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Kinetics and Mechanism of Interactions Between Iron(III) and Desferrioxamine B. The Formation and Hydrolysis of Ferrioxamine B in Acidic Aqueous Solution

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The kinetics of the formation and hydrolysis of ferrioxamine B complexes have been studied in acidic aqueous solution (0.001—1.0 M HCl) at 25.0 °C, $\mu = 1.0$ M (maintained by NaCl). Two stage kinetics have been observed in both the formation and in the hydrolysis reactions. The proposed reaction model involves formation/hydrolysis of bidentate, tetradentate, and hexadentate bonded desferrioxamine B to iron(III).

The formation of the bidentate complex occurs by two paths: one involving unhydrolysed ferric ion yields the rate constant $k_1 = 282$ M^{-1} s^{-1}, and the other via hydrolysed ferric species gives the rate constant $k_1' = 4.1 \times 10^3$ M^{-1} s^{-1}. The analogous rate constants for the reverse hydrolysis reactions were obtained; $k_{-1} = 0.65$ M^{-1} s^{-1} for the acid dependent and $k_{-1}' = 0.016$ s^{-1} for the acid independent path.

The formation and hydrolysis rate constants of the tetradentate linked complex have been calculated using the steady-state approximation as $k_2 = 9$ s^{-1} and $k_{-2} = 2$ M^{-1} s^{-1}, respectively.

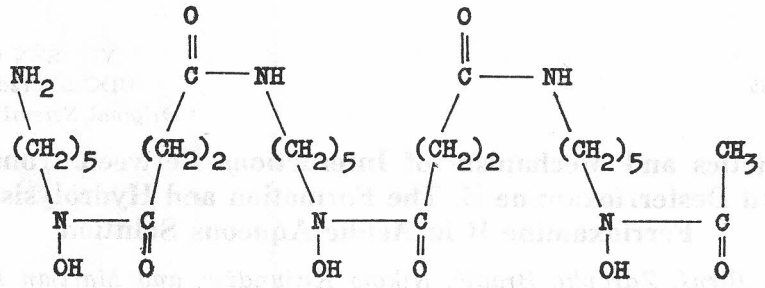
The conversion of tetradentate to hexadentate as well as hydrolysis of the hexadentate to tetradentate bonded species have been characterized by the rate constants $k_3 = 2.2$ s^{-1} and $k_{-3} = 18$ M^{-1} s^{-1}, respectively.

These results are compared with kinetic data previously reported. Chloride media increases the rate of formation and hydrolysis of the bidentate species, and possibly has the same effect on the tetradentate species but does not affect hydrolysis of the hexadentate complex. These effects were analyzed in terms of a labilization of iron(III) coordinated ligands by inner-sphere coordinated chloride.

INTRODUCTION

Hydroxamic acids have recently attracted a great deal of interest because of their biological and pharmacological roles.[1] The hydroxamic function is the active site of many siderophores which are known to mediate iron transport in biological systems.[2] In our previous work we have been interested in the kinetics and mechanism of iron(III)-hydroxamic acid interactions involving the formation of different monohydroxamatoiron(III) complexes and, more recently, in the interaction of ferric ions with desferrioxamine B (H_2DFB).[3-6]

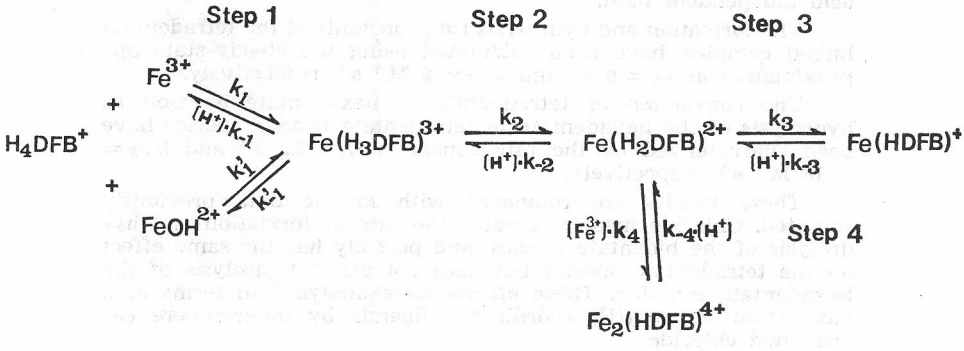
Naturally produced trihydroxamic acid desferrioxamine B is a linear siderophore (**I**) which has high specificity and affinity for iron(III).



H_3DFB (**I**)

The water soluble complex of ferric ion with desferrioxamine B, called ferrioxamine B, /Fe(HDFB)$^+$/, exhibits a high stability constant of the order of 10^{30} M^{-1}. These features have led to the clinical use of H_3DFB-methane-sulphonate salt[7] as a drug. The overload body iron in chronic or acute iron poisoning is reduced by excreting $Fe(HDFB)^+$ by the kidney.

Because of the importance of ferrioxamine B, several groups have investigated this system from thermodynamic and kinetic points of view.[6,8–14] The reported results may be accomodated by the reaction Scheme below. The symbols $Fe(H_3DFB)^{3+}$, $Fe(H_2DFB)^{2+}$, $Fe(HDFB)^+$ represent 1 : 1 complexes where desferrioxamine B is bonded to iron(III) by one, two and three hydroxamate functionalities, respectively.



SCHEME

In hexadentate bonded complex $Fe(HDFB)^+$ there is only protonated nitrogen of the terminal amino group, while in tetradentate $Fe(H_2DFB)^{2+}$ and bidentate linked species $Fe(H_3DFB)^{3+}$ the uncoordinated hydroxamate groups are also protonated in acidic media. The symbol $Fe_2(HDFB)^{4+}$ represents the binuclear diferrioxamine B complex.

The majority of the kinetic data refer to the Step 1. The equilibria of Steps 2 and 3 have been studied by Schwarzenbach,[13,14] and the kinetics of

Step 3 by Tufano and Raymond.[12] In an excess of the ferric ion over H_3DFB we have observed[6] the Step 4.

