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Siderophore analogues: a new macrocyclic tetraamine tris(hydroxamate) ligand; synthesis and solution chemistry of the iron(III), aluminium(III) and copper(II) complexes †

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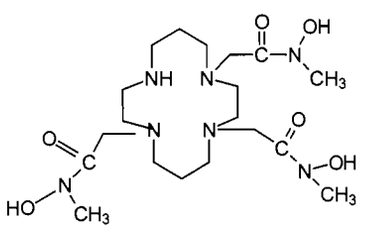
A new siderophore analogue TETMAHA (1,4,8,11-tetraazacyclotetradecane-*N*,*N*′,*N*″-tris(*N*-methylacetohydroxamic acid), a ligand with three hydroxamic acid groups bonded to the macrocycle CYCLAM, has been synthesized and characterized. Its acid–base and chelating properties towards iron(III), aluminium(III) and copper(II) have been studied by potentiometric and spectroscopic techniques. This ligand forms quite stable complexes with those metal ions and the formation constants have been determined. The mechanism of the electron transfer of the iron complexes, thought to be important in the biological activity shown by this ligand, has also been studied by cyclic voltammetry.

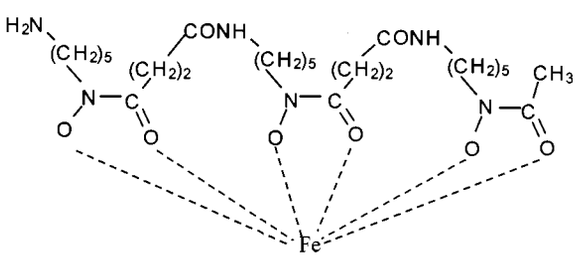
Introduction

Siderophores are naturally occurring chelators produced by microorganisms which use them as iron transporting agents. Their medical significance is due to the fact that iron is a limiting nutrient to bacterial growth, and also to their use as drugs to facilitate iron mobilization in man.[1] The most used clinical agent in cases of iron poisoning (Cooley's anemia) is desferrioxamine B (DFA), a tris(hydroxamate) ligand which has also been used as a mitigating drug for the Al^{3+} toxicity.[2] Accordingly, there has been a considerable interest in the development of synthetic siderophore analogues.[3–6] As part of our interest in this type of biomimetic ligands,[7–10] we have synthesized a new tris(hydroxamate) ligand, the 1,4,8,11-tetraazacyclotetradecane-*N*,*N*′,*N*″-tris(*N*-methylacetohydroxamic acid) (TETMAHA). This compound has three hydroxamate groups which provide the hexadenticity necessary for a complete octahedral encapsulation of the iron(III) ions with the FeL stoichiometry. These hydroxamate pendant groups are attached to a macrocycle backbone (CYCLAM), aimed at providing some preorientation of the chelating groups towards the metal complexation. The compound DOTRMAHA [1,5,9-triazacyclododecane-*N*,*N*′,*N*″-tris(*N*-methylacetohydroxamic acid)][7] is an earlier example of this type of macrocyclic tris(hydroxamate) ligand which has been prepared and studied in our group. However the new ligand has some advantages over the previous one, namely in terms of facility of preparation, since the macrocycle backbone is commercially accessible. On the other hand it has a free amine as with desferrioxamine B, which may improve its potential usefulness as a drug and which can be used as a point of attachment to a polymeric solid matrix, thus improving its properties for removing residual "hard" ions from water solutions.

We report herein the preparation and characterization of this new ligand, followed by equilibrium studies of its complexes with several metal ions (Cu^{II}, Fe^{III} and Al^{III}) in aqueous solution, using potentiometric and spectroscopic titrations (UV-VIS and NMR), as well as ESR studies. *In vivo* microbiological studies will be reported separately.



TETMAHA



FERRIOXAMINE B

Results and discussion

Synthesis

The preparation of the ligand TETMAHA involved the synthesis of *O*-benzyl-*N*-methylbromoacetohydroxamic acid and its coupling to the amine groups of the commercially available (Aldrich) macrocyclic backbone 1,4,8,11-tetraazacyclotetradecane, by using the method and conditions previously reported.[7] In the present synthesis we used about three equivalents of base (NaH) and α-bromohydroxamic acid "arm".

Protonation studies

The acid–base behaviour of TETMAHA was studied through

† *Supplementary data available*: potentiometric titration curves, absorption and ^{27}Al NMR spectra. Available from BLDSC (No. SUP 57485, 7 pp.). See Instructions for Authors, 1999, Issue 1 (http://www.rsc.org/dalton).

Table 1 Stepwise protonation constants of TETMAHA and other relevant analogous, global formation constants and electronic spectral data of corresponding copper(II) complexes

	H^+	Cu^{II}					
Ligand	log K_i	log $\beta_{Cu_pH_qL_r}$ [a]	λ_{max}/nm (ε/M^{-1} cm^{-1})	$g_{\parallel}$	$g_{\perp}$	$10^4A_{\parallel}$/cm^{-1}	$10^4A_{\perp}$/cm^{-1}
TETMAHA	11.47						
	9.72			pH 3.59		pH 3.59	
	8.78	(1,3,1) 45.11	590 (162)	2.237	2.079	181.88	15.01
	8.00	(1,2,1) 38.3	320 (4766)	pH 8.07		pH 8.07	
	6.64	(1,1,1) 30.8		2.247	2.090	171.88	43.34
	2.00						
	<2						
	b	*b*	*c*	*d*	*d*	*d*	*b*
CYCLAM	11.59						
	10.62	(1,0,1) 27.2	513 (100)	2.186	2.049	205.0	38.73
	2.42						
	1.61						
	b	*b*					
4MeCYCLAM	9.34		650 (275)				
	8.99	(1,0,1) 18.3	315 (5040)	2.240	2.072	162.08	50.28
	2.58						
	2.25						
	e	*e*	*e*				
DACHDMAHA	9.67	(1,2,1) 26.61					
	8.53	(1,1,1) 22.01	600 (101)	—	—	—	—
	7.30	(1,0,1) 17.33					
	3.97						
	d	*d*	*d*	*d*	*d*	*d*	*d*
CYCLEN	10.97						
	9.87	(1,2,1) 24.80	599 (257)	2.198	2.057	184.2	24.1
	<2						
	<2						
	f	*f*		*g*		*g*	
DFA	10.84	(1,3,1) 36.99					
	9.46	(1,2,1) 33.10	—	2.332	—	167	—
	9.00	(1,1,1) 23.98					
	8.3	(1,0,1) 13.73					
		(2,1,1) 32.09					

