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ScienceDirect
Coordination Chemistry Reviews 252 (2008) 1225–1240
COORDINATION CHEMISTRY REVIEWS
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Review

Iron chelating agents for the treatment of iron overload

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Received 19 July 2007; accepted 15 December 2007
Available online 23 December 2007

Contents

Abstract

The importance of iron chelators in medicine has significantly increased in recent years. Iron is essential for life but it is also potentially more toxic than other trace elements. This is because we lack effective means to protect human cells against iron overload and because of the role of iron in the generation of free radicals. In order to protect patients from the consequences of iron toxicity, iron chelating agents have been introduced in clinical practice. Unfortunately, the ideal chelator for treating iron overload in humans has not been identified yet. In this paper we examine a few characteristics of iron chelators, with some emphasis on the effects of redox cycling, on absorption mechanisms and on some properties of the pFe. A brief summary is then made of the chelators recently proposed or in development for the treatment of iron overload.

Keywords: Iron chelator; Beta-thalassemia; pFe; Hydroxypyridinone; Desferal

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doi:10.1016/j.ccr.2007.12.014

1. Introduction

A previous volume of this journal devoted to ISMEC'97 already contained a review by one of the authors with the title "Iron chelating agents in clinical practice" [1], this paper is an overview of the pertinent literature that appeared thereafter, and also gives some details on topics related to important aspects in the understanding of the mechanisms of action of iron chelators. In particular in this review we will deal with the application of iron chelators to iron overload diseases and some important aspects in their chemical design, and the different clinical applications such as cancer therapy, treatment of malaria, and so on. The study of iron chelators in cancer therapy has had its greatest development in the past decade, and has recently been discussed thoroughly [2–4]. Excessive amounts of iron may become very toxic to the human body due to the fact that it lacks effective mechanisms to protect cells against iron overload. Iron overload may be caused by the following:

- increase in iron absorption from the diet, genetically determined in hereditary haemochromatosis [5], in which genetic defects alter the regular absorption of iron from the gut or due to iron excess in the diet as in Bantu siderosis [6];
- parenteral administration of iron in transfusion-dependent anaemias (β-thalassemia, a genetic disease with a defect in haemoglobin synthesis, requires regular blood transfusions to prevent anaemia);
- pathological conditions characterized by increases in iron, above all in the brain.

In the following, after a reference to iron metabolism in humans and to iron overload diseases, we will discuss the chemical characteristics and requirements of iron chelators and the new molecules in clinical use or under clinical trials.

2. Iron distribution in humans

The total body iron content in normal human adults is about 40–50 mg/kg body weight, corresponding to 2.8–3.5 g in a 70 kg man (male: weight 70 kg, total blood 5200 mL/5500 g, total plasma 3000 mL/3100 g; female: weight 58 kg, total blood 3900 mL/4100 g, total plasma 2500 mL/2600 g) [7]. About 80% of the total iron content is found in haemoglobin in circulating red blood cells and in myoglobin in the muscle; the remaining 20% is distributed between storage proteins, ferritin and haemosiderin, a small amount in various iron-containing enzymes, and about 3–4 mg circulates in the plasma bound to transferrin. Iron in plasma is turned over about 10 times a day. Iron metabolism in the human body is essentially conservative: the average absorption is 1–3 mg/day, and almost the same amount is excreted by cell desquamation from gut and skin, and in women of fertile age, through menstruation or pregnancy. The delicate equilibria between iron uptake and iron loss, and the mechanisms regulating iron uptake are accurately discussed by Crichton and Ward [8].

2.1. Iron overload

Iron overload is the most serious complication of β-thalassemia and is the focal point of its management. A comprehensive overview on iron overload in β-thalassemia has been presented in a recent paper by Oliveri [9]. Also in patients that do not receive transfusions, abnormal iron absorption produces an increase in the body iron burden evaluated in the 2–5 g per year range [10]. Regular blood transfusions lead to double this iron accumulation. The annual iron load from blood transfusion can be estimated from the number of red cell units given. A unit (420 mL of donor blood) contains about 200 mg of iron. Iron accumulation introduces progressive damage in liver, heart, and in the endocrine system if a chelating therapy is not introduced. Iron is deposited in parenchymal tissues and in reticuloendothelial cells. When the iron load increases, the iron binding capacity of serum transferrin is exceeded and a non-transferrin-bound fraction of plasma iron (NTBI) appears, which generates free hydroxyl radicals and induces dangerous tissue damage. Iron accumulates at different rates in various organs, each of which react in a characteristic way to the damage induced by NTBI and by the intracellular labile iron pool (LIP). Based on these observations, chelators that remove iron from specific target organs would be desirable, above all in the heart, cardiac disease being the life-limiting consequence of iron overload. Chelators can act on different iron pools: (i) serum iron bound to transferrin; (ii) iron in the form of non-transferrin-bound iron (NTBI); (iii) when the transferrin is saturated, the iron stored in ferritin and in haemosiderin; (iv) the labile iron pool (LIP) that is found in the cytoplasm.

In the first two cases chelators act directly on the plasma, while in the second two it has to penetrate the cells and so the iron-chelate complex must leave the cell.

3. Chemistry and design of iron chelators

Iron chelators should reduce tissue iron levels, allowing efficient transport and excretion without iron redistribution, prevent excessive organ iron accumulation and neutralize toxic labile iron pools. Absorption from the gastrointestinal tract and cell penetration, both of which depend on diffusion through the biological membranes, are governed by molecular size, lipophilicity and net molecular charge, as will be discussed in Section 3.2. In recent years extensive research has been directed toward bidentate and tridentate ligands, which are more likely to be orally active and penetrate cells. The efficacy of the iron chelators used today is limited by the fact that they convert to glucuronidate metabolites: most of the DFO and Deferiprone (DFP also reported as L1 or CP20) is metabolized to an inactive glucuronide form and their chelating efficiency becomes less than 10%. A further step would then be to design compounds with a reduced metabolic rate. Let us first recall the main properties that an ideal iron chelator should have:

- high chelating efficiency
- specific affinity for Fe(III)

- suitable redox potential of complexes
- chemical structure of complexes
- fast complex-formation kinetics
- slow rate of metabolism
- oral bioavailability
- tissue and cell penetration
- no iron redistribution
- low toxicity
- it should reach a negative iron balance
- low cost

The iron paradox is what stops us from attaining the ideal iron chelator: in fact iron is essential for many metabolic functions (oxygen transport and utilization, DNA synthesis, electron transport), but it becomes toxic when accumulated. A chelator should therefore remove only iron in excess without interfering with iron homeostasis (absorption, distribution and utilization) and iron-dependent enzymes (ribonucleotide reductase, lipooxygenase), while leaving other essential metals, such as zinc, copper and calcium, unaffected. Properties such as molecular weight, lipo-hydrophilic balance, kinetics, distribution and metabolism are essential in determining the limit between safety and toxicity. In the chemical design of iron chelators for clinical application metal selectivity and the corresponding ligand–metal complex stability are paramount. In theory chelating agents can be designed for either the Fe(II) (ferrous) or Fe(III) (ferric) oxidation state. High-spin Fe(III) is a spherically symmetrical tripositive cation of radius 0.65 Å, and is classified as a hard Lewis acid by virtue of its high charge density. It forms most stable bonds with hard ligands, such as charged hydroxamate oxygen atoms. In contrast, the Fe(II) cation, which has a relatively low charge density, prefers chelators that contain soft donor atoms, exemplified by the nitrogen-containing ligands. Since ligands that prefer Fe(II) retain an appreciable affinity for other biologically important bivalent metals, such as Cu(II) and Zn(II) ions, a non-toxic Fe(II)-selective ligand is extremely difficult to design. In contrast, Fe(III)-selective ligands, typically oxyanions and notably hydroxamates and catecholates, are generally more selective for tribasic metal cations than dibasic cations. Many tribasic cations, for instance Al(III) and Ga(III), are not essential to living cells, thus in practice Fe(III) is the best target for an 'iron chelator' under biological conditions. We stress here that the research on chelators to treat the various Al(III)-dependent diseases has benefited from the studies on iron chelators [11]. Ligands can be classified structurally according to the number of donor atoms that each molecule possesses for co-ordinate bond formation. A factor of great importance in the stability of a metal complex is the number of chelate rings formed in the resulting complex: the more the rings, the greater the stability of the complex. The number of chelating rings can be enhanced by increasing the number of donor atoms attached to the ligand. A widely used standard to compare the "strength" of chelating agents under biological conditions is the pFe value [12] (see Section 3.3). Generally a pFe value ≥20 is required for efficient iron scavenging from biological matrices. A comparison of the pFe values for hexadentate and bidentate ligands suggests that hexadentate ligands are far better than their bidentate counterparts under *in vivo* conditions. Significantly, most natural siderophores are hexadentate ligands.

