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Chelating Agents Related to Ethylenediamine Bis(2-hydroxyphenyl)acetic Acid (EDDHA): Synthesis, Characterization, and Equilibrium Studies of the Free Ligands and Their Mg^{2+}, Ca^{2+}, Cu^{2+}, and Fe^{3+} Chelates

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Iron chelates such as ethylenediamine-*N*,*N*′-bis(2-hydroxyphenyl)acetic acid (EDDHA) and their analogues are the most efficient soil fertilizers to treat iron chlorosis in plants growing in calcareous soils. EDDHA, EDDH4MA (ethylenediamine-*N*,*N*′-bis(2-hydroxy-4-methylphenyl)acetic acid), and EDDCHA (ethylenediamine-*N*,*N*′-bis(2-hydroxy-5-carboxyphenyl)acetic acid) are allowed by the European directive, but also EDDHSA (ethylenediamine-*N*,*N*′-bis-(2-hydroxy-5-sulfonylphenyl)acetic acid) and EDDH5MA (ethylenediamine-*N*,*N*′-bis(2-hydroxy-5-methylphenyl)acetic acid) are present in several commercial iron chelates. In this study, these chelating agents as well as *p*,*p*-EDDHA (ethylenediamine-*N*,*N*′-bis(4-hydroxyphenyl)acetic acid) and EDDMtxA (ethylenediamine-*N*,*N*′-bis(2-metoxyphenyl)-acetic acid) have been obtained following a new synthetic pathway. Their chemical behavior has been studied to predict the effect of the substituents in the benzene ring on their efficacy as iron fertilizers for soils above pH 7. The purity of the chelating agents has been determined using a novel methodology through spectrophotometric titration at 480 nm with Fe^{3+} as titrant to evaluate the inorganic impurities. The protonation constants were determined by both spectrophotometric and potentiometric methods, and Ca^{2+} and Mg^{2+} stability constants were determined from potentiometric titrations. To establish the Fe^{3+} and Cu^{2+} stability constants, a new spectrophotometric method has been developed, and the results were compared with those reported in the literature for EDDHA and EDDHMA and their *meso*- and *rac*-isomers. pM values have been also determined to provide a comparable basis to establish the relative chelating ability of these ligands. The purity obtained for the ligands is higher than 87% in all cases and is comparable with that obtained by ^1H NMR. No significant differences have been found among ligands when their protonation and stability constants were compared. As expected, no Fe^{3+} complexation was observed for *p*,*p*-EDDHA and EDDMtxA. The presence of sulfonium groups in EDDHSA produces an increase in acidity that affects their protonation and stability constants, although the pFe values suggest that EDDHSA could be also effective to correct iron chlorosis in plants.

Introduction

Iron chelates (Figure 1) derived from ethylenediamine-*N*,*N*′-bis(2-hydroxyphenyl)acetic acids are the fertilizers of choice to treat iron chlorosis by soil applications.[1] This is a nutritional disorder in plants characterized by a significant decrease of chlorophyll in leaves that reduces the yield and quality of many crops.[2] Among other causes, iron chlorosis results from the low availability of Fe in alkaline soils where this metal is already present in the form of insoluble oxide/hydroxides.[3] Furthermore, the chlorosis can be induced by the presence of HCO_3^-,[4] and by high concentrations of metals such as Mn, Cu, Zn, Co, Ni, or Cd that may compete with

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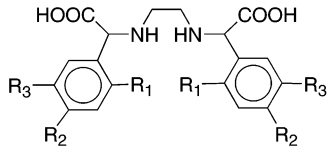

1a R_1 = OH; R_2 = R_3 = H; *o,o*-EDDHA
1b R_1 = H; R_2 = OH; R_3 = H *p,p*-EDDHA
1c R_1 = OH; R_2 = Me; R_3 = H EDDH4MA
1d R_1 = OH; R_2 = H; R_3 = Me EDDH5MA
1e R_1 = OMe; R_2 = H; R_3 = H; EDDMtxA
1f R_1 = OH; R_2 = H; R_3 = SO_3H EDDSHA
1g R_1 = OH; R_2 = H; R_3 = COOH EDDCHA

Figure 1. Structure of the chelating agents.

Fe at the level of plant uptake.[5] The accepted mechanism of remediation of iron chlorosis by Fe chelates involves the enzymatic reduction of Fe^{3+} to Fe^{2+} by an Fe(III) chelate reductase. The outcome of this reduction is the liberation of Fe^{2+} that is taken up by roots, producing a molecule of free ligand during the process.[6]

The efficacy of an iron chelate to solve iron chlorosis depends on several factors. The first one is the ability of the compound to chelate Fe^{3+} and other metals existing in soils, like Ca^{2+}, Mg^{2+}, and Cu^{2+}. Copper is generally present in low concentrations in soils, but it becomes a major competitor of Fe^{3+} due to its ability to be chelated by amino acids. The second factor is the ability of the plant roots to reduce Fe^{3+} from the chelating agent. This capacity is influenced by the stability of the Fe^{3+} and the Fe^{2+} chelates.[2] Other factors that have to be considered are the ability of the chelating agent to dissolve more native iron present in the soil and the adsorption processes of the chelates into soil materials.[7] To predict the reactivity of iron chelates in soil conditions and their efficiency as correctors of iron chlorosis, it is necessary to know their speciation.

The first set of data about the protonation constants of EDDHA **1a** and the stability constants for its Ca^{2+}, Mg^{2+}, and Cu^{2+} complexes was reported by Frost and Martell in 1957.[8] Since spectrophotometric methods were not used at that time, the stability constants of EDDHA/Fe^{3+} could not be determined, and the two highest protonation constants of **1a** became inaccurate. Further work by Bannochie reported the protonation constants and the stability constants of diastereomeric *meso*-EDDHA and *rac*-EDDHA free ligands and their Fe^{3+} chelates.[9] The analogous data for both diastereomers of EDDH4MA have been also reported by Ahrland et al.[10]

The synthesis of EDDHA was originally reported by Kroll in 1957, from a Strecker reaction on the diimine derived from ethylenediamine and salicylaldehyde (eq a in Scheme 1).[11] Although the procedure can be employed to obtain other substituted EDDHAs,[12] a major drawback of this method is the need for liquid HCN during the industrial process. Other approaches to the synthesis of EDDHAs are based on a Mannich-like reaction between phenol (or substituted phenols), ethylenediamine, and glyoxylic acid (eq b in Scheme 1).[13] This method is used for the preparation of all of the EDDHA derivatives currently in the market. However, this synthesis produces a mixture of regioisomers and polycondensation products in variable amounts depending on the reaction conditions.[14] Other approaches to obtain ethylenediamine-bis(hydroxyphenyl)acetic acids are less general.[15]

The first structural characterization of Fe chelates of EDDHA derivatives was made through the study of the Mg-[*rac*-Fe(III)-EDDHA]$_2$ salt. These studies showed that these chelates have an octahedral disposition with the EDDHA ligand **1a**, hexacoordinated to the Fe nucleus.[16] The ferric complexes of the *rac*- and *meso*-isomers of EDDHA **1a** are represented in Figure 2. This [6,5,6] arrangement of rings across the Fe center having the phenolic groups in equatorial positions is considerably more favored than the alternative [5,5,5] arrangement. Nevertheless, it has been calculated that 0.5% of the [5,5,5] complex could be present in the racemic mixture.[17] Analogous structures can be assumed for the Fe chelates of compounds **1c**, **1d**, **1f**, and **1g**, assuming a 1:1 stoichiometry of metal−ligand. Stoichiometries higher than 1:1 have been reported for compounds **1f**[18] and **1g**[19] due to the presence of additional coordinating groups.