However, owing to different reaction conditions or various approaches to the system, a lack of data coherency appeared. In this paper we report the kinetics of formation and hydrolysis of ferrioxamine B in acidic equeous solution of 1.0 M ionic strenght maintained by sodium chloride. In order to simplify the reaction model, Step 4 was eliminated ensuring that the ferric ion was never in excess over H_3DFB.

EXPERIMENTAL SECTION

Materials

A stock solution of ferric chloride (0.15 M in 0.1 M HCl) was prepared from ferric chloride hexahydrate (Merck), and standardized as described previously.[5] Reagent iron(III) solutions were prepared by dilution of the stock solution with aqueous HCl/NaCl, keeping the H^+ ion concentration high enough to prevent hydrolysis of iron(III) ion.

Desferrioxamine B methanesulphonate, (®DESFERAL) (Ciba-Geigy Corporation) was recrystallized from methanol and was dried over P_4O_{10} in a vacuum dessicator (m. p. 149—151 °C).

All solutions were prepared using water which was double distilled from alkaline $KMnO_4$ in an all-glass apparatus.

All other chemicals were of analytical grade, and were used without further purification.

Methods. Preparation of Solutions

In ferrioxamine B hydrolysis experiments, water solutions of 4×10^{-5} M iron(III) in $4—36 \times 10^{-4}$ M H_3DFB were mixed in stopped flow apparatus with 2 M HCl. In addition the solution of 3×10^{-4} M iron(III) in 4×10^{-4} M H_3DFB was prepared and mixed with 0.02—1 M HCl.

The formation of ferrioxamine B was studied by mixing 2×10^{-4} M $FeCl_3$ in 0.002—2 M HCl with 0.004 M H_3DFB. The ligand concentration dependance on the formation of ferrioxamine B was measured using 2×10^{-4} M $FeCl_3$ in 0.01 M HCl and 0.002—0.02 M H_3DFB.

All the experiments were performed in aqueous solutions of 1.00 M ionic strength maintained by NaCl. Ionic strength is defined as $\mu = 0.5 \Sigma C_i z_i^2$, where C_i and z_i are total concentrations and formal charge numbers of ions present in solution, respectively.

Kinetic Measurements

The kinetic experiments were performed on a Durrum D-110 stopped-flow and a Cary 16K UV-VIS spectrophotometer. The cuvette, mixing chamber, and drive syringes were thermostated at 25 ± 0.05 °C by water circulating from a constant temperature bath. From the oscilloscope tracings of photomultiplier output voltage (absorbance) as a function of time were collected and analysed in terms of mono- and/or two stage pseudo-first order kinetics.

All the computations were performed on a UNIVAC 1100 computer at the University Computing Center, Zagreb. The observed rate constants were fitted to the proposed reaction models as described elsewhere.[15]

The kinetics were followed by measuring the absorbance change at 300, 335, 440, 472, and 560 nm, respectively.

The total concentration of H^+ ion in the experiments was calculated by summation of added HCl and the protons released from hydrolysis of iron(III) ion, using the hydrolysis constants reported in the literature.[16]

RESULTS

Hydrolysis of Ferrioxamine B

(i) Faster Kinetic Stage

The hydrolysis of ferrioxamine B under experimental conditions described in Figure 1 is characterised by two well separated kinetic stages. The faster one exhibits a linear dependence on proton concentration, whereas the second stage appears to be proton independent under these conditions. In addition, the first process is evidently independent of ligand desferrioxamine B concentrations, since the data points at 0.2 mM and 1.0 mM H_3DFB lie on the same line in Figure 1.

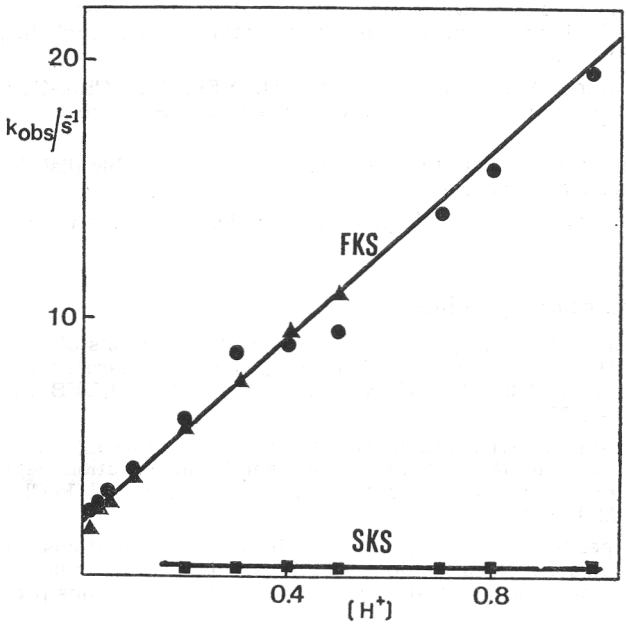


Figure 1. Acid dependence for the fast (FKS) and slow (SKS) kinetic stage of ferrioxamine B hydrolysis. ● and ■ total concentrations: 1×10^{-4} M $FeCl_3$ and 1×10^{-3} M H_4DFB^+. ▲ total concentrations: 1.5×10^{-4} M $FeCl_3$ and 2×10^{-4} M H_4DFB^+. The lines are computed from equations (1) and (4).

The faster stage was not observed at 472 nm where tetradentate and hexadentate ferrioxamine B have approximately the same molar absorptivity.[17] Therefore, this stage corresponds to the interconversion of hexadentate and tetradentate ferrioxamine B represented by Step 3 of the Scheme. This stage is at least ten times faster then the following slower one, therefore each of them can be separately analysed.

Assuming that Step 2 is much slower than Step 3 the observed rate constants can be described by the equation (1):

$$k_{obs} = k_{-3}[H^+] + k_3 \qquad (1)$$

On the other hand, if Step 2 is much faster than Step 3 the observed rate constant may be described by equation (2):

$$k_{obs} = k_{-3}[H^+] + k_3 \frac{K_2}{K_2 + [H^+]} \quad (2)$$

The data were analysed according to equation (1). This yields the values $k_3 = 2.2(1)\ s^{-1}$ and $k_{-3} = 18(1)\ M^{-1}\ s^{-1}$. (The numbers in parenthesis are standard deviations referring to the last significant digit). Data analysis based on equation (2) should give approximately the same values for k_{-3} and k_3. The highest value of the studied proton concentration ($[H^+] = 1$) is almost ten times lower than the value of $K_2 = 6$ as determined spectrophotometrically, giving the quotient $K_2/(K_2 + [H^+]) \simeq 1$. As a consequence, the resulting linear dependence of k_{obs} on proton concentration does not allow a prediction about the rates of Step 2. The calculated equilibrium constant $K_3 = k_3/k_{-3} = 0.12$ M is in good agreement with reported values (see Table).

