Siderophore analogues. Chelating properties of a new cyclic diaza-dihydroxamic acid

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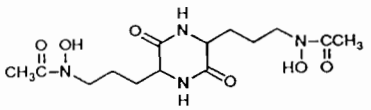
Abstract

A new cyclic diaza-dihydroxamic acid, 1,4-diazacycloheptane-*N,N'*-bis(*N*-methyl-acetohydroxamic acid) (DACHDMAHA) has been synthesized and studied. This paper is the second one devoted to finding synthetic ferric sequestering agents suitable for mimicking chemical and biological properties of the naturally occurring rhodotorulic acid. This ligand, having the hydroxamate arms bound to a seven-membered ring diamine, presents some biological activity, as was previously found for the corresponding six-membered compound (PIPDMAHA). Stability constants with iron(III) and iron(II) as well as with copper(II) and copper(I) have been determined by potentiometry, spectrophotometry and cyclic voltammetry, this latter technique being also useful for the interpretation of the electrochemical reaction and the determination of the rate constant of dissociation of iron(II) complexes. Comparing the complexes obtained with DACHDMAHA and PIPDMAHA it has been noticed that the complexes with the first ligand are more stable and that the rate constant of dissociation of the iron(II) complex is higher, showing that this ligand has more of the characteristics of a siderophore.

Introduction

Synthetic iron-sequestering agents [1–4] are of current interest for use as drugs, such as in the treatment of iron-overloaded patients, or models of siderophores, naturally occurring iron-chelating compounds. Hydroxamate type siderophores generally possess three or two hydroxamate functional groups which are able to form hexacoordinate stable iron(III) complexes (FeL or Fe_2L_3, respectively) in order to solubilize the iron and to transport it into the cell membrane. In the design and synthesis of new iron-sequestering agents, efforts have been made to utilize two of these functional groups [1, 5] in order to obtain analogues of the naturally occurring siderophore, rhodotorulic acid (**1**) (Fig. 1). We have also recently developed a new cyclic diamino-dihydroxamic acid (PIPDMAHA) (**2**) which has been proved to be a reasonable model for rhodotorulic acid in terms of physicochemical and biological properties [6]. In order to go further in our approach to the design and synthesis of new artificial siderophore analogues of rhodotorulic acid, we present and describe herein another new cyclic diamino-dihydroxamic acid, 1,4-diazacycloheptane-*N,N'*-bis(*N*-methyl-acetohydroxamic acid), (DACHDMAHA) (**3**), which aims at determining the effect of structural modifications, such as the size of the cyclic diamine backbone, on several important properties, namely the ability for iron chelation and biological activity. We report here the preparation and characterization of the ligand, including its acid–base properties and its ability for complexation with iron(III) and iron(II) and also Cu(II) and Cu(I) using Vis and NMR spectroscopy and potentiometry as well as cyclic voltammetry for the study of electron transfer processes.



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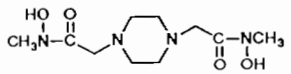

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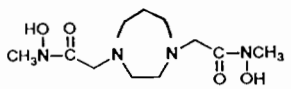

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Fig. 1. Structural formula of rhodotorulic acid (**1**), and synthetic analogues PIPDMAHA (**2**) and DACHDMAHA (**3**).

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Results and discussion

Acid–base properties of the ligand

The acid–base properties of the ligand DACHDMAHA have been studied by potentiometry. The fully protonated form of the ligand LH_4^{2+} can release four protons in the pH range 3–10: two from the hydroxamate groups and two from the amino groups. The completely protonated ligand LH_4^{2+} was titrated with sodium hydroxide (see Fig. 2, curve (1)) and it presents four partial ionization constants: $pK_1^H = 2.93 \pm 0.03$, $pK_2^H = 6.88 \pm 0.07$, $pK_3^H = 8.48 \pm 0.05$, $pK_4^H = 10.26 \pm 0.04$. The attribution of each of the other three ionization constants to individual acid/base centres presents some difficulty due to the proximity of the ionization constants and consequently some overlap of the dissociation processes [7]. Although these studies by NMR spectroscopy are still in progress, the two first ionization constants seem to be mainly due to the ionization of the protonated amino groups and the last two seem to be mainly attributed to the hydroxamate groups.

The high acidity of the ammonium protons in this ligand relative to the corresponding cyclic amine, 1,4-diazacycloheptane ($pK_1^H = 6.70$, $pK_2^H = 10.41$) [8], might be attributed to hydrogen bonding interactions involving the nitrogen atoms of amino groups and the nearby hydroxamate hydroxylic protons (Scheme 1), in a six-membered ring intermediate, and also to the electron withdrawing of the NROH group. Moreover, an identical behaviour was previously observed for an analogous ligand, PIPDMAHA [6], and, to a lesser extent, for amino acids [9].

In addition, the difference between the first and second protonation of the amine group might be attributed to coulombic repulsion effects between each positive site $\overset{+}{N}HR_2$ of the N,N-diprotonated species, LH_4^{2+}.

