Iron(III) Complexation by Desferrioxamine B in Acidic Aqueous Solutions. The Formation of Binuclear Complex Diferrioxamine B

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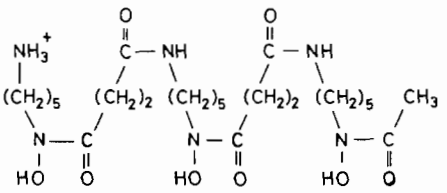
The iron(III) complexation by a trihydroxamic acid, desferrioxamine B ($DFBH_4^+$), in acidic aqueous solutions at 1.0 M ionic strength (NaCl) and 25 °C was studied spectrophotometrically. The results are discussed in terms of a competition between iron(III) and the proton for the hydroxamate oxygen. The equilibrium quotient for the formation of diferrioxamine B complex, $Q = [Fe_2(DFBH)^{4+}][H^+]^3/[Fe^{3+}]^2[DFBH_4^+]$, was calculated as being $(2.7 \pm 0.3) \times 10^5$ mol L^{-1}.

Introduction

The coordination chemistry of iron(III) with oxygen-containing ligands exhibiting wide-ranging importance has a long past in complex chemistry, and has recently attracted a great deal of attention because of the biological implications of iron [1, 2]. It has long been recognized [3] that iron(III) binds strongly to the hydroxamate group of synthetic hydroxamic acids [3, 4], and of naturally occurring siderophores [1, 3, 5]. Desferrioxamine B (I), one of the naturally occurring trihydroxamic acids, exhibits a high specificity for iron(III) over other biologically important metal ions [3]. Owing to its physicochemical properties, DFB marketed as ®Desferal (desferrioxamine B methanesulphonate) by the Ciba-Geigy Corporation, is used in the treatment of acute accidental iron poisoning, and in chronic iron overload [6–8] resulting as a side effect of transfusion therapy in patients suffering from Cooley's anemia (β-thalassemia), a genetic disease characterized by diminished synthesis of β-chains of hemoglobin [6]. This drug is capable of reducing an excess of body-iron by sequestering iron in the form of ferrioxamine B, which is then rapidly excreted by the kidneys [6–8]. The conditions under which Desferal is sequestering iron from body-pools are still doubtful, and its effectiveness has been discussed [9a].

Since the first use of Desferal in the clinical treatment of β-thalassemia major and related diseases [7], many other iron-chelating agents have been evaluated as potential drugs [7, 9, 10]. Among the siderophores



(I) $DFBH_4^+$

which have recently entered clinical trials to determine their effectiveness, there is a dihydroxamic chelator, rhodotorulic acid, H_2RA. Raymond and coworkers [11a, b] have reported that the predominant iron-rhodotorulate species at physiological pH is the dimer $Fe_2(RA)_3$ which below pH 4 dissociates into a monomer, Fe(RA).

The chemical properties of ferrioxamine B complex and of its trihydroxamic desferri derivative have been described earlier by Prelog [12] and others [3, 13]. There is no data on the formation of any binuclear chelate of desferrioxamines with iron [1, 3, 12] even though a binuclear complex of dicopper(II)–siderophore has been reported [3a]. Besides an equilibrium study [3], the chelation of iron(III) with desferrioxamine B has also been examined by kinetic methods [5a, 5c, 14], but no indication of any but the mononuclear iron(III) species were noted. Apart from that, our recent stopped-flow data [5b] have definitively indicated that desferrioxamine B, in an aqueous acidic solution, is able to chelate two iron(III) ions to form the binuclear diferrioxamine B complex, $Fe_2(DFBH)^{4+}$.

This paper presents the spectrophotometric characterization and the equilibrium quotient determination of diferrioxamine B complex.

Experimental

All the experiments were performed at 25 ± 0.1 °C, in an aqueous solution of 1.00 *M* ionic strength defined as $0.5 \Sigma C_i z_i^2$, where C_i and z_i are total concentrations and the formal charge numbers of the

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ions present in the solution, respectively. The ionic strength was maintained by NaCl, and double-distilled water in an all-glass apparatus was used throughout the experiments.

