A New Iron(III) Ion Sequestering Ligand: Synthesis, Solution Chemistry and Electrochemistry†

M. Amélia Santos,* M. Alexandra Esteves, M. Cândida T. Vaz and M. L. S. Simões Gonçalves
Centro de Química Estrutural, Complexo I, Instituto Superior Técnico, 1096 Lisboa Codex, Portugal

A new cyclic diaminodihydroxamic ligand, piperazine-1,4-bis(*N*-methylacetohydroxamic acid) (H_2L^2), presenting a reasonable analogy with the naturally occurring siderophore rhodotorulic acid (H_2L^1), has been prepared through an easy two-step process. This ligand, which has proved to be biologically active, forms a very stable complex $[Fe_2L^2{}_3]$. The ligand and its iron and copper complexes have been characterized by potentiometric and spectrophotometric techniques. The mechanism of electron transfer in the complexes has been studied by voltammetric methods, and the kinetics of dissociation of the iron(II) complex, which might be of crucial importance in the biological activity of this siderophore analogue, was also investigated.

Iron is an essential element of all living systems but its supply is restricted by the extreme insolubility of iron hydroxide in water at neutral pH. For this reason microorganisms produce a class of molecules, siderophores, which selectively bind and transport iron from the environment into the cell.[1] Generally, siderophores contain catecholate and hydroxamate ligands for chelation to iron(III). The hydroxamate group is one of the most common found in siderophores produced by molds, fungi and yeast. These compounds are predominantly trihydroxamic acids, such as ferrichrome and ferrioxamine which form very stable complexes with iron(III). However, some are dihydroxamic acids, as rhodotorulic acid (H_2L^1), forming very stable dinuclear complexes $[M_2L_3]$.

There has been considerable interest in the development of new siderophore analogues.[2–5] However, some of them have limitations as a consequence either of their water insolubility or/and considerable preparative difficulties. In this paper we report a new cyclic diaminodihydroxamic acid H_2L^2 which is biologically active[6] and seems to be a physicochemical model of rhodoturulic acid, a dihydroxamic acid produced by *rhodotorula pilimane*.[7] The inversion of the hydroxamate sequence relative to rhodotorulic acid seems to be practically irrelevant since the activity of the retro isomer of ferrichrome is similar to that of ferrichrome.[8] This work includes the synthesis and characterization of the ligand piperazine-1,4-bis(*N*-methylacetohydroxamic acid), the determination of its acid–base properties, the thermodynamic characterization of iron(III) and copper complexes by potentiometric and spectrophotometric titrations, the study of electron-transfer mechanisms and the kinetics of dissociation of the iron(II) complex by voltammetric methods. Molecular models suggest for the complex $[Fe_2L^2{}_3]$ the structure shown in Fig. 1, two piperazine rings having a distorted-boat conformation and the other one a chair conformation.

Results and Discussion

Acid–Base Properties.—The ligand H_2L^2 has four acid–base centres: two in hydroxamate groups and two in amino groups. From the potentiometric titration of the completely protonated form of the ligand with sodium hydroxide [Fig. 2(*a*)] it can be seen that four protons are being titrated by each molecule of the ligand, with the partial ionization constants pk_1^H = 2.44 ± 0.06, pk_2^H = 6.67 ± 0.02, pk_3^H = 8.45 ± 0.02 and pk_4^H = 9.53 ± 0.02.

Although preliminary ^1H NMR titration studies and

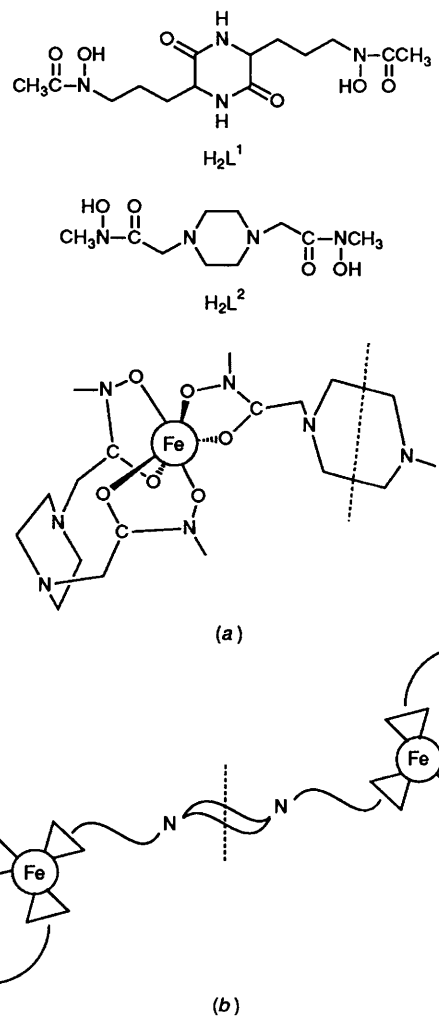

Fig. 1 Structural formulae of rhodotorulic acid (H_2L^1), the synthetic dihydroxamate ligand (H_2L^2) and the proposed structure for the iron(III) complex $[Fe_2L^2{}_3]$ [(*a*) molecular model, (*b*) schematic representation]

† *Non-SI unit employed:* G = 10^{-4} T.