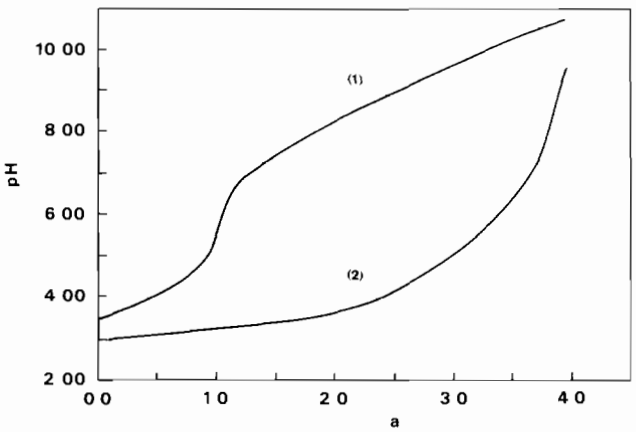
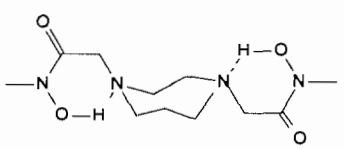


Fig. 2. Potentiometric titration curves with sodium hydroxide for the ligand DACHDMAHA (1) and for the copper(II)–ligand (1:1) system (2), at $\mu = 0.10$ M (KNO_3), 25 0 °C, a = moles of base added per mole of ligand present, $C_L = C_{Cu} = 1.5 \times 10^{-3}$ M.



Scheme 1

Copper complexation

Copper(II) complexation with DACHDMAHA was studied by potentiometry together with spectroscopic observations and the copper(I) species by cyclic voltammetry. A copper(II)–ligand 1:1 titration curve is shown in Fig. 2, curve (2). From the potentiometric titrations conducted at $C_L = 7.5 \times 10^{-4}$ and 1.5×10^{-3} mol dm^{-3} for the 1:1 copper-to-ligand ratio and $C_L = 1.0 \times 10^{-3}$ mol dm^{-3} for the 1:2 ratio, it was found that copper(II) can form three complexes (MH_2L, MHL and ML) and evaluation of the stability constants by SUPERQUAD program gave $\log \beta_{CuH_2L} = 25.01 \pm 0.08$ ($\beta_{CuH_2L} = [CuH_2L]/[Cu][H]^2[L]$), $\log \beta_{CuHL} = 22.43 \pm 0.01$ and $\log \beta_{CuL} = 16.85 \pm 0.02$. From speciation studies it can be seen that the protonated species CuHL reaches its maximum concentration at a pH around 3.8, for $C_L/C_M = 20$ (Fig. 3). Above that pH value another species (CuL) starts being formed reaching its maximum concentration at pH around 7.8, where it is the main species (> 95%), even at neutral pH. The absence of dinuclear species was suggested by EPR studies at different pHs (pH = 3.5–10.5), which did not show any disappearance of spectra or appreciable decreasing of their intensities.

Comparison of the stability constant calculated for ML species with that previously found for PIPDMAHA [6] (a homologous ligand resulting from subtracting a

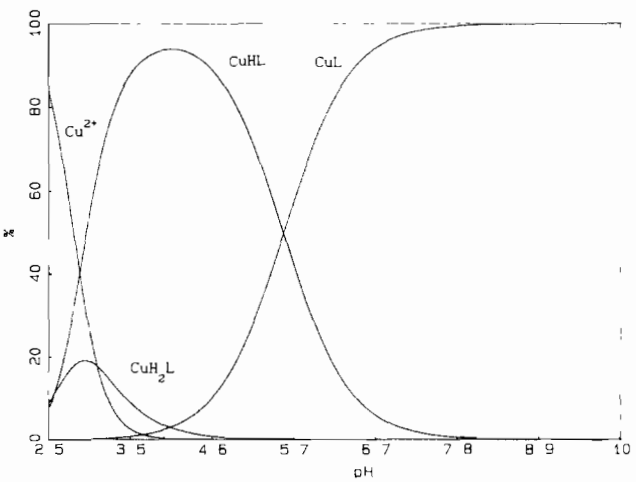


Fig 3 Speciation curves for Cu(II)–DACHDMAHA complexes as a function of pH $(C_L)/C_{Cu} = 20$; $C_{Cu} = 2.487 \times 10^{-4}$ M.

methylene group from the cyclic backbone) shows an increase of about four log units. The existence of an identical behaviour on the stability constants previously found for the copper complexes of the corresponding diazadiacetate ligands (DACHDA, PIPDA) [10] led us to attribute such difference to enthalpic effects as a result of the required preorganization of the piperazine ring in an unfavourable 'boat' conformation, for the formation of the complex. Also, the observed increase in the stability of the copper complex, CuL, as compared with that found for glycine hydroxamate complex (log $\beta_{CuL} = 10.682$ [11] might be attributed to the higher number of coordination sites of the first ligand.

Some typical absorption spectra, obtained for the Cu–DACHDMAHA system in the pH range 3–10 are reported in Fig. 4. The highest value of λ_{max} for the Cu^{2+}–DACHDMAHA system is *c.* 677 nm for pH ≃ 3, whereas it reaches *c.* 600 nm with maximum absorbance for the pH range 7–10. These observations seem to indicate the presence of more than one species, in accordance with the potentiometric results, which indicate the presence of two main complexes, $CuHL^+$ and CuL. The shift with pH of the only absorption band in the range 677–600 nm, as well as the corresponding change of solution colour, suggest a change in the coordination of each complex. Therefore in the acid region the solution presented a greenish colour whose absorption band ($\lambda_{max} = 677$ nm for pH ≃ 3) is attributed to d–d transition energy. Considering the similarity between this value and that found for the copper complex ($\lambda_{max} = 672$ nm) with a monohydroxamic acid without any amino group (*N*-methyl-α-chloroacetohydroxamic acid), it might be suggested that the coordination of the MHL complex involves hydroxamate groups and eventually one amino group, the other being protonated.