Ferric chloride stock solution (0.15 *M* in 0.1 *M* HCl) was prepared from ferric chloride hexahydrate (Merck), and standardized both spectrophotometrically and gravimetrically as Fe_2O_3. The methanesulphonate salt of desferrioxamine B (®Desferal) was kindly supplied by the Ciba-Geigy Corporation. The salt was recrystallized from methanol and was stored in a vacuum desiccator over $CaCl_2$ (m.p. 149–151 °C). All the other reagents were of analytical grade, and were used without further purification.

Physical Measurements and Computations

The spectrophotometric titrations and electronic spectra were recorded on a Cary 16 K spectrophotometer equipped with a thermostatted cell compartment. In these experiments the total proton concentration was calculated as the sum of added HCl and proton released from the iron species present in solution. On the other hand, in potentiometric titrations of ferrioxamine B, the proton concentration was calculated from pH readings, using an experimentally determined function $p[H^+] = a \cdot pH + b$, which is a characteristic of the combination glass electrode used. This function was always determined immediately before the main experiment, by titration of 0.33 *M* HCl (0.67 *M* NaCl) with 1.00 *M* NaOH at 25 °C. The titrations were carried out in a water-jacketed cell maintained at 25 ± 0.1 °C by an external circulation bath. A digital pH-meter, Orion Research Model 701 with combination glass electrodes (Radiometer GK 2322C) were used for pH reading. In all experiments volumes of titrants were delivered by an autoburette Radiometer ABU 12.

All the computations were performed on a UNIVAC 1100 computer at the University Computing Center, Zagreb. A modified version of the originally published [15] CORNEK computer program was used for the refinement of the formation quotients from potentiometric and spectrophotometric data, as well as for the refinement of absorption spectra.

Results

Schwarzenbach and Schwarzenbach [3c] have already pointed out that, as the pH is lowered below 2.4, the original color of the equimolar solution of iron(III) and desferrioxamine B changes from orange to reddish violet. On the other hand, we have observed that at constant acidity in the acidic pH range of the solution, the addition of an excess of iron(III) chloride to a solution of desferrioxamine B made the orange color turn to reddish violet. The

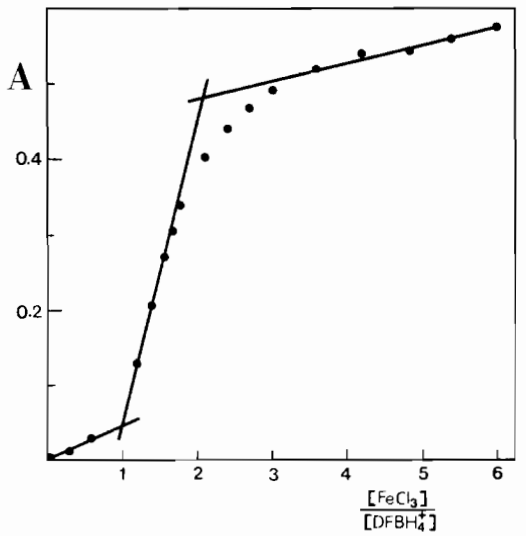


Fig. 1. Spectrophotometric titration of DFB with iron(III) ion at 25 °C, I = 1.0 *M*, λ = 650 nm, d = 0.1 cm. Total DFB and HCl concentrations are 1×10^{-2} *M* and 4×10^{-2} *M*, respectively.