During our ongoing work directed toward the development of new chelating agents for the treatment of iron chlorosis,[20] as well as toward the determination of the presence of impurities in commercial formulations,[14] we became aware of the existence of serious discrepancies between the reported data for EDDH4MA[10] (**1c**) and our own results. Additionally, data for its positional isomer EDDH5MA (**1d**), as well as

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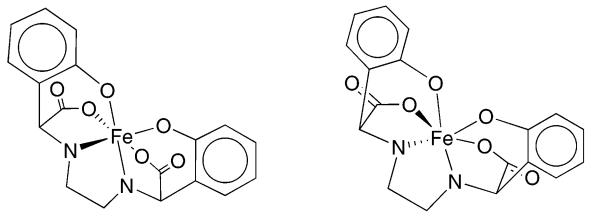
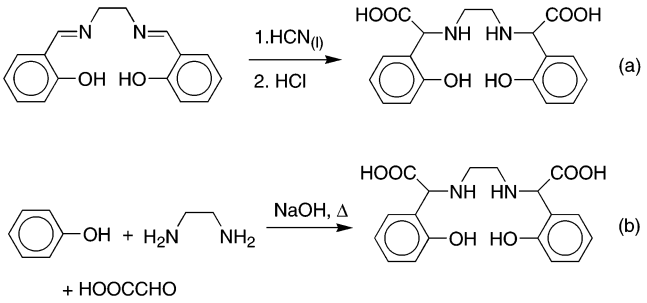


Figure 2. Structure of (A) *rac*-EDDHA and (B) *meso*-EDDHA.

Scheme 1



for other benzene-substituted analogues, are not known. Furthermore, our recently developed route to synthesize this type of ligand allows us the access to unambiguously characterized pure ligands.[20] Reported herein is a general method for the synthesis of EDDHA and other related derivatives starting from different hydroxybenzaldehydes and using trimethylsilylcyanide (TMSCN) as the cyanide transfer agent, as well as the characterization and equilibrium studies of the free ligands and their Mg^{2+}, Ca^{2+}, Cu^{2+}, and Fe^{3+} chelates using a novel methodology.

Experimental Section

General Procedures. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$, on a Varian XL-300S (299.94 MHz for ^{1}H and 75.43 MHz for ^{13}C), a Bruker 250-AC (250.13 MHz for ^{1}H and 62.90 MHz for ^{13}C), a Bruker 200-AC (200.13 MHz for ^{1}H and 50.03 for ^{13}C MHz), and a Bruker Avance-300 (300.13 MHz for ^{1}H and 75.48 MHz for ^{13}C) spectrometers. Chemical shifts are given in ppm relative to TMS (^{1}H, 0.0 ppm), or $CDCl_3$ (^{13}C, 77.0 ppm). 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), a Metrohm combined pH glass electrode, and an ion selective Cu^{2+} electrode. 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.[21] $CaCl_2$, $MgCl_2$, NaCl, NaOH, $CuSO_4$, $Cu(NO_3)_2$, HCl, and Fe^{3+} standard solution were obtained from Merck Chemical Co., and they were properly standardized. A Gran's plot analysis[22] was used to check for carbonate contamination of the standard aqueous NaOH and consistently revealed amounts of carbonate lower than 0.5%. The end points of all potentiometric titrations were determined by the Gran's function.[22] All titrations were made under inert atmosphere (99.9995 purity grade N_2, NaOH washed, 0.100 NaCl saturated).

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). The transformation of concentration constants (K^c) or thermodynamic constants (K^o) was made using the activity coefficients from Davies' equation.

All commercially available organic reagents were used without further purification. EDDHSA (**1f**) was supplied by DABEER (Barcelona, Cataluña, España).

Caution: HCN is produced by reaction of trimethylsilyl cyanide (TMSCN) with acid, water, or protic solvents. All reactions using this reagent should be carried out using the adequate precautions in well ventilated hoods.

General Procedure for the Synthesis of Imines 3a−e. Imines **3** (see Scheme 2 in Results and Discussion) were synthesized in quantitative yield by refluxing the corresponding aldehydes **2** with anhydrous ethylenediamine (2:1 molar ratio) in absolute ethanol.

General Procedure for the Synthesis of Aminonitriles 4a−e. To a solution of the corresponding imine **3** in anhydrous THF, under argon atmosphere and at 0 °C, was added TMSCN in a 1:6 molar ratio. The reaction was stirred at room temperature for 20 h and then quenched at 0 °C with NH_4Cl (sat. soln) and extracted with Et_2O (2×100 mL). The combined organic extracts were washed with water and dried over $MgSO_4$. The solution was filtered, and the solvent was removed under vacuum. Aminonitriles **4** were obtained in nearly quantitative yields as highly unstable oils and were hydrolyzed immediately after preparation, once their structure was confirmed by ^{1}H NMR spectroscopy.

Aminonitrile 4a. This material was obtained as a yellow oil from imine **3a**. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.68−2.95 (m, 4H), 4.58 (br s, 1H), 4.65 (br s, 1H), 6.73 (d, $J = 8.0$ Hz, 2H), 6.84 (t, $J = 7.4$ Hz, 2H), 7.10−7.23 (m, 4H), 8.25 (s, 1H).

Aminonitrile 4b. This material was obtained as a yellow oil from imine **3b**. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.73−2.88 (m, 4H), 4.59 (br s, 2H), 6.70 (d, $J = 8.5$ Hz, 4H), 7.20 (d, $J = 8.5$ Hz, 4H).

Aminonitrile 4c. This material was obtained as an orange oil from imine **3c**. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.17 (s, 6H), 2.64−2.92 (m, 4H), 4.55 (br s, 1H), 4.60 (br s, 1H), 6.52 (br s, 2H), 6.64 (d, $J = 7.6$ Hz, 2H), 7.03−7.14 (m, 2H).

Aminonitrile 4d. This material was obtained as a yellow oil from imine **3d**. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.15 (s, 6H), 2.54−3.01 (m, 4H), 4.56 (br s, 1H), 4.61 (br s, 1H), 6.62 (dd, $J = 8.2$, 3.6 Hz, 2H), 6.96 (dd, $J = 8.2$, 1.9 Hz, 2H), 7.01 (dd, $J = 3.6$, 1.9 Hz, 2H).

Aminonitrile 4e. This material was obtained as a brown oil from imine **3e** and TMSCN (1:3 ratio) after 40 h at room temperature. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.73−2.95 (m, 4H), 3.70 (s, 6H), 4.74 (br s, 1H), 4.75 (br s, 1H), 6.76−6.87 (m, 4H), 7.16−7.25 (m, 4H). ^{13}C NMR (50.03 MHz, $CDCl_3$) δ 46.1, 46.4, 49.5, 49.7, 55.5, 111.1, 118.9, 119.0, 120.8, 123.1, 128.5, 130.3, 156.6.

General Procedure for the Synthesis of Amino Acids 1a, 1c, and 1d. Concentrated aqueous HCl (12 M) was added over the freshly prepared aminonitrile **4** 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 **1** were obtained as a mixture of isomers by precipitation at pH 3−4 with 6 M NaOH. The solids were filtered, washed successively with H_2O, EtOH, and acetone, and finally dried under vacuum.

Ethylenediamine-*N*,*N*′-bis(2-hydroxyphenyl)acetic acid (EDDHA), 1a, was obtained as a pale pink solid in 62% yield by

(21) Standard Specification for Reagent Water. *Annual Book of ASTM Standards*; ASTM: West Conshohocken, PA, 1995; 11.01.
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precipitation at pH 3. ^{1}H NMR (200 MHz, (DMSO-d_6:TFA) δ 3.06−3.29 (m, 4H), 5.08 (br s, 2H), 6.84−7.00 (m, 4H), 7.24−7.31 (m, 4H). ^{1}H NMR (200 MHz, D_2O:Na_2CO_3) δ 2.46−2.63 (m, 4H), 4.14 (s, 1H), 4.24 (s, 1H), 6.59−6.65 (m, 4H), 6.89−7.03 (m, 4H). ^{13}C NMR (50.03 MHz, (DMSO-d_6:TFA) δ 41.4, 58.1, 115.5, 116.8, 123.6, 130.7, 131.2, 155.3, 169.2. IR(KBr) 3410, 3098, 1643 cm^{-1}. Anal. Calcd for $C_{18}H_{20}N_2O_6$: C, 59.99; H, 5.59; N, 7.77. Found: C, 59.65; H, 5.48; N, 7.70.