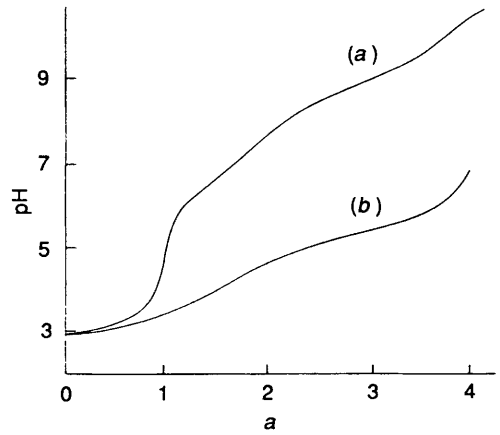

Fig. 2 Potentiometric titration curves for the ligand H_2L^2 (*a*) and its 1:1 copper complexes (*b*) at $I = 0.10$ mol dm^{-3} (KNO_3), 25.0 ± 0.1 °C; *a* = moles of base added per mol of ligand present, $c_L = c_{Cu} = 2 \times 10^{-3}$ mol dm^{-3}

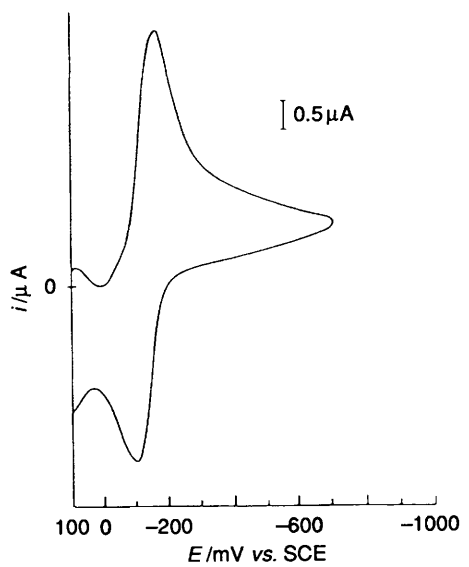

Fig. 3 Cyclic voltammograms for the Cu^{2+}–H_2L^2 complex at pH 7.0 in water [$c_L/c_{Cu} = 20$, $c_{Cu} = 2 \times 10^{-4}$ mol dm^{-3}, $v = 100$ mV s^{-1}, $I = 1$ mol dm^{-3} (KNO_3), 25 °C]

literature[9,10] pk^H values for molecules with some similarity suggest the attribution of the first two constants to the protonated amino groups, for the sake of clarifying this sequence of protonation ^{1}H NMR and potentiometric titrations of several model molecules are underway.

Copper Complexation.—The stability constants for the copper complexation were determined from potentiometric titration [Fig. 2(*b*)], using the SUPERQUAD program, at $c_M = c_L = 1 \times 10^{-3}$ mol dm^{-3} as well as other values such as $c_M = 5 \times 10^{-4}$, $c_L = 1 \times 10^{-3}$ and $c_M = 1 \times 10^{-3}$, $c_L = 2 \times 10^{-3}$ mol dm^{-3}. It was found that copper can form three complexes, $Cu(H_2L^2)$, $Cu(HL^2)$ and CuL^2, for which the stability constants $\log \beta_{Cu(H_2L^2)} = 23.66 \pm 0.06$, $\beta_{Cu(HL^2)} = 18.55 \pm 0.04$ and $\beta_{CuL^2} = 12.50 \pm 0.06$ have been determined {$\beta_{Cu(H_nL^2)} = [CuH_nL^2]\,[Cu][H]^n[L^2]$}.

Considering the similarity between $\lambda_{max} = 651$ nm of the copper complex of this ligand, and $\lambda_{max} = 672$ nm for the copper complex with a monomeric hydroxamic acid without any amino group (chloro-*N*-methylacetohydroxamic acid), both at pH ≈ 6–6.5, it might be suggested that the co-ordination of the CuL^2 species should involve the hydroxamate groups. However, a different type of co-ordination to Cu^{II} involving the amino groups and the N or O atoms of the

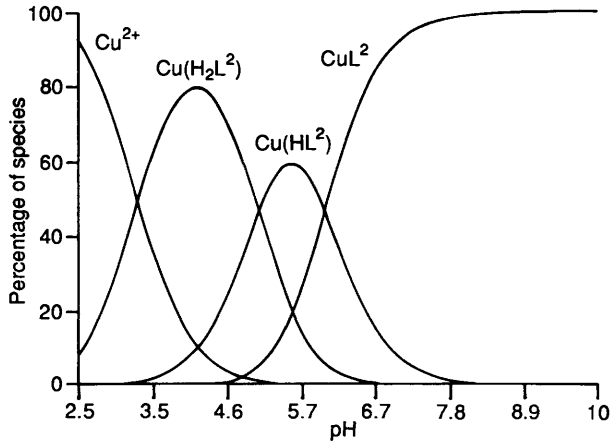

Fig. 4 Speciation curves for Cu^{2+}–H_2L^2 complexes as a function of pH ($c_L/c_{Cu} = 20$, $c_{Cu} = 2.5 \times 10^{-4}$ mol dm^{-3})

hydroxamate groups is also possible, as has been suggested[11] for other aminohydroxamic acids.

