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Effect of the tether on the Mg(II), Ca(II), Cu(II) and Fe(III) stability constants and pM values of chelating agents related to EDDHA†

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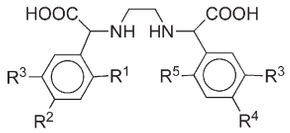
The effect of the length and the structure of the tether on the chelating ability of EDDHA-like chelates have not been established. In this work, PDDHA (propylenediamine-*N*,*N*′-bis(*o*-hydroxyphenyl)acetic acid), BDDHA (butylenediamine-*N*,*N*′-bis(*o*-hydroxyphenyl)acetic acid) and XDDHA (*p*-xylylenediamine-*N*,*N*′-bis(*o*-hydroxyphenyl)acetic acid) have been obtained and their chemical behaviour has been studied and compared with that of EDDHA following our methodology. The purity of the chelating agents, and their protonation, Ca(II), Mg(II), Fe(III) and Cu(II) stability constants and pM values have been determined. The stability constants and pM values indicate that EDDHA forms the most stable chelates followed by PDDHA. However, the differences among the pFe values are small when a nutrient solution is used, and in these conditions the XDDHA/Fe(III) chelate is the most stable. The results obtained in this work indicate that all the chelating agents studied can be used as iron chlorosis correctors and they can be applied to soil/plant systems.

Introduction

Iron chelates related to *o*,*o*-EDDHA/Fe(III) are used as fertilizers to correct the iron chlorosis in plants.[1–3] *o*,*o*-EDDHA **1** (ethylenediamine-*N*,*N*′-bis(2-hydroxyphenylacetic acid), *o*,*p*-EDDHA **2** (ethylenediamine-*N*-(2-hydroxyphenylacetic acid), *N*′(4-hydroxyphenylacetic acid), EDDH4MA **3** (ethylenediamine-bis(2-hydroxy-4-methylphenyl)acetic acid) and EDDCHA **4** (ethylenediamine-bis(2-hydroxy-5-carboxylphenyl)acetic acid) form very stable iron chelates (compounds **1–4** Fig. 1). All of them have two phenolate groups replacing two of the carboxylates of EDTA (ethylene diaminetetraacetic acid), which increases the stability of their iron chelates by more than 10^{10} times.[4] The *o*,*o*-EDDHA/Fe(III) chelate does not decompose even in strongly alkaline solutions.[5]



1 R^1 = R^5 = OH; R^2 = R^3 = R^4 = H — *o*,*o*-EDDHA
2 R^1 = R^4 = OH; R^2 = R^3 = R^4 = H — *o*,*p*-EDDHA
3 R^1 = R^5 = OH; R^2 = R^4 = Me; R^3 = H — EDDH4MA
4 R_1 = R^5 = OH; R^2 = R^4 = H; R^3 = COOH — EDDCHA

Fig. 1

The effect of the different substituents in the benzene ring of EDDHA-like chelating agents has been studied by us.[6] Thus we have confirmed the need of at least one *ortho*-hydroxyphenyl group to form Fe^{3+} chelates. This is exemplified by *o*,*p*-EDDHA **2** that is able to form Fe^{3+} complexes with stability constants lower than those of *o*,*o*-EDDHA/Fe(III) but higher than those of EDTA/Fe(III).[7]

Apart from the substituents in the benzene ring, other factors such as the size of the metal ion,[8–11] and the length of the alkyl chain connecting the hydroxyphenylglycine moieties can affect the stability of these metal chelates. White[12] has reported the synthesis of several chelating agents analogous to *o*,*o*-EDDHA with alkyl chains of different length linking the amino groups (from two to five carbon atoms) and the stability of their complexes with Fe(II), Fe(III), Mn(II), Mn(III), Gd(III) and Cr(III). On the other hand, it has been pointed out that the relative metal ion affinities of the two diastereomeric pairs of *o*,*o*-EDDHA and TMPHPG **5** (*N*,*N*′-trimethylenbis[2-(2-hydroxy-3,5-dimethylphenyl)glycine]) is related to the length of the tether joining both aromatic rings.[13] Thus, considering an octahedral coordination environment for a metal chelate, only the [6,5,6] pattern has been found in the solid state for *rac*-*o*,*o*-EDDHA complexes[14,15] whereas the [5,6,5] arrangement is probably the preferred configuration for *rac*-TMPHPG (Fig. 2).[13] On the other hand, [6,5,5] and [6,6,5] arrangements are the most likely for the *o*,*o*-EDDHA and TMPHPG *meso*-forms, respectively.[13,16,17] Additionally, the increase in the length of the diamine alkyl chain is reflected in the stability constants of the two diastereomeric pairs of these complexes. Hence, greater stability has been found for the *rac*-Fe–*o*,*o*-EDDHA compared to the *meso*-Fe–*o*,*o*-EDDHA on the basis of a more favorable octahedral geometry achieved by the ligand, whereas

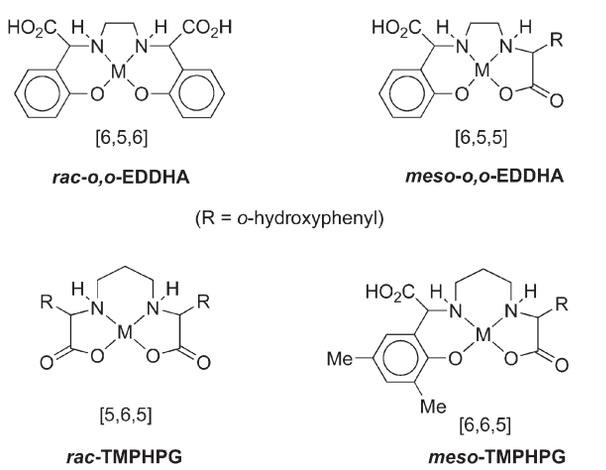


Fig. 2

† Electronic supplementary information (ESI) available: Figs. 1S–4S and Tables 1S–5S. See http://www.rsc.org/suppdata/dt/b4/b408730e/