[a] The (p,q,r) symbolism indicates a species with stoichiometry $M_pH_qL_r$. [b] From ref. 12. [c] From ref. 13. [d] From ref. 14. [e] From ref. 8. [f] From ref. 15. [g] From ref. 16.

potentiometric titration. The fully protonated form of the ligand can release seven protons: four from the amine groups and three from the hydroxamate groups. The corresponding protonation constants were determined through the analysis of the pH-titration curve (see SUP 57485) with the aid of the computer program SUPERQUAD[11] [log K_1 = 11.47(3); log K_2 = 9.72(3); log K_3 = 8.78(3); log K_4 = 8.00(3); log K_5 = 6.64(4); log K_6 = 2.00(9); log K_7 < 2]. The refined stepwise values are listed in Table 1, where log K values of some other structurally relevant ligands are also reported. Although the ligand has seven dissociable protons, potentiometric titration only allowed the accurate determination of six since under our experimental conditions this method is limited to the range pH 2–12. So, the seventh constant is too low to be accurately determined by potentiometry. Comparison of the values calculated for TETMAHA with those of CYCLAM[12] (see Table 1) strongly suggests the attribution of the first constant (K_1) to the unsubstituted amine of the macrocycle and the last two (K_6, K_7) to the protonation of two of the remaining three amine groups. However, the proximity between the range of values expected for the protonation constants of the remaining amine group and the hydroxamate groups (log K = 8–10)[17] makes difficult the attribution of the protonation constants to individual basic centres, thus suggesting there is some overlapping of these four protonation processes. A further analysis of the sequence of protonation and calculation of the corresponding microconstants is out of the scope of this work. Some general conclusions can still be derived from this set of results.

(a) Although the most basic centre of this ligand behaves like that of the macrocyclic CYCLAM and should correspond to the non-substituted amine, the other amine groups are less basic than those of the unsubstituted macrocycle. This also happens with 4MeCYCLAM[12] (see Table 1) and may be mainly attributed to the fact that tertiary amines are less basic than secondary amines. The high basicity of the first two sites (and the subsequent low basicity of the other two) of these macrocyclic amines is attributed to a hydrogen bond network.[12]

(b) The presence of the α-amino group in the hydroxamate side chains increases the acidic character of the hydroxamate group, as expected.[18] Conversely the hydroxamate groups induce a similar effect on the nearby amine group. Such behaviour could be due to electrostatic interactions between both these close basic sites as well as to internal hydrogen bonding between them, involving one six-membered ring intermediate (upon protonation of one of those two sites).[10]

Copper(II) complexation

The co-ordination tendencies of TETMAHA towards the copper(II) ion in aqueous solution were studied by potentiometry and UV-VIS spectrophotometry. The pH-metric titration obtained for the copper(II)–TETMAHA binary system with $C_L = 2.4 \times 10^{-3}$ M at a 1:1 ligand to metal molar ratio, in the range pH 2–11, shows the absence of insoluble species, in contrast to that for the 1:2 ligand to metal molar ratio which presented insoluble species even at acidic pH (≈5).

Analysis of the potentiometric titration curve for the 1:1 ligand to metal molar ratio, by the computer program SUPERQUAD,[11] showed that the best fitting model involves the formation of three copper(II) complex species $[Cu(H_3L)]^{2+}$, $[Cu(H_2L)]^{+}$, [Cu(HL)] in solution in the approximate range pH

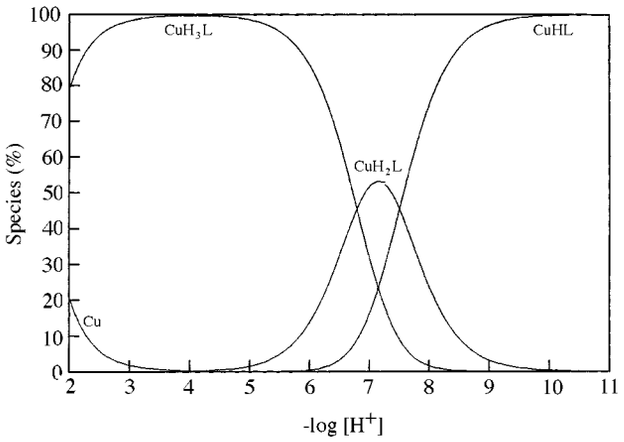


Fig. 1 Species distribution diagram for the system Cu^{II}–TETMAHA as a function of pH ($C_L/C_{Cu} = 1$; $C_L = 2.4 \times 10^{-3}$ M).

2–11. The calculated average formation constants are shown in Table 1. This model did not accept any binuclear species. On the other hand, since in the titration of a 1 : 2 ligand to metal molar ratio there was some precipitation we could not draw any conclusions about the existence of soluble dinuclear species. The values obtained for the formation constants of these copper(II) complexes suggest there is a quite strong metal–ligand interaction, when compared to copper(II) complexes of other synthetic trihydroxamate ligands and with desferrioxamine B (Table 1). The distribution of the complex species in solution, as a function of pH, is shown in Fig. 1. It can be seen that the complexation starts at acid conditions (pH 2), the $[Cu(H_3L)]^{2+}$ species being the major complex at acidic pH while [Cu(HL)] is the major complex in the basic region. At pH ≈ 7 the three complexes are present in solution.