***rac*-EDDHA and *meso*-EDDHA** were obtained using a modification of the method of Bailey.[16] EDDHA (4.5 g) was dissolved in refluxing EtOH (50 mL). Then, a solution of $MgCO_3$ (1.66 g in 150 mL of water) was added very slowly to the stirred solution of the chelating agent heating continuously. The resulting solution was filtered, and $Fe(NO_3)_3 \cdot 9H_2O$ (4.083 g in 100 mL of water) was added until pH 5. Precipitation was allowed to proceed overnight, and the volume was reduced to 200 mL by evaporation. The precipitate (*rac*-EDDHA/Fe^{3+}) was filtered after cooling. The filtrate was used to isolate the *meso*-EDDHA.

The resulting *rac*-EDDHA/Fe^{3+} precipitate was purified by refluxing in MeOH (200 mL), removing the solid phase by filtration, and reducing the volume by evaporation to 25 mL. *rac*-EDDHA/Fe^{3+} was obtained as red crystals. The *meso*-EDDHA/Fe^{3+} isomer was obtained by addition of $Fe(NO_3)_3 \cdot 9H_2O$ (1.21 g in 20 mL of water) and $MgCO_3$ (4.15 g) to the filtrate. The resulting solution was evaporated reducing the volume to 50 mL and then was filtered. The filtrate was left to stand to obtain the *meso*-isomer.

meso- and *rac*-EDDHAs can be obtained from their iron complexes by slow addition of KOH solution (1 M) over a solution of the complex until massive precipitation of iron oxide was observed. The addition must be done under inert atmosphere in order to avoid undesirable oxidation reactions. The resulting solution was quickly filtered, the pH lowered to 3.7 with HCl, and the solution left to stand for 30 min and filtered again. The solid phase was washed with deionized water until the solid was colorless. The purity of *rac*- and *meso*-EDDHA was checked by HPLC using the method developed by Lucena et al.[23] The results showed that the isomer *meso*-EDDHA contained less than 10% of the racemic form and the *rac*-EDDHA had less than 1% of the meso form.

Ethylenediamine-*N*,*N*′-bis(2-hidroxy-4-methylphenyl)acetic acid (EDDH4MA), 1c, was obtained as a pale pink solid in a 50% yield by precipitation at pH 3. ^{1}H NMR (200 MHz, DMSO-d_6:TFA) δ 2.29 (s, 6H), 3.10−3.33 (m, 4H), 5.23 (br s, 2H), 6.75 (m, 2H), 6.83 (br s, 2H), 7.20 (m, 2H). ^{1}H NMR (200 MHz, D_2O:Na_2CO_3) δ 2.10 (s, 6H), 2.49−2.72 (m, 4H), 4.20 (s, 1H), 4.27 (s, 1H), 6.46−6.53 (m, 4H), 6.83−6.90 (m, 2H). ^{13}C NMR (50.03 MHz, (DMSO-d_6:TFA) δ 20.7, 41.3, 58.3, 116.0, 118.4, 120.1, 130.4, 141.1, 155.6, 169.3. IR(KBr) 3415, 3095, 1618 cm^{-1}. Anal. Calcd for $C_{20}H_{24}N_2O_6$: C, 61.84; H, 6.23; N, 7.21. Found: C, 61.53; H, 6.47; N, 7.11.

***rac*-EDDH4MA and *meso*-EDDH4MA.** *rac*-EDDH4MA was obtained by precipitation of EDDH4MA at pH 2. The solid was filtered, washed with water, EtOH, and acetone, and dried. Further purification was achieved by dissolving the solid in 2 M HCl and adjusting the solution at pH 1.0 by addition of 6 M NaOH. The precipitate was filtered, washed with water, EtOH, and acetone, and dried. *rac*-EDDH4MA was obtained as a pale pink solid. ^{1}H NMR (200 MHz, D_2O:$NaCO_3$) δ 2.09 (s, 6H), 2.55−2.72 (m, 4H), 4.26 (s, 1H), 6.49−6.56 (m, 4H), 6.87−6.91 (m, 2H). ^{13}C NMR (50.03 MHz, (DMSO-d_6:D_2O:$NaCO_3$) δ 20.4, 44.9, 64.3, 117.7, 119.4, 120.9, 129.4, 139.7, 157.8, 177.7.

meso-EDDH4MA was obtained from the initial filtrate by precipitation at pH 3.5−4.0. The solid was purified as described. ^{1}H NMR (200 MHz, D_2O:$NaCO_3$) δ 2.10 (s, 6H), 2.61 (dd, J_1 = 9.79 Hz, J_2 = 18.17 Hz, 4H), 4.19 (s, 1H), 6.49−6.52 (m, 4H), 6.86−6.93 (m, 2H). ^{13}C NMR (50.03 MHz, (DMSO-d_6:D_2O:$NaCO_3$)) δ 20.4, 44.9, 64.3, 117.7, 119.4, 120.9, 129.4, 139.7, 157.8, 177.7.

Ethylenediamine-*N*,*N*′-bis(2-hidroxy-5-methylphenyl)acetic acid (EDDH5MA), 1d, was obtained as a pale pink solid in a 63% yield by precipitation at pH 4. ^{1}H NMR (200 MHz, DMSO-d_6:TFA) δ 2.26 (s, 6H), 3.10−3.42 (m, 4H), 5.24 (br s, 2H), 6.91 (m, 2H), 7.13 (s, 2H), 7.15 (m, 2H). ^{1}H NMR (200 MHz, D_2O:Na_2CO_3) δ 2.07 (s, 6H), 2.45−2.73 (m, 4H), 4.15 (s, 1H), 4.24 (s, 1H), 6.56 (d, J = 8.03 Hz, 2H), 6.87−6.92 (m, 4H). ^{13}C NMR (50.03 MHz, DMSO-d_6:TFA) δ 19.6, 41.7, 58.9, 115.6, 116.6, 128.1, 130.9, 131.8, 153.4, 169.4. IR (KBr) 3418, 3100, 1641 cm^{-1}. Anal. Calcd for $C_{20}H_{24}N_2O_6$: C, 61.84; H, 6.23; N, 7.21. Found: C, 61.59; H, 6.09; N, 7.09.

General Procedure for the Synthesis of Amino Acids 1b and 1e. Acetic formic anhydride freshly prepared by heating an equimolar mixture of Ac_2O and formic acid, at 60 °C for 1 h, was added over nitriles **4b** and **4e** at 0 °C.[24] The ratio of acetic formic anhydride/nitrile was 15:1. The mixture was stirred at 0 °C for 15 min and then poured into a mixture of ice−water and extracted with CH_2Cl_2 (2 × 100 mL), and the organic layers were dried over $MgSO_4$. The solvent was removed under reduced pressure. The obtained formyl acetonitrile was hydrolyzed without further purification by addition of concentrated HCl (ratio 30:1) and subsequent heating of the mixture at 50−60 °C for 2 h. The solvent was removed under vacuum, and the solid thus obtained was washed with EtOH and dried. Amino acids **1b** and **1e** were obtained as hydrochlorides and as a 1:1 mixture of isomers by precipitation.

Ethylenediamine-*N*,*N*′-bis(4-hydroxyphenyl)acetic acid (*p*,*p*-EDDHA), 1b, was obtained as a pale pink solid in a 20% yield. ^{1}H NMR (200 MHz, D_2O) δ 2.72−2.07 (m, 4H), 4.67 (s, 1H), 4.68 (s, 1H), 6.55−6.65 (m, 4H), 6.96 (d, J = 8.73 Hz, 4H). ^{13}C NMR (50.03 MHz, D_2O) δ 41.6, 63.3, 116.8, 121.5, 130.7, 158.2, 169.7. IR (KBr) 3404, 3323, 3286, 3197, 1697 cm^{-1}. Anal. Calcd for $C_{18}H_{22}Cl_2N_2O_6$: C, 49.90; H, 5.12; N, 6.47. Found: C, 49.55; H, 4.96; N, 6.19.