3.1. Effect of redox cycling

The chemical reactions between reactive oxygen species (ROS) and excess body iron, both as Fe(II) and Fe(III), may result in tissue or organ damage. Commonly ROS molecules are superoxide ($O_2^{\bullet -}$), hydroxyl radicals ($OH^{\bullet}$), alkoxyl radical ($RO^{\bullet}$), nitric oxide radical ($NO^{\bullet}$) and hydroperoxyl radical ($HO_2^{\bullet}$) [13].

To clarify the behaviour of these reactive species and their reactivity, we recall the electronic structure of the principal ROS molecules. Starting from the dioxygen molecule, this exists in its ground state as a triplet spin state, i.e. it has two unpaired electrons, each located in a different π* antibonding orbital. As reported by Crichton [14] "this imposes a restriction on oxidation by O_2, which means that dioxygen tends to accept its electrons one at the time slowing its reaction with non-radical species. Transition metals can overcome this spin restriction on account of their ability to accept and donate single electrons. The interaction of iron centres and oxygen is of paramount importance in biological inorganic chemistry."

Ground state O_2 ($^3\Sigma_g O_2$)	↑	↑
Singlet O_2 ($^1\Delta_g O_2$)	↑↓	
Singlet O_2 ($^1\Sigma_g{}^+$)	↑	↓
Superoxide $O_2^{\bullet -}$	↑↓	↑
Peroxide $O_2^{=}$	↑↓	↑↓

The addition of a single electron to the ground state O_2 molecule produces the superoxide radical $O_2^{\bullet -}$:

$$O_2 + e^- \rightarrow O_2^{\bullet -} \quad (1)$$

in which the electron enters one of the π^* antibonding orbital. The addition of a second electron gives the peroxide ion $O_2^{=}$ with no unpaired electrons:

$$O_2^{\bullet -} + e^- \rightarrow O_2^{=} \quad (2)$$

At physiological pH 7.4 this peroxide ion binds two protons to give H_2O_2:

$$O_2^{=} + 2H^+ \rightarrow H_2O_2 \quad (3)$$

A further reactive oxygen species in biological systems is the hydroxyl free radical, $OH^{\bullet}$, which can form by homolytic fission of the H_2O_2 molecule. This $OH^{\bullet}$ radical can also be produced according to the Fenton reaction (4) in presence of Fe^{2+}:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\bullet} \quad (4)$$

while the Fe^{3+} ion can be reduced by superoxide to give Fe^{2+} and molecular oxygen

$$Fe^{3+} + O_2^{\bullet -} \rightarrow Fe^{2+} + O_2 \quad (5)$$

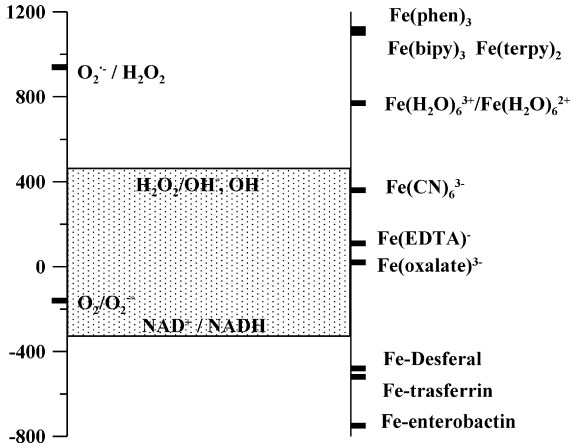


Fig. 1. Reduction potentials (mV) of important biological reactions, and of different iron complexes.

The sum of the last two reactions gives the Haber–Weiss reaction (6) by which superoxide and hydrogen peroxide produce the hydroxyl radical (together with molecular oxygen and hydroxyl anion) in the presence of a catalytic amount of iron:

$$O_2^{\bullet -} + H_2O_2 \xrightarrow{\text{iron}} O_2 + OH^- + OH^{\bullet} \quad (6)$$

The aptitude of an iron chelator to catalyze the Fenton reaction is a function of its redox potential. This potential should be in the proper range to satisfy two constraints: (1) the Fe(III)-complex must be reducible by the reductants in physiological environment (−0.16 V/NHE $O_2/O_2^{\bullet -}$, −0.28 V/NHE Ascorbate/Ascorbyl, −0.324 V/NHE $NADP^+$/NADPH), (2) the iron-chelate redox potential must be smaller than +0.46 V/NHE ($H_2O_2/OH^{\bullet}$, $OH^{\bullet}/OH^-$) [8,15], for the electron transfer from the Fe(II)-complex to H_2O_2 to be possible. These limit conditions can be observed at a glance in Fig. 1.

In order to catalyze a redox cycle, an iron complex must have a coordination site occupied by water or some other easily dissociable group that permits access to superoxide and hydrogen peroxide. From the redox potential of iron complexes with different ligands, also reported in Fig. 1, some considerations on their behaviour are possible:

- The redox potential of iron/EDTA complex (+0.12 V/NHE) allows both Fe(II) complex oxidation by hydrogen peroxide, and Fe(III) complex reduction by physiological reductants. Therefore this species can easily catalyze hydroxyl radical formation.
- The iron(II) form of iron–phenanthroline complex is so strongly stabilized (+1.15 V/NHE) that it cannot be oxidized by hydrogen peroxide.
- Desferrioxamine stabilizes the Fe(III) form (−0.40 V/NHE) so tightly that it cannot be reduced by physiological reductants.

The ROS defence mechanism in humans involves the enzymes glutathione peroxidase and catalase, which degrade H_2O_2 to water and oxygen. Iron binding proteins, such as lactoferrin, regulate the amount of unbound iron to prevent hydroxyl radical formation, and radical scavengers, such as vitamin E, interrupt the radical chain reaction. In an iron imbalance situation, iron is mobilized from proteins and can take part in redox reactions leading to ROS generation. When this exceeds the antioxidant defense, the cells undergo oxidative damage (oxidative stress).

To prevent iron participation in a catalytic cycle, with production of dangerous ROS, its redox potential must be controlled by proper chelation. In this way the redox potential of iron can be removed from the region in which it undergoes redox cycling, as illustrated in Fig. 1.

Significantly, the high selectivity of siderophores for Fe(III) over Fe(II) renders redox cycling unlikely under biological conditions. Chelators with nitrogen ligands tend to possess lower redox potentials and the coordinated iron can be reduced enzymatically under biological conditions. Boukhalfa and Crumbliss [16] have thoroughly discussed the correlation between the redox potential and the affinity for iron(II) and iron(III), the pH effect on redox potential and the redox facilitated iron exchange. In a recent paper, Merkofer et al. [17] took into account the redox properties of iron complexes with orally active chelators (hydroxypyridinones and ICL670): they measured the electrode potentials of −620 mV, −600 mV, −535 mV and −535 mV for CP20, ICL670, CP502 and CP509 complexes, respectively, but they remarked that, at lower chelator concentrations, the electrode potentials were significantly higher. Successively they pointed out [18] that the tight binding of iron(III) by hydroxypyridinones prevents redox cycling.

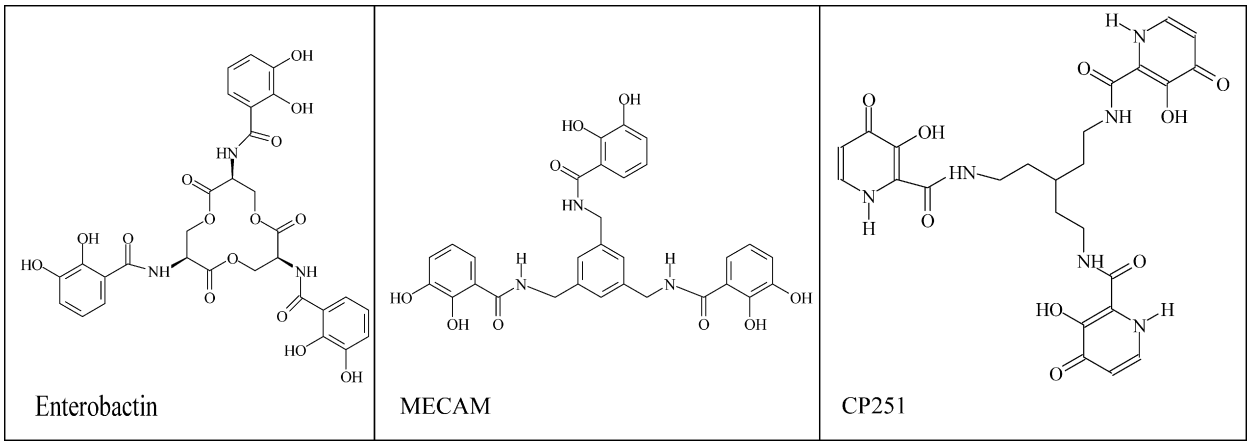
3.2. Absorption mechanism

In a recent work [19] Hider points out that the three major parameters governing diffusion through biological membranes are molecular size, lipophilicity and net charge. In particular the cut off molecular weight for drugs that have to be absorbed in the human intestine is approximately 500. Lipophilicity is generally estimated by the water–octanol partition coefficient (P). These general properties have been used by Lipinski et al. [20] to predict membrane permeability, adopting a four parameter analysis; their guidelines state that a poor absorption is likely when:

- molecular weight > 500
- log P > 5
- more than 10 hydrogen bond donors are present in the molecule (expressed as a sum of OH and NH groups)
- more than 10 hydrogen bond acceptors are present in the molecule (expressed as a sum of O and N atoms)

In Table 1, partially reproduced from Ref. [19], the Lipinski parameters for some common iron chelators are reported.