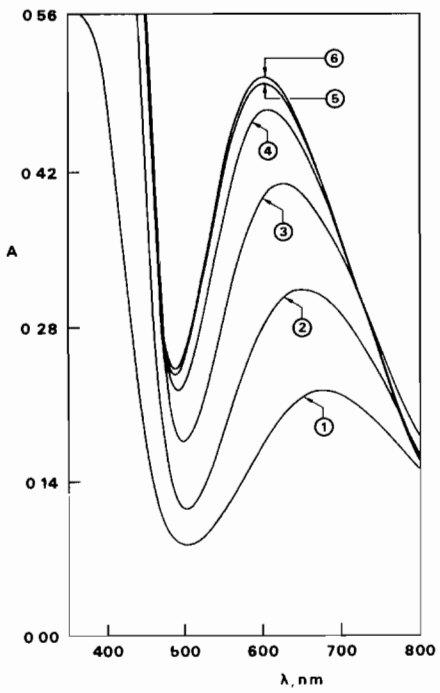


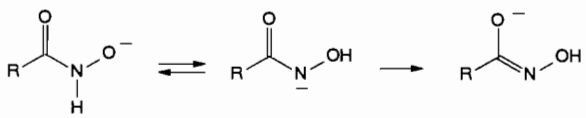
Fig 4. Absorbance spectra of the DACHDMAHA–Cu(II) complex ($C = 5 \times 10^{-3}$ M, $T = 25$ °C, $\mu = 0.10$ M KNO_3), as a function of pH. The equilibrium pH was as follows: (1) 3.07, (2) 4.09; (3) 5.07; (4) 6.34; (5) 7.44; (6) 8 48–10.01.

In the neutral or basic region (pH = 7–10) the solution becomes bluish and there is no change in energy or intensity of the absorption band ($\lambda = 600$ nm) in that range. These results suggest the existence of only one coordination type and one complex species, which, according to the species distribution curve, should be CuL. It is admitted for this complex that more amino groups and less oxygen atoms of the hydroxamate hydroxylic group should be involved than in the acid region.

Copper complexes involving chelation with hydroxamate groups via {O,O} coordination mode have also been found for N-alkyl dihydroxamic acids containing no α-heteroatom substitution, whose absorption bands may change between 757 and 640, depending on pH [12].

Copper complexes which involve both oxygen atoms of the hydroxamate moiety (in addition to the nitrogen atoms) taking part in the coordination were also found for the copper(II)–β-alaninehydroxamic acid system [13], which absorbs at $\lambda_{max} = 616$ nm at pH 4–9.

A type of 4N coordination, involving both N atoms of each hydroxamate moiety is not expected here, contrary to what happens with copper complexes of α-amino N-unsubstituted hydroxamic acids (e.g. glycinehydroxamic acid) [14], where it is possible to have an equilibrium hydroxamate–hydroximate, according to Scheme 2.



Scheme 2

Moreover such a 4N coordination would imply a λ_{max} in the range 535–540 nm, as has been found for the $[Cu(GLYHA)_2]$ complex [15], (GLYHA = glycinehydroxamate) and for the $[Cu(EN)_2]^{2+}$ complex [16] (EN = ethylenediamine).

The cyclic voltammetric behaviour of the Cu(II)/DACHDMAHA system was investigated in aqueous solution containing copper(II), ($C_{Cu} = 2.5 \times 10^{-4}$ M), an excess of ligand in different total concentrations ($C_L/C_M = 10$, 20, 50), buffered at pH = 7 with the ligand and using scan rates between 100 and 20 000 mV/s. It

has always been noted that the cathodic peak (Fig. 5) has a tendency to split into two peaks (I, II), the new peak (II), which appears at more cathodic potentials, having no corresponding anodic counterpart. Analysis of the effect of scan rate on the normalized intensity of the peaks shows that while $i_p^{II}/\sqrt{v}$ increases with scan rate, the opposite effect is observed for peak I, whereas $i_p^{III}/\sqrt{v}$ is approximately constant, and consequently the i_p^{I}/i_p^{III} ratio decreases with v. Also ΔE_p^{I-III} increases from 115 to 175 mV with the change of an order of magnitude of the scan rate, for $C_L/C_M = 30$. On the other hand E_p^{I} shifts in the negative direction about 20 mV for the same ratio and about 10 mV in the positive direction for $C_L/C_M = 10$. E_p^{II} has a cathodic shift of *c.* 50 mV for both ratios, whereas E_p^{III} always has an anodic shift (*c.* 25 mV). All of these results seem to suggest a typical CE [17] mechanism (see Scheme 3) where there is a quasi-labile dissociation of a complex at the double layer, the complex itself being directly reduced at more negative potentials.

$$A \rightleftharpoons O \overset{e}{\rightleftharpoons} R \quad (1)$$

$$A \overset{e}{\longrightarrow} R \quad (2)$$

Scheme 3.