From the voltammetric experiments (Fig. 3) at scan rates (v) between 100 and 900 mV s^{-1} it has been noticed that the copper complex at pH 7.0 exhibits a reversible one-electron couple ($\Delta E_p = 60$ mV) at $v < 500$ mV s^{-1}, but quasi-reversible at higher values of v, since ΔE_p increases and $i_p^c/i_p^a > 1$. So copper(II) complexes are being reduced to copper(I) complexes.

Since at $v = 100$ mV s^{-1} the electrochemical system is practically reversible, a study has been undertaken at these experimental conditions for different ligand concentrations, varying c_L/c_M from 10 to 40:1. It was observed that E_p^c and E_p^a do not change with this ratio, being −200 and −140 mV respectively. This seems to indicate that the copper is labile within the time-scale of the experiment, having the same co-ordination number for the oxidized and reduced forms, or that the charge-transfer reaction involves the complex itself.

Using the stability constants determined by potentiometry, a speciation calculation has been undertaken under the conditions of the voltammetric experiments ($c_L/c_M = 20:1$) for different pH values. From Fig. 4 it can be seen that at pH 7.0 there is about 88.7% CuL^2, 11.2% $Cu(HL^2)$ and 0.2% $Cu(H_2L^2)$ species.

It has also been checked that E_p^c is independent of pH at pH < 6.5 and shown that a plot of E_p^c *versus* pH is linear with slope −61 ± 3 mV for pH ≥ 7.0. Considering these variations and the fact that in terms of the ligand the co-ordination number is the same for copper-(I) and -(II), it can be concluded that the only complex of copper(I) is of the $Cu(HL^2)$ type. Indeed, under these experimental conditions, neglecting the presence of $Cu(H_2L^2)$ species, equation (1) is applicable where

$$E_p^c - E_p^s = -\frac{RT}{F}\ln\left(1 + \frac{1}{\beta^H[H]}\right) - \frac{RT}{F}\ln\frac{\beta^{II}_{Cu(HL^2)}}{\beta^{I}_{Cu(HL^2)}} \quad (1)$$

$\beta^{II}_{Cu(HL^2)}$ and $\beta^{I}_{Cu(HL^2)}$ are the stability constants of the copper-(II) and -(I) $Cu(HL^2)$ complexes and $\beta^H = 10^{6.1}$ dm^3 mol^{-1} is the formation constant of the copper(II) complex [$CuL^2 + H^+ \rightleftharpoons Cu(HL^2)$].

According to expression (1) it can be seen that E_p^c is independent of pH for pH < 6.1 where $1/\beta^H[H^+] < 1$, and is linearly dependent on pH with slope ≈ −60 mV for pH ≥ 6.1 where $1/\beta^H[H^+] > 1$, which agrees with the experimental results. On the other hand it seems reasonable that copper(I) predominantly forms a complex of $Cu(HL^2)$ type because, due to the soft character of this cation, it interacts more strongly with nitrogen than with oxygen atoms that can be protonated.

Since the reduction $Cu^{II} \longrightarrow Cu^{I}$ is not possible in a non-complexing medium, the value of the peak potential for this reduction has to be estimated from $E_p^s = E_{\frac{1}{4}} - (28.5/n)$ mV.

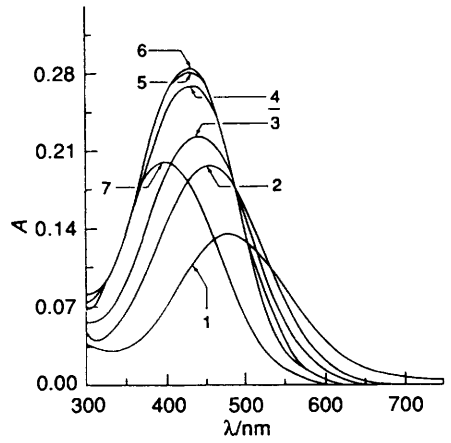

Fig. 5 Absorbance spectra of the Fe^{3+}–H_2L^2 solution as a function of pH; $c_{Fe} = 2 \times 10^{-4}$, $c_L = 2 \times 10^{-3}$ mol dm^{-3} (25 °C, 0.10 mol dm^{-3} KNO_3). Equilibrium pH values: 1.98 (1), 3.28 (2), 3.86 (3), 4.82 (4), 6.25 (5), 9.38 (6) and 11.02 (7)