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Table 1 Log protonation constants[a] for the chelating agents

	$\log K_1^H$	$\log K_2^H$	$\log K_3^H$	$\log K_4^H$
	[HL]/[H][L]	[H_2L]/[H][HL]	[H_3L]/[H][H_2L]	[H_4L]/[H][H_3L]
o,*o*-EDDHA[b]	11.94	10.73	8.66 ± 0.04	6.18 ± 0.06
PDDHA	12.17	11.08	8.79 ± 0.02	7.07 ± 0.11
BDDHA	11.96	10.89	8.73 ± 0.07	7.49 ± 0.23
XDDHA	11.70	10.86	8.50 ± 0.03	6.89 ± 0.20

[a] μ = 0.1 M (NaCl); T = 25 °C. [b] Ref. 6.

the differences found between *rac*- and *meso*-Fe–TMPHPG complexes were smaller.[13]

During our ongoing work directed toward the development of new chelating agents for the treatment of iron chlorosis,[18] as well as to determine the presence of impurities in commercial formulations,[19] several chelating agents **6–8** analogous to *o*,*o*-EDDHA with the amino groups linked by alkyl chains differing in length (ethylene, propylene and butylene) or structure (*p*-xylylene) have been synthesized (Fig. 3). Reported herein is the characterization and equilibrium studies of the free ligands and their Mg(II), Ca(II), Cu(II) and Fe(III) chelates, using the novel methodology previously described by us,[6] in order to test their potential as iron chlorosis correctors. Additionally, these chelating agents could provide novel complexes of Fe(II), Fe(III), Mn(II), Gd(III) and Cr(III) that would be of use to enhance magnetic resonance images of body organs and tissues.[12]

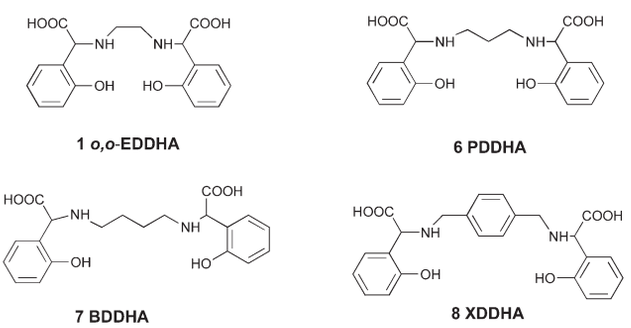


Fig. 3

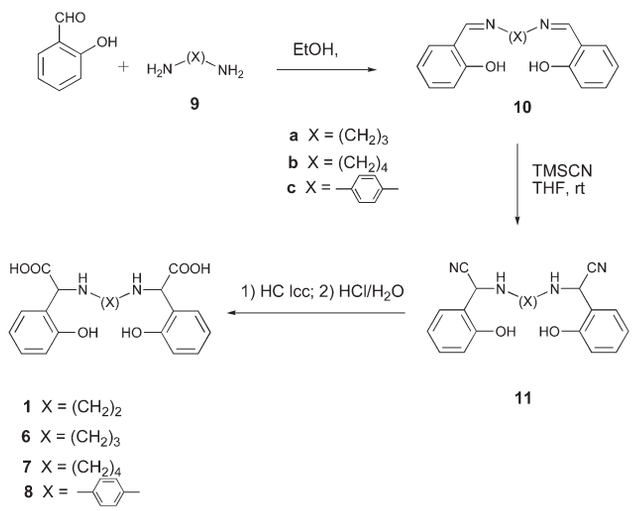
Results and discussion

Compound **1** was prepared as described previously by us.[6] The synthesis of ligands **6–8** was made in three steps starting by condensation of salicylaldehyde and the corresponding amines **9** in boiling absolute EtOH. The diimines **10** were obtained in almost quantitative yields as pure crystalline solids and were reacted with TMSCN in anhydrous THF at room temperature to yield the corresponding α-aminonitriles **11**. These compounds were submitted to acid hydrolysis by sequential treatment with concentrated HCl and dilute HCl (Scheme 1). The pure amino acids **6–8** were obtained as hydrochlorides directly from the hydrolysis step or as the free compounds by precipitation from the reaction mixture, by adjusting the solution to pH 4.3–4.7 with 6 M NaOH. Compounds **6–8** were obtained as a 1 : 1 mixture of *meso* and *racemic* diastereomers and their analytical and spectroscopic data were consistent with the proposed structures.

The titrimetric purities of the chelating agents **6–8** ranged from 70 to 95%. The molar absorptivities of all the Fe(III) chelates were determined at 480 nm. As the maximum of absorption for the Fe–phenolate bands of BDDHA and XDDHA are λ_{max} = 507.0 and 513.0 nm, respectively, their ε values at 480 nm are lower than those obtained for *o*,*o*-EDDHA and PDDHA (see Table 1S of ESI†).

Protonation constants

The first four protonation constants corresponding with the protonation of the two phenolate groups (K_1^H and K_2^H) and the two



Scheme 1

amino groups (K_3^H and K_4^H) for ligands **6–8** together with those previously reported by us for EDDHA **1** are shown in Table 1.

