JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Theoretical Speciation of Ethylenediamine-*N*-(*o*-hydroxyphenylacetic)-*N*′-(*p*-hydroxyphenylacetic) Acid (*o*,*p*-EDDHA) in Agronomic Conditions

FELIPE YUNTA, SONIA GARCÍA-MARCO, AND JUAN J. LUCENA*

Department of Agricultural Chemistry, Universidad Autónoma, 28049 Madrid, Spain

The presence of ethylenediamine-*N*-(*o*-hydroxyphenylacetic)-*N*′-(*p*-hydroxyphenylacetic) acid (*o*,*p*-EDDHA) as the second largest component in commercial EDDHA iron chelates has recently been demonstrated. Here is reported the speciation of *o*,*p*-EDDHA by the application of a novel methodology through the determination of the complexing capacity, protonation, and Ca^{2+}, Mg^{2+}, Cu^{2+}, and Fe^{3+} stability constants. The pM values and species distribution in solution, hydroponic, and soil conditions were obtained. Due to the para position of one phenol group in *o*,*p*-EDDHA, the protonation constants and Ca and Mg stability constants have different values from those of *o*,*o*-EDDHA and *p*,*p*-EDDHA regioisomers. *o*,*p*-EDDHA/Fe^{3+} stability constants are higher than those of EDTA/Fe^{3+} but lower than those of *o*,*o*-EDDHA/Fe^{3+}. The sequence obtained for pFe is *o*,*o*-EDDHA/Fe^{3+} ≥ *o*,*p*-EDDHA/Fe^{3+} > EDTA/Fe^{3+}. *o*,*p*-EDDHA/Fe^{3+} can be used as an iron chelate in hydroponic conditions. Also, it can be used in soils with limited Cu availability.

KEYWORDS: ***o*,*p*-EDDHA; *o*,*o*-EDDHA; iron chelates; fertilizers; formation constants; stability; speciation**

INTRODUCTION

Iron chlorosis is a nutritional disorder in plants that affects their development and decreases the yield of many crops growing on calcareous soils. Chlorosis results in a decrease in the amount of chlorophyll and is manifested by a gradual disappearance of the green color of the plants (*1*, *2*). Today, fertilization with synthetic iron chelates is the most common agricultural practice to relieve this problem. In Europe, the 98/3/EC directive regulates iron and other micronutrient chelates used as they are or incorporated in mixed fertilizers. Six chelating agents, all polyamine carboxylic acids, are permitted for this purpose: EDTA, DTPA, HEDTA, EDDHA, EDDH4MA, and EDDCHA; however, in the new regulation proposed by the EC, *o*,*p*-EDDHA is also included. Ethylenediamine-*N*,*N*′-bis(*o*-hydroxyphenyl)acetic acid (*o*,*o*-EDDHA) (**1** in **Figure 1**) and its analogues are among the most efficient iron chelating agents used (*3*).They have two phenolic groups replacing two of the carboxylates of EDTA, which increases the stability of the iron chelate by $>10^{10}$ (*4*). Therefore, EDTA and its analogues (**4** in **Figure 1**) are weaker than EDDHA and its analogues, and their use is restricted to neutral soils or to hydroponic-like cultures (*2*). The synthesis of *o*,*o*-EDDHA (**1** in **Figure 1**), originally reported by Kroll (*5*) in 1957, is a Strecker reaction on the imine derived from ethylenediamine and salicylaldehyde. Although the procedure can be employed to obtain other symmetrically substituted EDDHAs (*6*, *7*), the drawback is the need for the liquid HCN during the industrial process. Other approaches to the synthesis of ethylenediaminebis(*o*-hydroxyphenyl)acetic acids are based on a Mannich-like reaction between phenol (or substituted phenols), ethylenediamine, and glyoxylic acid (*8*, *9*). This method is used for the preparation of all of the EDDHA currently on the market. Some analytical methods for the quality control of such products have been developed and employed to determine the *o*,*o*-EDDHA/Fe^{3+} content in the product (*10*−*13*). Other important aspects related to the composition of the commercial chelates have been neglected with these analytical methods. Actual industrial synthesis of *o*,*o*-EDDHA (*6*, *7*) produces a mixture of three regioisomeric products, namely, *o*,*o*-EDDHA, *o*,*p*-EDDHA, and *p*,*p*-EDDHA (**1**, **2**, and **3**, respectively, in **Figure**