A and O are, respectively, the copper(II) complex and free copper(II) species in solution. So peak I is probably due to the quasi-reversible reduction of copper(II) (O), owing to the dissociation of the complex, peak II to the reduction of the non-dissociated complex (A) and peak III to the oxidation process. In fact, if this assumption is valid, $i_p^{I}/\sqrt{v}$ should decrease with v not only due to the quasi-reversible behaviour (checked by the increase of $E_p^{III} - E_p^{I}$ with scan rate) but also due to the CE mechanism, since at higher scan rates there is less time for the complex to dissociate. In terms of potentials, E_p^{I} should shift in the negative direction with v due to the quasi-reversibility and to the positive side due to the CE mechanism, since there is less free ligand in the interface for high scan rates. With a higher excess of ligand the dissociation of the complex is more difficult and this last effect is less pronounced. This explains why E_p^{I} shifts to the positive direction with v for lower values of C_L/C_M and in the negative direction for higher C_L/C_M ratios. Since there is less time for the complex to be dissociated $i_p^{II}/\sqrt{v}$ should increase with the scan rate and E_p^{II} should shift cathodically due to the irreversible behaviour.

In order to understand the electrochemical reaction corresponding to peak III, cyclic voltammograms have been obtained for the inversion potential between the two cathodic peaks (Fig. 6). From these results it can be noticed that in this situation $i_p^{I} = i_p^{III}$ for several scan rates. This means that the oxidation peak III can be attributed to oxidation of both species corresponding to the cathodic peaks. Since they have the same height when process II has no effect, this means that there is no amalgam effect and so we can conclude that Cu(II) is being reduced to Cu(I) as happens to the copper(II)–PIPDMAHA system.

From the potentiometric titrations three copper species have been detected, the stability constants (log $\beta_{ML} = 16.85$, log $\beta_{MHL} = 22.43$ and log $\beta_{MH_2L} = 25.01$) being higher than those obtained for the Cu(II)–PIPDMAHA system. This higher affinity of copper(III) for this ligand also contributes to the slower kinetics of dissociation of this complex comparative to

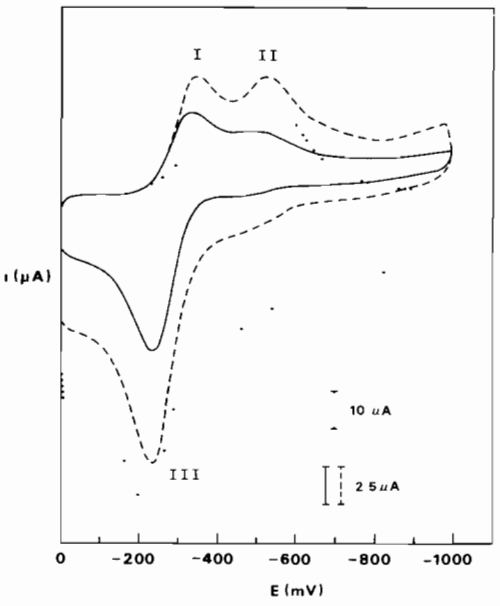


Fig. 5. Cyclic voltammograms for the DACHDMAHA–Cu system at pH = 7.0 and at different scan rates $v = 200$ (– – –), 800 (- - -), 5000 (. .) mV/s ($C_L/C_{Cu} = 20$, $C_{Cu} = 2.49 \times 10^{-4}$ M, $\mu = 1$ M KNO_3, $T = 25$ °C)

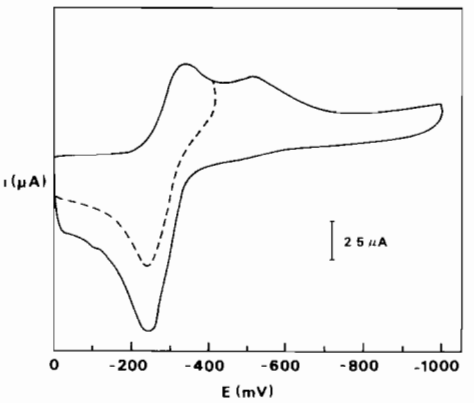


Fig. 6 Cyclic voltammograms for the DACHDMAHA–Cu system with different switch potentials at pH = 7.0, $v = 300$ mV/s ($C_L/C_{Cu} = 20$, $C_{Cu} = 2.49 \times 10^{-4}$ M, $\mu = 1$ M KNO_3, $T = 25$ °C)

the copper(II)–PIPDMAHA complex where the size of the ring is smaller and the complexes are labile.

A speciation calculation (Fig. 3) shows that under the conditions of the voltammetric experiments the dominant species is the ML complex ($C_{ML} > 95\%$).

From the variation of pH between 5.0 and 9.0 it has been found that for each scan rate the value of E_p^{II} does not change significantly and on the other hand E_p^{III} versus pH presents linear variation with slope -66 ± 3 for $v = 200$ mV/s, which means that the process of change of one electron involves also one proton, according to eqn. (3), as has also been previously postulated for the oxidation/reduction mechanism of the copper–PIPDMAHA system [6].

$$Cu(II)L + H^+ + e^- \rightleftharpoons Cu(I)HL \quad (3)$$

Even for the smallest scan rate the system is quasi-labile and so the stability constant of the copper(I) complex can be estimated from eqn. (4), according to the same procedure used in d.c. polarography [18].

$$(E_p)_c^I - (E_p)_s = -\frac{59.15}{\alpha n} \log \frac{\beta_{ML}^{II}}{\beta_{MHL}^{I}} - \frac{59.15}{\alpha n} pH + \frac{59.15}{\alpha n} \frac{(i_p)_d}{(i_p)_I} \quad (4)$$

where $(i_p)_d$ is the peak current for the reduction of the complex (i_p^{II}) for the highest scan rate where peak I practically does not exist. From $E_{p/2} - E_p = 48/\alpha n$ mV for peak I a value of $\alpha = 0.8$ has been determined [17].