In order to obtain an insight into the nature of the co-ordination involved in these copper(II)–TETMAHA complexes, UV-VIS spectra were recorded as a function of pH in the region 250–800 nm ($C_L/C_{Cu} = 3$; $C_L = 3 \times 10^{-3}$ M). These spectra exhibit a single broad band in the visible region ($\lambda_{max} = 590$ nm at pH 1.10, $\varepsilon_{max} = 194$ M^{-1} cm^{-1}; $\lambda_{max} = 600$ nm at pH 12.11, $\varepsilon_{max} = 147$ M^{-1} cm^{-1}) assigned to the copper d–d transition; an intense charge-transfer (c.t.) transition band in the near-UV region ($\lambda_{max} = 320$ nm at pH 1.10, $\varepsilon_{max} = 4734$ M^{-1} cm^{-1}; $\lambda_{max} = 320$ nm at pH 12.11 $\varepsilon_{max} = 4764$ dm^3 mol^{-1} cm^{-1}) and a shoulder, that appears as the pH increases, at around 420 nm. It can be seen that at very low pH (1.10) copper(II) is bound to the ligand, thus suggesting the existence of a very strong complex. The dependence on the pH gives support to the existence of more than one species in the range of pH.

Analysis of visible absorption spectra of the Cu^{II}–TETMAHA complexes shows that the d–d transition band is red shifted relative to that of the backbone macrocycle CYCLAM ($\lambda = 513$ nm, $\varepsilon_{max} = 100$ M^{-1} cm^{-1})[13] metal coordination of which involves four nitrogen atoms. In order to see the effect of the nitrogen substitution of CYCLAM on the co-ordination towards copper(II) we synthesized the corresponding methylated derivative, 4-MeCYCLAM. There was some red shift of the visible absorption maximum ($\lambda_{max} = 650$ nm, $\varepsilon_{max} = 275$ M^{-1} cm^{-1}, pH 7.0) relative to that of CYCLAM, presumably due to a change from four- to five-co-ordination with one water molecule in the fifth position.[19] In fact, the stepwise methylation of CYCLAM increases the red shift and the ε value ($\lambda_{max} = 526$ nm, $\varepsilon_{max} = 125$ M^{-1} cm^{-1} for 1-MeCYCLAM; $\lambda_{max} = 545$ nm, $\varepsilon_{max} = 138$ M^{-1} cm^{-1} for 2-MeCYCLAM).[20] Thus, nitrogen substitution of CYCLAM to give TETMAHA could be thought as responsible for some of the observed red shift. The UV peak at around 320 nm is similar to the c.t. band observed for 4-MeCYCLAM. However, TETMAHA has also a shoulder that appears at around 420 nm with increasing pH. Such a behaviour is characteristic of other α-aminohydroxamic acids which are known to form complexes with Cu^{II} involving both the amino and the hydroxamate chelating groups.[21,22] Thus, at higher pH where the hydroxamate arms are deprotonated it is probable that the hydroxamate oxygen atoms are also involved in the co-ordination. In fact some reported α-aminohydroxamic acids, when co-ordinated to copper(II), present one c.t. band centred around 325 nm which consists of three transitions at 430, 355 and 315 nm.[23,24] The 430 nm band was assigned as the hydroxamate oxygen to metal transition band and the other two to the hydroxamate nitrogen to metal c.t. band. Thus, the shoulder at around 420 nm which appears with increasing pH could be associated with the involvement of the hydroxamate oxygen. Moreover the Cu^{II}–4-MeCYCLAM complex does not present any absorption band at 420 nm. Therefore, the pH dependence observed in the c.t. and the d–d transition bands of the Cu–TETMAHA complex gives support to the existence of an interplay between the amine-*N* and hydroxamate-*O* co-ordination modes. In fact, as the pH increases there is a decrease in the intensity and a red shift of the d–d band that is accompanied by an increase in the c.t. band with the appearance of a shoulder in the 420 nm region that has its maximum at pH 8.9. This indicates that the increase in the pH should be accompanied by an increase in the hydroxamate co-ordination. Eventually, in addition to the co-ordination to the amine nitrogens of the macrocycle, we have, zero, one and two hydroxamates co-ordinated to Cu^{II} in the species $Cu(H_3L)$, $Cu(H_2L)$ and Cu(HL), respectively. The Cu(HL) species should have the same co-ordination as the $Cu(H_2L)$ but with the third hydroxamate arm deprotonated or could be a mixed-ligand hydroxo complex $Cu(H_2L)(OH)$. The program SUPERQUAD does not allow the distinction between these two complexes. A mixed co-ordination of amine-*N* atoms of substituted macrocycles and of hydroxamate-*O* atoms of the side arms has been proposed for other polyaminopoly(*N*-methylhydroxamate) ligands, such as DACHDMAHA [1,4-diaazacycloheptane-*N*,*N*′-bis(*N*-methylacetohydroxamic acid)][8] or DOCYDMAHA [12,14-dioxo-1,4,8,11-tetraazacyclotetradecane-4,8-bis(*N*,*N*′-methylacetohydroxamic acid)].[10]

In order to gain further insight on the type of co-ordination involved, ESR spectra were obtained for the copper(II) complexes with TETMAHA at different pH. Since no important variations were detected in the spectra along the titration, only two spectra were selected (pH 3.59 and 8.07) for the parameter analysis because under these conditions the major species in solution are $Cu(H_3L)$ and Cu(HL), respectively. Noteworthy is the fact that, in the range of pH studied, no ESR-silent pH region was detected, thus suggesting the absence of dinuclear species under our experimental conditions (or they are sufficiently separated to give spectral parameters indicative of magnetically non-interacting species). All the recorded spectra (Fig. 2) present four lines due to copper coupling. The corresponding ESR parameters (Table 1) ($g_3 > g_1$, g_2) indicate an axially elongated tetragonal copper(II) ion environment, as with many copper(II) complexes in solution.[25] Comparison of the ESR parameters, calculated for the copper(II)–TETMAHA complexes (pH 3.59) and the backbone macrocycle CYCLAM (Table 1),[14] shows that the introduction of the hydroxamate pendant arms in the macrocycle induces an increase on $g_{\|}$ and a decrease on $A_{\|}$. This seems to indicate that the planar ligand field becomes weaker while the axial ligand field becomes stronger,[25] as a result of the pyramidalization of the copper site, due to the displacement of the Cu from the 4N plane. Moreover, the ESR parameters, calculated for the copper(II) complexes with 4-MeCYCLAM, also present identical differences relative to those values for the complex with CYCLAM. The same happens with the [12]aneN_4 macrocycle CYCLEN,[14] due to the small size of the macrocycle, and also with some 14-membered tetraimine macrocycles (TIM), [Cu(TIM)X], where X is a neutral or charged (−1) ligand.[26] Thus, the ESR of $Cu(H_3L)$ could be rationalized in terms of a five-co-ordinated