Ethylenediamine-*N*,*N*′-bis(2-metoxyphenyl)acetic acid (EDDMtxA), 1e, was obtained as a beige solid in 89% yield. ^{1}H NMR (200 MHz, D_2O) δ 2.94−3.27 (m, 4H), 3.58 (s, 3H), 3.60 (s, 3H), 4.92 (br s, 1H), 5.05 (br s, 1H), 6.90−7.01 (m, 4H), 7.14−7.25 (m, 2H), 7.35−7.49 (m, 2H). ^{13}C NMR (50.03 MHz, D_2O) δ 41.5, 41.6, 55.6, 60.7, 60.9, 112.2, 117.0, 121.6, 131.5, 133.2, 157.2, 157.3, 170.2. IR (KBr) 3328, 3259, 3186, 1735, 1709 cm^{-1}. Anal. Calcd for $C_{20}H_{26}Cl_2N_2O_6$: C, 52.07; H, 5.68; N, 6.07. Found: C, 51.85; H, 5.46; N, 5.79.

Determination of the Purity of Chelating Agents. The solutions of chelating agents were prepared under purified N_2 atmosphere. The ligands were completely dissolved in a volume of 0.200 M NaOH calculated to be 4 times the molar amount of the ligand, and the pH was fixed at 6 by the addition of 2 mM MES buffer [2-(*N*-morpholino)ethanesulfonic acid]. Ionic strength was adjusted to 0.100 M by addition of NaCl. The concentrations of the chelating agent solutions prepared were low enough (about 1 × 10^{-4} M) to comply with Beer's law.

The experimental solution (60 mL) was placed in a 150 mL thermostated jacketed reaction vessel provided with airtight cap

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fitted with a gas inlet and outlet tubes, combined pH glass electrode, white light spectrode, two piston burets (tips placed below the surface of the solution), and a magnetic stirrer. The photometric titration (carried out at 25.0 ± 0.5 °C) consists of the addition of 4.47×10^{-4} M Fe^{3+} standard solution to the chelating agent (samples of about 1×10^{-4} M) until the absorbance at 480 nm presented no changes.

A discussion of the mathematical procedure followed to analyze the obtained data as well as the photometric curves is fully developed in the Supporting Information.

Since no iron complexation was observed for the *p,p*-EDDHA and EDDMtxA, their purities were determined by potentiometric titrations with Cu^{2+} ion selective electrode in the same conditions as before. A 25 mL portion of an approximately 1×10^{-3} M solution of the chelating agents, prepared as before, was titrated with a 4×10^{-3} M Cu^{2+} standardized solution until constant potential was observed.

Determination of Stability Constants by Potentiometry. Potentiometric titrations were described in detail elsewhere.[25] Titrations of chelating agent solutions were made at 25.0 ± 0.5 °C in a sealed, water-jacketed glass vessel, which was maintained under purified N_2 atmosphere. Ionic strength was maintained at 0.100 M with reagent grade NaCl. Due to the ion 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 agent was weighed to the nearest 0.01 mg and was dissolved under N_2 atmosphere using 4 or 6 equiv of NaOH (0.200 M). When needed, Ca^{2+} or Mg^{2+} solution was added in appropriate ligand/metal (1:1 and 1:10) ratios. The solutions were diluted to a final volume of 50.0 mL. A volume of 25 mL 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 with phenol dissociation, were calculated using the FORTRAN program BEST[25] described in detail elsewhere.[26]

Determination of Stability Constants by Spectrophotometry. Except for EDDMtxA, the first and second protonations occur at pH values where potentiometric measurements become inaccurate. Then, the stability constants were measured spectrophotometrically,[27] since the combination of protons with the phenolic groups are accompanied by extensive changes in the absorption spectra. For each ligand, 10 1×10^{-4} M solutions were prepared with the ionic strength adjusted at 0.100 M with NaCl and pH adjusted from 10.5 to 13.5 within 0.3−0.5 pH intervals. Spectra (250−400 nm) were obtained in a Shimazdu UV−vis spectrophotometer. Wavelengths for 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 seed for the calculations. The mathematical procedure, spectroscopy equilibrium curves, and wavelengths chosen for the determination of the first two phenolate protonations are shown in the Supporting Information.

Stability constants for the Fe^{3+} and Cu^{2+} chelates were calculated from spectrophotometric data obtained after base titration. Portions containing 4 or 6 equiv of standard base (0.200 M) were added to the chelating agent, and then, the ionic strength was maintained at 0.100 M with reagent grade NaCl. Solutions of iron and copper chelates (1:1 metal/ligand ratio) were prepared under N_2 at 25.0 ± 0.5 °C, by slow addition of Fe^{3+} or Cu^{2+} standard solutions. When the iron chelate was formed, hydrochloric acid was added until the solution was colorless (the final pH depends on the chelating agent, sometimes it is as low as pH 1.5−3). The experimental solution was diluted to 500 mL with type I water[21] to be 1×10^{-4} M in iron chelate. A 25 mL portion was placed in a 50-mL thermostated jacketed reaction vessel as described previously. For the Fe^{3+} 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.

For the Cu^{2+} chelate, the pH was raised to 12 by addition of NaOH. The experimental solution was diluted to 500 mL with type I water[21] to be 1×10^{-3} M in copper chelate. A 25 mL portion of the experimental solution, under the same conditions used for the iron complex, was 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 stability constants (K_{FeL}, K_{FeHL}, $K_{Fe(OH)L}$, K_{CuL}, K_{CuHL}, and K_{CuH_2L}) were calculated from the data by the use of an in-house program using Microsoft Excel Solver (the Solver options used were already described elsewhere)[28] utilizing mass balance and known equilibrium constant constraints[29] while minimizing the least-squares absorbance fit to the observed spectral curves. The mathematical procedure as well as the photometric curves with Fe^{3+} and Cu^{2+} are described in the Supporting Information.

pM Values and Species Distribution. A more reliable parameter for ligand effectiveness is the pM value, where pM = −log [M], which 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. In the first model, the calculation of [M], the concentration of free aquo metal ion was made taking account only the proton affinities of the ligand and other chelate species such as protonated metal complexes, in accordance with Bannochie and Martell.[30] These values were computed for the pH range from 4.0 to 12.0, with strength ionic fixed at 0.100 M, using a 10% excess of ligand. In the second model, pM values, where pM = −log[M], were calculated in a nutrient solution system in order to know the total sequestering ability of iron chelates in agronomic conditions. For this, both component and thermodynamic databases of the equilibrium speciation model MINTEQA2 program were modified in order to include each ligand and their formation constants (as activity constants).[31] Each ligand was introduced as iron chelate in the Hoagland nutrient solution, whose composition was the following: $[Fe^{3+}] = [\text{ligand}] = 1 \times 10^{-4}$ M; $[Cu^{2+}] = 3.15 \times 10^{-7}$ M; $[Ca^{2+}] = 1.6 \times 10^{-3}$ M; $[Mg^{2+}] = 8 \times 10^{-4}$ M. pFe values were calculated in the 4−12 pH range.

The distribution of species was determined by means of theoretical models considering the conditions in which they are applied. With this aim, a model was employed to know the behavior of the chelating agents in solution in the 4−13 pH range. Species distribution was established using the same methodology as that used to calculate pFe in agronomic conditions.