(ii) Slower Kinetic Stage

According to the Scheme the fast stage could be followed by two other kinetic stages corresponding to Steps 2 and 1. However, the change in absorbance following the faster stage monitored at five different wavelengths (300, 335, 440, 472 and 560 nm) gives a good straight lines for a plot of $\log (A_t - A_\infty)$ vs, time indicating only one reaction. Assuming that the faster stage involves Step 3, for the reasons mentioned above, the combination of events involved in Steps 1 and 2 gives rise to the slow kinetic stage.

Under the experimental conditions defined in Figure 1. the slow stage is essentially independent of proton concentration. This is not expected from the model proposed by the Scheme, since all of the hydrolysis steps are proton dependent. The effect of the change of proton concentration on the hydrolysis rate might be depressed by the reverse complexation reaction as it becomes more and more dominent as the proton concentration decreases. This assumption is substantiated by pH-jump experiments at much lower total concentrations of reactants, allowing the hydrolysis to go to completion. The results of such experiments are shown in Figure 2. It is obvious that under these experimental conditions a nonlinear dependence of k_{obs} on $[H^+]$ is obtained.

In contrast to the faster kinetic stage, the slower kinetic stage exhibits a dependence on the concentration of H_4DFB^+ as illustrated in Figure 3. It confirms that Step 1 is involved in the slower stage. If Step 1 is much faster than Step 2 only one kinetic stage would be observed (involving these two steps) giving the following expression for the corresponding observed rate constant:

$$k_{obs} = \frac{k_{-2}[H^+]^2}{[H^+] + K_3} + \frac{k_2 K_1 [H_4DFB]^+}{[H^+] + K_h + K_1 [H_4DFB^+]} \quad (3)$$

The hydrolysis constant K_h of ferric ions was used as earlier reported.[16]

TABLE I

Rate and Equilibrium Constants of 1 : 1 Complexes of Ferric Ions with Desferrioxamine B at 25 °C[a]

Step 1					Step 2			Step 3			Ionic Strength	Reference
k_1 / $M^{-1}s^{-1}$	k_{-1} / $M^{-1}s^{-1}$	k_1' / $M^{-1}s^{-1}$	k'_{-1} / s^{-1}	K_1	k_2 / s^{-1}	k_{-2} / $M^{-1}s^{-1}$	K_2 / M	k_3 / s^{-1}	k_{-3} / $M^{-1}S^{-1}$	K_3 / M	/ M	
282	0.65	4.1×10^3 ($\simeq10^5$)[b]	0.016	430	≈9	≈2	5.8	2.2	18	0.12	1.0 (NaCl)	This work
0.2	1.9×10^{-3}	200	2.1×10^{-3}	125							2.0 ($NaClO_4$)	8
								3.4	34	0.10	0.2 (KNO_3)	12
394		(4—40)10^3									0.1 (Formate buffer)	11
								(2.5×10^3)[c]			0.1 (KCl)	9
										0.11	0.1 ($NaClO_4$)	13
										0.08	1.0 (NaCl)	19

[a] All constants are defined as in the Scheme.
[b] The pathway referring to the $FeClOH^+$. For explanation see the text.
[c] At 36 °C.

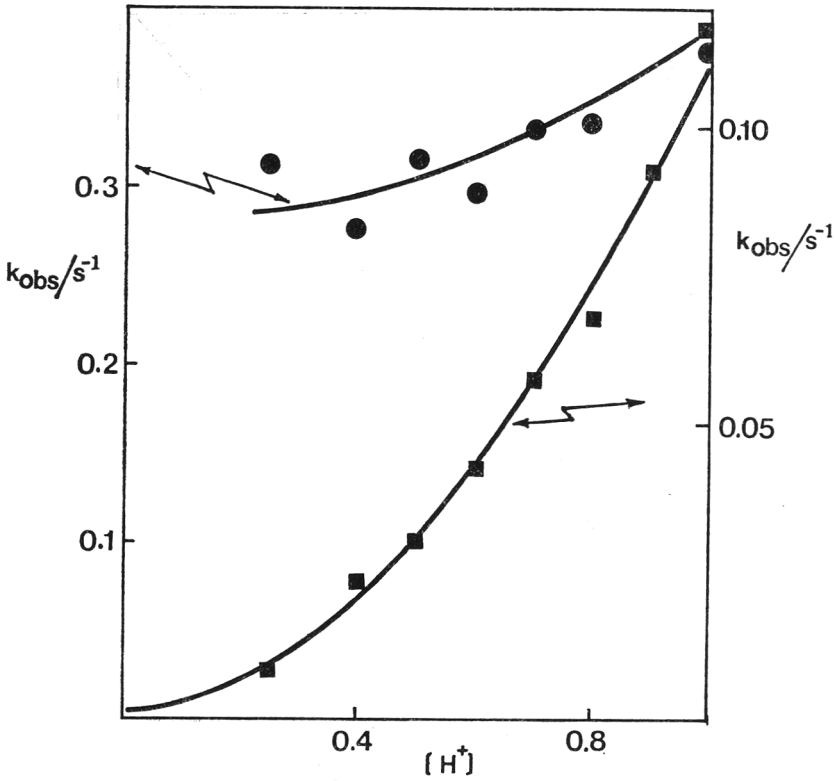


Figure 2. Acid dependence for the slow kinetic stage of ferioxamine B hydrolysis. ● total concentrations: 1×10^{-4} M $FeCl_3$ and 1×10^{-3} M H_4DFB^+. ■ total concentrations: 5×10^{-6} M $FeCl_3$ and 5×10^{-6} M H_4DFB^+. The curves are computed according to equation (4).