The protonation constants corresponding to the carboxylate groups (K_5^H and K_6^H) could not be determined because in the back-titration the precipitation of the ligands occurred after the addition of the fourth equivalent of acid.[17]

As the length of the tether chain increases, *i.e.* from two (*o*,*o*-EDDHA) to four methylene units (BDDHA), the two amino groups are more independent from each other and there is less charge repulsion between them. This fact is reflected in the values of $\log K_4^H$ for PDDHA BDDHA and XDDHA, which are higher than that of *o*,*o*-EDDHA (Table 1). The difference between the values of $\log K_3^H$ and $\log K_4^H$ (the protonation constants for the nitrogen atoms) could be used as a reference of the degree of independence between the amino groups (the smaller the difference the higher the independence between the two amino groups). These values are 1.24 (BDDHA), 1.61 (XDDHA) and 1.72 (PDDHA), all considerably smaller than the difference observed between $\log K_3^H$ and $\log K_4^H$ for *o*,*o*-EDDHA (2.48) the ligand that bears the shorter tether. In the case of PDDHA, the difference between both constants is similar to that reported for TMPHPG,[12] a chelating agent in which the amino groups are also linked by three methylene groups.

Ca(II) and Mg(II) stability constants

Ca(II) and Mg(II) stability constants for compounds **6–8** are shown in Table 2. From the Ca(II) and Mg(II) potentiometric curves, it may be presumed the existence of at least three species of the metal chelates: MH_2L, MHL^- and ML^{2-}. In order to compare the stability of such metal–ligand complexes, the equilibrium constant K_{ML} and therefore $\log K_{ML}$, are used.[20] The magnitudes of Mg(II) stability constants are higher than those of Ca(II) for all the products studied. This behaviour is in good agreement with that found for *o*,*o*-EDDHA and their analogues,[6,21] but is the opposite to that observed for EDTA and EDTA-like ligands.[20] Metal properties[22,23] (size of the metal, charge, ionic radius and so on) and ligand architecture[10] were already used to explain this fact.

Table 2 Log stability constants[a] for Ca(II) and Mg(II) chelates

	Ca(II)			Mg(II)		
	[ML]/[L][M]	[MHL]/[H][L][M]	$[MH_2L]/[H]^2[L][M]$	[ML]/[L][M]	[MHL]/[H][L][M]	$[MH_2L]/[H]^2[L][M]$
o,*o*-EDDHA[b]	7.29 ± 0.30	16.77 ± 0.33	25.95 ± 0.50	9.76 ± 0.05	18.18 ± 0.15	25.36 ± 0.24
PDDHA	6.17 ± 0.19	16.43 ± 0.40	25.86 ± 0.40	8.81 ± 0.08	17.33 ± 0.27	25.57 ± 0.64
BDDHA	7.16 ± 0.06	16.31 ± 0.38	25.47 ± 0.14	7.52 ± 0.44	16.15 ± 0.54	25.14 ± 0.61
XDDHA	6.05 ± 0.22	16.55 ± 0.33	25.07 ± 0.24	7.35 ± 0.49	16.59 ± 0.53	24.85 ± 0.51

[a] μ = 0.1 M (NaCl); T = 25 °C. [b] Ref. 6.

Table 3 Log stability constants[a] for Cu(II) and Fe(III) chelates

	Cu(II)			Fe(III)		
	[ML]/[L][M]	[MHL]/[H][L][M]	$[MH_2L]/[H]^2[L][M]$	[ML]/[L][M]	[MHL]/[H][L][M]	$[MOHL]/[H]^{-1}[L][M]$
o,*o*-EDDHA[b]	25.13 ± 0.00	32.61 ± 0.01	37.31 ± 0.01	35.09 ± 0.28	36.89 ± 0.21	23.66 ± 0.27
PDDHA	22.31 ± 0.14	31.73 ± 0.08	38.88 ± 0.05	33.54 ± 0.23	35.84 ± 0.22	21.74 ± 0.14
BDDHA	19.55 ± 0.16	28.68 ± 0.05	35.38 ± 0.05	29.69 ± 0.17	33.66 ± 0.08	18.75 ± 0.25
XDDHA	17.52 ± 0.13	26.59 ± 0.09	-[c]	30.13 ± 0.18	34.12 ± 0.19	-[c]

[a] μ = 0.1 M (NaCl); T = 25 °C. [b] Ref. 6. [c] Not determined.

Mg(II) is more affected than Ca(II) by the increase of the number of methylene units in the linker between the two amino groups (Table 2). This fact is contrary to several theories that suggest that an increase in the chelate ring size destabilizes better complexes of larger metal ions (*i.e.* Ca(II)) than complexes of smaller metal ions (*i.e.* Mg(II)).[9] Bearing in mind the structure of the complexes discussed in Fig. 2, we could consider that in the case of Mg(II), the central of the three chelate rings defining the plane is more stable for *o*,*o*-EDDHA (five-membered ring) than in the case of PDDHA (six-membered ring) or BDDHA (seven-membered ring). However, for Ca(II) (a larger metal ion), an increase in the length of the alkyl chain does not produce a clear effect over the stability constants, possibly due to the poor affinity between calcium and phenolic ligands.[9,10]