The value calculated for the stability constant of the reduced complex ($\log \beta_{MHL}^{I} = 19.9 \pm 0.2$) shows that this Cu(I) complex species is less stable than the corresponding Cu(II) complex.

If the limiting step in the formation of the copper complex of ML type is the dehydration of the metal ion with a rate constant of $k_f \sim 10^9$ M^{-1} s^{-1} [18], a value for the rate constant of dissociation of the complex, 10^{-7} s^{-1}, should be anticipated [19], considering the stability constant of the complex of about 10^{16}. In this case the complex should be inert and not quasi-labile in terms of cyclic voltammetry. The increase of the rate constant can be due to adsorption of the ligand at the positive side of the electrocapillarity curve, where copper(II) is being reduced (probably due to interaction of amino groups with mercury) and to the subsequent induced adsorption of copper(II), as has been verified for copper(II) complexes with other amino acids such as proline, hydroxyproline and D-allohydroxyproline [20, 21].

Iron complexation

Interactions between DACHDMAHA and iron(III) were studied by spectrophotometry and with iron(II) by cyclic voltammetry.

Examination of the pH dependence of the visible spectra of the ferric complex (Figs. 7 and 8) reflects the presence of sequential complexation reactions. Maximum absorbance is observed for the pH range 5–8, with $\lambda_{max} = 426$ nm and ϵ_{max} about 2800 M^{-1} cm^{-1}, in terms of iron concentration. As the pH is lowered or increased a decrease in the absorbance and a bathochromic or hypsochromic shift are observed, respectively. Comparison of these results with those found for other similar hydroxamate ligands [6], suggests that in the range of pH corresponding to the maximum absorbance

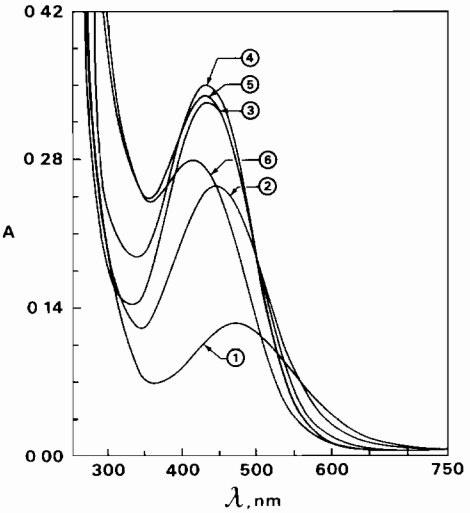


Fig. 7. Absorbance spectra of the Fe(III)–DACHDMAHA solution as a function of pH. $C_L = 1\,29 \times 10^{-3}$ M, $C_L/C_M = 10$ (25 °C, 0.10 M KNO_3) The equilibrium pH for curves (1)–(6) are: (1) 3.1; (2) 3.5; (3) 4.1, (4) 7 0, (5) 9.9; (6) 11.0

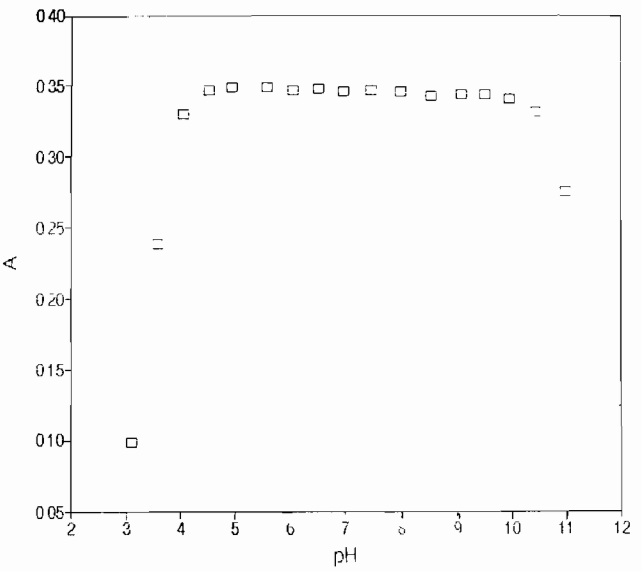


Fig. 8. Curve of the variation of absorbance of Fe(III)–DACHDMAHA solutions as a function of pH at $\lambda_{max} = 426$ nm, $C_L = 1\,29 \times 10^{-3}$, $C_L/C_M = 20$ (25 °C, 0.10 M KNO_3).

three hydroxamate groups might be involved in the coordination with each ferric ion, this feature suggesting the existence of a Fe_2L_3 complex.

Coordination involving the amino group is not expected taking into account the similarity of λ_{max} and ϵ_{max} between this complex and others described in the literature for ferric hydroxamate complexes without any amino group in the ligand [4, 22, 23]. Also considering the high affinity of ferric ion to harder bases such as the hydroxamate anion relative to the softer neutral tertiary amine, and on the other hand the increased stability of complexes containing five-membered chelated ring relatively to six-membered chelates [24], the amino group of our ligand is not likely to be involved in the coordination, as was proposed by Brown *et al.* for iron(III) glycine hydroxamate.