HBED fails to be correctly described (it is not in fact efficiently absorbed via the oral route) because the Lipinski criteria do not take into account the zwitterionic nature of this molecule.



3.3. Use of pFe

A further point that will be discussed is the use of pFe to describe the efficiency of a chelator. This is defined [12] as the negative logarithm of the concentration of the free Fe(III) in solution, calculated for total [ligand] = 10^{-5} M and total [iron] = 10^{-6} M at pH 7.4. A comparison of ligands under these conditions is useful, as the pFe value takes into account the effects of ligand protonation and denticity, as well as differences in metal–ligand stoichiometries. In fact, it is not formally correct to make a simple numerical comparison between the formation constant of a 1:1 iron complex ([ML]) of a hexadentate chelator (K_{11}) and the overall formation constant (β_{13}) for a 1:3 iron complex (ML_3) of a bidentate chelator. The two constants are differently related to the concentrations:

$$[ML]_h = K_{11}[L]_h[M]_h, \qquad [ML_3]_b = \beta_{13}[L]_b^3[M]_b$$

where subscripts h and b refer to hexadentate and bidentate ligands, respectively.

Table 1
Parameters of iron chelators for their evaluation according to the Lipinski criteria

	Molecular weight	log P	H bond donors (OH and NH)	H bond acceptors (O and N)
Lipinski "cut off" values	500	5	5	10
Hexadentate ligands				
DFO	560	<1	6	14
DFO prodrug	737	<2	3	20
Enterobactin	669	<3	9	18
MECAM	576	<4	9	12
CP251	557	<1	9	16
HBED	388	<1	4	8
Monoethyl HBED	416	<1	3	8
Dimethyl HBED	416	<2	2	8
Tridentate ligands				
Desferriothiocin	238	<2	2	5
ICL670	373	3.8	3	7
Bidentate ligands				
Deferiprone	139	−0.77	1	3
CP502	196	−1.36	2	5

When the ligand is in a large excess, e.g. $L_{Tot} = 10M_{Tot}$, and the complex formation is almost complete ([ML] = M_{Tot}), the above equations reduce to

$$M_{Tot} = K_{11}(0.9L_{Tot})[M]_h, \qquad M_{Tot} = \beta_{13}(0.7L_{Tot})^3[M]_b$$

The two ligands can be considered of the same chelating efficiency if the same value of free metal ($[M]_h = [M]_b$) is obtained. This happens when

$$K_{11} = \beta_{13} \times 0.381L_{Tot}^2$$

Alternatively, it can be also deduced that, with numerically identical constants, the efficiency of a 1:3 mode of chelation decreases when the square of the total ligand concentration decreases:

$$[M]_b = \frac{[M]_h \times 2.62}{L_{Tot}^2}$$

It was therefore proposed to use the pFe value, taking into account ligand denticity as well as proton competition. Nevertheless, in many situations these assumptions may be not realistic. In fact, iron concentration in plasma and other fluids can be estimated [8] as $\sim 2 \times 10^{-5}$ M, which increases remarkably in pathological situations. Similar conclusions can be drawn if we consider the quantity of iron excreted per day > 0.5 mg/kg body weight necessary to obtain a negative iron balance in transfused patients. The above iron concentration is significantly higher than the 10^{-6} M used in pFe definition.

Moreover, if we take into account the daily doses clinically used for different iron chelators (DFO: daily doses 40 mg/kg, MW 570 Da and DFP): 100 mg/kg, MW 139 Da), the corresponding concentrations range from 10^{-3} to 10^{-5} M according to the mode of administration, the absorption and metabolic properties.

The definition of pFe can therefore be useful for a first insight of the chelating properties of a ligand, but we can make a more realistic evaluation and comparison based on the knowledge of the real conditions of use. In particular, different chelators are normally compared based on their pFe values calculated from data at 25 °C and 0.1 M ionic strength. The passage at 37 °C and 0.15 M could alter the ligand ionization constant and the iron complex formation constants in a different way, and

Table 2
Literature protonation ($\log K_1$, $\log K_2$) and complex formation constants for some pyridinones and catechols

Ligand	$\log K_1$	$\log K_2$	$\log K_{11}$	$\log K_{12}$	$\log K_{13}$	Ref.
Pyridinone						
1-Hydroxy-2-	5.86	1.2	10.61	9.5	7.1	[22]
1-Hydroxy-2-	5.78	–	10.3	9.0	7.6	[23]
3-Hydroxy-4-	9.01	3.34	14.2	11.6	9.3	[23]
1,2-Dimethyl-3-hydroxy-4-	9.64	3.56	14.92	12.23	9.79	[24]
1,2-Diethyl-3-hydroxy-4-	9.93	3.81	15.21	11.76	9.78	[25]
Catechol						
Catechol	13.0	9.22	20.01	14.69	9.09	[26]
3-Nitro-	11.20	6.65	15.71	13.21	–	[27,28]
4-Nitro-	10.83	6.70	15.53	13.10	9.59	[29]
3,4-Dihydroxyphenylethanoic ac	13.7	9.49	20.1	14.8	9.0	[26]
2,3-Dihydroxy-*N*,*N*-dimethylbenzam	12.1	8.42	17.77	13.96	8.51	[30]

lead to noticeable changes in pFe. Moreover, mere knowledge of the pFe value is insufficient to describe the speciation of the metal/ligand system and to suggest the biologically active species at physiological pH. It does not contain any information on the donor-atom set or on the complex structure in solution. It is of no use in predicting the competition of other ligands or metal ions that are also present in the system. On the contrary, if we know the speciation model under defined experimental conditions we can easily calculate the pFe value as well. Unfortunately, in spite of the great interest in iron-chelation therapy, thermodynamic knowledge on complex-formation equilibria in these systems is still rather poor.

A linear correlation between stability constants of 1:1 iron complexes, such as $\log K_{11}$, and the first ionization constants of the ligand, $\log K_1$, was presented by Crisponi et al. [21] based on literature data of carboxylic acids, phenols, amino acids and hydroxo acids, hydroxamic acids, salicylic acids, catechols and pyridinones. In particular, if we examine single classes of ligands, for example pyridinones and catechols (Table 2), good linear correlations can be observed between the first protonation constant and the iron complex formation constants $\log K_{11}$, $\log K_{12}$ and $\log K_{13}$ (Fig. 2).

These correlations are a clear indication that the same properties determine proton and iron binding. Let us assume that we can modulate the $\log K_1$ values of a class of ligands with proper substituents: the $\log K_{11}$, $\log K_{12}$ and $\log K_{13}$ values can be estimated by the parameters of the above straight lines. The pFe values of catechols and pyridinones, calculated from the set of constants thus obtained as a function of $\log K_1$, are reported in Fig. 3.