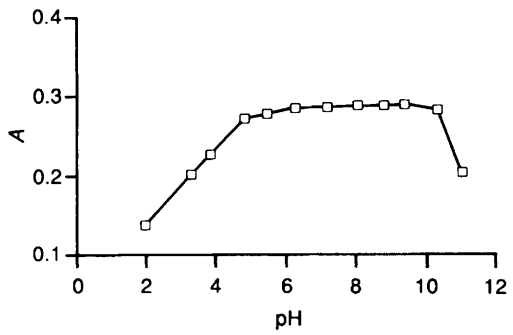

Fig. 6 Variation of the absorbance of Fe^{3+}–H_2L^2 solution as a function of pH at $\lambda_{max} = 425$ nm; $c_{Fe} = 2 \times 10^{-4}$, $c_L = 2 \times 10^{-3}$ mol dm^{-3}

$E_{\frac{1}{2}}$, the halfwave potential of d.c. polarography, can be expressed as in equation (2), where D_O and D_R, f_O and f_R are,

$$E_{\frac{1}{2}} = E° - 2.3\frac{RT}{nF}\log\frac{f_R}{f_O} - 2.3\frac{RT}{nF}\log\left(\frac{D_O}{D_R}\right)^{\frac{1}{2}} \quad (2)$$

respectively, the diffusion coefficients and activity coefficients of the oxidized and reduced species, $E_{\frac{1}{2}}$ and $E°$ being referred to the same reference electrode. Assuming $D_O = D_R$, which is reasonable since both species are in solution, calculating the activity coefficients f_R and f_O according to the Davies[12] expression at an ionic strength of 1 mol dm^{-3}, and using the standard potential from the literature, we get $E_p^s = -134$ mV in relation to the calomel electrode. As $E_p^c = -200$ mV, from equation (1) and using $\beta^{II}_{Cu(HL^2)}$ and β^H from potentiometric results, upon making the necessary corrections for the ionic strength we calculate $\log \beta^{I}_{Cu(HL^2)} = 16.5 \pm 0.2$. So, the copper(I) complex is less stable than the copper(II) complex.

Iron Complexation.—In order to study the interaction of iron(III) with H_2L^2 the absorption spectra in the visible range of the complexes generated *in situ* ($c_{Fe} = 2 \times 10^{-4}$ and $c_L = 2 \times 10^{-3}$ mol dm^{-3}) at pH values between 1.98 and 11.62 have been studied. Fig. 5 suggests the presence of different complexed species at different pH values. However, between pH 3 and 9 an isosbestic point seems to suggest that only two species exist in solution. Between pH *ca.* 6.3 and 9.4 there is a dominant species in solution with $\lambda_{max} = 425$ nm and with ε_{max} about 2880 dm^3 mol^{-1} cm^{-1}, in terms of iron concentration (Fig. 6). These data compare well with literature values for co-ordination of three hydroxamate groups, including trihydroxamate ligands ([FeL], $\lambda_{max} = 423$ nm, $\varepsilon_{max} = 2700$ dm^3 mol^{-1} cm^{-1} per Fe),[13] dihydroxamate ligands ($[Fe_2L_3]$, $\lambda_{max} = 424$ nm, $\varepsilon_{max} = 3000$ dm^3 mol^{-1} cm^{-1} per Fe)[2] or monohydroxamate ligands ($[FeL_3]$, $\lambda_{max} = 426$ nm, $\varepsilon_{max} = 2410$ dm^3 mol^{-1} cm^{-1} per Fe),[14] which suggest that three hydroxamate groups might be involved in the complex of Fe^{III} with H_2L^2. Besides, a general rule has been accepted[2] according to which absorptivity of an iron(III) hydroxamate is about $1000n$ dm^3 mol^{-1} cm^{-1}, n being the number of hydroxamate groups found per iron(III) ion. Therefore we can assume that the dominant absorbing species for $c_L/c_M = 10:1$ and pH between 6.3 and 9.4 is probably of the type $[M_2L_3]$.

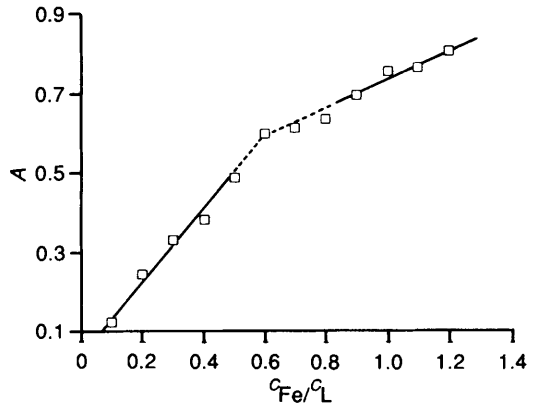

Fig. 7 Plot of the absorbance of the iron(III) complex at $\lambda_{max} = 425$ nm as a function of the ratio of c_{Fe}/c_L at pH 7.0