The decrease of the absorbance in acid or basic conditions might be due to the lowering of the number of hydroxamate groups that are coordinated to the metal ion, according to a known general rule [23] stating that the absorptivity of a ferric hydroxamate is about $1000 \times n\ M^{-1}\ cm^{-1}$, n being the number of hydroxamate groups bound per ferric ion. Therefore in acid conditions (pH = 3.5, $\epsilon = 1918\ M^{-1}\ cm^{-1}$) we might have only two hydroxamate moieties coordinated to the metal ion, due to the competition between the metal ion and proton, and probably the following equilibria:

$$Fe_2L_3 + 2H^+ \rightleftharpoons Fe_2H_2L_3^{2+} \quad (5)$$

The stoichiometry of the dominant species at pH = 7.0 (close to the physiological value) was also studied by plotting the absorbance at λ_{max} as a function of the molar ratio of iron(III) per ligand (Fig. 9). It is shown

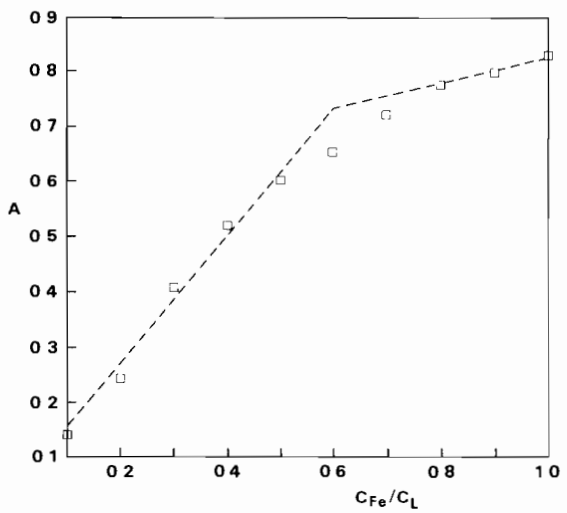


Fig. 9. Plot of the absorbance of the ferric complex at $\lambda_{max} = 426$ nm, as a function of the ratio of C_M/C_L at pH = 7.0 (L = DACHDMAHA).

that there is an intersection of the two straight lines at a ratio of about 0.6, indicating the formation of 2:3 complex of iron(III) which gives support to the hypothesis of the formation of Fe_2L_3 complexes at this pH.

The structure proposed for the Fe_2L_3 complex should be similar to the lowest energy conformer calculated for the corresponding PIPDMAHA complex [26]. The ferric ions are coordinated to the oxygen atoms of hydroxamate moieties. This complex has two ligand molecules, each one with both hydroxamate arms coordinated to the same ferric ion and the piperazine ring in a distorted boat conformation; the other ligand molecule, having a chair conformation for the cyclic diamine and each hydroxamate arm binding each ferric centre, completes the octahedral hexacoordination for metal ions.

Regarding the diprotonated species $Fe_2H_2L_3^{2+}$, we proposed the opening of each ten-membered ring, $Fe(O–C–C–N–C)_2$, associated with the boat conformation, due to protonation of one hydroxamate group, as suggested by the observed affinity with pH of the intensity of the band corresponding to the hydroxamate coordination.

The stability constant for the Fe(III)/DACHDMAHA complex was not determined by potentiometry because this method is not quite adequate for the determination of very high stability constants. Therefore we determined the stability constant of Fe_2L_3 species by making use of the competition between EDTA and DACHDMAHA for the ferric ion, at pH = 7.0. In this method we have always started with an excess of ligand ($C_L/C_M = 10$) and then added EDTA (C_L/C_{EDTA} was about 5, 6 and 10). Spectral changes observed when EDTA is added to a solution of DACHDMAHA are shown in Fig. 10. Assuming that Fe_2L_3 is the dominant species in solution, before the addition of EDTA, the decrease of the absorbance of the complex at λ_{max} is attributed to the chemical reaction (6)

$$Fe_2L_3 + 2HEDTA^{3-} + 4H^+ \rightleftharpoons 2FeEDTA + 3LH_2 \quad (6)$$

Once the equilibrium has been attained (time elapsed about 2–3 days) we have calculated the remaining Fe_2L_3 complex concentration from absorbance measurements. From the known protonation constants of DACHDMAHA and EDTA, the measured pH values and the mass balances, a value for $\log \beta_{23} = 65.4 \pm 0.3$ was obtained for all EDTA concentrations. The fact that this complex is about four log unities more stable than the similar PIPDMAHA–Fe(III) complex (about the same difference that has been found between the corresponding copper complexes) may be due to the absence of the strain conformation ('boat') which is dic-

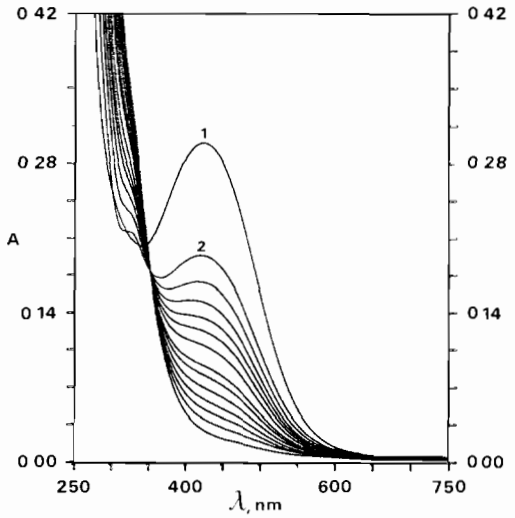


Fig. 10. Absorbance spectra of the Fe_2L_3 complex (L= DACHDMAHA). $C_L/C_{Fe}=10$, $C_{Fe}=1\,1\times10^{-4}$ M, pH=7 0 (1), before addition of EDTA; (2), after addition of EDTA at different times ($C_L=C_{EDTA}$).

tated by the formation of the PIPDMAHA complex, as was mentioned above.