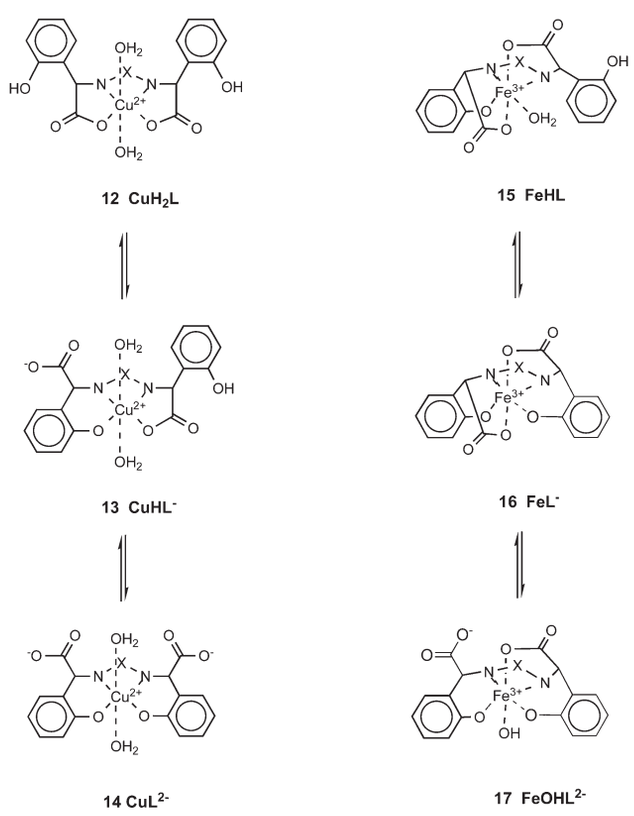
In both Ca(II) and Mg(II) systems, XDDHA has the lowest stability constants. When XDDHA is compared with *o*,*o*-EDDHA both, the increase in the chelate ring size and the higher preorganization of the molecule could be affecting its ability to form complexes. Several authors have pointed out that the increase in preorganization level leads to an higher stability of the complexes (*i.e.* EDTA *vs.* CDTA, *trans*-1,2-diaminocyclohexane, *N*,*N*,*N*′,*N*′-tetraacetic acid).[9,10] However, it is more likely that the low stabilities of XDDHA Ca(II) and Mg(II) complexes are related to the presence of the 4,4′-substituted benzene ring that imposes a conformational strain to the complex that is not present in the other ligands studied. The presence of the aromatic ring in the ligand may thwart the formation of the three chelate rings defining the plane of the complex and, in consequence, the stability of the chelate is greatly diminished.

Cu(II) and Fe(III) stability constants

The Cu(II) and Fe(III) stability constants are presented in Table 3.

The different Cu(II) chelate species are represented in Scheme 2. At low pH values, a blue Cu(II) complex is observed, which no doubt involves only coordination to the ethylenediamine nitrogens, the carboxylate oxygens and two molecules of water (**12** in Scheme 2).[24] The upper pH value for the range at which this species is predominant is different depending on the chelating agent (4.70, 7.15 and 6.70 for *o*,*o*-EDDHA/Cu(II), PDDHA/Cu(II) and BDDHA/Cu(II)), respectively. Since a precipitate appears at pH below 8 for XDDHA/Cu(II), the stability constant for the diprotonated species could not be determined in this case. A new green complex is formed as the pH rises. This complex involves the coordination of the Cu(II) with the phenolate groups (**14** in Scheme 2) at pH above 7.48, 9.42, 9.13 and 9.07 for *o*,*o*-EDDHA/Cu(II), PDDHA/Cu(II), BDDHA/Cu(II) and XDDHA/Cu(II), respectively. Finally, the species **13** appears at intermediate pH values and involves the two amino groups, one carboxylate group and one phenolate group. When comparing the Cu(II) stability constants in Table 3 it is clear that an increase in the distance between the two amino groups reduces the stability of the Cu(II) chelate, the *o*,*o*-EDDHA/Cu(II) complex being the most stable (Table 3). In the case of XDDHA/Cu(II) both the distance between the two amino groups and the rigidity imposed by the benzene ring make this complex the least stable of the series.

The Fe(III) chelate species are also represented in Scheme 2. The predominant species **16** involves the coordination with the nitrogen atoms, the carboxylate oxygens and the phenolate groups and this occurs at pH above 1.89, 3.42 and 3.97 for *o*,*o*-EDDHA/Fe^{3+}, PDDHA/Fe(III) and BDDHA/Fe(III),



Scheme 2

Table 4 pFe[a] *vs* pH for iron chelates[b]

	7	7.5	8	8.5
o,*o*-EDDHA[c]	23.7	25.2	26.7	28.0
PDDHA	21.2	22.8	24.4	25.8
BDDHA	17.5	19.3	20.9	22.4
XDDHA	18.8	20.4	21.9	23.3

[a] Calculated for $[L_T] = 1.1 \times 10^{-6}$ M, $[Fe_T] = 1.0 \times 10^{-6}$ M. [b] For pFe data in the whole pH range see Table 4S in ESI.† [c] Ref. 6.

Table 5 pCu[a] *vs*. pH for copper chelates[b]

	7	7.5	8	8.5
o,*o*-EDDHA[c]	14.3	15.5	16.8	18.1
PDDHA	13.1	14.3	15.6	16.8
BDDHA	9.7	10.9	12.0	13.0
XDDHA	8.3	9.4	10.4	11.3

[a] Calculated for $[L_T] = 1.1 \times 10^{-6}$ M, $[M_T] = 1.0 \times 10^{-6}$ M. [b] For pCu data in the whole pH range see Table 5S in ESI.† [c] Ref. 6.

Table 6 pFe values against pH in agronomic conditions[a]

	7	7.5	8	8.5
o,*o*-EDDHA	15.09	16.28	17.67	19.27
PDDHA	15.01	16.23	17.65	19.27
BDDHA	14.99	16.21	17.63	19.25
XDDHA	15.38	16.59	18.01	19.58

[a] $[Fe(III)] = [Ligand] = 1.0 \times 10^{-4}$ M; $[Ca(II)] = 1.6 \times 10^{-3}$ M; $[Mg(II)] = 8.0 \times 10^{-4}$ M; $[Cu(II)] = 3.15 \times 10^{-7}$ M.

respectively. The hydroxylated species (**17**), where one carboxylate is replaced by an OH group, occurs at pH above 11. The protonated species (**15**) involves the coordination with a water molecule instead of the phenolate group.