As the pH is lowered, from about 5 to 2, a bathochromic shift is observed (the solution became more reddish) as well as a decrease in the maximum absorbance (*e.g.* for pH 3.3, $\lambda_{max} = 456$ nm and $\varepsilon = 2020$ dm^3 mol^{-1} cm^{-1}). An isosbestic point is observed in the range pH *ca.* 3–9, at about $\lambda_{max} = 475$ nm. These results seem to suggest that the two species present in solution are $[Fe_2L^2{}_3]$ and a protonated species having λ_{max} higher than 425 nm (λ_{max} of $[Fe_2L^2{}_3]$). So when the pH decreases the equilibrium (3) is possible.

$$[Fe_2L_3] + 2H^+ \rightleftharpoons [Fe_2H_2L^2{}_3]^{2+} \quad (3)$$

At pH 11 another species is formed with $\lambda_{max} = 400$ nm and $\varepsilon_{max} = 2040$ dm^3 mol^{-1} cm^{-1}, perhaps due to hydrolysis, where only two hydroxamates are bound to the metal, since the spectrum does not cross the others at $\lambda = 475$ nm (isosbestic point).

The stoichiometry of the dominant iron complex at pH 7.0 was studied by plotting the absorbance at λ_{max} 425 nm as a function of the mole ratio of iron(III) per ligand between 0.3 and 1.5, for $c_L = 10^{-4}$ mol dm^{-3}, at pH 7.0 and ionic strength $I = 0.1$ mol dm^{-3} (KNO_3) (Fig. 7). It can be seen that under these experimental conditions the dominant species is of the type 2:3, $[Fe_2L^2{}_3]$.

The formation constant of the iron(III) complex of H_2L^2 cannot be determined directly from potentiometric titration data, since Fe^{III} forms very stable complexes with dihydroxamates and iron(III) hydroxo-species are probably formed together with the H_2L^2 complexes, consistent with the fact that better statistical parameters are obtained if the last points at higher pH values are neglected. So we tried to determine the stability constants of $[Fe_2L^2{}_3]$ by visible spectrophotometry using a competiton reaction with ethylenediaminetetraacetate (edta) at a fixed pH (7). The advantage of this procedure is that there is always an excess of ligand and so the formation of iron(III) hydroxo-species is less probable.

First we have used a ratio $c_L/c_{Fe} = 10:1$ and added edta (c_L/c_{edta} was about 7, 8 and 9:1). After each addition there is a decrease in the absorbance at the λ_{max} of the iron(III) complex with H_2L^2 ($\approx$425 nm) due to the competition of edta (Fig. 8). If we assume that $[Fe_2L^2{}_3]$ is the dominant species in solution before adding edta, after the addition of this ligand the

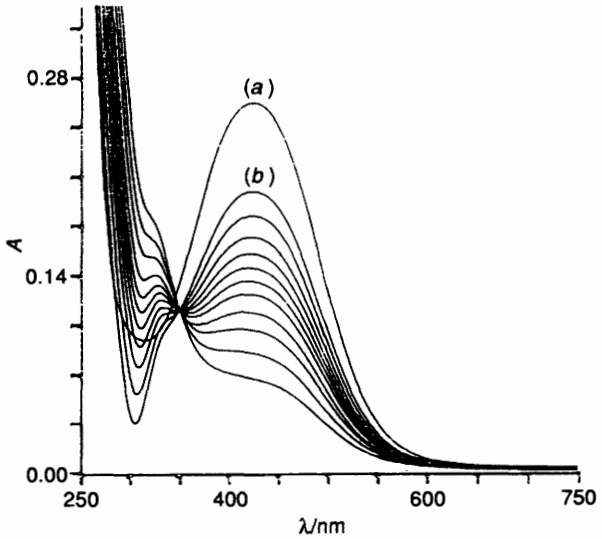

Fig. 8 Absorbance spectra of the $[Fe_2L^2{}_3]$ complex; $c_L/c_{Fe} = 10:1$, $c_L = 9 \times 10^{-4}$ mol dm^{-3}, pH 7.0. (*a*) Before addition of edta, (*b*) after addition of edta at different times ($c_{edta}/c_L = 1.5:1$)

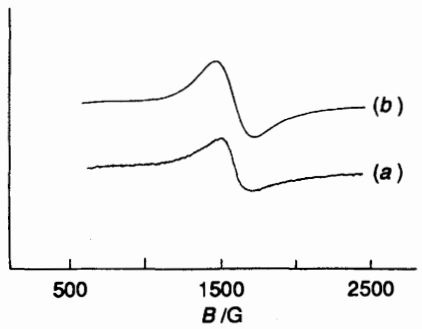

Fig. 9 The EPR spectra of iron(III) complexes with (*a*) H_2L^2 (Fe: L = 2:3), (*b*) chloroacetohydroxamic acid (Fe: L = 1:3) in water; $c_{Fe} = 1$ mmol dm^{-3}, pH 7.0, microwave power = 0.24 mW, temperature = 4.2 K, microwave frequency = 9.42 GHz

chemical reaction is (4). When the equilibrium was attained

$$[Fe_2L^2{}_3] + 2\,Hedta^{3-} + 4H^+ \rightleftharpoons 2[Fe(edta)]^- + 3H_2L^2 \quad (4)$$

(within 2–3 d) we estimated the concentration of the complex $[Fe_2L^2{}_3]$ from the absorbance at $\lambda = 425$ nm. Considering the mass balances, a value of $\log \beta_{23} = 61.7 \pm 0.3$ was obtained for all of the edta concentrations used, which gives support to our assumption. The order of magnitude of this value agrees with the literature[2] for some iron(III) dihydroxamate complexes where $\log \beta^{III}_{Fe_2L_3}$ is between 62.1 and 62.4.