kinetics of these processes has been studied by the stopped-flow technique [5b]. The absorbance data at 650 nm, obtained under experimental conditions in Fig. 1, show that two types of iron(III) complexes exist in the solution. The results are in accord with the assumption that mononuclear ferrioxamine B and binuclear diferrioxamine B are formed, containing 1:1 and 2:1 molar ratio of iron(III) to desferrioxamine B, respectively. The complete system could be described by the following equations (1–6), where water molecules and chloride as ligands are omitted in the presentation of iron species:

$$Fe^{3+} + H_4DFB^+ \rightleftharpoons Fe(DFBH_3)^{3+} + H^+ \quad (1)$$

$$Fe^{3+} + H_4DFB^+ \rightleftharpoons Fe(DFBH_2)^{2+} + 2H^+ \quad (2)$$

$$Fe^{3+} + H_4DFB^+ \rightleftharpoons Fe(DFBH)^+ + 3H^+ \quad (3)$$

$$2Fe^{3+} + H_4DFB^+ \rightleftharpoons Fe_2(DFBH)^{4+} + 3H^+ \quad (4)$$

$$Fe^{3+} \rightleftharpoons Fe(OH)^{2+} + H^+ \quad (5)$$

$$2Fe^{3+} \rightleftharpoons Fe_2(OH)_2^{4+} + 2H^+ \quad (6)$$

The above equilibria are characterized by the appropriate equilibrium quotients, Q_i, generally presented as (7),

$$Q_i = \frac{[Fe_m(H_4DFB)_nH_p]}{[Fe]^m[(H_4DFB)]^n[H]^p} \quad (7)$$

where the symbols in square brackets denote concentration.

The formation constants of the hexadentate and tetradentate bound complexes have already been

TABLE I. Equilibrium Quotients for the Formation of $Fe_m(DFBH_4)_nH_p$ Complexes at 25 °C, 1.0 *M* Ionic Strength, Determined by Spectrophotometric Titration at 500 nm.

i	m	n	p	Q_i	log Q_i	pQ_a[a]
1	1	1	−1	$(4.3 \pm 3.4) \times 10^2$	2.64 2.01[b] 1.89[c]	−0.77
2	1	1	−2	$(2.5 \pm 0.2) \times 10^3$	3.40 4.51[e]	1.12 1.19[d] 0.94[e]
3	1	1	−3	$(1.9 \pm 0.3) \times 10^2$	2.28 3.57[e]	
4	2	1	−3	$(2.7 \pm 0.3) \times 10^5$	5.43	

[a]pQ_a has the meaning of a formal −log of the acid dissociation constant of the complex involved in equilibrium (i). [b]Kinetically determined at 2.0 *M* ionic strength (ref. 5a). [c]Determined in 4.8 *M* HCl. [d]Determined by pH titration (this work). [e]Determined at 20 °C in 0.1 *M* $NaClO_4$ (ref. 3c).

determined (see Table I), however, at 0.1 *M* ionic strength [3c]. In order to obtain the formation quotient of the binuclear species, the spectrophotometric titration at 500 nm was performed within the concentration ranges of iron(III) from 1×10^{-5} to 0.12 *M*, desferrioxamine B from 10^{-4} to 0.1 *M*, and HCl from 10^{-3} to 0.98 *M*. Because of the iron(III) chloride concentration used, since it should be high enough to assure formation of diferrioxamine B in a desired extent, the ionic strength was maintained at 1.0 *M*. At this ionic strength the concentration of chloride differs more than 10% from 1.0 *M* in only two experiments. Furthermore these two experiments fall in the concentration range of iron(III) where absorbance is independent of iron(III) concentration and the medium effect may not affect the determination of the formation quotient.