When the full expression (3) is taken into account e. g. under conditions of Figure 3. the initial linear dependence of k_{obs} on H_4DFB^+ should be distorted at high concentrations of H_4DFB^+. Figure 3 shows that this is not the case and equation (3) does not fit the experimental data.

The second term of expression (3) is neglected when hydrolysis goes to completion as e. g. in experiments in Figure 2. Under such conditions the plot of $[H^+]/k_{obs}$ vs. $1/[H^+]$ exhibits acceptable linearity. From the intercept and slope, $K_3 = 2.8$ M was calculated. This value does not agree with the K_3 value calculated from the data of the faster kinetic stage, $K_3 = 0.12$ M, nor with earlier reported values (see Table). Therefore this model cannot accomodate the available data and should be abandoned.

Another plausible reaction variant is the model proposing that Step 1 is slow and Step 2 is a fast preequilibrium. This assumption leads to the following equation for the observed rate constant:

$$k_{obs} = \frac{(k_{-1}[H^+] + k'_{-1})[H^+]^2}{[H^+]^2 + K_2[H^+] + K_2K_3} + \frac{(k_1[H^+] + k'_1K_h)[H_4DFB^+]}{[H^+] + K_h} \quad (4)$$

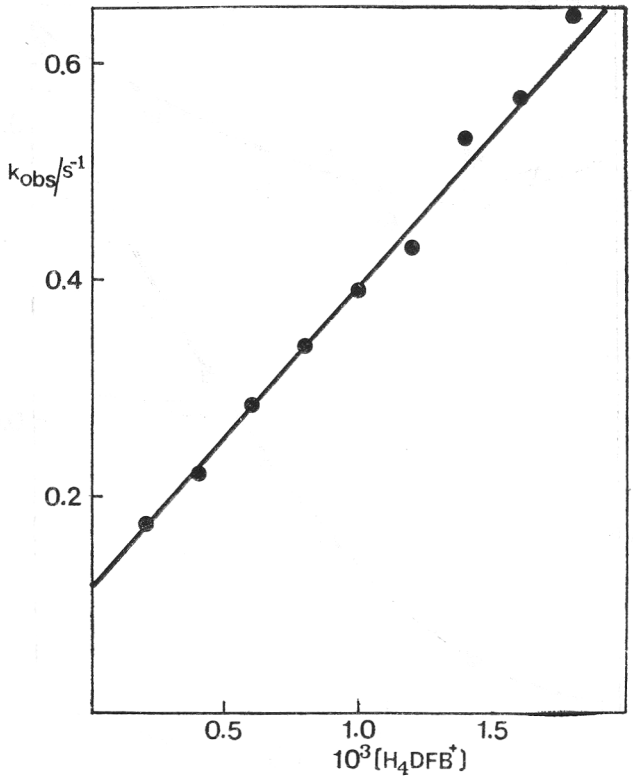


Figure 3. The ligand dependence for the slow kinetic stage of ferrioxamine B hydrolysis. Total concentrations: 2×10^{-5} M $FeCl_3$ and 1.0 M HCl. The line is calculated from equation (4).

A non linear least squares analysis applied to equation (4) gives the following values: $k_1 = 282(3)$ M^{-1} s^{-1}, $k_{-1} = 0.65(3)$ M^{-1} s^{-1}, $k_1' = 4.1(2) \times 10^3$ M^{-1} s^{-1}, $k_1' = 1.6(1) \times 10^{-2}$ s^{-1} and $K_2 = 5.8(3)$ M. However, it should be noted that the value of k_1' was poorly defined when only hydrolysis data was analysed. A possible reason for this is the very small contribution of that path at the experimental conditions where the second stage of the hydrolysis is measurable. On the other hand, below 0.1 M HCl, where this reaction path may contributte significantly, the amplitude of the second stage is too small to be measured.

Ferrioxamine B Formation Kinetics

The value of k_1' given above was calculated by using both the hydrolysis as well as the formation kinetic data (a series of data obtained at 560 nm is presented in Figure 4 and 5) since in this case the acidity range may be expanded up to 10^{-3}M H^+. The formation kinetics exhibit one or two stage characteristics depending on proton and H_4DFB^+ concentrations. The two

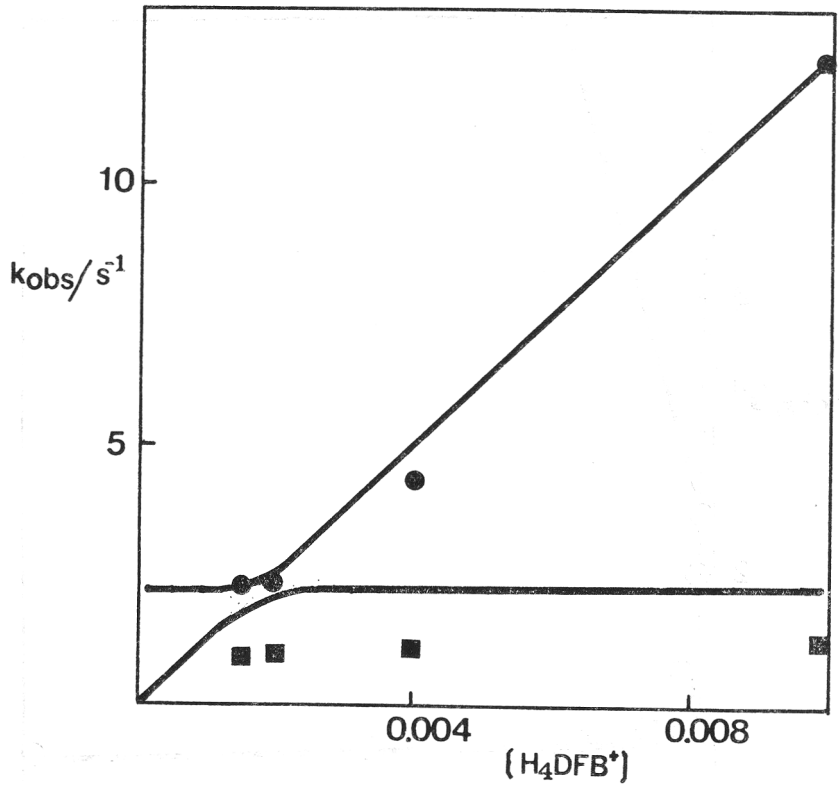


Figure 4. The ligand dependence for the fast (●) and the slow (■) kinetic stage of ferrioxamine B formation. Total concentrations: 1×10^{-4} M $FeCl_3$ and 5×10^{-3} M HCl. The lines are computed solving the secular equation given in the Results section.

kinetic stages appear to be well separated at higher concentrations of H_4DFB^+ and/or as the acidity of the reaction media is decreased. Therefore, only these data points were included in computer analysis. Nevertheless, only the first stage of complexation exhibits a H_4DFB^+ dependence indicating that in the formation kinetics the faster stage involves the interactions depicted in Step 1.