In the cases represented in Fig. 3 a break point at $\log K_1$ 7.4 can be observed. This is accountable based on the conditional constants (calculated according the definitions in the book of the IUPAC-SC database) which show a maximum at $\log K_1$ 7.4. These observations stress the important role of proton competition: within a class of chelators the best results are obtained with that ligand whose pK is nearest to the physiological pH 7.4. An example of this is the behaviour of 4-nitrocatechol with respect to catechol. The introduction of a nitro group lowers both protonation and iron complex formation constants, but the pFe value of 4-nitrocatechol is four orders of magnitude higher than that of catechol. In this way Hider [31], by introducing an amido function in the 2-position of 3-hydroxypyridin-4-one, reduced both the pK and the overall

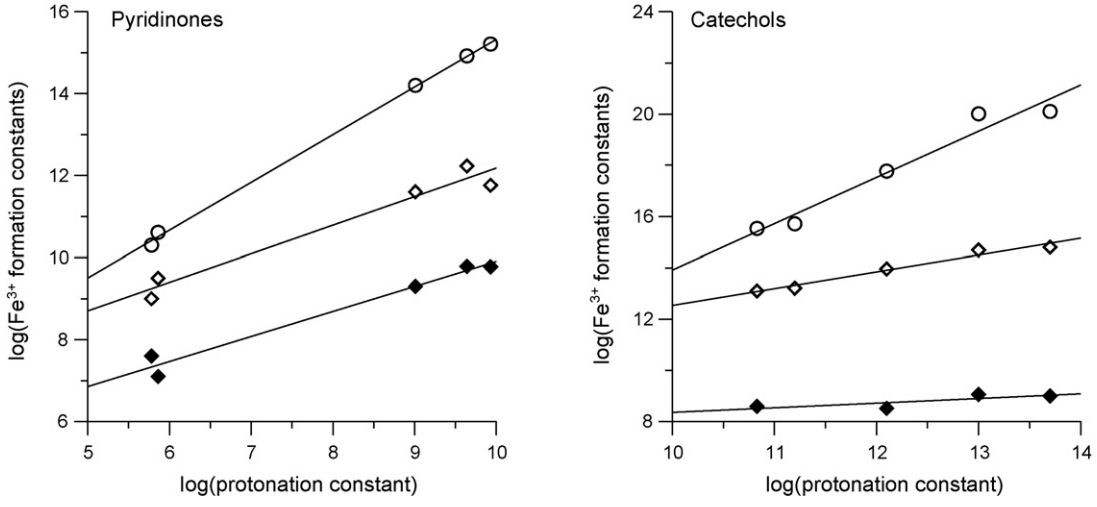


Fig. 2. Correlations between the first protonation constant and the iron complex formation constants for some pyridinones (left) and catechols (right).

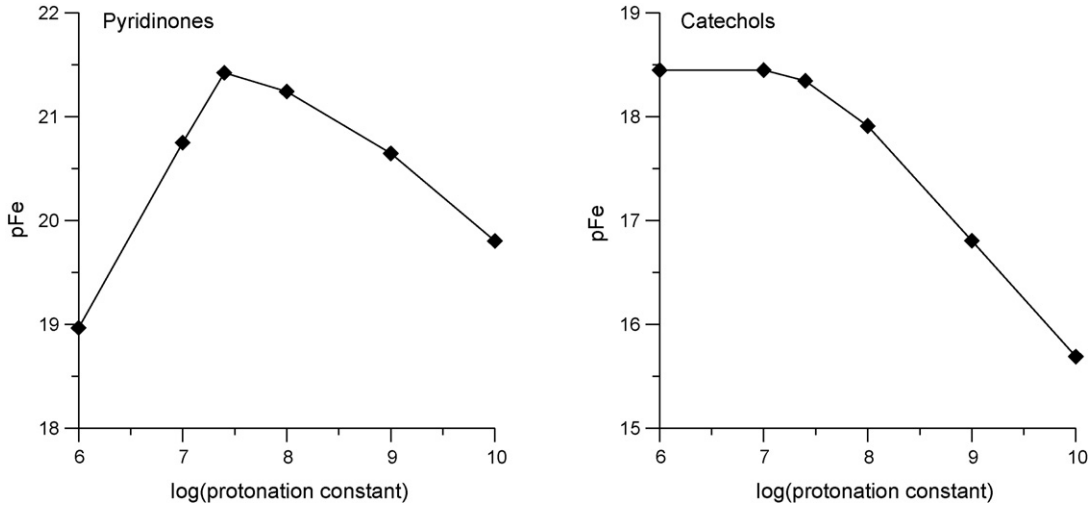
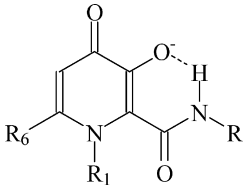


Fig. 3. pFe values of pyridinones (left) and catechols (right) *vs.* the first protonation constant; pFe were calculated from the set of constants obtained as a function of log K_1.

iron stability constants, while increasing the corresponding pFe value.



3.4. Ligands and denticity

3.4.1. Hexadentate ligands

Besides DFO, a large number of hexadentate ligands, mimicking the natural siderophores, have been proposed to date [32–55]. They are all characterized by high pFe values (up to 32.2 in the case of Ref. [40], compared to the value of 26.5–26.8 accepted for DFO [56,57]). At neutral pH, only the 1:1 complex, where Fe is hexacoordinated, is normally present in solution, and the use of pFe values can be sufficient to describe the system. However, all exceed the molecular weight allowed for oral absorption.

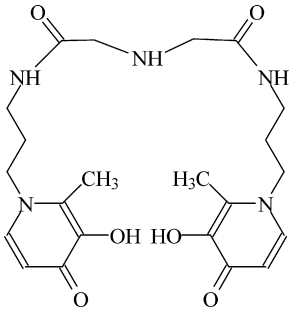
3.4.2. Pentadentate ligands

In the authors' knowledge, only two potentially pentadentate ligands for ferric ion are reported in literature [58]: 2,3- and 3,4-Dicatecholspermidine. These ligands have a good affinity for iron(III) (pFe values = 25 and 22, respectively), but different complex species (with various protonation degrees) are present at neutral pH. Moreover, at least one coordination position is always occupied by a water molecule, leaving the way open to the Fenton reaction. This new series of chelators was aimed at antibiotic targeting.

3.4.3. Tetradentate ligands

Many tetradentate ligands have been investigated to date as possible iron(III) chelators for oral use. They are most often made up of dihydroxamic acids [59–63], but disphosphonates [64] and a bis(3-hydroxy-4-pyridinone)-IDA derivative [65] have also been described. The molecular weight of these ligands is generally below the limit of 500 suggested by Lipinski (see Section 3.2) for oral absorption and pFe values are in the range of 18–21 log units. In order to completely saturate the six coordination positions on ferric ion, the denticity of these ligands requires formation of polynuclear species, which are invariably found in these systems. In particular, the most common polynuclear complex is the dimer Fe_2L_3, with a charge depending on ligand structure. A common feature of these ligands is that a small amount of mono-nuclear complexes containing an unsaturated ferric ion is always present under the experimental conditions considered for the calculation of the pFe value. An especially high affinity for the ferric ion has been found by Santos et al. [65] concerning the new bis(3-hydroxy-4-pyridinone) derivative of iminodiacetic acid, imino-bis(acetyl(1-(3′-aminopropyl)-3-hydroxy-2-methyl-4-pyridinone)), IDAPr(3,4-HP)$_2$.

The calculated pFe value is 25.8, of the same order of magnitude as that of DFO. However, in the case of IDAPr(3,4-HP)$_2$, up to eight complex species form in the explored pH range (0.4–9), and their relative amount at a defined pH (e.g. 7.4) depends on the total concentration of both the metal ion and the ligand, as shown in Fig. 4.



IDAPr(3,4-HP)$_2$

3.4.4. Tridentate ligands

The best way to reduce the molecular weight of the ligand and at the same time ensuring the saturation of the coordina-

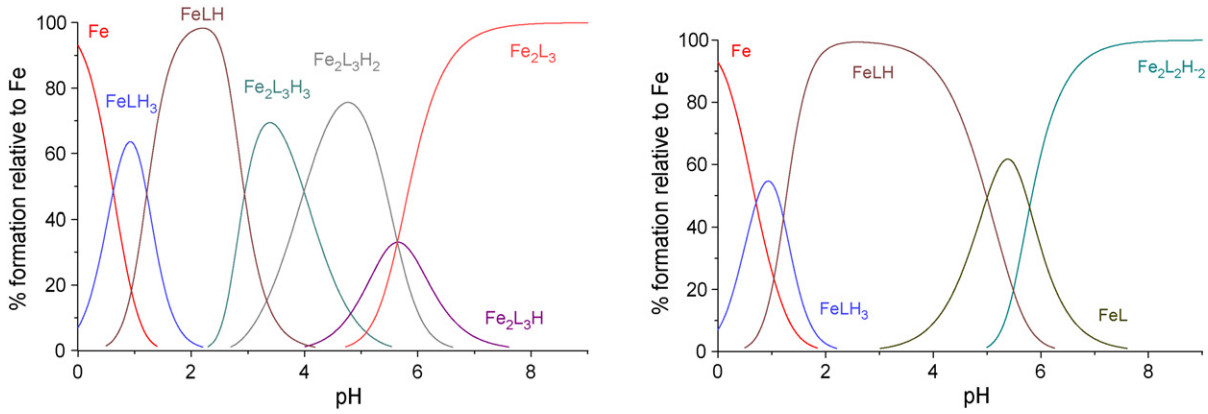
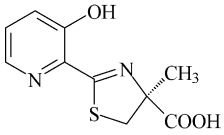
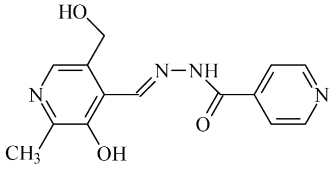


Fig. 4. Distribution of Fe(III)/IDAPr(3,4-HP)$_2$ complexes *vs.* pH at different metal-to-ligand ratios: M/L = 1:2 (CM = 2.8×10^{-4} M) on the left; M/L = 1:1 (CM = 5.9×10^{-4} M) on the right. Data from Ref. [65].