Electron paramagnetic resonance (EPR) results (Fig. 9) also suggest the existence of a dimeric diiron(III) complex with the dihydroxamate ligand H_2L^2. Its spectrum shows a peak at $g = 4.3$, as for iron(III) tris(chloroacetohydroxamate). However, the linewidth for the piperazinedihydroxamate complex (280 G) is larger than that found for the acetohydroxamate complex (200 G), indicating some dipole–dipole interaction, thus supporting a dimeric structure. For the simulated dimer we evaluated an approximate distance Fe ··· Fe of 9.5 Å,* close to the limit (9 Å) proposed by Aasa *et al.*[16] for the existence of significant interactions between high-spin iron(III) ions.

Voltammetric studies of a solution of iron(III) (2.5×10^{-4} mol dm^{-3}) with a ten-fold excess of ligand at pH 7.0 were carried out (Fig. 10). The scan rate effect on the peak height showed that at

* Calculated distance between both hydroxylic hydrogen atoms of the *Z*(*cis*) hydroxamic acid arms of H_2L^2 in the chair conformation.[15]

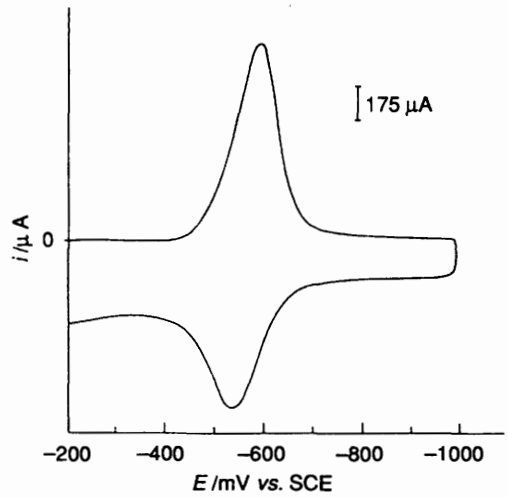

Fig. 10 Cyclic voltammograms for the $[Fe_2L^2{}_3]$ complex at pH 7.0 in water [$c_L/c_{Fe} = 10:1$, $c_{Fe} = 2 \times 10^{-4}$ mol dm^{-3}, $v = 10$ V s^{-1}, $I = 1$ mol dm^{-3}, (KNO_3), 25 °C]

lower scan rates ($v < 1000$ mV s^{-1}) $i_p/v^{\frac{1}{2}}$ is constant, although at higher scan rates an increase was observed, typical of a weak adsorption of the complex. There is also adsorption in the anodic reaction, although less important. Thus $E_p{}^c$ changes cathodically by about 30 mV when v increases to 10 v and $\Delta E_p = 60$ mV at higher scan rates ($v = 20\,000$ mV s^{-1}). On the other hand, at scan rates between 200 and 5000 mV s^{-1}, $i_p{}^a/i_p{}^c$ changes from 0.4 to 0.6, being closer to 1 for $v = 20\,000$ mV s^{-1}.

So, the global mechanism should be of the electrochemical–chemical type[17] with $n = 1$, represented by equations (5) and (6) for the species in solution and by (5a) and (6a) for the

$$O + ne \rightleftharpoons R \quad (5)$$

$$R \longrightarrow Z \quad (6)$$

$$O_{ads} + ne \rightleftharpoons R_{ads} \quad (5a)$$

$$R_{ads} \longrightarrow Z \quad (6a)$$

adsorbed species, the chemical step being practically negligible at higher scan rates ($v = 20\,000$ mV s^{-1}). Therefore, probably iron(III) is reduced to iron(II), both being in complexed form, the latter species being irreversibly dissociated. Since at higher scan rates $\Delta E_p = 60$ mV ($c_M = 2.5 \times 10^{-4}$, $c_L = 2.5 \times 10^{-3}$ mol dm^{-3}, pH 7.0), this suggests the existence of a non-interacting change of two electrons occurring at a similar potential to that corresponding to independent one-electron reduction of each metal site of $[M_2L_3]$ species.[18,19]

On the other hand, under these experimental conditions the slope of $E_p{}^c$ *versus* pH (between 5 and 9) for scan rates between 5000 and 10 000 mV s^{-1} is about 30 mV, which agrees with the stoichiometry (7). This means that the iron(III) complex that is

$$[Fe_2L^2{}_3] + H^+ + 2e^- \rightleftharpoons [Fe_2HL^2{}_3]^- \quad (7)$$

reduced is of the type $[M_2L_3]$, as confirmed by spectrophotometry, and that the iron(II) complex is protonated, therefore less strongly bound to the ligand and afterwards irreversibly dissociated.