However, the discussion about the structure of the different XDDHA/Fe(III) species is not so clear. The predominant species appears at pH above 3.99 and no XDDHA/Fe(III) species have been observed at pH above 11 due to the decomposition of the chelate. A study of molecular mechanics for the XDDHA/Fe(III) complex has indicated that a closed octahedral environment for the iron, involving the two amino, two carboxylate and two phenolate groups (as in FeL^- **16**), necessarily requires the complete distortion of the benzene ring. This fact makes very unlikely a closed octahedral coordination for the XDDHA/Fe(III) species present in solution. Interestingly, the planarity of the benzene ring is maintained if one of the nitrogen atoms is not involved in the coordination of the metal. Considering that the stability constants of the complexes BDDHA/Fe(III) (with no special conformational restrictions) and XDDHA/Fe(III)$^+$ are similar, a strained closed octahedral structure seems to be very unfeasible for this later chelate, whereas a structure in which one of the six coordination sites available is not used on behalf of the stability of the complex should be much more likely. The decomposition of the XDDHA/Fe(III) chelate at pH above 11 is a fact that supports this argument. To maintain the planarity of the benzene ring, the predominant species ($FeOHL^{2-}$ **17**) would only involve two carboxylate oxygens, two phenolate groups and one of the two nitrogen atoms. As we have commented above, complexes with only four chelation sites such as *p*,*p*-EDDHA/Fe(III) or EDDMtxA/Fe(III) are so unstable that they can not be formed.[6]

In view of the values of the Fe(III) stability constants in Table 3, EDDHA with a two-methylene group tether is the best chelating agent. This is in agreement with the data reported by White.[12] The effect of the size of the chelate ring in the stability of metal complexes has been studied in other chelating agents with similar structures. The stability constant of HBTDA/Fe(III) (*N*,*N*′-bis-(2-hydroxybenzyltrimethylenedinitrilo)-*N*,*N*′-diacetic acid) ($\log K_{FeL} = 37.8$)[4] is lower than that of HBED/Fe(III) ($\log K FeL = 39.7$).[21] The difference between the two constants is similar to that obtained in this work for EDDHA and PDDHA (see Table 3). A further increase in the length of the alkyl chain results in an additional decrease in the stability with metal ions such as Fe(III).

pM values and species distribution: agricultural relevance

pFe and pCu values were determined using the first model[6] in a 4–12 pH range. Tables 4 and 5 only show the pM values at agronomically relevant pH values.

From the pFe values listed in Table 4, *o*,*o*-EDDHA and PDDHA are the most effective ligands. BDDHA is the poorest ligand in all pH range. In fact, the pFe values follow the same sequence observed for the Fe(III) stability constants (see Table 3). *o*,*o*-EDDHA and PDDHA could be applied to soil systems and be used as iron chlorosis correctors. However, XDDHA and BDDHA show pFe values lower than those of EDTA at pH 7.5 (pFe = 22.3) and their possible application into soil systems is questionable until further experimental evidence could be collected.

The sequence obtained for pCu (Table 5) is similar to that obtained for pFe and it is in agreement with the Cu(II) stability constants shown in Table 3. *o*,*o*-EDDHA and PDDHA are the most effective ligands and XDDHA has the lowest pCu values in all pH range.

pFe and pCu values obtained in pure solutions are not very useful in physiological studies and agronomic use, since the iron chelates are employed in systems where several other metals (*i.e.* Cu(II), Ca(II), Mg(II), *etc*) are present. The presence of those metals can modify the relative effectiveness of the iron chelates and, therefore, the pFe values could vary. For this reason and as an example, the pFe values were also obtained in the second model for the chelating agents under study in a nutrient solution system (Table 6).

These pFe values resulted to be lower than those collected in Table 4, due to the competition between iron and the other metals. All the phenolic chelating agents considered presented similar pFe values in nutrient solution conditions and hence all could be used as chlorosis correctors. However, their behavior will depend on other external factors as soil properties, method of application, culture type, weather conditions, solubility, *etc.*

In Fig. 4 the species distribution curves for the chelating agents in Hoagland nutrient solution are shown together with those already calculated for *o*,*o*-EDDHA.[6] For all phenolic ligands the FeL species is the predominant in the whole agronomic pH range (5–9). Thus, 100% of the iron chelate remains as FeL^- species at pH below 11 for *o*,*o*-EDDHA and PDDHA and at pH below 10 for BDDHA and XDDHA. The hydroxylated $FeOHL^{2-}$ species appear at pH around 11.0 in those ligands in which it has been possible to determine it. Only at pH above 11.5, the calcium and magnesium chelates become predominant species. Due to the low concentration of Cu(II) in Hoagland nutrient solution, copper chelates are not predominant in either pH range, although their stability constants are higher than those of Ca(II) and Mg(II) that are present in larger concentrations. The curves are in good agreement with the pFe values, because all phenolic ligands are able to form very stable iron chelates in solution conditions.

Considering now the data in Table 6, XDDHA is not only agronomically valid but also shows the highest affinity for Fe(III). If the structure of XDDHA/Fe(III) complexes did not necessarily involve the six binding groups of the XDDHA around the metal, this result could be interpreted in the way that it is not necessary a close octahedral environment to be effective in chelating iron. In fact, a similar behaviour has been reported by us for *o*,*p*-EDDHA as chlorosis corrector.[7]

In conclusion, the Ca(II), Mg(II), Cu(II) and Fe(III) stability constants of *o*,*o*-EDDHA analogs **6–8** are affected by the