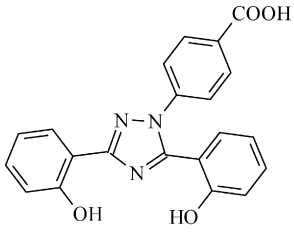
tion sphere of Fe(III) is by using tridentate or bidentate ligands with high affinity for the metal ion. The first tridentate ligand described was Desferrithiocin (DFT) [2-(3-hydroxypyridin-2-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylic acid [66,67], which binds iron through its thiazole nitrogen, phenolic and carboxylate oxygen atoms. Desferrithiocin forms a bis-complex with Fe(III), characterized by an overall formation constant log $\beta = 29.60$ as reported by Anderegg [68]. DFT nephrotoxicity encouraged the research of safe derivatives like GT56-252 (see Section 4.2.1), whose complex-formation equilibria have not yet been thoroughly investigated. The promising qualities of this molecule have led to extensive structure–activity studies by the group of Bergeron [69–74] in order to develop non-toxic (or less toxic) derivatives of this pharmacophore.



Desferrithiocin



PIH



ICL670

PIH acts as a tridentate chelator with an iron affinity similar to that of DFO [75,76], which coordinates Fe(III) in an octahedral mode through phenolic oxygen, imine nitrogen and carbonyl oxygen; it was synthesised by Ponka in 1979 from pyridoxal and isonicotinic acid hydrazide via a Schiff base condensation [77]. Starting from this molecular basis an exceptional quantity of molecules has been synthesized and tested for their potential applications in the treatment of iron overload disease and cancer. The evolution of this line of research is thoroughly reported in a review by Kalinowski and Richardson [4] and in some recent papers [78,79] where the authors discuss the structure–activity relationships and point out that redox-inactive iron chelators are well suited for the treatment of iron overload diseases, whereas iron chelator complexes with high redox activity show promising results as chemotherapeutics against cancer.

ICL670 (Deferasirox, Exjade) is a bis-hydroxyphenyl-triazole ligand that behaves like a tridentate chelator. It is characterized by a triazolyl nitrogen and two phenolic oxygen atoms as donor groups. With its rigid structure and its constraint of two six-membered chelate rings, ICL670 has a strong preference to bind small metal cations. Complex-formation equilibria of ICL670 and some analogues with different transition metal ions have been studied by Heinz et al. [80] and Steinhauser et al. [81] in water/DMSO 80:20, 0.1 M KNO_3 at 25.0 °C. As shown in Fig. 5, in the presence of a ligand-to-metal excess of 2:1 the percentage of the $[Fe(III)L_2]^{3-}$ species is close to 100%. The data reported by these authors (Table 3) point out selectivity of ICL670 towards Fe(III). This compound has a relatively high affinity towards Al(III), which makes it potentially interesting for a selective sequestration of aluminum.

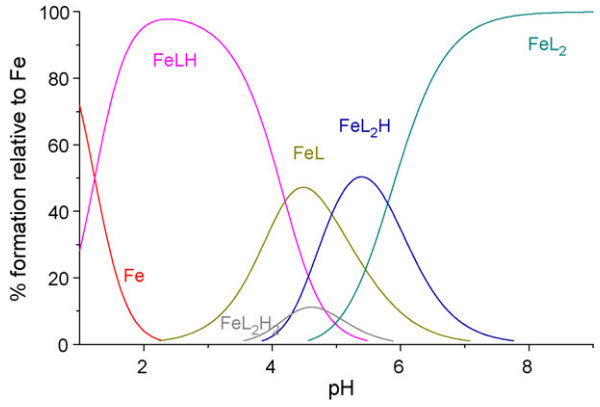
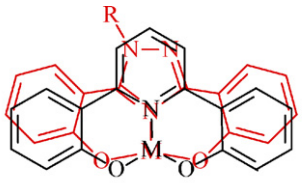


Fig. 5. Distribution of Fe(III)/ICL670 complexes *vs.* pH at M/L = 1:2 ($C_M = 5.0 \times 10^{-4}$ M). H_2O/DMSO 80:20, 25 °C and $I = 0.1$ M (KNO_3). Data from Ref. [81].

Table 3
Overall formation constants for the binary complexes between ICL670 and different metal ions, in H_2O/DMSO 80:20, at 25 °C and $I = 0.1$ M (KNO_3)

	Mg(II)	Ca(II)	Cu(II)	Zn(II)	Al(III)	Fe(III)
[ML]	7.6	5.5	18.8	13.3	19.8	23.3
[M(HL)]					24.1	27.5
[ML_2]			23.9	17.5	34.0	38.6
[ML(HL)]					39.4	44.4
[$M(HL)_2$]					44.7	48.7

Used pK_a values: 4.62, 10.13 and 12.09. Data from Ref. [80].



Hypothetical planar structure of 2,6-bis-(2'-hydroxyphenyl)pyridine (black) superimposed to ICL670 (red)

More recently, Steinhauser et al. [82] synthesized and characterized the pyridine-based analogue of ICL670 2,6-bis-(2′-hydroxyphenyl)pyridine, expecting that the presence of pyridine nitrogen donor, which is more nucleophilic than the triazole nitrogen atom, would enhance the stability of Fe(III) complexes. Moreover, since "the presence of the central, six-membered pyridine unit further reduces the O···O separation of a hypothetical planar configuration, an even more pronounced selectivity for small metal cations" could be expected. Potentiometric and spectrophotometric investigations on solutions containing the ligand and either Fe^{3+} or Cu^{2+} have been performed in H_2O/DMSO medium (80:20). Preliminary results showed the formation of 1:1 and 1:2 complexes for both cations, and a rough estimate of log β of 36 and 24 was proposed for the cumulative formation-constant of the respective bis-complexes. However, a precise determination of these values has not been possible due to precipitation at pH values higher than 5.

Very recently a series of new bis(hydroxyamino)-1,3,5-triazines (BHTs) has been proposed for selective iron(III) chelation [83]. Protonation equilibria were investigated by titrimetry in a 50% water/methanol solvent. Cumulative formation constants of the $Fe(BHT)_2$ complexes were determined by competition tests against EDTA and successive spectrophotometric analysis in hydro-alcoholic solution after 24 h equilibration. Computed pFe values were as high as 22.3–23.3, which is comparable to the value of 22.5 reported for ICL670 [84]. The species suggested at neutral pH is a FeL_2 complex, but a complete speciation study still has to be performed.

3.4.5. Bidentate ligands

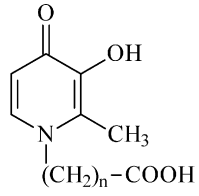
Though hexadentate ligands show the highest affinity and selectivity for Fe(III) ion, there is a growing awareness that small bidentate ligands have many advantages in the research of new chelators for the treatment of iron overload. In fact, concerning the distribution properties of both the free ligand and the corresponding iron complexes, it is relatively simple to design a bidentate ligand following the Lipinski guidelines, especially in terms of molecular weight and H-bond donors and acceptors. On the other hand, one drawback is that the complete saturation of the Fe(III) coordination sphere requires formation of a FeL_3 species, implying that bidentate ligands generally present pFe values significantly lower than those of siderophores (see Section 3.3).

As already pointed out by Hider and Liu [19], only 3-hydroxypyridin-4-ones possess the required high affinity for Fe(III) ion. In the presence of adequate ligand excess, Deferiprone forms the neutral 1:3 complex, which is stable over a wide pH range. The complex completely envelops the coordinated iron and the redox potential is −828 mV (this value, reported by El Jammmal [113], seems really too low with respect to the reduction potentials in Fig. 1, and in contrast with the value −0.620 mV reported by Merkofer [17].): there is virtually no ability to redox cycle under physiological conditions (see Section 3.1). At low concentrations of Deferiprone, the 1:2 complex may also exist at neutral pH. However, recent mass spectroscopic studies have documented that this species is relatively stable and a low ability to generate hydroxyl radicals in the presence of hydrogen peroxide and vitamin C was observed.

A detailed thermodynamic study, recently performed on the Fe(III)/Deferiprone complex-formation equilibria [85], has pointed out that species distribution is practically unaffected by a change in temperature (25 or 37 °C) and ionic strength (0.1 or 1.0 M, KCl).

In order to improve chelation efficacy, considerable effort has been applied to the design of novel hydroxypyridinones with enhanced pFe values.

The most recent results are those reported by Santos et al. [86] who investigated a set of three *N*-carboxyalkyl 3-hydroxy-4-pyridinones.