Electrochemical studies of the iron(III)–DACHDMAHA system with $C_M=2.5\times10^{-4}$ M and excess of ligand ($C_L/C_M=10$–40) at pH≃7.0 were carried out by cyclic voltammetry. The obtained voltammograms (Fig. 11) have shown one cathodic peak (III) and two anodic peaks (I and II) that are clearer at higher scan rates where the normalized peak height of the cathodic peak ($ip/\sqrt{v}$) is approximately constant for $v\leqslant1000$ mV/s and increases for the highest scan rates used (up to 20 000 mV/s). So it seems that at least peak III is affected by weak adsorption superimposed to the process of diffusion in solution. Our results show that E_p^{III} shifts 30 mV for each ten-fold increase in the scan rate, the difference between potentials of peaks I and III (ΔE_p) is about 60 mV, $i_p^I/\sqrt{v}$ increases with v in a regular way, and i_p^I/i_p^{III} changes from *c.* 0.4 to *c.* 1.0 when v changes from 100 to 20 000 mV/s if i_p^{III} is corrected for adsorption. So, according to Nicholson and Shain [17], the global mechanism that is responsible for peaks III and I should be of the EC type, involving the global change of two electrons at a similar potential, with the change of one electron per each non-interacting ferric site of M_2L_3 species [27, 28], as happened for the PIPDMAHA–Fe(III) system and other dimeric complexes [23].

In the DACHDMAHA–Fe(III) system another anodic peak appears for higher scan rates at a more positive potential (peak II), which suggests that it is due to the oxidation of the iron(II) complex in the adsorbed state; in that case, adsorption of the iron(II)

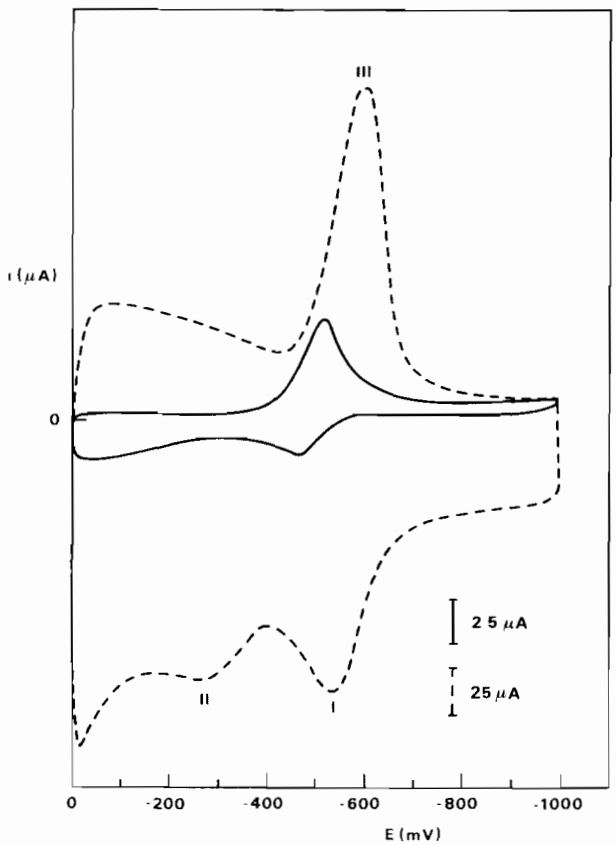


Fig. 11 Cyclic voltammograms for the DACHDMAHA/Fe system at different scan rates: (——) 100, (– – –) 20 000 mV/s $C_L=5\,0\times10^{-3}$ M, $C_L/C_M=20$, $T=25$ °C, $\mu=1$ M (KNO_3), pH=7.0.

complex is quite strong since there is a clear difference between the energies involved in the two processes. Such an adsorption effect was not observed on the PIPDMAHA system, which might be attributed to the fact that the DACHDMAHA ligand has a bigger aliphatic chain and therefore more affinity for the hydrophobic surface of mercury.