Several models involving the binuclear complex of various degrees of protonation have been checked. The goodness of the fit has been the criterion of the plausibility of the particular model. The best fit was obtained by the model involving only one binuclear complex, releasing three protons as in equation (4). The equilibrium quotients, obtained by fitting more than 60 data points, simultaneously refining all the quotients as well as the molar extinction coefficients of $Fe(DFBH_2)^{2+}$, and $Fe_2(DFBH)^{4+}$ complexes, are presented in Table I. The quotients Q_5 and Q_6 were used as determiend by Milburn and Vosburgh [16]. It should be noted that no improvement of the fit was obtained with the models involving (as well as $Fe_2(DFBH)^{4+}$) the other binuclear species of various degrees of protonation. Therefore, our model proposes the existence of only one type of binuclear complex, *i.e.*, $Fe_2(DFBH)^{4+}$, at the studied acidity. The molar extinction coefficients of the hexadentate and bidentate complexes were kept constant throughout the computation. The former coefficient was easily determined in the solution of low acidity, whereas the latter was obtained in a separate spectrophotometric titration, in 4.8 *M* HCl. In such an acidic solution the iron is assumed to be predominantly bonded to the ligand in the bidentate mode. From the absorbance increase at 500 nm (λ_{max}), when H_4DFB^+ ranged from 0.017 to 0.122 *M*, the Q_1 and molar extinction coefficient of the bidentate complex were calculated as 77 ± 6 and 1250 ± 50 L mol^{-1} cm^{-1}, respectively. Ignoring the effect of ionic strength on the molar extinction coefficient* the value obtained was used in the treatment of the data at 1.0 *M* ionic strength. Throughout the computation this molar extinction coefficient was held constant, but the value of Q_1 was determined with a large standard deviation and must be considered as an upper limit. On the other hand, the obtained Q_1 seems reasonable in respect to the values obtained at 2.0 *M* and 4.8 *M* ionic strength (see Table I).

By fitting the data at different wavelengths it was possible to construct the absorption spectrum of the $Fe(DFBH_2)^{2+}$ complex ion. This spectrum cannot be directly determined in solution, due to the interference of either the hexadentate complex, free iron(III) ion, or the bidentate complex. In Fig. 2 the spectra of all these species, as well as the spectrum of binuclear diferrioxamine B, are presented.

Discussion

Our spectrophotometric results show that in an aqueous acidic solution, at an excess of iron(III) ion over desferrioxamine B, several mononuclear (as well as binuclear) complexes can be present, depending on the total iron(III) concentration and pH. The species distribution at $-\log[H^+] = 2$, 0.01 *M* $DFBH_4^+$ and various iron(III) concentrations is shown in Fig. 3. The predominant species in the neutral or slightly acidic solution of equimolar reactants is the hexadentate complex, $Fe(DFBH)^+$, with the absorbance maximum at 425 nm (Fig. 2). As the pH of such solution is decreased, λ_{max} shifts to longer wavelengths due to the formation of the tetradentate complex, $Fe(DFBH_2)^{2+}$. When a solution of the tetradentate species is made highly acidic, *i.e.* 4.8 *M* HCl, a new band with an absorbance maximum at 500 nm appears, corresponding to the formation of the bidentate chelate, $Fe(DFBH_3)^{3+}$. This behavior fits well the

*We have observed no effect of ionic strength, varied from 0.1 to 1.8 *M*, on the molar extinction coefficient of bidentate monoacethydroxamatoiron(III) complex.