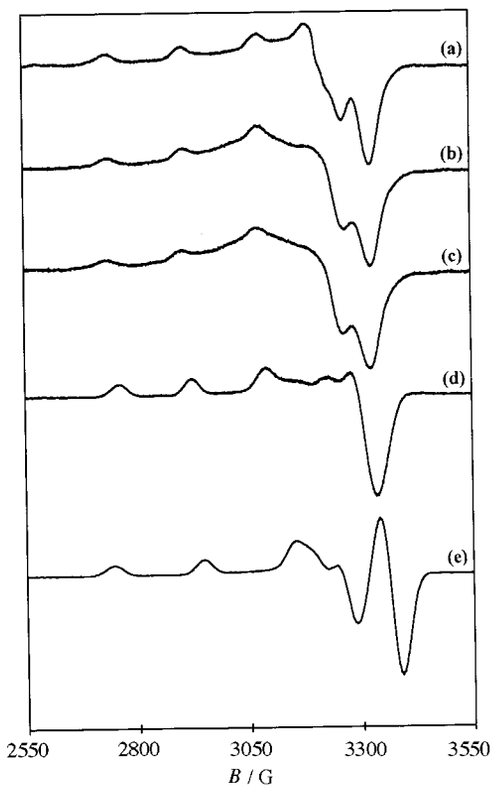


Fig. 2 The ESR spectra of frozen D_2O solutions with 20% ethylene glycol, containing the copper(II) complexes of the following ligands: TETMAHA, pH 3.59 (a), 6.10 (b), 8.07 (c); 4-MeCYCLAM, 7.22 (d); CYCLAM, pH 7.77 (e). ($C_L/C_{Cu} = 1$, $C_{Cu} = 6.0 \times 10^{-3}$ M; $T = 100$ K, frequency 9.34 GHz and modulation frequency 100 kHz).

copper(II) complex (square-pyramidal geometry) in which the Cu^{II} lies above the four nitrogen atoms of the macrocycle, and one water molecule is in the axial position.

The deprotonated complex species $Cu(H_2L)$ and Cu(HL) should have hydroxamate groups free for a fifth or sixth internal co-ordination to the metal ion. Although the involvement of these chelating groups in the co-ordination was suggested by the UV-VIS studies in the neutral–basic pH range (see above), it is also suggested by the ESR spectra. Comparison of the spectrum registered at pH 8.07 with the previous one (pH 3.59) shows there is an increase in $g_{\|}$ (2.247) accompanied by a concomitant decrease in $A_{\|}$ (171.88×10^{-4} cm^{-1}) which may be attributed to an increase of the hydroxamate co-ordination mode. In fact, the ESR spectra of copper(II) complexes involving $\{O,O\}$ co-ordination to hydroxamate moieties, such as *N*-phenylbenzohydroxamic acid (PBHA),[27] desferrioxamine (DFA)[16] and *N*-methylacetohydroxamic acid (MAHA)[27] having $g_{\|} \approx 2.33$–2.27 and $A_{\|} \approx 167 \times 10^{-4}$–$189 \times 10^{-4}$ cm^{-1}, depending on the number of chelating groups.

Iron complexation

The interaction between TETMAHA and iron(III) was studied by UV-VIS spectroscopy. The set of visible spectra obtained from a pH titration of the Fe^{III}–TETMAHA system (Fig. 3) shows that the complex formation begins at a very low pH (<2), thus suggesting the existence of high stability constants for the corresponding complexes. Since iron(III) is a typical "hard" ion, the corresponding complex with TETMAHA should have $\{O,O\}$-hydroxamate co-ordination. As the pH is raised there is a blue shift of the λ_{max} with concomitant increase in the intensity of the absorption band: for pH 1.1 $\lambda_{max} = 470$ nm, $\varepsilon_{max} = 1750$ M^{-1} cm^{-1} per Fe; for pH 4.74–10.5 $\lambda_{max} = 425$ nm, $\varepsilon_{max} = 2939$ M^{-1} cm^{-1} per Fe. This d–d transition band is typical of iron(III) hydroxamates and the increase in the intensity and

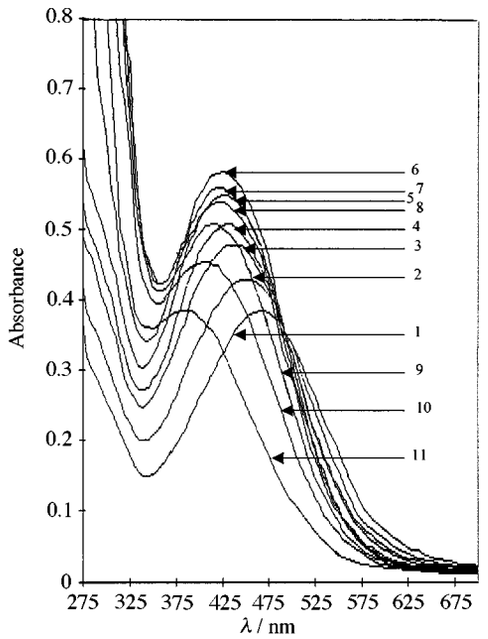


Fig. 3 Absorbance spectra of Fe^{III}–TETMAHA as a function of pH: (1) 1.10, (2) 2.29, (3) 3.09, (4) 3.61, (5) 4.74, (6) 7.04, (7) 10.05, (8) 10.94, (9) 11.47, (10) 11.91 and (11) 12.56. $C_L/C_{Fe} = 10$, $C_{Fe} = 1.99 \times 10^{-4}$ M; $I = 0.1$ M (KNO_3); $T = 25.0$ °C.