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Table 1. log Protonation Constants[a] for Chelating Agents **1** with Reference Values Given in Brackets)

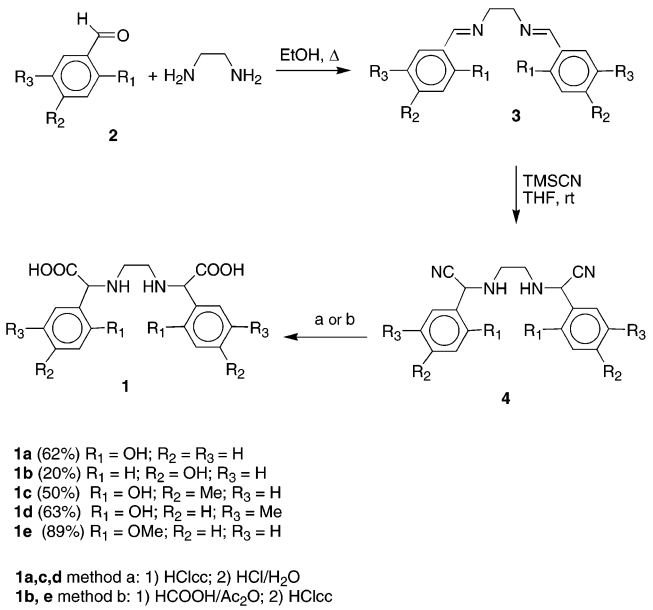
	K_1^H [HL]/[H][L]	K_2^H [H_2L]/[H][HL]	K_3^{Hp} [H_3L]/[H][H_2L]	K_4^H [H_4L]/[H][H_3L]	K_5^H [H_5L]/[H][H_3L]	K_6^H [H_6L]/[H][H_3L]
EDDHA	11.94 (11.68)[b]	10.73 (10.24)[b]	8.66 ± 0.04 (8.64)[b]	6.18 ± 0.06 (6.32)[b]		
rac-EDDHA	11.88 (12.05)[c]	10.80 (10.87)[c]	8.67 ± 0.01 (8.79)[c]	6.28 ± 0.11 (6.33)[c]		
meso-EDDHA	11.90 (11.90)[c]	10.89 (10.85)[c]	8.58 ± 0.04 (8.76)[c]	6.16 ± 0.02 (6.36)[c]		
p,p-EDDHA	9.94 ± 0.04	9.07 ± 0.02	6.85 ± 0.06	4.36 ± 0.07		
EDDH4MA	11.63	10.48	8.73 ± 0.01	6.40 ± 0.13		
rac-EDDH4MA	11.83 (11.53)[d]	10.71 (10.79)[d]	8.58 ± 0.15 (8.95)[d]	6.17 ± 0.03 (nd)[d]		
meso-EDDH4MA	11.97 (11.46)[d]	10.75 (10.75)[d]	8.72 ± 0.13 (9.11)[d]	6.35 ± 0.02 (6.63)[d]		
EDDH5MA	11.89	10.78	8.71 ± 0.11	6.55 ± 0.13		
EDDMtxA			7.61 ± 0.09[e]	4.92 ± 0.47[e]		
EDDHSA	10.43 ± 0.08	8.90 ± 0.03	7.45 ± 0.04	5.90 ± 0.06	2.63 ± 0.09	1.93 ± 0.16

[a] This work; $\mu = 0.1$ M (NaCl); $t = 25$ °C. [b] Ref 8; $\mu = 0.1$ M (KNO_3); $t = 25$ °C. [c] Ref 9; $\mu = 0.1$ M (KCl); $t = 25$ °C. [d] Ref 10; $\mu = 1.0$ M (NaCl); $t = 25$ °C. [e] Although these protonation constants correspond to K_1^H and K_2^H, they are shown as K_3^H and K_4^H respectively as they involve the protonation of the secondary nitrogens.

Results and Discussion

The synthesis of ligands **1** was done starting from the corresponding aldehydes **2** by condensation with ethylenediamine in boiling absolute EtOH. The diimines **3** were obtained either as pure compounds from the reaction medium or recrystallized from EtOH. Imines **3** were reacted with TMSCN in anhydrous THF at room temperature to yield the corresponding α-aminonitriles **4**. The presence of an *o*-hydroxy group in the aromatic ring made compounds **4a**, **4c**, and **4d** highly reactive, and they were submitted to acid hydrolysis without further purification by sequential treatment with concentrated HCl and diluted HCl (Scheme 2). In contrast, α-aminonitriles **4b** and **4e** lacking an *o*-hydroxy group were considerably more stable but gave retrocondensation products when they were hydrolyzed in concentrated HCl. To avoid this undesirable reaction, the hydrolysis of nitriles **4b** and **4e** was made by previous formylation of the amino groups in the nitriles (HCOOH/Ac_2O) followed by acid hydrolysis.[24] The pure amino acids **1a**, **1c**, and **1d** were obtained by precipitation from the reaction mixture, by adjusting the solution to pH 3 with 6 M NaOH. Amino acids **1b** and **1e** were obtained as their hydrochlorides. Compounds **1** were isolated as a 1:1 mixture of diastereomers in all cases, and their analytical and spectroscopic data were consistent with the proposed structures. The purity of the chelating agents synthesized was higher than 90% by ^{1}H NMR.

Scheme 2



1a (62%) R_1 = OH; R_2 = R_3 = H
1b (20%) R_1 = H; R_2 = OH; R_3 = H
1c (50%) R_1 = OH; R_2 = Me; R_3 = H
1d (63%) R_1 = OH; R_2 = H; R_3 = Me
1e (89%) R_1 = OMe; R_2 = H; R_3 = H

1a,c,d method a: 1) HClcc; 2) HCl/H_2O
1b, e method b: 1) HCOOH/Ac_2O; 2) HClcc

NMR has been frequently used to determine the purity of the free chelating agents.[14,29] However, despite its accuracy, inorganic impurities and water content cannot be evaluated by this technique. Titrimetric techniques[32,33] are alternative techniques to determine the purity of ligands such as EDDHA and their analogues.

The titrimetric purity of the chelating agents **1a**−**f**, determined by photometry (with Fe^{3+}) or potentiometry (with Cu^{2+}), was higher than 87% in all cases, which is in good agreement with the estimated purity obtained by ^{1}H NMR (considering that the error of this technique is ±4%). The molar absorptivities of all the Fe^{3+} chelates are of the same order of magnitude, as both phenolate groups are coordinated to Fe^{3+} (see Supporting Information). The molar absorptivities of *p,p*-EDDHA/Fe^{3+} and EDDMtxA/Fe^{3+} could not be determined since the formation of these Fe chelates is not possible. For this reason, the purities of ligands **1b** and **1e** were determined using the potentiometric method with Cu^{2+} solution.

Protonation Constants

As stated, except for *p,p*-EDDHA (**1b**) and EDDHSA (**1f**), the spectrophotometric constants K_1^H and K_2^H were used as seed and then refined together with the protonation constants (that were determined by potentiometric methods) using the program BEST.[25] The protonation constants for ligands **1a**−**f** are shown in Table 1. With the exception made for EDDMtxA, which bears two methoxy groups, all chelating agents tested are hexadentate, having two phenolates, two nitrogens, and two carboxylates.[34]

The two highest protonation constants of EDDHSA are considerably lower than those of EDDHA and EDDH4MA (Table 1). A similar trend is observed for the dissociation constants of *p*-hydroxybenzenesulfonic acid ($pK_a = 8.82$), phenol ($pK_a = 10.00$), and 3-methylphenol ($pK_a = 10.09$). Significantly, the magnitude of K_1^H and K_2^H for *p,p*-EDDHA

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Table 2. log Stability Constants for Ca^{2+} and Mg^{2+} Chelates of Ligands **1**