In the case when two stages are observable but not well separated the Lowry and John method[18] was applied to the model in which Step 2 of the Scheme is considered as a fast equilibrium. Step 1 cannot feature a fast pre-equilibrium because the rate of the first kinetic stage corresponds to the rate of disappearance of the iron(III) ion band at 335 nm. On the other hand, from the hydrolysis data, Step 3 appears under present conditions to be too slow to feature a fast equilibrium.

The calculated rate and equilibrium constants of equations (2) and (4) have been inserted in the solution of a secular equation of the form:

$$\begin{vmatrix} a_{11} - \lambda & a_{12} & 0 \\ a_{21} & a_{22} & a_{23} \\ 0 & a_{32} & a_{33} - \lambda \end{vmatrix} = 0$$

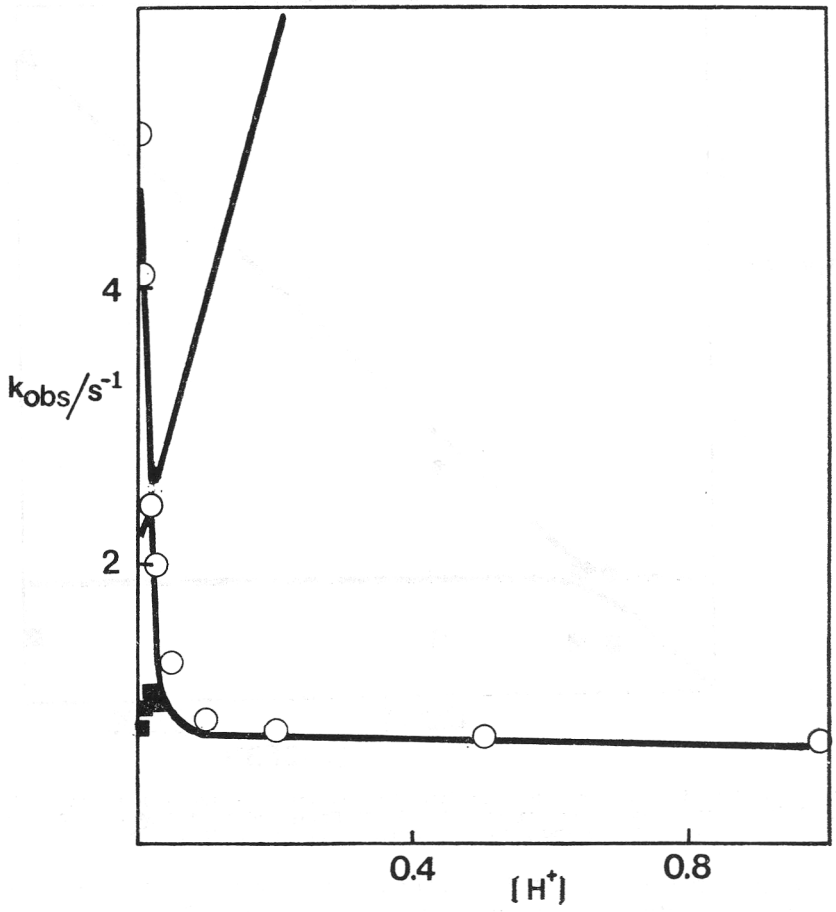


Figure 5. Acid dependence for ferrioxamine B formation. Total concentrations: 1×10^{-4} M $FeCl_3$ and 2×10^{-3} M H_4DFB^+. ■ the slower kinetic stage when two stage kinetics is observed. The lines are computed solving the secular equation given in the Results section.

where coefficients a_{ij} are given as follows:

$$a_{11} = \frac{k_1 [H^+] + k_1' K_h [H_4DFB^+]}{[H^+] + K_h}$$

$$a_{12} = -(k_{-1} [H^+] + k'_{-1}) \frac{[H^+]}{[H^+] + K_2}$$

$$a_{21} = -a_{11}$$

$$a_{22} = a_{12} + k_3 \frac{K_2}{[H^+] + K_2}$$

$$a_{32} = -k_3 \frac{K_2}{[H^+] + K_2}$$

$$a_{33} = a_{23}$$

The calculated values of k_{obs} for the first stage agree well with the measured values. However, the calculated values for the second stage predicted by this procedure are almost twice as high as the measured ones. This will be discussed later.

Steady-state Approximation Approach

A steady-state approximation with the bidentate species as an unstable intermediate appears to be the best model for the slower hydrolysis reaction. This assumption may arise from the fact that the intermediate is not observed spectrophotometrically. In 0.25 M HCl the slower hydrolysis reaction proceeds by exhibiting an isosbestic point at 366 nm (Figure 6) indicating the absence of a measurable concentration of bidentate linked ferrioxamine B. The starting spectrum of tetradentate-hexadentate mixture (equilibrated during the faster relaxation) has been changed directly to the final products i. e. a mixture of hydrolysed and unhydrolysed ferric ions. Unfortunately this spectral change cannot be followed in more acid media because the reaction rate is too fast. However, the values of the stability constants of the ferrioxamine B complex of various degrees of denticity indicate a low concentration of bidentate species in comparison with tetradentate and hexadentate linked complex under most of our experimental conditions. Thus, when the steady-state approximation is applied to the slower stage the following expression holds:

$$k_{obs} = \frac{(k_{-1}[H^+] + k'_{-1})\, k_{-2}[H^+]^2}{(k_{-1}[H^+] + k'_{-1} + k_2)([H^+] + K_3)} + \frac{k_2 (k_1[H^+] + k'_1 K_h)[H_4DFB^+]}{(k_{-1}[H^+] + k'_{-1} + k_2)([H^+] + K_h)} \qquad (5)$$