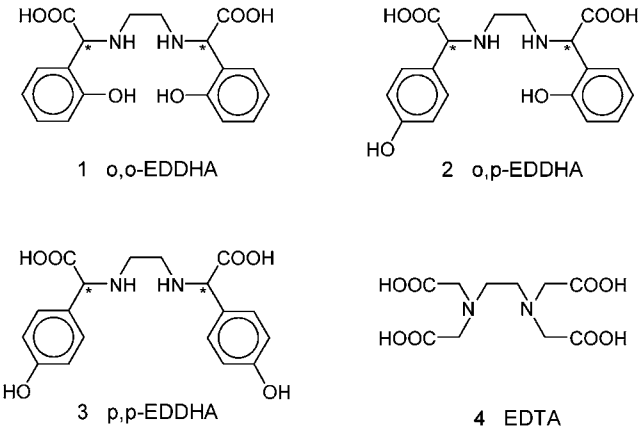

Figure 1. Chelating agents and chelates described in the text. *, Chiral carbons.

* Author to whom correspondence should be addressed (e-mail juanjose.lucena@uam.es).

10.1021/jf034304r CCC: $25.00
Published on Web 08/05/2003

1) in variable amounts. To study the single components of this mixture and to know their ability to form ferric complexes, a general method for the synthesis of *o*,*o*-EDDHA, *p*,*p*-EDDHA (**1** and **3**, respectively, in **Figure 1**) has already been reported (*14*). Also in our research, 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, have been developed. It has been claimed that the second main component present in *o*,*o*-EDDHA commercial iron chelates is the *o*,*p*-EDDHA isomer (**2** in **Figure 1**) (*15*, *16*). Two alternative routes for the synthesis of *o*,*p*-EDDHA were developed (*17*). Furthermore, having obtained a pure sample of *o*,*p*-EDDHA, we have been able to confirm unambiguously that the *o*,*p*-EDDHA/Fe^{3+} complex is actually present in considerable amounts in commercial samples of *o*,*o*-EDDHA iron chelates (*17*). When *p*,*p*-EDDHA was characterized and compared with its *o*,*o*-EDDHA regioisomer (*14*), the relative position of the hydroxyl group into the benzene ring was revealed as a very important factor in the formation of the ferric complex. *p*,*p*-EDDHA/Fe^{3+} does not form in aqueous solution because the two *p*-hydroxyphenyl groups are sterically impaired to bind Fe^{3+} (*14*).

Several authors evaluate the stability, reactivity, and sorption processes of the iron chelates with differents soil components (peat, oxyhydroxides, calcium carbonate, and clay minerals) and agronomic soils. Thus, Hernández-Apaolaza et al. (*18*) reported that EDDHA/Fe^{3+} chelate was more retained in acid peat than EDDH4MA/Fe^{3+}, but in montomorillonite, at low concentration, the retention of EDDH4MA/Fe^{3+} is greater than that of EDDHA/Fe^{3+}. The *meso*-EDDHA/Fe^{3+} isomer was the most retained. The absorption was very low for EDDH4MA/Fe^{3+} isomers. EDDHA/Fe^{3+}, EDDH4MA/Fe^{3+}, EDDHSA/Fe^{3+}, and EDDCHA/Fe^{3+} presented high stability at alkaline pH values and low reactivity with alkaline soils and soil components (*19*). The presence of other secondary products such as *o*,*p*-EDDHA/Fe^{3+} and *p*,*p*-EDDHA modified decisively the behavior of commercial formulations containing EDDHA/Fe^{3+}. To determine the stability of each secondary product in agronomic conditions, it is necessary to evaluate the reactivity using standards.

Chemical speciation has been profusely used in mineral nutrition. Computerized chemical equilibrium models are powerful tools for studying the relationships between chemical speciation and the uptake, transport, and function of elements (*20*). Halvorson and Lindsay (*21*) developed computer programs to calculate the equilibrium levels of chelate metals in nutrient solutions and soils systems considering solid phases and the presence of oxides. This type of modelization has been already applied to study the stability of *o*,*o*-EDDHA/Fe^{3+} chelate showing the good correlation with its behavior in agronomic conditions.

The aim of this research work is to study the binding capacity of *o*,*p*-EDDHA chelating agent. Thus, the following has been determined: chelating capacity, formation constants, pM determination, and the species distribution in nutrient solution and soil conditions.

MATERIALS AND METHODS

General. The methodology has been described elsewhere (*14*). Potentiometric measurements were performed with a Metrohm 719 or 721 potentiometer (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.