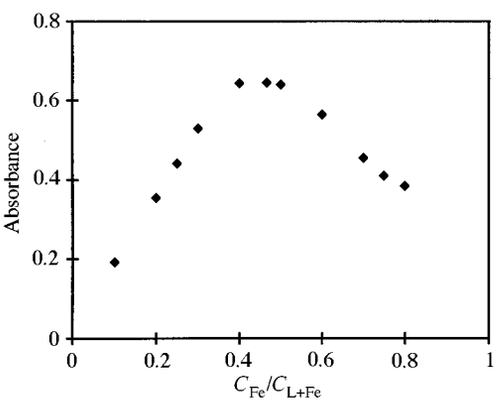


Fig. 4 Job's plot for determination of stoichiometry of the Fe^{III}–TETMAHA complex in aqueous solution at pH 7. $C_L + C_{Fe} = 6 \times 10^{-3}$ M; $I = 0.1$ M (KNO_3); $T = 25.0$ °C.

energy of the absorption band with increasing pH indicates greater co-ordination. The constancy of the λ_{max} and ε_{max} for pH 4.7–10.5 strongly suggests the existence of one predominant complex species in this pH range. On the other hand, taking into account that ε_{max} of the iron(III) hydroxamate complexes is approximately 3000 M^{-1} cm^{-1}, we may assume that such major species should have three co-ordinated hydroxamate moieties.[28] Similarly at pH ≈ 2 ($\varepsilon_{max} \approx 2000$ M^{-1} cm^{-1} per Fe) the corresponding complex species should have only two co-ordinated hydroxamate moieties. The isosbestic point at $\lambda = 495$ nm (in the pH range 2.2–7.4) suggests the existence of only two major species in that pH range. For pH above 10.5, the corresponding iron(III) complexes present a decrease in the absorption and λ_{max} (orange colour lightens to yellowish) with increasing pH, which may be attributed to mixed hydroxo–hydroxamate complexes (hydrolytic processes).

Analysis of Job's plots,[29] carried out at pH 7.0 ($\lambda_{max} = 425$ nm) (Fig. 4), clearly indicates that the corresponding major metal complex species should have 1:1 ligand:metal ratio. Thus, similarly to what has been found for desferrioxamine[16] and other trihydroxamate siderophore analogues[7,30] (Table 2) the interaction of TETMAHA with iron(III) seems to involve

Table 2 Stepwise protonation constants of TETMAHA and other relevant analogues, global formation constants and electronic spectral data of the corresponding iron(III) and aluminium(III) complexes and reduction potential (*vs.* SCE) of iron complexes

	H^+	Fe^{III}				Al^{III}
Ligand	log K_i	log $\beta_{Fe_pH_qL_r}$	λ_{max}/nm (ε/M^{-1} cm^{-1})	p[M]	$E_{1/2}$/mV	log $\beta_{Al_pH_qL_r}$
TETMAHA	11.47					(1,4,1) 44.17
	9.72	(1,2,1) 41.6				(1,3,1) 40.71
	8.78	(1,1,1) 37.8	425 (2939)	22.8	−602	(1,2,1) 35.60
	8.00	(1,0,1) 25.5				(1,1,1) 30.72
	6.64					(1,0,1) 21.02
	2.00					
	<2					
	a	*a*	*a*		*a*	
DOTRMAHA	>12					
	9.20					
	8.52	(1,2,1) 27.5	425 (3050)	21.7	−585	—
	7.68	(1,1,1) 24.2				
	4.69					
	<2					
	b	*b*	*b*			
DOTRMPHA	>12					
	9.58					
	8.77	(1,2,1) 28.8	425 (2960)	21.7	—	—
	7.90	(1,1,1) 25.0				
	5.35					
	<2					
	c	*d*	*d*			*e*
DFA	10.84					(1,2,1) 35.11
	9.46	(1,1,1) 30.5	440 (2640)	26.3	−698	(1,1,1) 33.93
	9.00					(1,0,1) 24.50
	8.3					

[a] From ref. 7. [b] 1,5,9-Triazacyclododecane-*N*,*N*′,*N*″-tris(*N*-methylpropionohydroxamic acid), from ref. 30. [c] From ref. 16. [d] From ref. 15. [e] From ref. 31.

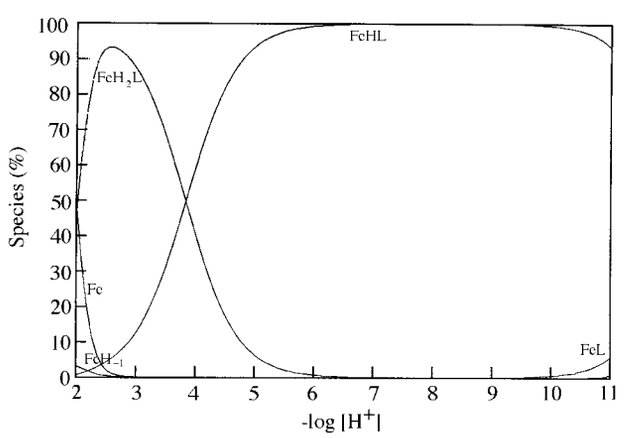


Fig. 5 Species distribution diagram for the system Fe^{III}–TETMAHA as a function of pH ($C_L/C_{Fe} = 10$; $C_{Fe} = 1.99 \times 10^{-4}$ M).

mainly a monomeric species, in a wide range of pH centred around the neutral region.

The stability constants for iron(III) complexes were determined by spectrophotometric titration (Fig. 3), aided by the PSEQUAD[32] program for the data analysis, under conditions of $C_{Fe} = 1.99 \times 10^{-4}$ M and a ten-fold excess of ligand to prevent potential precipitation of iron(III) hydroxides. In the range pH 2–11 the best-fitting model for the interpretation of the spectrophotometric titration data includes the monomeric species $Fe(H_2L)$, Fe(HL) and FeL. We have also included the binary hydroxo complexes $[Fe(OH)_x]^{3-x}$ ($x = 1$ or 2), $[Fe_2(OH)_2]^{4+}$ and $[Fe_3(OH)_4]^{5+}$with fixed constants, according to those previously reported.[31,33] The calculated stability constants are shown in Table 2 and the distribution of the complex species in solution, as a function of pH, is in Fig. 5. It can be seen that the Fe(HL) species is the major complex in a wide range of pH centred around the neutral region, $Fe(H_2L)$ exists at acidic pH and FeL in the basic region.