N-carboxyalkyl 3-hydroxy-4-pyridinones

Results indicated that the binding affinity and the hydrophilic character decrease on increasing the size of the alkyl chain. The stability constants of the complexes in this study are in the order $\beta_{Fe} > \beta_{Ga} > \beta_{Al}$, and the same trend stands for the corresponding pM values. Among these chelating agents, the *N*-carboxyethyl derivative has the highest affinity towards Fe(III) metal ions (pFe = 21.3), and it could compete with transferrin (pFe = 20.3). However, the exemplificative distribution diagram reported in Fig. 6 clearly shows that a large percentage of bis-complex is present in dilute solution, even with a 10-fold molar excess of the ligand.

The same authors [87] also investigated a series of extra-functionalized 3-hydroxy-4-pyridinone chelators of hard metal

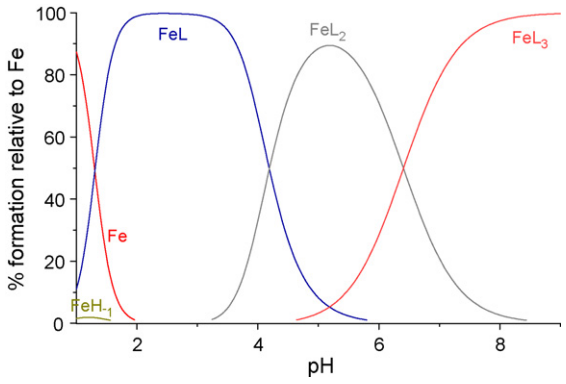


Fig. 6. Distribution of Fe(III)/*N*-carboxyethyl 3-hydroxy-4-pyridinone complexes *vs.* pH at M/L = 1:10 ($C_M = 1.0 \times 10^{-6}$ M). Data from Ref. [86].

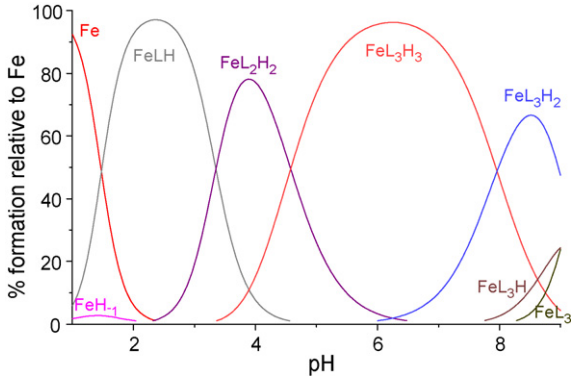
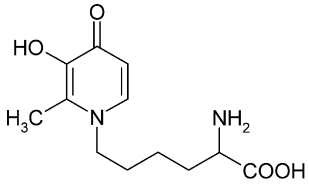


Fig. 7. Distribution of Fe(III) complexes with H_2L^9 *vs.* pH. M/L = 1:10 ($C_M = 1.0 \times 10^{-6}$ M). $T = 25\,^{\circ}C$ and $I = 0.1$ M (KNO_3). Data from Ref. [87].

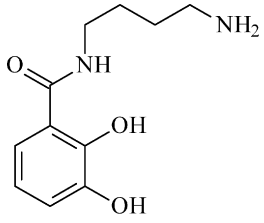
ions, containing different side-chains with peptidomimetic groups. Their first aim was to obtain information about the changes induced by these groups on the physical–chemical properties of the ligands (lipophilicity, acid–base properties and metal-binding affinity towards Fe(III), Ga(III) and Al(III)). These studies were followed by bio-evaluation in mice, either as hard metal chelators or as complexes with [67] Ga. Among the ligands studied in this work, the most promising was 1-(4,4-aminocarboxy-butyl)-3-hydroxy-2-methyl-pyridin-4-one (H_2L^9), which showed the fastest blood clearance, and the highest excretion and *in vivo* stability.



1-(4,4-aminocarboxy-butyl)-3-hydroxy-2-methyl-pyridin-4-one

It presents a pattern quite similar to that of Deferiprone, although with slightly slower excretion through the kidneys probably due to the higher metal affinity. The speciation diagram shows that at neutral pH the most abundant species possess the 1:3 stoichiometry and the Fe(III) metal ion is completely coordinated (see Fig. 7).

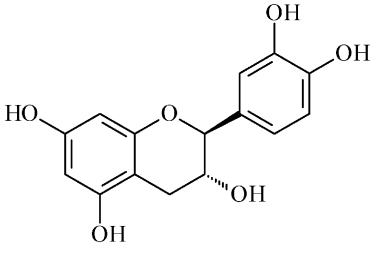
Ligands containing the catechol moieties possess a high affinity for iron(III), but the high charge density of the ionized phenolic functions is associated with high affinity for protons (pK_a values 12.1 and 8.4); therefore the binding of cations by catechol has a marked pH sensitivity. Moreover the complexes forming at pH 7.0 bear a net charge and are consequently unlikely to permeate membranes by simple diffusion. For simple bidentate catechols, the 1:2 complex is the dominant form in the pH range 5.5–7.5. Unsubstituted catechols possess pFe values in the region of 15; hence they are not effective scavengers under *in vivo* conditions. However when two adjacent amide functions are introduced in the dihydroxyterephthalamide derivatives, intramolecular hydrogen bonding generates molecules with pFe values in the range in which iron scavenging is possible. An additional problem with catechol-based ligands is their susceptibility towards oxidation [53].



Aminochelin

A recent study [88] on derivatized catechols concerned aminochelin, a siderophore produced by *Azotobacter vinelandii*. The three protonation constants of aminochelin were determined by simultaneous spectrophotometric and potentiometric titrations ($\log K_1 = 12.1$, $\log K_2 = 10.22$ and $\log K_3 = 7.04$), and the overall stability constant of the 1:3 iron complex was found to be $\log \beta_3 = 41.3$, resulting in a pFe value of 17.6.

Elhabiri [89] reported the results of a study on the complexation of iron(III) by catechin, an abundant catecholate-type polyphenol present in green tea.

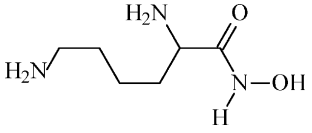


Catechin

Using a combination of electrospray mass spectrometry, absorption spectrophotometry and potentiometry, the authors characterized three ferric complexes of catechin as well as a ternary complex with nitrilotriacetic acid (NTA) added to the medium as an exogenous ligand. The mass spectrometric study clearly pointed out the formation of three mononuclear ferric complexes of catechin, with, one, two and three molecules of catechin, respectively. The values of the stability constants obtained for the iron(III)–catechin complexes are comparable to those determined for iron(III)–catechol complexes. This fea-

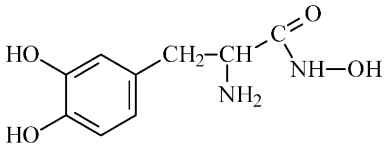
ture strongly suggests that the dihydrobenzopyrane moiety in catechin does not significantly affect the stability of the metal complexes and therefore has no particular electronic or steric effect. The affinity of catechin (pFe = 17.2) for iron(III) is much lower than that of natural siderophores. The presence of NTA leads to the formation of ternary species which improve the global sequestering ability (catechin + NTA: pFe = 18.9). NTA can be considered a good model of citric acid, which is a physiological iron chelator. Hydroxamates show a lower affinity for iron(III) than catechol. However, it has the advantage of forming neutral *tris*-complexes with iron(III), capable in principle of permeating membranes by non-facilitated diffusion [19]. The selectivity of hydroxamates, like catechols, favours tribasic cations over dibasic cations. Because of the relatively low protonation constant ($pK_a \sim 9$), hydrogen ion interference at physiological pH is less pronounced than that of catechol ligands; consequently the 1:3 complex predominates at pH 7.0, when sufficient ligand is present. However, the affinity of a simple bidentate hydroxamate ligand for iron is insufficient to solubilize iron(III) at pH 7.4 at clinically achievable concentrations. As the pFe of bidentate hydroxamates is very low, it is likely that only tetradentate and hexadentate hydroxamates will be effective iron(III) scavengers under *in vivo* conditions.

Wirgau et al. [90] reported a deep investigation on the behaviour of L-lysinehydroxamic acid (LysHA) towards the Fe(III) ion. *N*-Methylacetohydroxamic acid and acetohydroxamic acid are characterized by overall formation constants with Fe(III) much lower than L-LysHA, due to one or more of the five major differences among the two hydroxamic acids, and LysHA. These are: (i) the lower pK_a value for the chelating hydroxamate group in LysHA, (ii) the higher overall positive charge on the Fe-LysHA complex, (iii) the presence of an α-amine moiety in the Fe-LysHA complex, and (iv) the *N*-substituent, (v) the *tris*-complexes for N-methylacetohydroxamic acid and acetohydroxamic acid have a neutral charge, whereas the tris complex for LysHA has a large positive charge (+6).