Inspection of equations (5) and (4) reveals that they are identical in proton and H_4DFB^+ concentration dependence, if $k_{-1}[H^+] < (k_{-1}' + k_2)$. This means that the values of k_2 computed according to (5) will represent the lowest limit (all the larger values will satisfy the inequality). Analysis of the experimental data according to equation (5) gives the same values of k_1, k_{-1}, and k_{-1}' as were obtained by applying equation (4). However instead of equilibrium constant K_2 from equation (4) the rate constants of Step 2 were calculated as $k_2 = 9(7)\ s^{-1}$ and $k_{-2} = 2(1)\ M^{-1}\ s^{-1}$.

A large standard deviation of k_2, expected because of the limitation mentioned above, shows that this reaction path is not well characterized under our experimental conditions. However, the obtained relatively high value of k_2 ensures the preequilibrium condition required by the model represented by equation (4). The analyses of equation (4) and (5) show that both models represented by these two equations could be equally well applied to our experimental conditions.

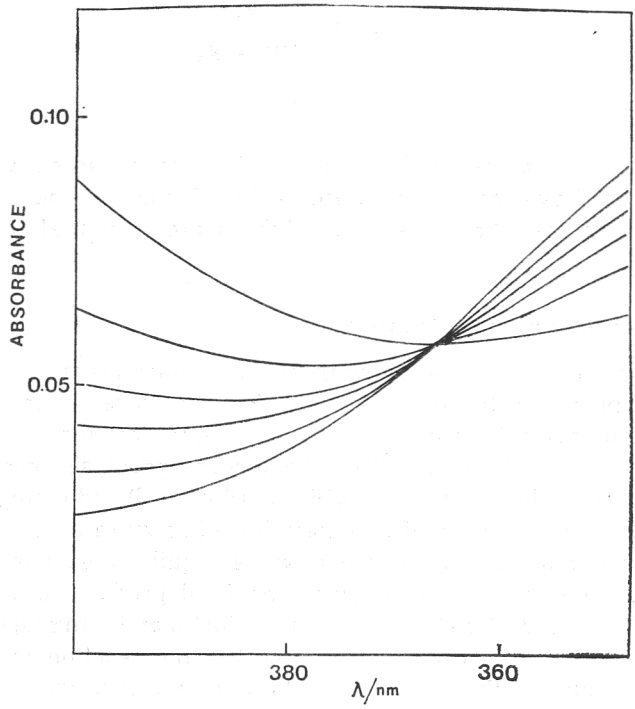


Figure 6. Spectral changes accompanying the slow kinetic stage of ferrioxamine B hydrolysis. Total concentrations: 8×10^{-6} M $FeCl_3$, 8×10^{-6} M H_4DFB^+, and 0.25 M HCl. Optical path 10 cm.

Kinetic Effects of Perchlorate Media

The calculated values referring to Step 1 are widely different from those in perchlorate media reported by Monzyk and Crumbliss[8] (see Table). The effect of the change from chloride to perchlorate media on both stages are illustrated in Figure 7. The observed decrease in the rate of the slower hydrolysis stage with an increase in perchlorate concentration exhibits nonlinearity which may be caused by the presence of several reacting species containing different numbers of coordinated chloride ions. This is supported by the fact that in 1 M chloride most of iron(III) exists in form of mono-and dichloroaquo complexes.[19]

DISCUSSION

The Scheme represents an attempt at a kinetic description of the interaction between ferric ions and desferrioxamine B. When there is no excess of ferric ion over H_4DFB^+, three kinetic steps involving two intermediates [$Fe(H_3DFB)^{3+}$ and $Fe(H_2DFB)^{2+}$] may be expected, assuming that hydrolysis of ferric ions is a diffusion controlled process, and that the five-membered ring closure is much more rapid than the coordination of the first hydroxamato oxygen. The constants describing the system are presented in the Table, and the obtained results are paralelled by the values already reported.

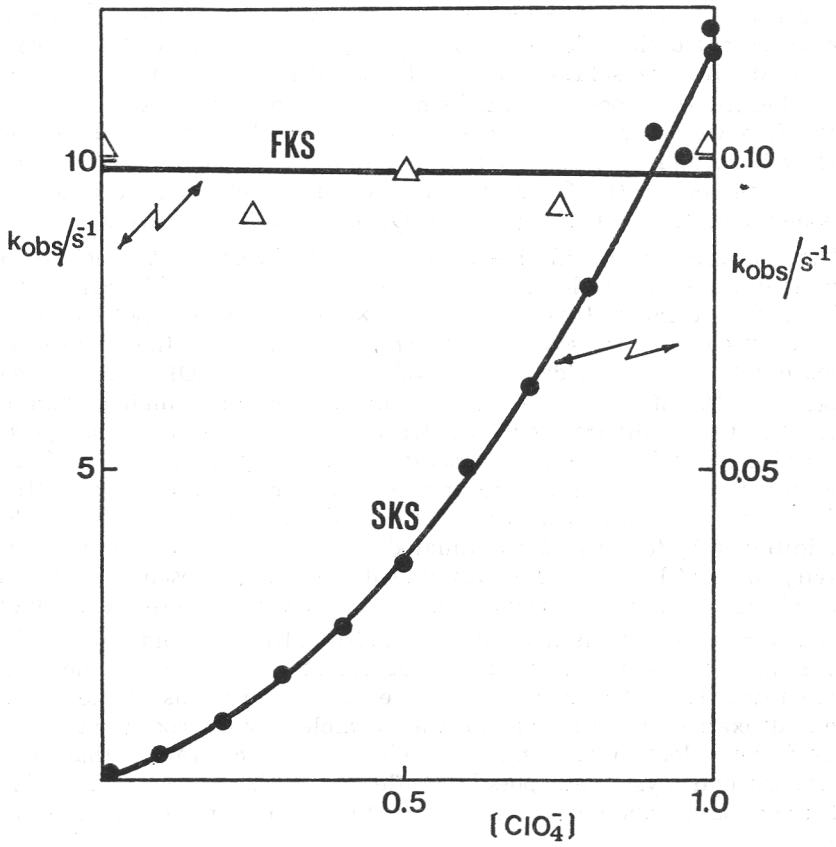


Figure 7. Perchlorate dependence for the fast (FKS) and slow (SKS) kinetic stage of ferrioxamine B hydrolysis.Total concentrations: 2×10^{-4} M $FeCl_3$, 1×10^{-3} M H_4DFB^+, and 0.5 M H^+. Total concentrations: 1×10^{-5} M $FeCl_3$, 1×10^{-5} M H_4DFB^+, and 1.0 M H^+.