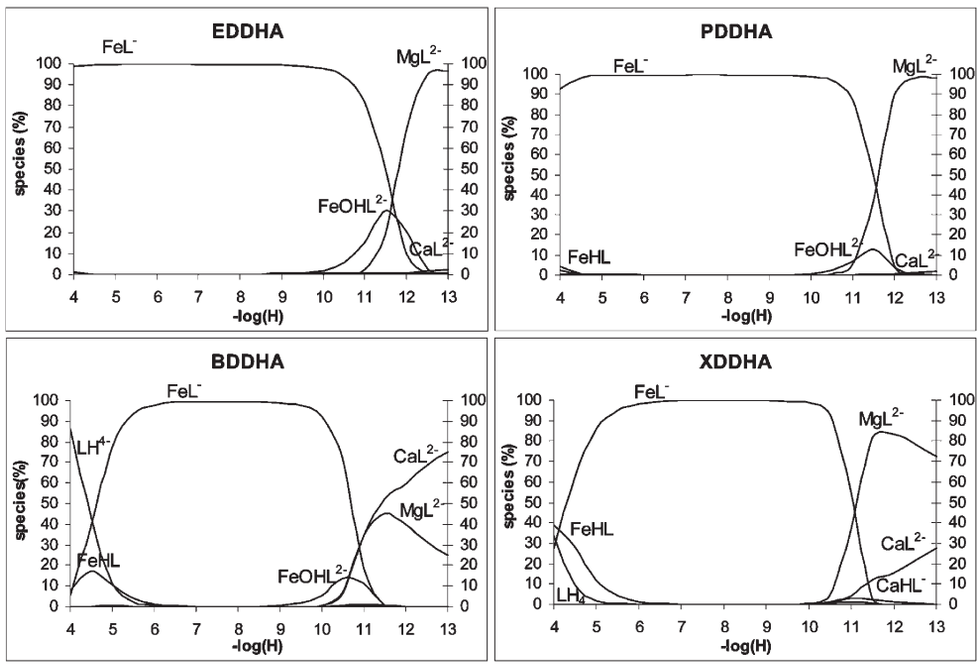


Fig. 4 Species distribution against pH using Hoagland nutrient solution composition for ligands **1**, **6**, **7** and **8**. $[Fe^{3+}] = [Ligand] = 1.0 \times 10^{-4}$ M; $[Ca^{2+}] = 1.6 \times 10^{-3}$ M; $[Mg^{2+}] = 8.0 \times 10^{-4}$ M; $[Cu^{2+}] = 3.15 \times 10^{-7}$ M.

length and the structure of the tether linking the two amino groups. The sequence obtained for each metal is similar and the general trend is that the higher the distance between the two amino groups the lower the value of the corresponding stability constant. The pM values of ligands **6–8** calculated using the first model show the same tendency. However, when pFe is calculated using a nutrient solution composition, the differences among pFe values decrease and XDDHA shows the highest affinity to Fe(III). With independence of the length and the complexity of the tether linking the amino groups, all the chelating agents studied can be used as iron chlorosis correctors and they can be applied into the soil/plant system.

Experimental

General procedures

^{1}H and ^{13}C NMR spectra were recorded on a Bruker 200-AC (200.13 MHz for ^{1}H and 50.03 MHz for ^{13}C) spectrometer. Chemical shifts are given in ppm relative to TMS (^{1}H, 0.0 ppm), or $CDCl_3$ (^{13}C, 77.0 ppm) or otherwise stated. IR spectra were taken on a Perkin-Elmer 781 spectrometer. Potentiometric measurements were performed with a Metrohm 719 and/or 721 potentiometers (precision of 0.1 mV) and a Metrohm combined pH glass. Photometric titrations were carried out using a Metrohm 662 photometer (resolution of 10 ± 0.1 nm) with a white-light spectrode of path length 2 × 10 mm. Both potentiometers were controlled by a TiNet 2.4 software program for PC.

Flame-dried glassware and standard Schlenk techniques were used for oxygen- or water-sensitive reactions. All reagents used in this work were of analytical grade. All aqueous solutions were prepared with CO_2-free, water type I grade.[25] $CaCl_2$, $MgCl_2$, NaCl, NaOH, $Cu(NO_3)_2$, HCl and Fe^{3+} standard solutions were obtained from Merck Chemical Co and they were properly standardized. A Gran's plot analysis[26] was used to check for carbonate contamination of the standard aqueous NaOH and consistently revealed less than 0.5% of carbonate. All titrations were made under N_2 inert atmosphere (99.9995 purity grade N_2, NaOH washed, 0.100 NaCl saturated), ionic strength fixed at 0.1 M with NaCl and at 25 ± 0.5 °C. Commercial buffer solutions were used to calibrate the combined pH glass electrode in order to read $-\log(H^+)$. Therefore, all equilibrium constants are calculated as mixed constants (K^m). They have been readily transformed to concentration constants (K^c) or thermodynamic constants (K^o) using the activity coefficients from Davies' equation.

All commercially available organic reagents were used without further purification.

General procedure for the synthesis of imines 10

Imines **10** were synthesized in quantitative yield by refluxing salicylaldehyde and the corresponding amine **9** (2 : 1 molar ratio) in absolute ethanol for 2 h.

Imine, 10a. Starting from 2.44 g (20 mmol) of salicylaldehyde and 0.83 cm^3 (10 mmol) of **9a**, 2.0 g (71%) of **10a** was obtained; mp 64–66 °C (yellow crystals from EtOH). δ_H ($CDCl_3$) 2.05 (2 H, q, J = 6.6 Hz), 3.63 (4 H, t, J = 6.6 Hz), 6.76–6.91 (4 H, s), 7.14–7.28 (4 H, m), 8.30 (2 H, s), 13.36 (1 H, br s); δ_C ($CDCl_3$) 31.7, 56.8, 117.0, 118.6, 118.7, 131.3, 132.3, 161.1, 165.4. Found: C, 71.39; H, 5.80; N, 10.18. Calc. for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44%.

Imine, 10b. Starting from 2.44 g (20 mmol) of salicylaldehyde and 0.88 g (10 mmol) of **9b**, 2.5 g (84%) of **10b** was obtained; mp 91–93 °C (yellow crystals from EtOH). δ_H ($CDCl_3$) 1.67–1.81 (4 H, m), 3.58 (4 H, m), 6.76–6.90 (4 H, m), 7.15–7.27 (4 H, m), 8.28 (2 H, s), 13.46 (1 H, br s); δ_C ($CDCl_3$) 28,6, 59.4, 117.1, 118.6, 118.9, 131.3, 132.2, 161.3, 165.0. Found C, 71.39; H, 6.15; N, 9.90. Calc. for $C_{17}H_{18}N_2O_2$: C, 72.32; H, 6.43; N, 9.92%.