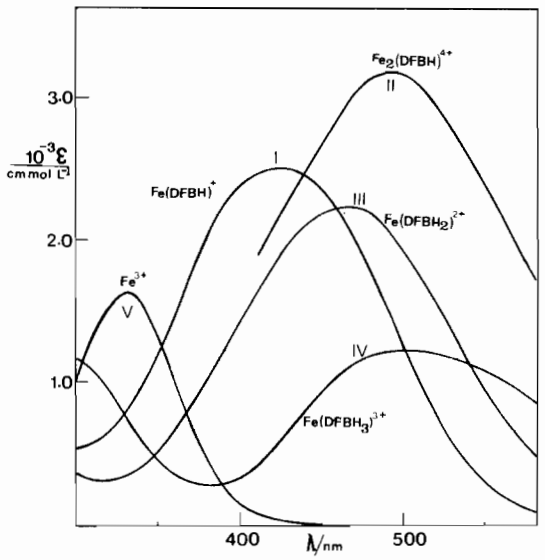


Fig. 2. Electronic spectra of different species involved in the reaction between iron(III), DFB and the proton, at 25 °C, I = 1.0 *M* (except for spectrum IV, I = 4.83 *M*). Curve I: Spectrum of the equimolar solution of $FeCl_3$ and DFB, in 2×10^{-4} *M* HCl. Curve II: Spectrum of the solution consisting of 3×10^{-4} *M* DFB, 0.12 *M* $FeCl_3$ and 0.1 *M* HCl, taken *vs.* 0.12 *M* $FeCl_3$, 0.1 *M* HCl. Curve III: Spectrum calculated fitting the spectrophotometric data at various wavelengths. Curve IV: Spectrum calculated using the value of $Q_1 = 77$, and the spectrum of a solution consisting of 0.01 *M* DFB, 6×10^{-4} *M* $FeCl_3$ and 4.83 *M* HCl. It is corrected for the absorbance of free iron and DFB. Curve V: Spectrum of the solution consisting of 3×10^{-4} *M* $FeCl_3$ and 0.1 *M* HCl.

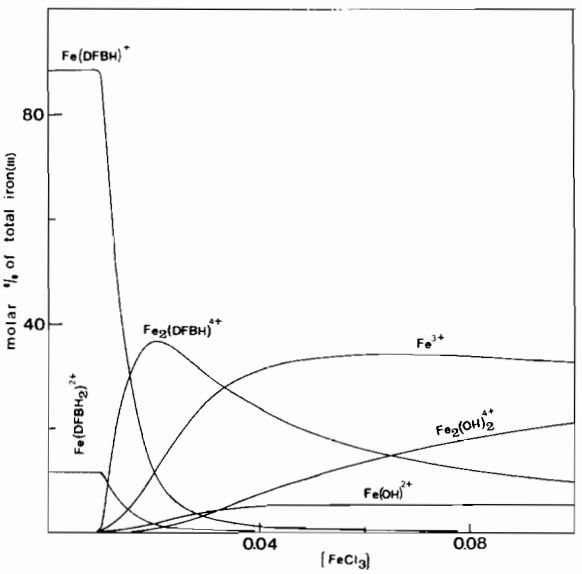


Fig. 3. Distribution of different iron(III) species in a solution of 0.01 *M* DFB, $-\log[H^+] = 2$, as a function of total iron(III) concentration (the possible condition of the gastric juice in the treatment of accidental poisoning by iron(III) containing drugs).

general feature of iron(III) interactions with monohydroxamic acids. On the other hand, the reddish-violet color, with λ_{max} at 495 nm, is exhibited by a moderately acidic solution of the binuclear complex ion, $Fe_2(DBH)^{4+}$, a species not observed nor characterized earlier [1, 3, 5c, 12].

The equilibria between iron(III) and $DFBH_4^+$ as well as between iron(III) and monohydroxamic acids, have been elucidated by combining redox potentials, pH and spectrophotometric measurements [3]. In the formation of ferrioxamine B two types of chelate effect may be considered: one arising from the formation of five-membered rings and the other from the formation of fourteen-membered rings. The existence of a small chelate effect related to the formation of two fourteen-membered rings in ferrioxamine B (defined in relation to acethydroxamic acid) has been already noticed [3b]. However for the determination of this effect, precise knowledge of the ligand pK_a values is required. Since the three pK_a values of $DFBH_4^+$ fall within a narrow range ($\Delta pK_a \simeq 0.7$[3c]), their determination might be quite inaccurate.*

On the other hand the exact value of the chelate effect due to the formation of the five-membered ring is not easy to estimate since the choice of the true reference ligands is very difficult. Therefore, our results of the equilibria studies will be discussed in terms of a competition between the proton and an iron(III) ion (bound in $Fe(H_2O)_6^{3+}$, Fe-bidentate- or Fe-tetradentate-hydroxamate complexes), for a hydroxamate oxygen (-NHO^-) of desferrioxamine B or of simple monohydroxamates. This approach encompasses the chelate effects, but rather in a qualitative way.

In Scheme I, the interactions of interest between iron(III), the proton, and $DFBH_4^+$ are depicted. A similar scheme (Scheme II) is drawn for the interactions of monohydroxamic acids, and the results of the analyses of these Schemes are presented in Table II. The above mentioned competitions are expressed by the competition factors defined as the log of equilibrium quotients $R_n = K_{nFe}/K_{nH}$.