Under our experimental conditions, no matter whether the complexation or the hydrolysis reaction is studied, two kinetic stages appear to be observable at the most. The same kinetic behaviour was reported in formate-ion media by Kazmi and McArdle[11], however, Lentz et al.[9] as well as Monzyk and Crumbliss[8] studying the same system under different conditions noted more than two kinetic stages.

Monzyk and Crumbliss have studied the hydrolysis of ferrioxamine B complex, therefore a stage involving Step 4 may be eliminated. In their work the uncoordinating perchlorate ion was used as the supporting electrolyte in contrast to the coordinating formate used by Kazmi and McArdle or to the chloride ions used in this work. It seems that the presence of the coordinating ions somehow reduces the number of the observed kinetic stages. An inspection of the rate constants in the Table reveals the high accelerating effects of the chloride and formate anions on Step 1. A simple explanation of this effect is the labilisation of the water molecule coordinated to iron(III) by chloride and formate ligands. The value of $k_1' = 4.1 \times 10^3$ M^{-1} s^{-1} was calculated neglecting the coordination of chloride to ferric ions. The reported equilibrium constants

of the formation of $FeCl^{2+}$ [22] and $FeCl_2^+$ [19] allow the estimation of about 10% of non-coordinated chloride ferric ions /$Fe(H_2O)_6^{3+}$ and $Fe(H_2O)_5OH^{2+}$ related by K_h/ in 1 M chloride solution. If the $Fe(H_2O)_5OH^{2+}$ ion is the most reactive nonchelated iron(III) species, it might have an estimated value of $k_1' = 4 \times 10^4$ M^{-1} s^{-1}. This value strikingly exceeds $k_1' = 200$ M^{-1} s^{-1} obtained by Monzyk and Crumbliss and cannot be explained simply by taking into account the ionic strength effect (1 M vice versa 2 M). Therefore this reaction path is abandoned and that involving $FeCl(H_2O)_4OH^+$ should be invoked.

The coordination of chloride could markedly decrease the acidity of the coordinated water molecules as was found[21] in case of $Fe(SCN)(H_2O)_5^{2+}$ ($K_h = 6.5 \times 10^{-5}$ M compared to the $K_h = 1.7 \times 10^{-3}$ M for $Fe(H_2O)_6^{3+}$). Assuming that equally charged thiocyanato and chloride ions have the same effect on the coordinated water acidity, k_1' involving $FeCl(H_2O)_4OH^+$ species may be estimated as 10^5 M^{-1} s^{-1}. This value is about 350 times higher than the k_1 referring to the nonhydrolysed iron(III) species, whereas the analogous ratio $k_1'/k_1 = 1500$ holds for perchlorate media.[8] Apparently the presence of chloride in the inner coordination sphere increases the reactivity of iron(III) ($k_1 = 282$ M^{-1} s^{-1} in comparison with $k_1 = 0.2$ M^{-1} s^{-1} as is cited in the Table). The additional hydrolysis of coordinated water molecules also increases the reactivity of iron(III) but not as dramatically as in the absence of the coordinated chloride. A similar observation was reported for formate media[11].

The comparison of the hydrolysis of $Fe(H_3DFB)^{3+}$ (k_{-1} and k_{-1}') in chloride and perchlorate media shows again that chloride dramatically increases the reaction rates. This acceleration may be explained in terms of the weakening of the hydroxamate bond in the bidentate complex by the coordinated chloride. The acid dependent path exhibits much higher acceleration than the acid independent hydrolysis path possibly reflecting a charge effect. Namely, only the former path involves interaction between the species of the positive charges.

The mode of activation in substitution reactions at the iron(III) center has been a subject of controversy. Recently published results[22,23] of kinetics of water exchange on iron(III) as well as complexation of iron (III) by the highly charged nonbasic ligands[24] seem to be consistent with dissociative activation for substitution on $Fe(H_2O)_5OH^{2+}$ and associative activation for $Fe(H_2O)_6^{3+}$. On the contrary, activation parameters reported[25] for the formation and dissociation of a series of monohydroxamatoiron(III) complexes indicate associative activation for both ferric ions. However, the strict criteria for an I_a mode of activation have not probably been met in $Fe(H_2O)_5OH^{2+}$ path. It has been indicated[25] that the associative character is a result of H-bonding between the entering hydroxamic acid and the coordinated hydroxo group rather than of direct interaction of hydroxamic acid with the iron(III) center. In such a case the rate of substitution at the iron center can not exceed the dissociation of metal coordinated water molecules. The chloride acceleration effect may be explained in the light of this model either by lowering the positive charge of iron(III) ion and hence increasing the probability of its association via H-bonding with the positively charged ligand H_4DFB^+, or by labilization of the coordinated water molecule when its dissociation appears as the rate determining step (H-bonding of hydroxamic acid to the iron coordinated hydroxy group may significantly decrease the labilizing effect of the coordi-

nated hydroxo group presumably causing the dissociation of coordinated water as the rate determining step).

An eight fold increase of k_{-1}' observed in chloride media is consistent with the assumption that coordinated chloride labilizes the bond of hydroxamate oxygen to the ferric center. This observation can be indicative of an I_d mechanism.