Imine, 10c. Starting from 2.44 g (20 mmol) of salicylaldehyde and 1.36 g (10 mmol) of **9c**, 3.03 g (88%) of **10c** was obtained; mp 147–149 °C (yellow crystals from EtOH). δ_H ($CDCl_3$) 4.71 (4 H, s), 6.78–6.92 (4 H, m), 7.11–7.33 (8 H, m), 8.37 (2 H, s), (s, 2H), 13.28 (1H, br s); δ_C ($CDCl_3$) 63.2, 117.0, 118.6, 126.1, 126.5, 127.1, 128.9, 131.4, 132.3, 138.6, 161.0, 165.7. Found C, 76.53; H, 6.01; N, 8.18. Calc. for $C_{22}H_{20}N_2O_2$: C, 76.72; H, 5.85; N, 8.13%.

General procedure for the synthesis of aminonitriles 11

To a solution of the corresponding imine **10** in anhydrous THF, under argon atmosphere and at 0 °C, TMSCN was added in a 1 : 6 molar ratio. The reaction was stirred at room temperature for 20 h, then quenched at 0 °C with NH_4Cl (sat. soln.)

and extracted with Et_2O (2 × 100 cm^3). The combined organic extracts where washed with water and dried over $MgSO_4$. The solution was filtered and the solvent was removed *in vacuo*. Aminonitriles **11** were obtained in nearly quantitative yields as highly unstable oils and were hydrolysed immediately after preparation, once their structure was confirmed by ^1H NMR spectroscopy.

Aminonitrile, 11a. This was obtained as a yellow oil from 2.0 g (7.08 mmol) of imine **10a**. δ_H ($CDCl_3$) 2.02–2.10 (2 H, m), 2.59–2.93 (4 H, m), 4.59 (1 H, br s), 4.63 (1 H, br s), 6.72 (2 H, dd, J_1 = 8.1 Hz, J_2 = 1.2 Hz), 6.82 (2 H, m), 7.08–7.21 (4 H, m).

Aminonitrile, 11b. This was obtained as a yellow oil from 2.49 g (8.40 mmol) of imine **10b**. δ_H ($CDCl_3$) 1.59–1.78 (4 H, m), 2.52–2.95 (4 H, m), 4.62 (1 H, br s), 4.95 (1H, br s), 6.71–6.88 (4H, m), 7.08–7.27 (4 H, m).

Aminonitrile, 11c. This was obtained as a yellow oil from 3.03 g (8.80 mmol) of imine **10c**. δ_H ($CDCl_3$) 3.74–3.96 (4 H, m), 4.58 (1 H, br s), 4.62 (1 H, br s), 6.73 (2 H, m), 6.85 (2 H, m), 7.09–7.23 (4 H, m), 7.24 (4 H, s).

General procedure for the synthesis of amino acids 6–8

Concentrated aqueous HCl (12 M) was added over the freshly prepared aminonitrile **11** in a 30 : 1 molar ratio. The mixture was heated at 50–60 °C for 6 h and then water was added in a volume equal to the acid. The resultant mixture was refluxed for 6 h. Amino acids **6–8** were obtained as hydrochlorides and as a mixture of *meso* and *racemic* diastereomers. The free amino acids could be obtained also as a diastereomeric mixture by precipitation at pH 3.0 to 4.5 with 6 M NaOH. The solids were filtered, washed successively with H_2O, EtOH and acetone, and finally dried *in vacuo*.

Propylenediamine-*N*,*N*′-bis(2-hydroxyphenyl)acetic acid (PDDHA) 6

The hydrolysis of 2.93 g (8.0 mmol) of nitrile **11a** yielded 3.1 g (86% yield) of **11a** as its hydrochloride. The free amino acid (1.04, 11%) was isolated as a white solid by precipitation at pH 4.3. ν_{max}/cm^{-1} (KBr) 3402, 3066, 1632; δ_H (DMSO-d_6–TFA) 2.12–2.21 (2 H, m), 2.85–3.00 (4 H, m), 5.15 (2 H, br s), 6.89 (2 H, m), 7.00 (2 H, m), 7.24–7.35 (4 H, m); δ_C (DMSO-d_6–TFA) 21.7, 43.0, 58.0, 115.5, 117.2, 119.1, 130.0, 130.9, 155.5, 169.2. Found: C, 60.71; H, 5.80; N, 7.31. Calc. for $C_{19}H_{22}N_2O_6$: C, 60.95; H, 5.92; N, 7.48%.

Butylenediamine-*N*,*N*′-bis(2-hydroxyphenyl)acetic acid (BDDHA) 7

The hydrolysis of 3.17 g (9.0 mmol) of nitrile **11b** yielded 3.1 g (71% yield) of **7** as its hydrochloride. The free amino acid (1.89 g, 54%) was isolated as a white solid by precipitation at pH 4.4. ν_{max}/cm^{-1} (KBr) 3404, 3045, 1626; δ_H (DMSO-d_6–TFA) 1.69 (4 H, m), 2.91 (4 H, m), 4.95 (2 H, br s), 6.85–6.91 (4 H, m), 7.19–7.27 (4 H, m); δ_C (DMSO-d_6–TFA) 21.9, 45.0, 58.1, 115.5, 117.0, 119.2, 129.9, 130.9, 155.3, 169.1. Found: C, 61.54; H, 6.10; N, 7.13. Calc. for $C_{20}H_{24}N_2O_6$: C, 61.84; H, 6.23; N, 7.21%.