At lower scan rates, between 100 and 500 mV s^{-1}, and for $5 < pH < 7$, the slope of $E_p{}^c$ *vs.* pH is higher, increasing to about 60 mV at 100 mV s^{-1}, which means that $E_p{}^c$ is more positive than it should be according to the previous mechanism. The reduction is therefore easier than expected, probably due to the decomposition of the reduced form.

From the constancy of $E_p{}^c$ and $E_p{}^a$ with the change of

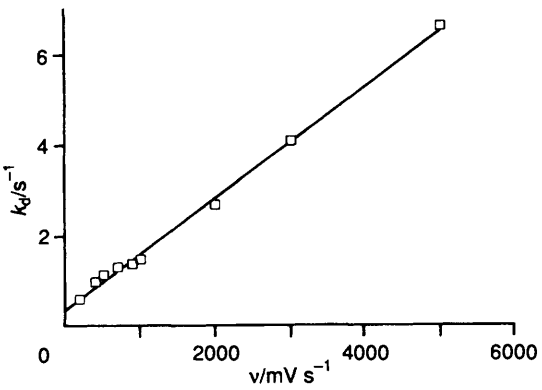

Fig. 11 Variation of the rate constant of dissociation of the Fe^{2+}–H_2L^2 complex with scan rate

concentration of the ligand for ratios c_L/c_M between 10 and 40:1, as was the case with copper, we can conclude that the co-ordination number of the iron-(III) and -(II) complexes is the same in relation to the ligand. At the highest scan rates, where the chemical reaction practically does not occur, we have expression (8) where $\beta^{III}_{Fe_2L^2_3}$ and $\beta^{II}_{Fe_2HL^2_3}$ are, respectively, the

$$\Delta E_p = -\frac{59.15}{2}\log\frac{\beta^{III}_{Fe_2L^2_3}}{\beta^{II}_{Fe_2HL^2_3}} - \frac{59.15}{2}\,pH \qquad (8)$$

global stability constants of the iron-(III) and -(II) complexes.

$$10.69 = \frac{59.15}{2}\left(\log\frac{\beta^{III}}{\beta^{II}} + pH\right) \qquad (9)$$

$$\log(\beta^{III}/\beta^{II}) = 29.14 \qquad (10)$$

Since at 1 mol dm^{-3} ionic strength, $\log \beta^{III}_{Fe_2L^2_3} = 61.7 \pm 0.3$, we have $\log \beta^{II}_{Fe_2HL^2_3} = 32.6 \pm 0.3$, this constant being defined as $[Fe_2HL^2{}_3]/[Fe]^2[H][L^2]^3$.

Kinetic Results.—In order to determine the rate constant of the irreversible dissociation of the iron(II) complex with H_2L^2 it was not possible to use the treatment of Nicholson and Shain[20] since the results at higher scan rates are influenced by adsorption. So a semiempirical method was used according to Polcyn and Shain[19] and the apparent rate constant k_d^{app} estimated at each scan rate. From the extrapolation of k_d^{app} to $v = 0$ mV s^{-1} (see Fig. 11), a value of $k_d = 0.35$ s^{-1} has been determined for the rate constant of dissociation of the iron(II) complex.

The fact that the iron(II) complex with H_2L^2 is quickly dissociated in an irreversible way may be favourable for the mechanism of iron release and might account for the biological activity that has already been found,[6] albeit further studies on the mechanism involved are still in progress. Indeed it has been suggested that for some important siderophores, such as ferrichrome[21] and iron(III) rhodotorulate,[22] the uptake of iron from iron(III) complexes by microorganisms should be favourable through a mechanism, proposed by Leong and Neilands,[23] involving the microorganism cell reduction of iron-(III) to -(II) siderophores with subsequent re-excretion of the free ligand. This mechanism seems to be reasonable in our case, owing to the weakly bound iron(II) complex and its dissociation.

Experimental

Synthesis.—The synthesis to be described here employs an approach whereby chloroacetohydroxamic acid is first obtained in high yield using literature methods[24] and then two hydroxamic acid arms are attached to the cyclic diamine (piperazine) *via* the amine groups. All the reactions were TLC controlled. Analytical reagents were used as supplied. The solvents were dried according to standard methods.[25]

Chloro-N-methylacetohydroxamic acid. A suspension of sodium hydrogencarbonate (1.260 g, 0.015 mol) and *N*-methylhydroxylamine hydrochloride (1.253 g, 0.015 mol) in dry tetrahydrofuran (30 cm^3) was cooled in an ice-bath with stirring under N_2. One hour later chloroacetyl chloride (1.446 g, 0.015 mol) in dry tetrahydrofuran (10 cm^3) was dropwise added over a period of 30 min. The reaction mixture was stirred at 0 °C under N_2 for 1 h and then allowed slowly to reach room temperature. After 4 h the mixture was filtered and the filtrate evaporated under reduced pressure. Recrystallization of the solid residue from diethyl ether gave white crystals (1.183 g, 72%), m.p. 69–71 °C (lit.,[26] gives 70 °C). ^1H NMR (D_2O): δ 3.19 (s, 3 H) and 4.36 (s, 2 H). IR (KBr): 1630 cm^{-1} (C=O).