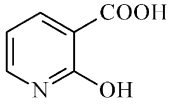
L-lysinehydroxamic acid

This net positive charge leads to a significant charge–charge repulsion and to an overall decrease in the stability of its *tris*-complex. LysHA was found to bind Fe(III) exclusively through the hydroxamate moiety, leaving the two amine groups free to potentially act as recognition agents.

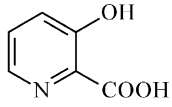


Dopaha

A direct comparison between the complexing ability of catecholate and hydroxamate moieties has been possible by inspecting the solution behaviour of 2,3-dihydroxyphenylalanine-hydroxamic acid (Dopaha) [91]. Potentiometric, spectrophotometric and ^{1}H NMR measurements have been reported for the complexes of Fe(III), Al(III) and Mo(VI) with Dopaha as well as for binary (acetohydroxamic acid, α-alaninehydroxamic acid and 1,2-dihydroxy-3,5-benzenedisulphonate) and ternary model systems. With Fe(III), Al(III) even at a 1:10 metal-to-ligand ratio, precipitation occurs below pH 5.5 but the precipitates dissolve above pH 9. Even if precipitation prevented the calculation of the stability constants of metal complexes, some information about the coordination modes was provided by spectroscopic measurements: amine-type coordination was not detectable at all, while hydroxamate-type coordination was found with both metals below the precipitation pH, and catecholate-type coordination was exclusively found after dissolution.

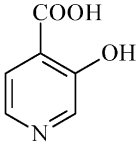


2-hydroxynicotinic acid (HNic)

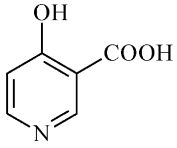


3-hydroxypicolinic acid (HPic)

Hydroxynicotinic (HNic) and hydroxypicolinic (HPic) acids have been investigated as possible iron-chelating agents by Di Marco et al. [92]. Both ligands form very stable complexes with iron at acidic pH values. HPic can inhibit the hydrolysis of the metal also at physiological pH values, while for HNic the precipitation of hydroxide begins at pH 5. A large number of mononuclear complexes are formed in both cases.



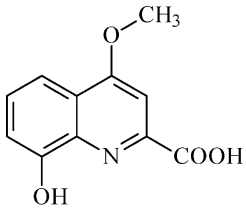
3-hydroxy-4-pyridinecarboxylic acid (3H4P)



4-hydroxy-3-pyridinecarboxylic acid (4H3P)

The investigation was extended to 3-hydroxy-4-pyridinecarboxylic acid (3H4P) and 4-hydroxy-3-pyridinecarboxylic acid (4H3P) [93]. The chemical interactions of these ligands with Fe(III) were investigated by means of potentiometric titrations and UV–vis spectrophotometry. A large number of mononuclear complexes were formed in solution; one of the Fe–3H4P species was

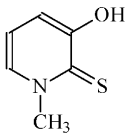
obtained as a solid compound and characterised by elemental analysis. Both 3H4P and 4H3P form strong complexes in solution with iron(III). The speciation in the two iron–ligand systems is very similar at acidic pH values. At neutral pH values the presence of FeL_3 is significant for the Fe–3H4P system, while FeL_3H_3 and FeL_3H_2 still prevail for the Fe–4H3P system. The precipitation of iron hydroxide started at neutral pH in all titrations. The speciation patterns of the two ligands with iron(III) are similar to each other and also to those previously obtained for aluminium(III). However, the relative complexation strength of 3H4P and 4H3P in comparison with other hydroxypyridinecarboxylic acids was unexpectedly low, and in any case much lower than that of Deferiprone.



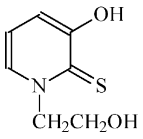
Quinolobactin

Quinolobactin [94] is a new siderophore produced by a pyoverdine deficient mutant of *Pseudomonas fluorescens*. The protonation constants of quinolobactin were determined by potentiometric titrations (5.50 and 10.30). Its complex-formation equilibria with iron(III), studied by means of spectrophotometric and potentiometric titrations, gave the following overall stability constants: $\log \beta_{111} = 18.60$, $\log \beta_{121} = 32.60$, $\log \beta_{120} = 28.20$, resulting in a pFe value of 18.2. Quinolobactin forms a complex of the 1:2 stoichiometry at physiological pH. The UV–vis spectroscopic parameters of FeL_2 agree with a complex containing two ligands coordinated to one Fe^{3+} cation through the oxygen and nitrogen quinoline atoms. There is no evidence of coordination by the carboxylate group, presumably because it could be unfavoured to achieve an octahedral environment around Fe(III). The carboxyl group do not enhance the efficiency of the ligand to chelate Fe(III), but it can favour aqueous solubilization both of the ligand and of its ferric complex.

Two kinds of 3-hydroxypyridine-2(1*H*)-thiones were recently synthesized by Katoh et al. [95].

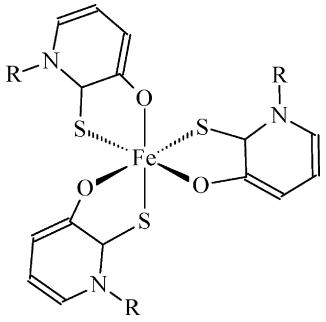


(**a**) 3-hydroxy-1-methylpyridine--2(1H)-thione



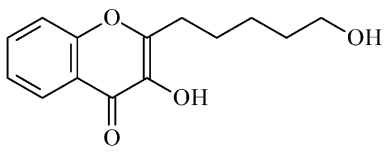
(**b**) 3-hydroxy-1-(2-hydroxyethyl) pyridine--2(1H)-thione

The pK_a of compound **a** was calculated to be 9.7 from the pH at the midpoint of neutralization. The visible spectroscopic analysis indicated that both **a** and **b** formed stable 1:3 Fe(III) complexes. The stability constant of the Fe(III)–**b** complex was estimated from the competitive reaction with EDTA and was found to be $\log \beta_3 = 36.7$. A structural hypothesis for this species is



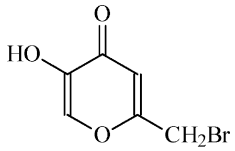
Unfortunately, a stability constant could not be measured for the Fe(III)–**a** complex due to its low solubility in water. 3-Hydroxypyranones are strong iron(III) chelators and like hydroxypyridinones they form neutral 1:3 complexes with iron(III). As the electron density is lower than the corresponding 3-hydroxypyridin-4-ones, the affinity for iron is somewhat lower; typically the pFe values falling close to 15 [19].

The complexing properties towards the Fe(III) ion of the potentially tridentate 3-hydroxy-2-(5-hydroxypentyl)-4*H*-chromen-4-one have recently investigated by Kong et al. [96]. The pK_a value and iron affinity constants have been determined by spectrophotometry using aqueous methanol solutions. The extrapolated affinity constants $\log \beta_1$, $\log \beta_2$, and $\log \beta_3$ for iron(III) in aqueous solution were 9.95, 18.69, and 26.02, respectively, with a corresponding pFe value of 14.6. Job plot and mass spectra data demonstrated that the 1:3 species is favoured at pH 7.4, under high excess of ligand. These results indicate that 3-hydroxy-2-(5-hydroxypentyl)-4*H*-chromen-4-one acts as a bidentate ligand when coordinated to iron(III). No evidence has been obtained for the tridentate mode over the pH range 2–9. The suggested reason for this observation was that the side chain aliphatic oxygen remained protonated over this pH range, thus being ineffective as an iron(III) ligand. Spectroscopic data showed that the iron(III) complex is relatively unstable at pH 7.0. Presumably, the hydroxyl anion slowly competes with the ligand, leading to the formation of iron hydroxides.



3-hydroxy-2-(5-hydroxypentyl)-4H-chromen-4-one

A further hydroxypyranone, the bromokojic acid (5-hydroxy-2-(bromomethyl)-4*H*-pyran-4-one), has recently been investigated [97].



bromokojic acid

The formation of iron(III) complexes was investigated in aqueous solutions as a function of the pH and by UV–vis titrations. The investigation was performed only in the acidic pH range, most likely due to precipitation. Only variously hydroxylated mono- and bis-complexes were found. The FeL_3 complex was never detected in a measurable concentration even using a high ligand/Fe(III) molar ratio (30:1).

4. Development of iron chelators

The development of iron chelators involves particular and rigorous steps. The first step is the definition of the chemical properties of the ligand and of the ligand–iron complex, while the second consists of cellular studies using red blood cells, reticulocytes, cell lines and primary cell cultures (hepatocytes, myocardial and reticuloendothelial cells). The safety and the efficacy of iron chelators are then tested on animal models, both non-iron and iron-overloaded. Acute and long-term toxicity studies in different species of animals are essential for the planning of clinical trials.