The values calculated for the stability constants of these complexes seem to be higher than those of ferrioxamine B (see Table 2).[15] This could be as a result of some pre-orientation of the chelating groups to their co-ordination environment induced by the macrocycle backbone. However, comparison between stability constants of different siderophores or analogues must be made with caution due to differences in the protonation constants. Thus, for a better comparison of the capacity of different compounds to complex iron, p[M] values are used. In fact the Fe^{III}–TETMAHA complex presents lower p[M] than ferrioxamine (Table 2), besides its apparent higher stability constant. Furthermore, analysis of the set of p[M] values in Table 2 shows that TETMAHA is the best iron chelator, among all the tris(hydroxamate) siderophore analogues synthesized in our laboratory. This may be attributed to the fact that this ligand has lower coulombic repulsions, between the positively charged metal ion and the protonated amine of the macrocycle, as compared with DOTRMAHA, due to the expected longer distance between these centres.

In summary, the Fe(HL) species should have the iron(III) ion co-ordinated to the three $\{O,O\}$-hydroxamate moieties and contain one amino group in its protonated form. The $Fe(H_2L)$ species should have two hydroxamate groups co-ordinated to iron(III), as indicated by the UV-VIS spectra. Since it is not enough for a complete wrapping of the metal ion, this complex should also have two water molecules in apical positions to satisfy the octahedral configuration.

Electrochemistry of the FeHL complex

In order to determine the usefulness of TETMAHA as a siderophore model, the electrochemical properties of the iron(III) complex were studied at neutral pH. Thus, cyclic voltammetric studies of an aqueous solution containing iron(III) and a twenty-fold excess of ligand ($C_{Fe} = 2.5 \times 10^{-4}$ M) were carried out at pH 7; the results are summarized in Table 3. There is an increase in i_p^a/i_p^c with the scan rate (v), which becomes near unity for $v = 20$ V s^{-1}; there is also a decrease in the

Table 3 Voltammetric data for the Fe^{III}–TETMAHA complex Fe(HL) ($C_{Fe} = 2.5 \times 10^{-4}$ M, $C_L/C_{Fe} = 20$, pH 7.0, $I = 0.1$ M)

v/V s^{-1}	$10^6 i_p^c$/A	i_p^a/i_p^c	E_p^c [a]/mV	ΔE_p/mV
0.1	0.06	0.47	−610	86
0.5	1.17	0.71	−625	95
1	1.53	0.54	−628	73
5	5.24	0.77	−624	71
10	7.62	0.94	−639	67
20	15.20	1.03	−642	57

[a] The potentials are referred to the SCE.

cathodic shift with increasing scan rate (E_p^c changes cathodically by about 18 mV, when the scan rate changes from 0.1 to 1 V s^{-1}, and only about 11 mV for v between 1 and 10 V s^{-1}). This set of data suggests that the electron transfer process may be interpreted in terms of a two-step reaction with an EC mechanism:[34] a reversible electrochemical step (E) followed by an irreversible chemical reaction (C), presumably a dissociation process. On the other hand, when the chemical reaction is negligible ($v = 20$ V s^{-1}) ΔE_p is 57 mV, thus indicating that the reversible redox process is a one-electron transfer. The reduction potential of the complex TETMAHA–iron(III) ($E_{1/2} = -0.671$ V) is in the range found for physiological reductants such as NADH (reduced nicotinamide adenine dinucleotide).[35] Furthermore, this complex seems to be easier reduced than some natural occurring siderophores, as suggested by their lower potentials: $E_{1/2} = -0.690$ V for ferrichrome and −0.698 V for ferrioxamine B.[36] Such an electrochemical behaviour, namely the easy reduction accompanied by the dissociation of the reduced species,[37] gives some support to the biological activity demonstrated by this ligand with several bacteria.[38]

Aluminium(III) complexation

The co-ordination tendencies of TETMAHA toward the aluminium(III) ion in aqueous solution were studied by potentiometry and NMR spectrophotometry. Analysis of the potentiometric titration curve, by the computer program SUPERQUAD, showed that the best fitting model involves the formation of five main Al^{III}–TETMAHA species, presented in Table 2: log $\beta[Al(H_4L)]^{4+} = 44.17(7)$; log $\beta[Al(H_3L)]^{3+} = 40.71(4)$; log $\beta[Al(H_2L)]^{2+} = 35.6(1)$; log $\beta[Al(HL)]^{+} = 30.72(3)$; log $\beta[AlL] = 21.02(4)$. In this calculation we have included the binary hydroxo complexes $[Al(OH)_x]^{3-x}$ ($x = 1$ or 4), $[Al_2(OH)_2]^{4+}$ and $[Al_3(OH)_4]^{5+}$ with the corresponding stability constants. The stability constants of these hydroxo species were determined in former work by Öhman and Forsling[39] and these values have been used in our laboratory for many years as constant data.[31] The speciation study shows that, under our experimental conditions, only $[Al(OH)_4]^-$ is relevant, but just under basic conditions.

The distribution of the complex species in solution, as a function of pH is shown in Fig. 6. It can be seen that at pH 2 there is almost 95% free aluminium which can only be completely co-ordinated to the ligand at pH 5; at pH ≈ 9.5 it begins to form $[Al(OH)_4]^-$. The main complex in the neutral region is [Al(HL)]. These data demonstrate that the interaction of the ligand with aluminium(III) is weaker than with iron(III) and that the hydroxo complexes play an important role in this system. In fact, to calculate the species involved in this system, we only used titration data with pH less than 10 because there was some difficulty in stabilizing the potential at upper pH values, probably due to the hydroxo species. To confirm this distribution of the complex species some ^{27}Al NMR spectra were obtained. The bands corresponding to the Al^{III} co-ordinated to the ligand, in the pH *ca.* 3–10 region, were very broad and overlapped, and so they do not allow any conclusion. Only the sharp line at δ 0 of the standard $[Al(H_2O)_6]^{3+}$ appears at low pH and that of $[Al(OH)_4]^-$ near δ 80 appears at basic pH. Thus,