Piperazine-1,4-bis(N-methylacetohydroxamic acid), H_2L^2. A solution of chloro-*N*-methylacetohydroxamic acid (0.350 g, 3.2 mmol) in water (20 cm^3) was cooled in an ice-bath with stirring and pH neutralized by 3 mol dm^{-3} potassium hydroxide. Then a solution of piperazine (0.138 g, 1.6 mmol) in water (10 cm^3) was added dropwise, with stirring, followed by basification of the reaction mixture (pH ≈ 10). This was then left at room temperature with stirring and continuously adding base solution in order to maintain the pH at around 9.5, for 3 d. The reaction solution was then acidified to pH 6 in a cool medium with 3 mol dm^{-3} hydrochloric acid and concentrated in vacuum with isolation of successive fractions of inorganic precipitates. The final organic solid residue was dried in vacuum and extracted with dry methanol. Removal of solvent under reduced pressure and drying of the residue under vacuum gave a crude white solid (0.255 mg, 53%) which was flash chromatographed on silica gel eluting with CH_2Cl_2–MeOH (50:20). The compound was recrystallized from methanol–diethyl ether. Yield 0.101 g (21%), m.p. 176–179 °C (decomp.). IR (KBr): 1660 cm^{-1}. ^1H NMR (D_2O): δ 2.78 (s, 8 H), 3.23 (s, 6 H) and 3.54 (s, 4 H). Mass spectrum: *m/z* 260 (M^+), 243 $(M - 17)^+$ and 226 $(M - 34)^+$ (Found: C, 43.90; H, 7.40; N, 20.25. Calc. for $C_{10}H_{20}N_4O_4 \cdot 0.4HCl$: C, 43.70; H, 7.45; N, 20.40%).

Potentiometric Titrations.—Potentiometric measurements were carried out with a Crison Digilab 517 instrument using an Ingold U 1330 glass electrode and a U 1335 saturated calomel reference electrode (SCE), with a Wilhelm-type salt bridge containing 0.10 mol dm^{-3} NMe_4NO_3 solution. The ionic strength of the titrated solutions was kept at 0.10 mol dm^{-3} using KNO_3 and the temperature was controlled at 25.0 ± 0.1 °C by circulating thermostatted water through the jacketed titration cell. The potentiometric data were refined by using the SUPERQUAD program.[27]

Electrochemical Measurements.—The electrochemical measurements were performed with a Princeton Applied Research PAR 173 instrument coupled with a Nicolet 309 oscilloscope for high scan rates. Cyclic voltammetry was conducted at a hanging mercury drop working electrode with a platinum auxiliary electrode and a SCE as reference. All potentials, when not specified, were referred to the latter. The supporting electrolyte was KNO_3 (1.0 mol dm^{-3}). All solutions were thermostatted at 25 ± 0.1 °C and degassed with N_2.

Spectrophotometric Measurements.—All spectra were measured on a Lambda 9 Perkin Elmer spectrophotometer. Quartz cells with 1 cm optical pathlength were thermostatted at 25.0 ± 0.1 °C for all experiments. Solutions of the iron(III) complex were generated *in situ*, by addition of a standardized iron(III) nitrate solution to an excess of ligand. The pH measurements were done using a pH M63 Digital Radiometer

pH meter equipped with an ORION 91-03 combination pH electrode. The pH was adjusted with KOH or HNO_3.

Spectrophotometric competition experiments were performed with edta solution, and the reaction mixtures were allowed to equilibrate at 25 °C for several days until a constant reading was obtained. The supporting electrolyte was KNO_3 (0.1 mol dm^{-3}).

Other Measurements.—Proton NMR spectra were measured in a Varian Unity 300 or in a Bruker CXP 300 spectrometer at 25 °C. All the solutions were in D_2O (2×10^{-2} mol dm^{-3}) with sodium 3-(trimethylsilyl)[2,2,3,3-$^2H_4$]propionate as reference. The EPR spectra were obtained in methanol or water solutions on a Bruker ER 200-FRC spectrophotometer. The complexes were generated *in situ* by addition of iron(III) nitrate to an excess of ligand. The solutions were 1 mmol dm^{-3} in metal ion. Melting points were taken on a Buchi 530 capillary apparatus. Infrared spectra were recorded on a Perkin Elmer 683 spectrophotometer and mass spectra using a modified double-focusing AEI-MS9 spectrometer updated with a VG-Micromass ZAB console and dual EI/IC source. Elemental analyses were done with a Perkin Elmer 240B elemental analyser.

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