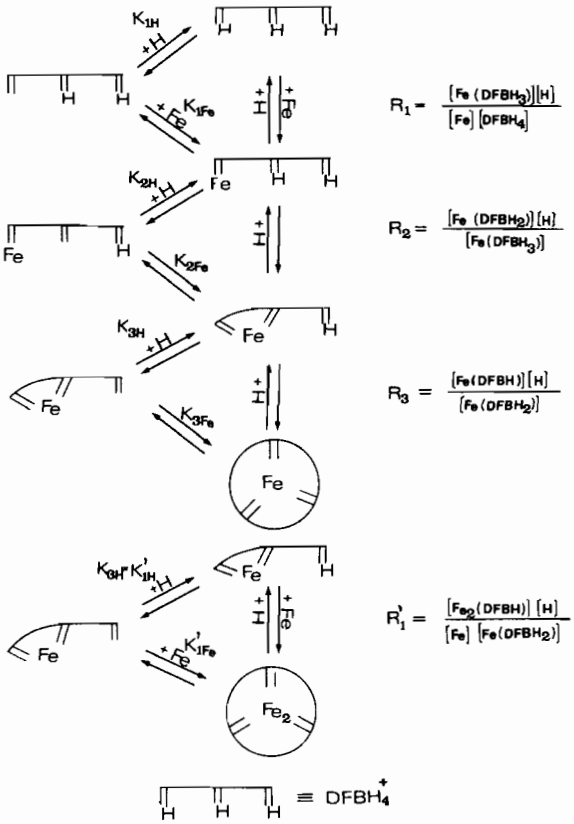
Schemes I and II show that in each equilibrium defined by R_n one proton is released from a hydroxamic group. However, R_n cannot be considered as being the acid dissociation constant of any particular species. Formally, R_n is to be regarded as a hypothetical acid dissociation constant of a given reaction ($1/K_{nH}$ being the real acid dissociation constant of the involved species, which for ferrioxamine B cannot be determined).

*The potentiometrically obtained pK_a values of H_4DFB^+ under experimental conditions utilised exhibit very high standard deviation (clearly, numerous solutions will approximately equally well fit the experimental titration curve) and for that reason they are not reported in the paper.

TABLE II. The Competition Between the Proton and the Iron(III) Centers for Various Hydroxamates.

log R_n[a]						log $\bar{R}_n \pm$ st. dev.	
n	DFB[b]	AHA[c]	BHA[c]	PIHA[d]	$Fe(DFBH_2)^{2+}$		
1	2.64 2.10[e]	2.05 2.04[f]	2.27	1.49	2.05	2.09	0.39
2	0.77	0.31	0.58	0.06	–	0.43	0.31
3	−1.16	−2.17	−1.39	−1.34	–	−1.52	0.45

AHA = acethydroxamic acid, BHA = benzhydroxamic acid, PIHA = pyrrolidone-5-hydroxamic acid. [a]For the explanation of log R_n see the Schemes and text. [b]This work. [c]From ref. 3c. [d]M. Pećar, N. Kujundžić, B. Mlinarević, D. Čerina, B. Horvat, M. Verić, *J. Pharm. Sci., 1975,* 64 , 970. [e]From ref. 5a. [f]From ref. 4b.



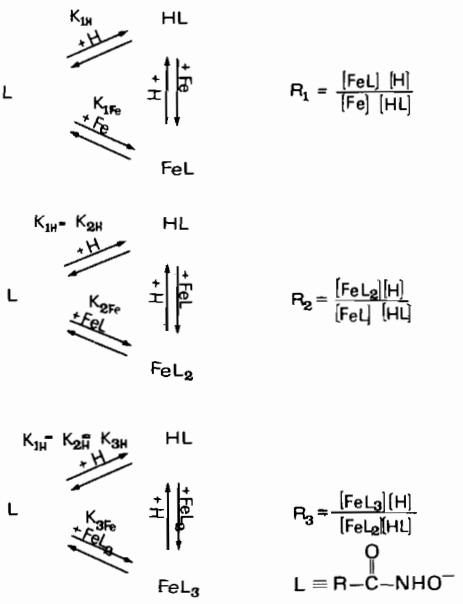
Scheme I. (Water molecules and charges are omitted).