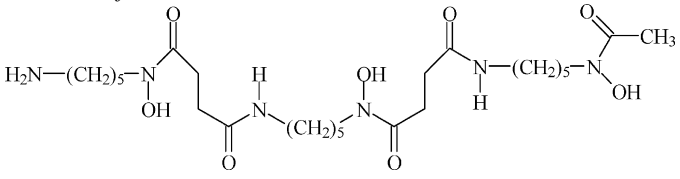
When the above steps have been successful, registration as a new drug requires, Phases I, II and III clinical trials, performed according to rigorous requirements established by national health agencies, in order to define the safety and pharmacokinetic and pharmacodynamic characteristics of the chelator in humans, to find the therapeutic dose, and to evaluate its tolerability and efficacy compared with the reference drug [98].

Once a product is marketed, pharmacovigilance evaluates and improves the safety of drugs, to establish and review the risks and benefits of the new iron chelators.

4.1. Current drugs in use

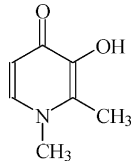
Nowadays iron overload diseases are treated with three different drugs, Desferal, which is given by subcutaneous infusion, and the two oral drugs Deferiprone and Exjade.

4.1.1. Desferal



Desferal was the first drug. It was introduced in the 1970s to treat iron overload. Its advantages and drawbacks are thoroughly illustrated elsewhere [1].

4.1.2. Deferiprone



Deferiprone, also known as L1 or Ferriprox, has been the first orally active iron chelator available for clinical use. It was licensed in India in 1995 and then in Europe in 2000. At present, DFP is available in about 50 countries. DFP is given by mouth two or three times per day, taking into account its partial transformation to the non-chelating *o*-glucuronide form. A stable decrease in serum ferritin and liver iron concentration in long-term therapy has been demonstrated in most transfusion-dependent patients [99]. Negative side effects are gastrointestinal symptoms (e.g., nausea, vomiting, gastric discomfort), arthralgia, zinc deficiency and, more seriously but with an incidence of 0.6 per 100 patient-years, agranulocytosis [100]. Treatment with DFP has shown an improvement in cardiac magnetic resonance imaging, due to a reduction in cardiac iron overload and improved cardiac function compared to patients treated with DFO [101–103].

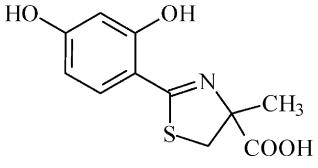
4.1.3. Exjade

After its approval in November 2005, Exjade, *N*-substituted bis-hydroxyphenyl-triazole (ICL670) became the second oral iron chelator in clinical use. It emerged from among more than 700 chelators using vigorous selection criteria as the compound that best combines high oral potency and tolerability in animals [104]. From a Phase I clinical evaluation, its good tolerability emerged with no major safety alarms up to 80 mg/(kg day). Iron excretion, almost entirely in the faeces, is dose-dependent. Its plasma half-life (11–19 h) allows a once daily oral dose. A 12-month Phase II study assessed the tolerability and efficacy of ICL670 compared to DFO. From Phase III clinical trials it emerged as an effective oral iron chelator suitable for long-term use [105].

4.2. Drugs in progress

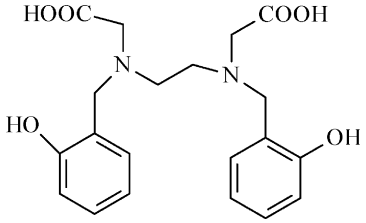
4.2.1. Desferrithiocin and analogs

Desferrithiocin, siderophore originally isolated from *Streptomyces antibioticus* in 1980, is now produced by chemical synthesis. DFT is a tridentate oral chelator with high affinity for Fe(III). The tests on iron overloaded rats demonstrated an effective reduction in liver ferritin iron. No relevant acute toxic effects were shown, but longer exposure in rats produced degenerative changes in the kidney, probably due to toxic effects of the Fe(III) complex. Among several DFT analogs synthesized and tested in animals, 4-OH-desaza-desmethyldesferrithiocin (Deferitrin or GT56-252) appeared the less toxic while remaining biologically active.



In Phase I trials in adults with β-thalassemia, Deferitrin promoted iron excretion in a dose-related manner and was well tolerated. There were no serious adverse events. Deferitrin may be useful in chelation monotherapy or in combined chelation therapy if its favourable pharmacokinetic profile, efficacy, safety and tolerability are confirmed by the more extensive Phase II clinical trials [106,107].

4.2.2. Hydroxybenzyl-ethylenediamine diacetic acid (HBED)



HBED is a hexadentate phenolic aminocarboxylate chelator, synthesized more than 30 years ago, that forms a 1:1 complex with Fe(III) with high affinity and selectivity. Initial studies in rodents showed its capacity to excrete radiolabeled iron both when administered parenterally and orally. Subsequent evaluation in iron-loaded primates and humans revealed low oral activity, too low to be of value for oral treatment of iron overload. A recent preclinical evaluation by Bergeron et al. [108] on the efficacy and safety of its monosodium salt showed an almost twice efficiency compared to DFO in promoting iron excretion. Na-HBED may prove to be an alternative to DFO in the treatment of iron overload, although it too requires parenteral administration.

4.2.3. Pyridoxal isonicotinoyl hydrazone (PIH)

Pyridoxal isonicotinoyl hydrazone is an aromatic hydrazone produced by chemical synthesis and identified as an effective iron chelator in 1979. PIH and some of its analogues were shown to be potent inhibitors of ROS production. Various PIH analogues, such as pyridoxal-benzoyl hydrazones, are more effective (280%) than PIH [109]. Progress in the development of PIH and its derivatives was in the beginning very slow because of the discouraging results in humans (“. . .patients treated with 30 mg/(kg day) of PIH have shown a modest net iron excretion of 0.12 ± 0.07 mg/(kg day), which is much less than the 0.5 mg/(kg day) that is required, on average, to achieve negative iron balance in regularly transfused patients” [99]) and for the lack of patent protection [110]. A remarkable growth of interest due to their properties as antiproliferative agents started successively, as discussed in Section 3.4.4.

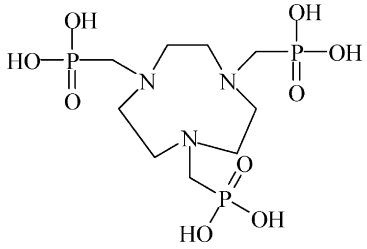
4.2.4. 40SD02

40SD02 is a new drug that results from attaching DFO to a modified starch polymer. This change gives a prolonged half-life, while preserving its affinity and specificity for Fe(III). It prevents the acute toxic effects of DFO, such as hypotension [111].

A Phase I study on 10 thalassemic patients with chronic iron overload showed that single doses, up to 600 mg/kg, were safe and well tolerated, and stimulated significant iron excretion. Average total iron excretion over 7 days was 0.46 mg/kg and 0.72 mg/kg in the 150 and 300 mg/kg dose groups, respectively. In a successive Phase Ib clinical trial 12 patients were treated, 4 patients at 150 mg/kg, 4 at 300 mg/kg, and 4 at 600 mg/kg: no adverse effects were described. At the highest dose level of 900 mg/kg, a single infusion induced a cumulative urinary iron excretion of 0.84–1.93 mg/kg for 7 days following drug administration.

4.2.5. IRC011

IRC011, synthesized in Israel in 1996, is a hexadentate chelator with stability constant 1000 times greater than DFO. Ten times less toxic than L1, it resists *in vivo* biotransformation [112]. Its water



solubility and limited membrane permeability are negative points. It may therefore be regarded as a parent compound.

5. Conclusions

We concluded our previous review [1] suggesting that: “The failure to find the ideal iron chelator can in our opinion be ascribed to difficulties inherent in the problem due to biological and clinical restraints. This is true despite the fact that it is a project capable of bringing together researchers in basic sciences and medical doctors involved in clinical practice. Chemists have used their knowledge to synthesize a large variety of iron chelators according to the structural requisites for their introduction in clinical practice. Nevertheless the clinical results have not been satisfactory. We hope that the dialogue among chemists and clinicians will lead to the common target of prolonging survival and improving the quality of life of iron-loaded patients.” We think that significant improvements have been made in the cure of iron overload with the introduction of Deferiprone and Exjade in the last decade, and with that of combined chelation therapy, but above all with a deeper knowledge of iron metabolism, drug targets, drug absorption mechanisms, the relationships between structure and physical–chemical properties, and the basic requirements for the different clinical purposes. Applications as anticancer drugs and against neurodegenerative disease will be the great challenge for the future which will certainly give a synergistic development in the search for the best iron chelators.

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