-piroxicam system at 250 nm, curves are for the 1:1 and 1:2 molar ratios at [Fe(III)] = 6.0×10^{-5} M.
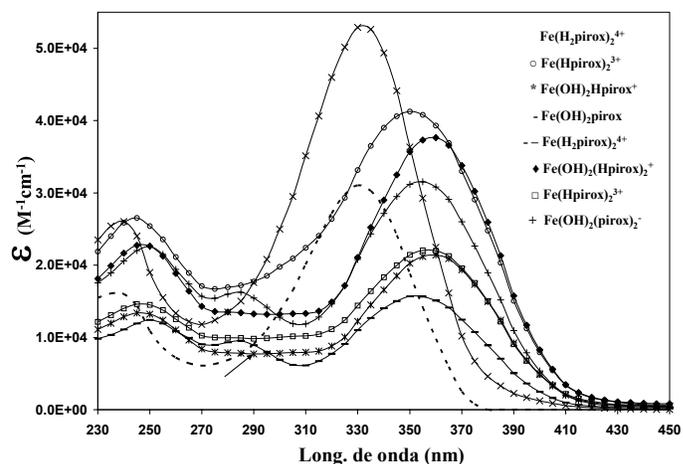
Fig. 8. Molar absorptivities for the species of the Fe(III)-piroxicam system.

Table 3: Fe(III)-piroxicam refined equilibrium constants, calculated from experimental data under pseudophysiological conditions.

Species	log β (standard deviation)	σ	U
$Fe(H_2pirox)^{4+}$	12.27 (0.01)	2.88×10^{-3}	5.56×10^{-3}
$Fe(Hpirox)^{3+}$	10.5*		
**$Fe(OH)_2Hpirox^+$	5.14 (0.03)		
$Fe(OH)_2pirox$	0.17 (0.04)		
$Fe(H_2pirox)_2^{5+}$	23.0 (0.008)	5.73×10^{-3}	2.94×10^{-2}
$Fe(Hpirox)_2^{3+}$	19.5 *		
***$Fe(OH)_2(Hpirox)_2^+$	14.25 (0.01)		
$Fe(OH)_2(pirox)_2^-$	4.57 (0.02)		

* It was fixed during the refining process.
** The $Fe(OH)pirox^+$ species was introduced to SQUAD.
*** The $Fe(pirox)_2^+$ was introduced to SQUAD.

(a)

(b)
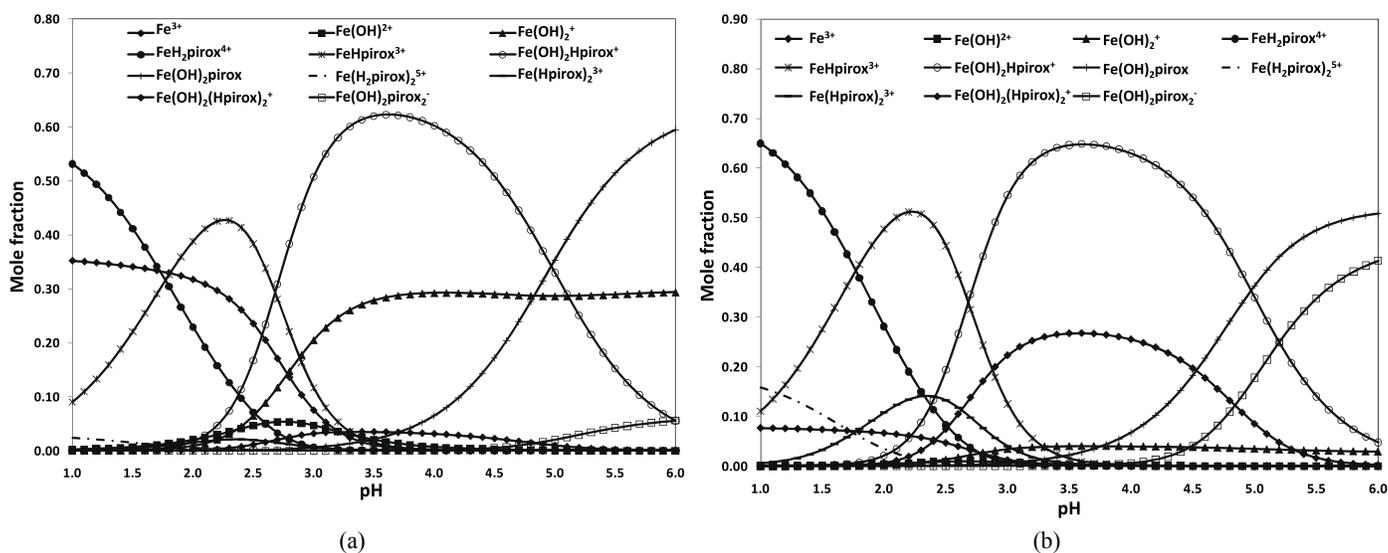
Fig. 9. Fe(III) species distribution diagrams a) 1:1 and b) 1:2 molar ratio at [Fe(III)] = 6.0×10^{-5} M.

Acknowledgements

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