The observed decrease of the competitive capability of iron(III) in the order

$$Fe(H_2O)_6^{3+} > Fe\left(\begin{matrix}O-N-\\ |\\ O-C-\end{matrix}\right)(H_2O)_4^{2+} > > Fe\left(\begin{matrix}O-N-\\ |\\ O-C-\end{matrix}\right)_2(H_2O)_2^{+},$$

i.e., $R_1:R_2:R_3 \simeq 10^2:10^0:10^{-2}$, could be a consequence either of the lowering of the effective charge at the iron center, or of the hydroxamate–hydroxamate mutual repulsions at the iron center. These effects obviously exceed the chelate effect in the closure of the third five-membered hydroxamate ring, which is proposed by Monzyk and Crumbliss [4b] as a driving force in the formation of monohydroxamatoiron(III) complexes.

In addition, Table II reveals that the competition factors for the same 'n' are essentially of the same order of magnitude. The small differences could be attributed to the variations in the electron donor/acceptor ability of the -C and -N substituents of the hydroxamate functionality rather than to a specific behavior of desferrioxamine B in comparison with the monohydroxamic acids. This phenomenon has already been thoroughly discussed by Monzyk and Crumbliss [4b]. The obtained similarity of log R_n (n = 2 and 3) may suggest that the 'effective



Scheme II. (Water molecules and charges are omitted).

molarity' [17] of iron(III) bonded to desferrioxamine B is approximately 1 mol L^{-1}, indicating low strain energies induced by the complexation*. It is worth noting that the affinity of the $Fe(H_2O)_6^{3+}$ ion for the hydroxamate functionality is slightly affected by the total charge or size of the hydroxamate molecule. The second iron(III) is nearly as tightly bound into diferrioxamine B as the first iron into ferrioxamine B. Stabilisation of the resonance form corresponding to nitrogen lone pair of electrons delocalization into the carbonyl function stabilises the iron–hydroxamato group bond [4b]. However, in our case a positively charged ammonium group at the end alkyl chain may exhibit a field effect rather then the inductive effect through the six σ-bonds, opposing the stabilisation of the complex. This is reflected in a slightly lower value of the formation constant of the second iron.

Diferrioxamine B has an almost three times higher molar extinction coefficient ($\epsilon_{495} = 3.25 \times 10^3$ L mol^{-1} cm^{-1}) in the absorbance maximum than bidentate linked ferrioxamine B. Based on these observations the structure of diferrioxamine B in which both irons are bidentate-bonded with one of the hydroxamate group uncoordinated, may be ignored. Therefore, it can be speculated that either one iron is bidentate- and another tetradentate-bonded, or that both irons are tridentate-bonded in the complex. In the latter case the middle group of the three hydroxamate groups of desferrioxamine B would feature a bridge, since it would be shared by two iron(III) ions. The fact that the second iron bonded into diferrioxamine B has essentially the same formation quotient as the iron bidentate bound into $Fe(DFBH_3)^{3+}$ ion, favours the former structure of the binuclear complex. However, a definitive assignment of the structure is beyond the scope of this paper.

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*The R_n values are dimensionless unless n = 2 and 3 in the iron(III)–DFB system, when their molar unity is mol L^{-1}. This difference arises from the fact that in Fe(III)–DFB complexes both reactants are part of the same molecule. Therefore, a procedure similar to that used in the comparison of the first- and second-order rate constants of related intra- and inter-molecular kinetics, respectively, may be adopted. Since the R_n values for DFB and the monohydroxamates do not essentially differ, the 'effective molarity' of iron(III), either bidentate or tetradentate bonded to DFB, must be approximately 1 mol L^{-1}.

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