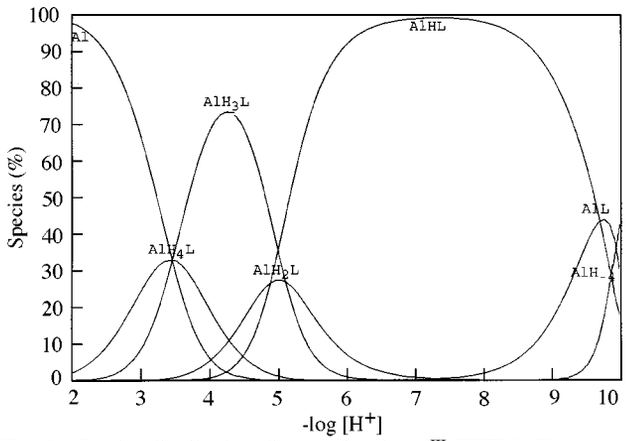


Fig. 6 Species distribution diagram for the Al^{III}–TETMAHA system as a function of pH ($C_L/C_{Al} = 2$; $C_L = 4 \times 10^{-3}$ M).

the ^{27}Al NMR spectra show the disappearance of the hexahydrated aluminium species $[Al(H_2O)_6]^{3+}$ and the appearance of $[Al(OH)_4]^-$, as a function of the pH, in accord with the potentiometric results.

Since Al^{3+} is a typical hard ion it is evident that the oxygen donor atoms of the hydroxamate groups are involved in the co-ordination and, consequently, [Al(HL)] should have one amino group protonated. This kind of co-ordination with aluminium was also found in other hydroxamates.[31,40] The stability constants of the aluminium(III) complexes with TETMAHA are lower than those with Fe^{III}, as expected, because Al^{III} has a lower ionic radius and consequently higher repulsion between the oxygen charges of chelating groups.

Conclusion

A new trihydroxamate siderophore analogue with biological activity[38] was synthesized and its chelating properties towards iron(III), copper(II) and aluminium(III) were characterized by potentiometric and spectroscopic techniques. These studies show that this ligand complexes Fe^{III} with 1:1 stoichiometry and hydroxamate-*O* co-ordination, in a similar way to that of ferrioxamine. The electron transfer process involves a one-electron two-step reaction with an irreversible chemical reaction (presumably a chemical dissociation of the reduced species) following the reversible electrochemical process. The fact that this global redox process is easier than that of the natural siderophore may contribute to the biological activity for several bacteria. Concerning the behaviour of this ligand towards copper(II), it was found that this metal ion co-ordinates to the amine nitrogen atoms of the macrocycle although the hydroxamate-*O* co-ordination may also be involved in deprotonated species. The Al^{III}–TETMAHA complexes have a behaviour similar to those of Fe^{III}, although with lower stability constants.

Experimental

Chemicals

Analytical grade reagents were used as supplied. Whenever necessary, solvents were dried according to standard methods.[41]

Synthesis

1,4,8,11-Tetraazacyclotetradecane-*N*,*N*′,*N*″-tris(*O*-benzyl-*N*-methylacetohydroxamic acid). To a suspension of the macrocycle 1,4,8,11-tetraazacyclotetradecane (0.15 g, 0.75 mmol) in dry dimethylformamide (dmf) (40 ml), sodium hydride was added (0.062 g, 2.6 mmol) under nitrogen. That mixture was left stirring for 15 min and then a solution of *O*-benzyl-*N*-methyl-2-

bromoacetohydroxamic acid[7] (0.67 g, 2.6 mmol) in dmf (10 ml) was added dropwise with stirring. After the addition the solution was stirred at 80 °C for 5 h. This mixture was then cooled until room temperature, taken up into ethyl acetate (150 ml) and washed with brine. The organic phase was dried over anhydrous Na_2SO_4. Evaporation of the solvent gave an oil which was purified by "flash" chromatography (silica gel, eluted with dichloromethane–methanol, 7.5:1) to give the compound as pure oil (0.32 g, 53%). IR (KBr): 1660 cm^{-1} (C=O). ^1H NMR ($CDCl_3$, TMS): δ 1.63 (m, 4 H, $NCH_2CH_2CH_2N$), 2.31 (m, 4 H, $N^8CH_2CH_2N^{11}$; $N^1CH_2CH_2CH_2N^{11}$), 2.44 (t_b, 2 H, $N^1CH_2CH_2CH_2N^{11}$), 2.64 (m, 6 H, $N^4CH_2CH_2CH_2N^8$; $N^8CH_2CH_2N^{11}$), 2.89 (t_b, 4 H, $N^1CH_2CH_2N^4$) 3.17 (s, 9 H, CH_3), 3.17 (m, 6 H, NCH_2CON), 4.83 (s, 6 H, $PhCH_2$) and 7.38 (m, 15 H, aryl *H*). *m*/*z* (FAB-MS) 733 (M + 1).

1,4,8,11-Tetraazacyclotetradecane-*N*,*N*′,*N*″-tris(*N*-methylacetohydroxamic acid). To a solution of 1,4,8,11-tetraazacyclotetradecane-*N*,*N*′,*N*″-*tris*(*O*-benzyl-*N*-methylacetohydroxamic acid) (0.32 g, 0.4 mmol) in dry methanol (30 ml) was added 10% Pd/C (100 mg) and the mixture was stirred under H_2 (1 atm) for 4 h at room temperature. After filtration of the solid residue, the solvent was evaporated under reduced pressure and the product obtained as a white powder which was then recrystallized from methanol–diethyl ether (150 mg, 70%), mp 187 °C (decomp.). IR (KBr): 1630 cm^{-1} (C=O). ^1H NMR [D_2O, sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS)]: δ 1.66 (m, 2 H, $N^8CH_2CH_2CH_2N^4$), 1.82 (m, 2 H, $N^{11}CH_2CH_2CH_2N^1$), 2.66 (m, 4 H, $N^8CH_2CH_2N^{11}$; $N^1CH_2CH_2CH_2N^{11}$), 2.79 (t, 2 H, $N^1CH_2CH_2CH_2N^{11}$), 2.90 (t, 2 H, $N^8CH_2CH_2CH_2N^4$), 2.95 (t_b, 2 H, $N^8CH_2CH_2CH_2N^4$), 3.07 (t, 2 H, $N^8CH_2CH_2N^{11}$), 3.20 (t, 4 H, $N^4CH_2CH_2N^1$), 3.23 (s, 9 H, CH_3), 3.58 (s, 4 H, N^8CH_2CO; N^1CH_2CO) and 3.80 (s, 2 H, N^4CH_2CO). *m*/*z* (FAB-MS): 462 (M + 1) (Found: C, 46.62; H, 8.12; N, 19.60. Calc. for $C_{19}H_{39}N_7O_6 \cdot 1.75H_2O$: C, 46.28; H, 7.97; N, 19.88%).