Accordingly, the global mechanism seems to be of the EC type, as mentioned above, with $n=1$, being represented by eqns. (7-1) and (8-1) for the species in solution, and by eqns. (7-2) and (8-2) for the adsorbed species, the chemical reaction being irreversible and practically negligible for higher scan rates.

$$O+ne \rightleftharpoons R \quad (7\text{-}1) \qquad O_{ads}+ne \rightleftharpoons R_{ads} \quad (7\text{-}2)$$

$$R \longrightarrow Z \quad (8\text{-}1) \qquad R_{ads} \longrightarrow Z \quad (8\text{-}2)$$

On the other hand E_p^{III} has a cathodic shift with increasing pH, presenting in the pH range 5–8 a linear variation with slope *c.* 30 mV, which agrees with the stoichiometry of the reduction (eqn. (9)).

$$Fe_2^{III}L_3+H^++2e^- \rightleftharpoons Fe_2^{II}HL_3^- \quad (9)$$

Also the constancy of E_p^{III} and E_p^I with concentration of the ligand ($C_L/C_M=10$–40) gives support to the

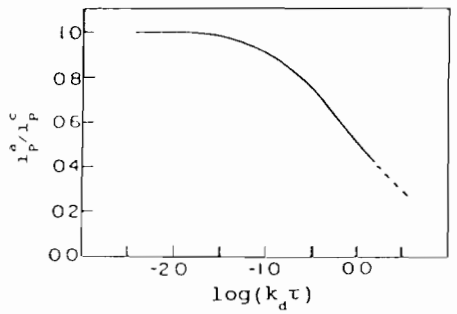


Fig. 12. Ratio of anodic to cathodic peak current as a function of $k_d\tau$ [17].

existence of an identical coordination number of oxidized and reduced iron complexes, in relation to the ligand.

For higher scan rates, where the dissociation or decomposition of the complex is negligible, it is possible to calculate the stability constant of the ferrous complex from the difference between the peak potentials for reduction of a simple ferric ion and of its complex, according to the following equation:

$$E_p^c - E_p^s = -\frac{59.15}{2}\log\frac{\beta_{203}^{III}}{\beta_{213}^{II}} - \frac{59.15}{2}\,pH \qquad (10)$$

where β_{203}^{III} and β_{213}^{II} are the global stability constants of the iron(III) complex (Fe_2L_3) and the iron(II) complex (Fe_2HL_3), respectively.

Using the value of $\log \beta_{Fe_2L_3}^{III} = 65.4 \pm 0.3$ obtained by spectrophotometry a value of $\log \beta_{Fe_2HL_3}^{II} = 36.2 \pm 0.3$ has been obtained.

Kinetic results

The rate constant for the irreversible dissociation decomposition of the iron (II) complex following the reversible reduction of the iron(III) complex (eqns. (7) and (8)) has been determined using the Nicholson and Shain [17] theoretical treatment. The working curve of i_p^a/i_p^c as a function of $\log k_d\tau$, presented in Fig. 12, has been used where τ is the time in seconds required to scan from $E_{1/2}$ to the switching potential. For scan rates between 1 and 10 V/s a value of $k_d = 6 \pm 1\ s^{-1}$, has been calculated, under the experimental conditions for the voltammetric studies, referred to in Fig. 11, and using i_p^a corrected for adsorption. So this compound seems to be more effective either as a drug for removal of iron excess from mammals (due to the higher stability constant of the complexes) or as a siderophore mimic (due to the increased lability of the reduced species).

Experimental

Synthesis of the ligand DACHDMAHA

The method of preparation of the ligand involved the condensation of the corresponding cyclic diamine DACH with chloroacetohydroxamic acid, as described in the literature [6]. DACHDMAHA was obtained as colourless crystals upon crystallization from ethanol/diethyl ether, yield 23%, m.p. 181–183 °C. IR (KBr, cm^{-1}): 1640. ^1H NMR (D_2O): δ (ppm) 2.05 (m, 2H), 3.08 (t, 4H), 3.17 (s, 10H), 3.82 (s, 2H). MS (*m/e*): 275 $(M+1)^+$, 257 $(M-17)^+$, 240 $(M-2\times17)^+$. *Anal.* Calc. for $C_{11}H_{22}N_4O_4 \cdot HCl$: C, 42.50; H, 7.40; N, 18.00. Found: C, 42.41; H, 7.31; N, 17.90%.

Solution studies

Potentiometric, voltammetric and spectrophotometric measurements were carried out as described in a previous paper [6].

For potentiometric and spectrophotometric measurements, the ionic strength was kept constant at 0.10 mol dm^{-3}, using KNO_3, and the temperature was 25.0 ± 0.1 °C. The concentration of the ligand in potentiometric titrations was 7.5×10^{-4} and 1.5×10^{-3} mol dm^{-3} for the 1:1 copper-to-ligand ratio and 1.0×10^{-3} mol dm^{-3} for the 1:2 ratio.

Refinement of the potentiometric data was obtained from the experimental titration with the aid of the SUPERQUAD PROGRAM [29]. The stability constants for the DACHDMAHA–Cu(II) complexes were obtained from titrations with C_M/C_L ratios 1:1 and 2:1.

The voltammetric studies were performed on a PAR 173 instrument with a Nicolet 305 oscilloscope. We have used a three electrode system with a hanging mercury drop working electrode, a platinum auxiliary electrode and an SCE as reference. The supporting electrode was KNO_3 (1.0 mol dm^{-3}) and degassed with N_2.

Other measurements

IR spectra were obtained as KBr pellets on a Perkin-Elmer 683 spectrophotometer. ^1H NMR spectra were recorded on a Varian Unity 300 spectrophotometer. EPR studies were performed in water solutions on a Bruker ER 200-FRC spectrophotometer. Melting points were taken in open capillaries on a Buchi 530 apparatus. The mass spectrum was obtained using a modified double focusing AEI-MS9 spectrophotometer updated with a VG-Micromass ZAB console. Microanalyses were performed with a Perkin-Elmer 240B elemental analyser. The complex was generated *in situ* by addition of ferric nitrate to excess of ligand, the solution of metal concentration being 1 mM.

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