	Ca^{2+}			Mg^{2+}		
	[ML]/[L][M]	[MHL]/[H][L][M]	$[MH_2L]/[H]^2[L][M]$	[ML]/[L][M]	[MHL]/[H][L][M]	$[MH_2L]/[H]^2[L][M]$
EDDHA	7.29 ± 0.30	16.77 ± 0.33	25.95 ± 0.50	9.76 ± 0.05	18.18 ± 0.15	25.36 ± 0.24
rac-EDDHA	7.99 ± 0.42	17.42 ± 0.39	26.87 ± 0.37	10.13 ± 0.03	nd	nd
meso-EDDHA	7.56 ± 0.49	17.10 ± 0.65	26.41 ± 0.64	9.44 ± 0.08	17.51 ± 0.25	26.56 ± 0.35
p,p-EDDHA	3.54 ± 0.52	12.93 ± 0.58	21.21 ± 0.65	3.74 ± 0.57	12.89 ± 0.39	20.79 ± 0.57
EDDH4MA	5.84 ± 0.28	15.86 ± 0.07	24.58 ± 0.17	8.00 ± 0.02	17.06 ± 0.15	24.81 ± 0.06
rac-EDDH4MA	7.18 ± 0.11	16.66 ± 0.36	26.31 ± 0.44	7.82 ± 0.18	17.01 ± 0.11	25.42 ± 0.34
meso-EDDH4MA	6.85 ± 0.24	16.89 ± 0.63	26.31 ± 0.73	9.65 ± 0.11	17.88 ± 0.05	26.33 ± 0.10
EDDH5MA	6.65 ± 0.57	16.76 ± 0.58	26.22 ± 0.78	7.83 ± 0.08	16.88 ± 0.10	25.28 ± 0.37
EDDMtxA	3.19 ± 0.40	10.64 ± 0.38	nd	3.93 ± 0.49	10.87 ± 0.39	nd
EDDHSA	5.40 ± 0.07	13.15 ± 0.27	21.75 ± 0.21	6.95 ± 0.12	14.29 ± 0.07	21.44 ± 0.13

[a] μ = 0.1 M (NaCl); t = 25 °C.

Table 3. log Stability Constants[a] for Cu^{2+} and Fe^{3+} Chelates of Ligands **1** with Reference Values Given in Brackets

	Cu^{2+}				Fe^{3+}			
	[ML]/[L][M]	[MHL]/[H][L][M]	$[MH_2L]/[H]^2[L][M]$	$[MH_3L]/[H]^3[L][M]$	[ML]/[L][M]	[MHL]/[H][L][M]	$[MH_2L]/[H]^2[L][M]$	$[MOHL]/[H]^{-1}[L][M]$
EDDHA	25.13 ± 0.00 (23.90)[c]	32.61 ± 0.01 (31.94)[b]	37.31 ± 0.01 (36.92)[b]		35.09 ± 0.28 (33.91)[d]	36.89 ± 0.21		23.66 ± 0.27
rac-EDDHA	24.94 ± 0.05 (25.27)[b]	32.87 ± 0.04 (32.99)[b]	37.33 ± 0.07 (37.43)[b]		35.86 (35.54)[b]	35.08		13.12 (23.76)[b]
meso-EDDHA	23.68 ± 0.02 (23.68)[b]	32.30 ± 0.00 (32.05)[b]	37.25 ± 0.01 (37.19)[b]		34.15 (33.28)[b]	36.56 (36.00)[b]		22.81 (22.83)[b]
p,p-EDDHA	14.74 ± 0.06	22.39 ± 0.06	28.50 ± 0.04	31.09 ± 0.04				
EDDH4MA	23.94 ± 0.03	31.06 ± 0.01	36.07 ± 0.01		34.44 ± 0.06	36.59 ± 0.07		22.81 ± 0.13
rac-EDDH4MA	22.67 ± 0.07	31.49 ± 0.02	36.62 ± 0.03		33.75 ± 0.05 (37.9)[e]	36.31 ± 0.05 (40.4)[e]		22.34 ± 0.05 (26.04)[e]
meso-EDDH4MA	24.47 ± 0.05	32.38 ± 0.05	37.21 ± 0.01		35.54 ± 0.07 (39.0)[e]	36.85 ± 0.03 (40.9)[e]		23.45 ± 0.17 (25.55)[e]
EDDH5MA	23.46 ± 0.02	32.21 ± 0.01	37.36 ± 0.01		33.66 ± 0.01	36.41 ± 0.10		21.98 ± 0.01
EDDMtxA	15.73 ± 0.12	23.65 ± 0.12	29.91 ± 0.19	32.38 ± 0.19				
EDDHSA	21.62 ± 0.29	29.61 ± 0.19	33.99 ± 0.12	35.48 ± 0.19	32.79 ± 0.16	34.63 ± 0.17	36.15 ± 0.18	21.91 ± 0.17

[a] μ = 0.1 M (NaCl); t = 25 °C. [b] Ref 9; μ = 0.1 M (KCl); t = 25 °C. [c] Ref 28; μ = 0.1 M (KCl); t = 25 °C. [d] Ref 27; μ = 0.1 M (KNO_3); t = 20 °C. [e] Ref 10; μ = 1.0 M (NaCl); t = 25 °C.

is lower than in the case of EDDHA. This is probably due to the stabilization of the *o*-hydroxyphenyl glycine moiety in EDDHA by formation of a hydrogen bond between the basic nitrogen atom and the phenolic group. This interaction is not possible in *p,p*-EDDHA, making the K^H values closer to the pK_a of phenol.

K_3^H and K_4^H correspond to the protonation constants of the secondary amine nitrogens, and K_5^H and K_6^H, to the protonation of the carboxylate oxygens. Bannochie and Martell[9] related the impossibility of getting the protonation constants of the carboxylate oxygens because of the precipitation caused by the addition of the fourth equivalent of acid in the back-titration. However, in a later work,[30] these authors considered that although the precipitation occurs, it is possible to titrate a supersaturated solution of these ligands quickly beyond this point. In this work, K_5^H and K_6^H are only determined for EDDHSA due to its higher solubility.

The protonation constants obtained for EDDHA and their isomers have comparable values and are very similar to those already published by Bannochie and Martell.[9] However, the magnitudes of our K_1^H and K_2^H for EDDHA are higher than those reported by Frost et al.,[8] since he used a potentiometric method that was limited at pH over 11. The methyl group in the benzene ring could slightly increase the basicity of the phenol (phenol, pK_a = 10.00; 3-methylphenol, pK_a = 10.09; 4-methylphenol, pK_a = 10.26). The effect of the sulfonic groups in increasing the acidity of the phenolic groups is reflected in the values of all the protonation constants of EDDHSA.

Ca^{2+} and Mg^{2+} Stability Constants. Ca^{2+} and Mg^{2+} stability constants for ligands **1a**−**g** are shown in Table 2. From the Ca^{2+} and Mg^{2+} potentiometric curves, the existence may be presumed of at least three species of the metal chelates: MH_2L, MHL^-, and ML^{2-}. The magnitudes of Mg^{2+} stability constants are higher than those of Ca^{2+} for all the products. This behavior is the opposite to that found for other hexadentate ligands such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), *trans*-1,2-ciclohexylenedinitrilotetraacetic acid (CDTA), *N*-(hydroxyethyl)ethylenediamine-*N,N′*-triacetic acid (HEDTA), and ethyleneglycolbis(ethylamine)tetraacetic acid (EGTA),[29] although it can be easily explained by considering both ligand architecture and metal properties (size of the metal, charge, ionic radius, etc.). Both Ca^{2+} and Mg^{2+} have been classified as hard acids, according to the HSAB principle of Pearson,[35] but the ionic radius of Mg^{2+} (0.7 Å) is smaller than that of Ca^{2+} (1.0 Å). As a conclusion, Mg^{2+} can form chelates more effectively than the larger Ca^{2+} with this type of phenolic ligand.[36] This phenomenon has also been observed for other phenolic ligands (e.g., *N,N′*-bis(2-hydroxybenzyl)ethylene-