1,4,8,11-Tetramethyl-1,4,8,11-tetraazacyclotetradecane (4MeCYCLAM). This compound was synthesized according to the literature.[20] Recrystallization from water–ethanol gave white crystals (60%). ^1H NMR (D_2O): δ 2.14 (q, 4 H, $NCH_2CH_2CH_2N$), 2.89 (s, 12 H, CH_3), 3.31 (m, 8 H, $NCH_2CH_2CH_2N$) and 3.54 (s, 8 H, NCH_2CH_2N). *m*/*z* (FAB): 257 (M + 1).

Potentiometric measurements

The pH potentiometric titrations were conducted at 25.0 ± 0.1 °C, at an ionic strength of 0.1 M (KNO_3) using a Crison Digital 517 instrument with an Ingold U1330 glass electrode and an Orion 90-00.11 Ag–AgCl reference electrode. The electrode calibration was carried out daily from a titration of a strong acid (HNO_3 0.1 M) with a strong base (KOH 0.1 M) at the same ionic strength to assure that we got adequate responses in the studied pH range and to control the exact concentration of the ligand (Gran's method).[42] The ligand was weighed directly into the potentiometric cell and the Cu^{II} pipetted from a stock solution of 0.1 M $Cu(NO_3)_2$; the Al^{III} was pipetted from a 5×10^{-2} M stock solution of $Al(NO_3)_3$ in 2.5×10^{-2} M HNO_3, the exact amount of aluminium being determined by inductively coupled plasma emission (Perkin-Elmer Plasma 400). Calculations from potentiometric data were performed with the SUPERQUAD[11] program and speciation curves with the SPEA program.[43]

Spectrophotometric measurements

All spectra were measured on a Lambda 9 Perkin-Elmer spectrophotometer at 25 °C and at a constant ionic strength (I = 0.1 M, KNO_3). Solutions of the metal complexes were generated *in situ* by addition, to an excess of the ligand, of a standard metal ion solution: $Cu(NO_3)_2$ 5×10^{-2} M in HNO_3 (1 M) and $Fe(NO_3)_3$ 1000 ppm in HNO_3 (0.5 M). The pH measurements were carried out using a 420A Orion pH-meter, equipped with an Orion 91-03 glass calomel combination electrode. The stability constants for the iron(III) complexes were evaluated from the spectrophotometric titration data, using the PSEQUAD[32] computer program.

Job method. Solutions of the metal complexes were generated *in situ*, by addition of a solution of 0.001 M $Fe(NO_3)_3$ in HNO_3 (0.5 M) to a solution of 0.001 M of ligand, buffered with Tris pH 7.0 (0.1 M) and ionic strength 0.1 M (KNO_3). Measurements were made at 425 nm with pH 7.0. All the solutions contained variable concentrations of ligand and Fe^{III} such that $C_L + C_{Fe} = 6.0 \times 10^{-4}$ M.

Electrochemical measurements

Cyclic voltammograms were recorded using a three-electrode system with a hanging mercury drop working electrode, a platinum auxiliary electrode, and a saturated calomel reference electrode (SCE). All measurements were performed with an AUTOLAB, ECOCHEMIE instrument coupled with a computer equipped with the GPES-3 program. The complex was generated *in situ*, in a solution of iron(III) ($C_{Fe} = 2.5 \times 10^{-4}$ M) with a 20-fold excess of ligand, at pH 7.0, at constant ionic strength (I = 1.0 M, KNO_3), thermostatted at 25 °C and degassed with N_2. This study involved varying the scan rates (v = 0.1–20 V s^{-1}). The pH measurements were performed with a 420A Orion pH meter equipped with an Orion 91-03 combined electrode.

Other measurements

The ^1H NMR spectra were recorded on a Varian Unity 300 spectrometer at 25 °C. Chemical shifts are reported in ppm (δ) from internal references {tetramethylsilane (TMS) in $CDCl_3$ solutions and sodium 3-(trimethylsilyl)[2,2,3,3-$^2H_4$]propionate in D_2O solutions}. The following abbreviations are used: s = singlet; t = triplet; t_b = broad triplet, m = multiplet. The ^{27}Al NMR spectra were recorded on a Bruker DRX500 spectrometer, with a 10 mm BB probe head at room temperature. Solutions were prepared in D_2O. A solution of $C_{Al} = 2 \times 10^{-3}$ M was used for pulse calibration and as a reference for 0.00 ppm. The acquisition parameters were as follows: spectrometer frequency, SF = 130.32 MHz; spectral window, SW = 9470 Hz; pulse width, P1 = 20 μs (flip angle *ca.* 45°); relaxation delay, D1 = 0.1 s; number of scans, NS = 16; digitizer resolution, DR = 18 Hz per point. Integrated intensities were calculated using the Bruker WIN-NMR program.

The ESR studies were performed on a Bruker ESP ER 200D spectrometer (X-band) in frozen D_2O solutions with 20% ethylene glycol to have a good glass ($C_L/C_{Cu} = 1$; $C_{Cu} = 6.0 \times 10^{-3}$ M; T = 100 K; modulation frequency 100 kHz). The spectra were simulated with the ESR program (version 1.0) developed by Frank Neese[44] and the g and A parameters were calculated. The IR spectra were recorded on a Perkin-Elmer 683 spectrophotometer. Melting temperatures were measured with a Leica Galen III hot stage apparatus and are uncorrected. Elemental analyses were performed on a Fisons EA1108 CHNF/O instrument. Mass spectra were recorded on a VG TRIO-2000 GC/MS instrument.

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