In the case of the proton dependent pathway the labilizing effect of the coordinated chloride is accompanied by the entropy effect due to the change of charge of the interacting species as has already been pointed out.[11]

It is reasonable to suppose that the accelerating chloride ion effect in bidentate bonded species operates also in the conversion of bidentate to the tetradentate linked complex. Unfortunately the value of k_2 is not available in perchlorate media and no comparison can be made.

Referring to the Step 2 it should be pointed out that the steady-state approximation considered above does not necessarily hold through the entire acid concentration range studied. The value of constant K_1 predicts the appreciable concentration of the bidentate complex (at least in equilibrium, almost 10% of total Fe) at the highest acidity used in this work. That could also be a reason why k_2 and k_{-2} are computed with very large uncertainties.

The values of the rate constants of Step 3 are in good agreement with those reported by Tufano and Raymond who studied iron removal from ferrioxamine B by EDTA at physiological acidities.[12] The obtained slight difference is probably due to the different ionic strengths used. The similarity of Tufano and Raymond's rate constants with those from this work indicates that a proton independent path of hydrolysis of $Fe(HDFB)^+$ does not operate, and the tetradentate hydroxo complex as the direct product can be excluded.

Furthermore, the indepedence of $Fe(HDFB)^+$ hydrolysis of chloride concentration (see Figure 7.) indicates the absence of seven coordinated iron(III) species under our experimental conditions. This type of complex with EDTA is described elsewhere[12,26].

The reported value[9] of $k_3 = 2.5 \times 10^3\ s^{-1}$ is obviously in large disagreement with other cited values of k_3 in the Table. Lentz et al.[9] proposed Step 3 as the rate determining step leading to the wrong assignament of the calculated value to k_3 instead of to the k_1' of Step 1.

Kazmi and McArdle reported biphasic kinetics of the formation of ferrioxamine B at high H_4DFB^+ concentration[11]. They assigned the second relaxation to the dissociation of the preformed 1 : 2 complex of ferric ion and desferrioxamine B ligand. This assignament is not acceptable in our reaction scheme for several reasons. To the best of our knowledge the 1 : 2 complex of ferric ion and desferrioxamine B has never been characterized. Furthermore, at H_4DFB^+ concentration as low as 2×10^{-3} M ($[H^+] = 10^{-2}$ M), two stage kinetics is still observed. It is unlikely to suppose the formation of $Fe(H_3DFB)_2^{3+}$ at such low concentration of ligand. On the other hand, the appearance of two distinct kinetic stages can be interpreted without invoking the proposed bis(desferrioxamine B) iron(III) complex. Namely, the attachment of the first chelate ring is dependent on H_4DFB^+ concentration, while the closure of the second and third chelate ring is not dependent. From the rate data of the Table it is easy to predict that at low ligand concentration Step 1 will be rate

determining and only one kinetic stage will be observed. At the other extreme i. e. at very high ligand concentration Step 3 will be rate determining and again only one kinetic stage will be observed. It is obvious that at some intermediate ligand concentration Steps 1 and 3 should be coupled (assuming that Step 2 is a fast equilibrium), and two kinetic stages will be observed. Nevertheless, the calculated values of k_2 and k_3 do not assure a complete separation of Steps 2 and 3, leading eventually to a partially coupling of these steps which may cause some error in the calculation of the slower observed rate constants. Such a coupling may be responsible for the disagreement in the computed and measured observed rate constants of the second kinetic stage of the formation as pointed out above.

Substantiation of the proposed kinetic model of the formation and hydrolysis of ferrioxamine B finds also its support in the consistency of the kinetically obtained equilibrium constants ($\log \beta_1 = 2.63$, $\log \beta_2 = 3.40$, and $\log \beta_3 = 2.48$) with those determined by spectrophotometric titration ($\log \beta_1 = 2.64$, $\log \beta_2 = 3.40$, and $\log \beta_3 = 2.28$).

The comparison is made by using cumulative equilibrium constants since the formation constant of bidentate linked complex was determined with a large standard deviation.[17]

Our results show that Kazmi and McArdle's suggestion of the attachment of the first chelate to the iron(III) center as the rate limiting step in the stepwise formation of ferrioxamine B complex does not necessarily hold under accesible concentrations. At physiological acidities having millimolar local concentration of H_4DFB^+ the rate of the first ring formation may exceed the rate of the third ring closure.

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SAŽETAK

Kinetika i mehanizam kompleksacije željezo(III)-iona sa desferioksaminom B. Nastajanje i hidroliza ferioksamina B u kiseloj vodenoj otopini

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Nastajanje heksadentano vezanog ferioksamina B iz željezo(III)-iona i desferioksamina B kao i hidroliza nastalog kompleksa odvija se preko dva međuspoja i to bidentano i tetradentatno vezanog ferioksamina B.

Kod 25 °C u kiselom i kloridnom mediju ionske jakosti 1,0 mol/L dobivene su slijedeće konstante brzine nastajanja (k_i) i hidrolize (k_{-i}) bidentanog (1), tetradentanog (2) i heksadentatnog (3) kompleksa:

$$k_1 = 282\ \mathrm{M^{-1}s^{-1}}\ \text{i}\ k_{-1} = 0{,}65\ \mathrm{M^{-1}s^{-1}}\ (\text{za}\ \mathrm{Fe^{3+}}),$$

odnosno

$$k'_1 = 4100\ \mathrm{M^{-1}s^{-1}}\ \text{i}\ k'_{-1} = 0{,}016\ \mathrm{s^{-1}}\ (\text{za}\ \mathrm{Fe(OH)^{2+}}); \quad (1)$$

$$k_2 = 9\ \mathrm{s^{-1}}\ \text{i}\ k_{-2} = 2\ \mathrm{M^{-1}\,s^{-1}}; \quad (2)$$

$$k_3 = 2{,}2\ \mathrm{s^{-1}}\ \text{i}\ k_{-3} = 18\ \mathrm{M^{-1}s^{-1}}. \quad (3)$$

Klorid-ioni ubrzavaju reakcije bidentatne i teradentatne specije, a nemaju utjecaja na reaktivnost potpuno kompleksiranog ferioksamina B. Raspravlja se o kinetičkoj labilizaciji liganada koordiniranim klorid-ionima.