(*p*,*p*-Xylylene)diamine-bis(2-hydroxyphenyl)acetic acid (XDDHA) 8

The hydrolysis of 6.22 g (15.6 mmol) of nitrile **11c** yielded 7.7 g (98% yield) of **8** as its hydrochloride. The free amino acid (4.18 g, 62%) was isolated by precipitation at pH 4.7. ν_{max}/cm^{-1} (KBr) 3423, 3115, 1618; δ_H (DMSO-d_6–TFA) 4.02 (4 H, br s), 4.87 (2 H, br s), 6.71–6.85 (4 H, m), 7.07–7.18 (4 H, m), 7.30 (4 H, s); δ_C (DMSO-d_6–TFA) 48.9, 58.6, 117.0, 117.4, 123.1, 155.0, 157.4, 158.2, 159.0, 159.7, 168.8. Found: C, 66.34; H, 5.41; N, 6.29. Calc. for $C_{24}H_{24}N_2O_6$: C, 66.04; H, 5.54; N, 6.42%.

Determination of the purity of chelating agents

The details of the method employed to determine the titrimetric purity of the free chelating agents, using spectrophotometric titration with Fe(III) has been described in an earlier paper.[6] The free ligands were previously dissolved in a volume of 0.200 M NaOH calculated to be four times the molar amount of the ligand. The pH was fixed at 6 by the addition of 2 mM MES buffer [2-(*N*-morpholino)ethanesulfonic acid]. The experimental solution (60 cm^3) was placed in a 150 cm^3 thermostated jacketed reaction vessel provided with airtight cap fitted with a gas inlet and outlet tubes, combined pH glass electrode, white-light spectrode, two piston burettes (tips placed below the surface of the solution) and magnetic stirrer. The photometric titration consists on the addition of 4.47 × 10^{-4} M Fe(III) standard solution to the chelating agent (samples of about 1 × 10^{-4} M) until the absorbance at 480 nm presented no changes. Molar absorptivities were also calculated at 480 nm for each chelating agent (see Table 1S in ESI†). The photometric curves, used to calculate the end point using the linear segments intersection method,[26] are shown in Fig 1S of ESI.†

Determination of stability constants by potentiometry

Potentiometric titrations were described in detail elsewhere.[27] Due to the low solubility of ligands in acid medium, all data were obtained by back-titration with aqueous 0.0500 M HCl standardized titrant. Approximately 10–20 mg of chelating agents were weighted to the nearest 0.01 mg and were dissolved using four or six equivalents of NaOH (0.200 M). When appropriate, Ca(II) or Mg(II) solutions were added in ligand : metal (1 : 1) and (1 : 10) ratio. The solutions were diluted to a final volume of 50.0 cm^3. A volume of 25 cm^3 of the experimental solution was back-titrated to pH 2.5 or until precipitation of ligand occurred.

All formation constants, except for the protonation constants corresponding to phenol dissociations, were calculated using the FORTRAN program BEST.[27,28]

Spectrophotometric equilibrium measurements

The first and second protonation constants were measured spectrophotometrically,[21] since the combination of protons with the phenolic groups are accompanied by extensive changes in the absorption spectra. For each ligand, ten-to-twelve 1 × 10^{-4} M solutions were prepared and pH adjusted from 10.0 to 13.8 with in 0.3–0.5 pH intervals. 250–400 nm spectra were obtained for each free ligand in a Shimazdu UV-VIS spectrophotometer. The wavelength on the maximum absorbances and molar absorptivities of L^{4-} and LH_2^{2-} species were initially estimated at pH 13.5 and 10, respectively, for each chelating agent (at these pHs the other species are in low concentration) and used as imput for the calculations. The spectroscopy equilibrium curves and wavelength chosen for the determination of the first two phenolate protonations are shown in Fig. 2S and Table 2S of ESI.†

Stability constants (K_{FeL}, K_{FeHL}, $K_{Fe(OH)L}$, K_{CuL}, K_{CuHL} and K_{CuH_2L}) for the chelates were calculated from spectrophotometric data obtained after base titration and using the theoretical model presented by Yunta *et al.*[6] The experimental iron-chelate solution (1 × 10^{-4} M; 25 mL) was placed in a 50-cm^3 thermostated jacketed reaction vessel. For the Fe(III) chelate, the experimental solution was titrated with aqueous 0.200 M NaOH titrant to pH 12. The absorbance of the solution was measured at 480 nm at each 0.05–0.1 pH interval, depending on the curve zone.

25 cm^3 of Cu(II)/chelate 1 × 10^{-3} M experimental solution, in the same conditions used for the iron complex, were titrated with aqueous 0.200 M HCl titrant until the solution was colorless or precipitation was observed. The absorbance of the solution was measured at 650 nm at 0.05–0.1 pH intervals, depending on the curve zone. The potentiometric curves with Fe(III) and Cu(II) are shown in Figs. 3S and 4S of ESI.†

pM Values and species distribution

A more reliable parameter for ligand effectiveness is the pM value, where pM = −log[M], is similar to the "chemical potential" of the aquo metal ion. A comparison of the total sequestering ability of ligands can be made through the determination of pFe and pCu values using two different models.[6] In the first model, the calculation of [M] was made considering only the proton affinities of the ligand and other chelate species such as protonated metal complexes, according to Bannochie *et al.*[13] These values were computed using a 10% excess of ligand. These conditions are far apart from agronomic reality. Therefore, in a second model, pM values were calculated using the standard Hoagland nutrient solution (for the composition of the standard Hoagland nutrient solution see Table 3S of ESI†) and the equilibrium speciation model MINTEQA2 program.[29] pFe values were calculated at 4–12 pH range.

The species distribution is commonly determined by means of theoretical models considering the conditions in which they are applied. The conditions of the second model were also employed to know the behavior of the chelating agents in nutrient solution at 4–13 pH range. Species distribution was established using the same methodology as that used to calculate pFe in agronomic conditions.[6]

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