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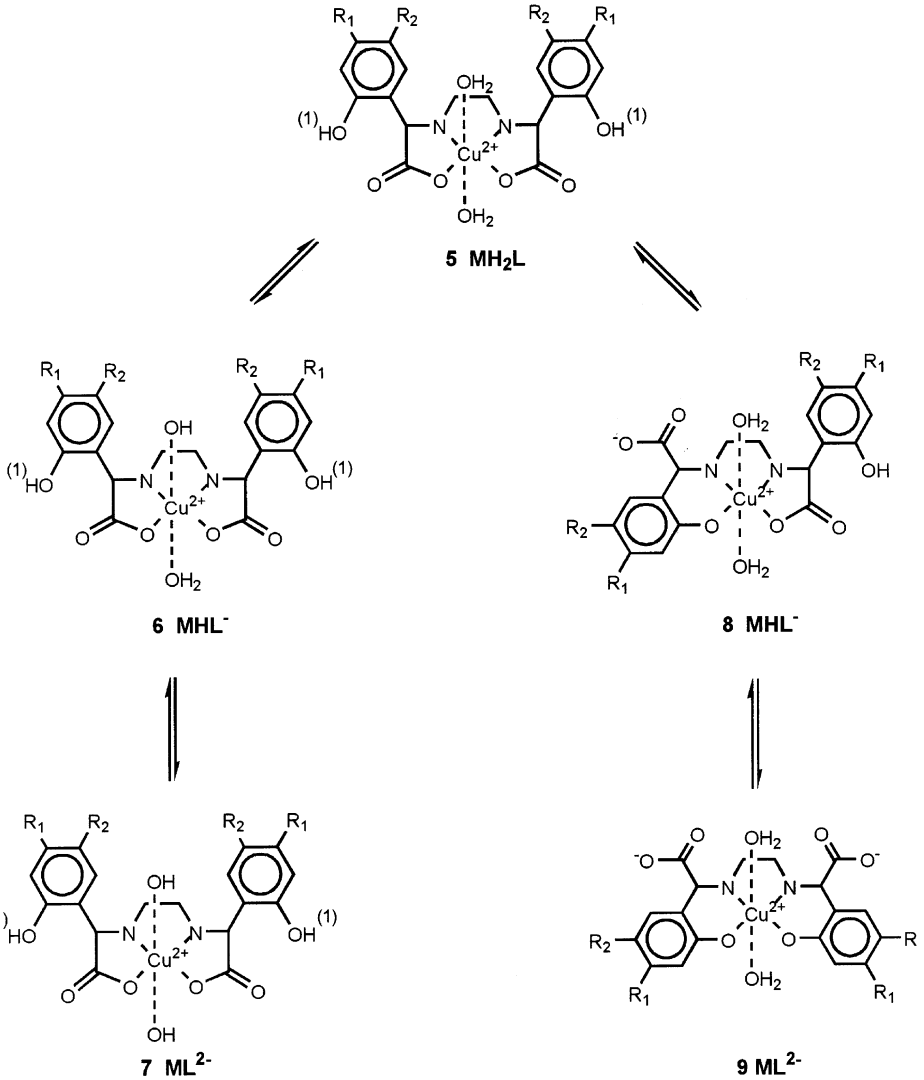
Scheme 3. Cu^{2+} Species Formed



	Ligand	R_1	R_2	(1)
1a	EDDHA	H	H	
1b	*p,p*-EDDHA	OH	H	H
1c	EDDH4MA	CH_3	H	
1d	EDDH5MA	H	CH_3	
1e	EDDMtxA	H	H	OCH_3
1f	EDDHSA	H	SO_3H	
1g	EDDCHA	H	COOH	

diamine-*N*,*N*′-diacetic acid, HBED).[27] In general, the presence of methyl and sulfonate substituents in the benzene ring has little effect on the Ca^{2+} and Mg^{2+} stability constants. Again, the lowest values correspond to *p*,*p*-EDDHA and EDDMtxA that have the phenolate groups unavailable for coordination with the metals.

Fe^{3+} and Cu^{2+} Stability Constants. For each ligand **1**, the Cu^{2+} and Fe^{3+} stability constants shown in Table 3 were determined from plots of absorbance against pH at 480 nm for iron and at 650 nm for copper. The observed values for EDDHA (and their *rac*- and *meso*-forms) are in good agreement with those previously reported.[9] This fact validates the spectrophotometric methodologies proposed and developed through this work. Therefore, spectrophotometric titrations can be used to determine Cu^{2+} and Fe^{3+} stability constants with phenolic ligands. Additionally, this methodology could be applied to determine any metal−ligand affinity if the formation of a chelate can be measured photometrically.[8,9,27,30]

The different Cu^{2+} chelate species are represented in Scheme 3. At neutral pH or below, a typical blue Cu^{2+} complex is observed, which no doubt involves only coordination to the ethylenediamine nitrogens, the carboxylate oxygens, and two molecules of water (**5** in Scheme 3).[37] For *p*,*p*-EDDHA and EDDMtxA, these are the only possible ways of binding Cu^{2+} due to the absence of the *ortho*-

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Table 4. pFe[a] against pH for Iron Chelates[b]

ligand	7	7.5	8	8.5
EDDHA	23.7	25.2	26.7	28.0
rac-EDDHA	24.4	26.0	27.4	28.8
meso-EDDHA	22.7	24.2	25.7	27.0
EDDH4MA	23.5	25.0	26.5	27.9
rac-EDDH4MA	22.6	24.1	25.5	26.9
meso-EDDH4MA	24.0	25.5	27.0	28.4
EDDH5MA	22.1	23.7	25.2	26.6
EDDHSA	25.9	27.2	28.3	29.3

[a] Calculated for [Lt] = 1.1×10^{-6} M, [Mt] = 1.0×10^{-6} M. [b] For pFe data in the whole pH range, see the Supporting Information.

Table 5. pCu[a] against pH for Copper Chelates[b]

ligand	7	7.5	8	8.5
EDDHA	14.3	15.5	16.8	18.1
rac-EDDHA	14.5	15.6	16.8	18.0
meso-EDDHA	13.9	14.9	15.9	16.9
EDDH4MA	13.4	14.7	16.1	17.4
rac-EDDH4MA	13.3	14.3	15.3	16.3
meso-EDDH4MA	13.9	15.0	16.2	17.4
EDDH5MA	13.7	14.8	15.8	16.8
EDDHSA	15.7	16.6	17.4	18.2
p,p-EDDHA	9.2	9.9	10.7	11.6
EDDMtxA	15.1	14.9	14.8	14.8

[a] Calculated for [Lt] = 1.1×10^{-6} M, [Mt] = 1.0×10^{-6} M. [b] For pCu data in the whole pH range, see the Supporting Information.

hydroxy groups. Consequently, their solutions remain blue until pH 12. Furthermore, for these chelating agents, the other Cu^{2+} stability constants must involve exclusively water molecules binding the metal (see **6** and **7** in Scheme 3). For the rest of the chelating agents, a new green complex is formed as the pH rises. This complex involves coordination of the Cu^{2+} by the phenol groups (**9** in Scheme 3). Finally, species **8** appear at intermediate pH values and involve the two amino groups, one carboxylate group and one phenolate group.

The Fe^{3+} chelate species are represented in Scheme 4. The predominant species involves the coordination with the nitrogen atoms, the carboxylate oxygens, and the phenolate groups (**11** in Scheme 4) except for *p,p*-EDDHA and EDDMtxA that are not able to form the chelate. The protonated (**10**) and hydroxylated (**12**) species are predominant at pH below 3 and above 10, respectively.

The differences in stability between iron and copper complexes of both isomers of EDDHA have been explained by a geometric selectivity effect.[9] However, it is not yet clearly understood why Cu^{2+} and Fe^{3+} *meso*-EDDH4MA complexes are more stable than those obtained from *rac*-EDDH4MA.

pM Values and Species Distribution. pFe and pCu values were determined using the first model (see the Experimental Section) in a 4−12 pH range. Tables 4 and 5 only show the pM values at agronomically relevant pH values.

The pFe values obtained for all the phenolic ligands **1b**−**f** in Table 4 are comparable in magnitude with the pFe's of EDDHA, supporting that all of them could be applied into a soil system as iron chlorosis correctors in calcareous soils. EDDHSA, *rac*-EDDHA, and *meso*-EDDH4MA are the most effective ligands for binding Fe^{3+}. On the other hand, EDDH5MA is the weakest ligand throughout the pH range used.

The pCu values are shown in Table 5. The pCu values for all the phenolic ligands (except for *p,p*-EDDHA and EDDMtxA) are comparable in magnitude. EDDHSA is the most effective at pH below 9. On the other hand, *p,p*-EDDHA has the lowest pCu value throughout the pH range used, due to the fact that the phenolic groups cannot bind copper. pCu values from EDDMtxA vary slightly with pH showing the highest values in acid pH. The relative behavior of EDDHSA with respect to EDDHA is similar to that of SHBED with respect to HBED.[38,39] The higher acidity provided by the sulfonic groups results in ligands that are more effective at lower pH values. Additionally, as indicated by the values of the protonation constants, the competition among metal and protons is higher in EDDHA than in EDDHSA, and therefore, EDDHSA is more effective than EDDHA for Fe^{3+} chelation. pFe and pCu trends for the isomers of EDDHA and EDDH4MA correspond with their stability constants, and *rac*-EDDHA and *meso*-EDDH4MA are more effective in binding metals than *meso*-EDDHA and *rac*-EDDH4MA, respectively.

pFe and pCu are very useful to compare effectiveness of these ligands when applied in solution containing only one metal. However, in physiological studies and agronomical use, iron chelates are employed in systems where several other metals (i.e., Cu^{2+}, Ca^{2+}, Mg^{2+}, 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, pFe values were also obtained in a second model for the chelating agents in a nutrient solution system (Table 6). These pFe values are lower than those of pFe presented in Table 4 for single metal chelation, due to the competition between Fe and the other metals. In general, all phenolic chelating agents considered present similar pFe values in nutrient solution conditions, supporting that they can be used as chlorosis correctors. Therefore, in order to determine their effectiveness, their behavior will depend on other external factors such as soil properties, way of application, culture type, meteorological conditions, solubility, and factors involving plant uptake processes.

In Figure 3, the species distribution curves for some chelating agents in Hoagland nutrient solution are shown. For all phenolic ligands (with exception made for *p,p*-EDDHA and EDDMtxA), the FeL species is predominant in the whole physiological pH range. Thus, 100% of the iron chelate remains as FeL^{-} species at pH below 11. The hydroxylated $FeOHL^{2-}$ species appear at pH around 11.5 in those ligands in which it has been possible to determine it. Only at pH above 11.5 do the calcium and magnesium chelates become predominant species. Due to the low concentration of Cu^{2+} in Hoagland nutrient solution, copper chelates are not predominant in either pH range, although their stability constants are higher than those of Ca^{2+} and

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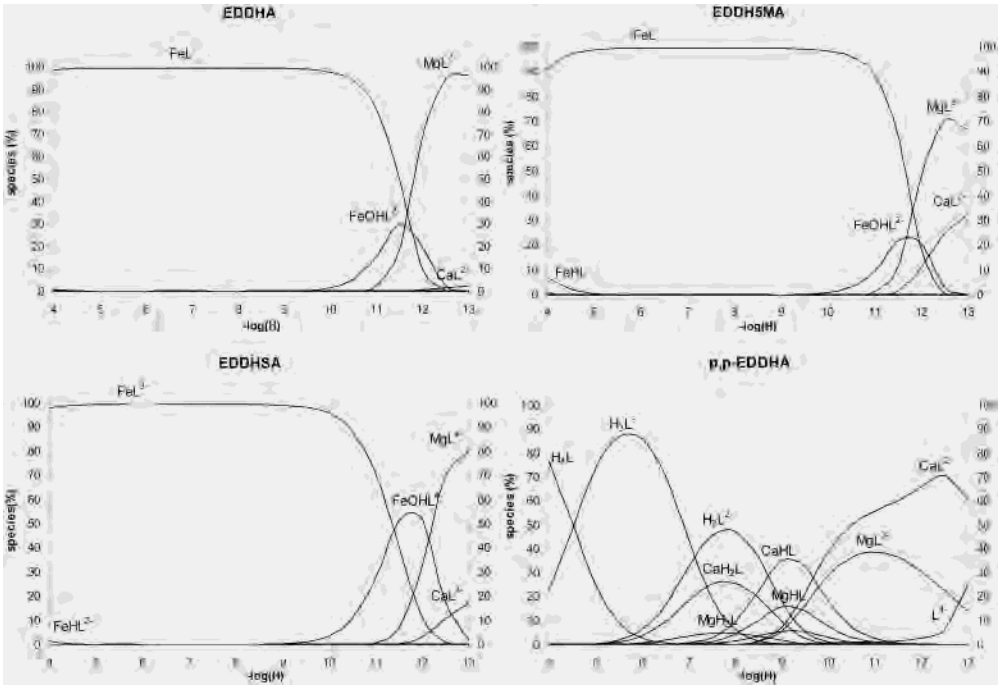
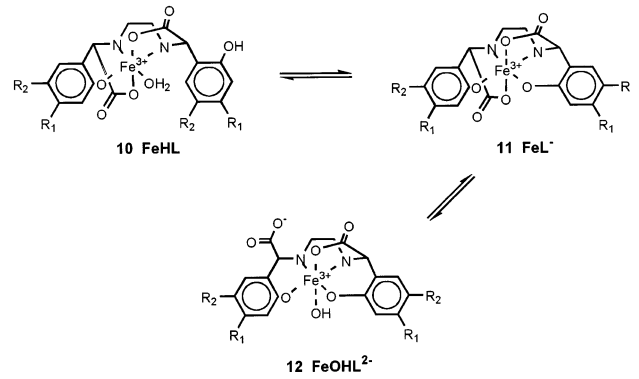


Figure 3. Species distribution against pH using Hoagland nutrient solution composition. $[Fe^{3+}] = [\text{ligand}] = 1.0 \times 10^{-4}$ M; $[Ca^{2+}] = 1.6 \times 10^{-3}$ M; $[Mg^{2+}] = 8.0 \times 10^{-4}$ M; $[Cu^{2+}] = 3.15 \times 10^{-7}$ M.

Scheme 4



	Ligand	R_1	R_2
1a	EDDHA	H	H
1b	*p,p*-EDDHA	OH	H
1c	EDDH4MA	CH_3	H
1d	EDDH5MA	H	CH_3
1e	EDDMtxA	H	H
1f	EDDHSA	H	SO_3H
1g	EDDCHA	H	COOH

Table 6. pFe Values against pH in Agronomic Conditions[a]

ligand	7	7.5	8	8.5
EDDHA	15.09	16.28	17.67	19.27
rac-EDDHA	15.20	16.40	17.79	19.34
meso-EDDHA	15.03	16.24	17.65	19.27
EDDH4MA	15.28	16.43	17.77	19.31
rac-EDDH4MA	15.07	16.27	17.69	19.30
meso-EDDH4MA	15.25	16.44	17.83	19.36
EDDH5MA	15.02	16.22	17.63	19.26
EDDHSA	15.26	16.46	17.86	19.41

[a] $[Fe^{3+}] = [\text{ligand}] = 1.0 \times 10^{-4}$ 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.

Mg^{2+}, which are present in larger concentration. The curves are in good agreement with the pFe values, because all phenolic ligands are able to form very stable iron chelates.

Conclusions

The proposed methodology involving analytical determinations (titrimetric purity, protonation and stability constants, pM) and speciation distribution in agronomic conditions has been developed and applied to phenolic ligands used as fertilizers. The ligands studied through this work have been obtained by means of a new general methodology of synthesis. Given that the procedure allows the synthesis of the chelating as pure compounds, these ligands can be used as standards to identify and quantify commercial chelates (EDDHA, EDDH4MA, and their geometric isomers) as well as to detect the presence of undesirable compounds in fertilizers of general use (EDDH5MA and *p,p*-EDDHA). The results obtained in this work indicate that EDDHA and its analogues (EDDH4MA, EDDH5MA, and EDDHSA) present comparable chelating ability, measured through divalent and trivalent metal affinities together with pFe and pCu values.

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Supporting Information Available: Equations related to the determination of purity of the chelating agents, spectrophotometric measurements for protonation constants and Fe^{3+} and Cu^{2+} stability constants, and pM determination, including figures and tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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