All reagents used in this work were of analytical grade. All aqueous solutions were prepared with CO_2-free, water type I grade (*22*). $CaCl_2$, $MgCl_2$, NaCl, NaOH, $Cu(NO_3)_2$, HCl, and Fe^{3+} standard solution were obtained from Merck Chemical Co. Gran plot analysis was used to check for carbonate contamination of the standard aqueous NaOH and consistently revealed <0.5% of carbonate (*23*). The $Cu(NO_3)_2$ solution was standardized with Na_2-H_2EDTA salt solution by potentiometric titration. The end points of all potentiometric titrations were determined by the Gran function (*23*). Fe^{3+}, Ca^{2+}, Mg^{2+}, and Cu^{2+} solutions were standardized by atomic absorption spectroscopy with a Perkin-Elmer Analyst 800 spectrophotometer. All titrations were made under inert atmosphere (99.9995 purity grade N_2, NaOH washed, 0.100 M NaCl saturated). All commercially available organic reagents were used without further purification. *o*,*p*-EDDHA (**2** in **Figure 1**) was supplied by Syngenta Crop Protection.

Purity and Complexing Capacity. *o*,*p*-EDDHA was characterized by spectroscopic techniques. ^{1}H NMR spectra, recorded on a Bruker 200-AC (200.13 MHz for ^{1}H), were kindly provided by Dr. Sierra's group and compared to those of *o*,*o*-EDDHA and *p*,*p*-EDDHA.

The complexing capacity of the *o*,*p*-EDDHA ligand has been determined using both photometric [with Fe(III) solution as titrant] and potentiometric [with Cu(II) solution as titrant] methods (*17*). The titrations were carried out at 25.0 ± 0.5 °C, and the ionic strength was fixed at 0.1 M with NaCl. The concentrations of the chelating agent solutions were $\sim 1 \times 10^{-4}$ M.

The end points of the photometric titrations were determined by both linear segments' intersection (*24*) and the smoothed second-derivative method (*25*). The Fe(III) solution was used as a titrant. pH was fixed at 6.0 with 2 mM MES and was maintained at this pH during the titration with NaOH solution. The *o*,*p*-EDDHA/Fe^{3+} molar absorptivity was also obtained at 480 nm.

The end points of the potentiometric titration of *o*,*p*-EDDHA with Cu(II) were determined by both Gran's function (*23*) and the first-derivative methods. The Cu^{2+} solution (5.062×10^{-4} M) was used as a titrant solution. A Cu^{2+} selective electrode with reference electrode was used to measure Cu^{2+} free cation concentration.

Protonation Constants. The protonation constants have been determined by both photometric and potentiometric methods. The first two association steps of the ligand were measured spectrophotometrically (*26*), because the combination of protons with the phenolic groups is accompanied by extensive changes in the absorption spectra (at 295 nm). Twelve 1×10^{-4} M solutions were prepared with the ionic strength adjusted to 0.100 M with NaCl and pH adjusted from 8.20 to 13.30 at 0.3−0.5 pH intervals. Spectra (250−500 nm) were obtained with a Shimazdu UV−vis spectrophotometer. The wavelength of the maximum absorbance and molar absorptivities of L^{4-} and LH_2^{2-} species were initially estimated at pH 13.5 and 9, respectively, and used as seeds for calculations. The calculations themselves involved a least-squares minimization of calculated versus observed absorbances varying the first and second protonation constants and the molar absorptivities (*14*).

The lowest protonation constants, corresponding to the two amino groups and two carboxylic groups, were determined by potentiometric titration (*27*). *o*,*p*-EDDHA chelating agent solution [1×10^{-3} M; $\mu =$ 0.1 M (NaCl)], previously dissolved in 4 equiv of NaOH per equivalent of chelating agent, was back-titrated with HCl (0.05009 ± 0.00005) M solution at 25 °C under N_2 atmosphere. The third through sixth ligand protonation constants were calculated using the FORTRAN program BEST (*28*).

Ca and Mg Stability Constants. The methodology used for protonation constants was also applied to determine the Ca^{2+} and Mg^{2+} stability constants. *o*,*p*-EDDHA/Ca and *o*,*p*-EDDHA/Mg chelates in 1:1 and 1:10 rates were previously formed and then were back-titrated with HCl (0.05009 ± 0.00005) M solution at 25 °C under N_2 atmosphere.

Total Ca^{2+} and Mg^{2+} concentrations were measured by atomic absorption spectrophotometry. The Ca^{2+} and Mg^{2+} stability constants were calculated using the FORTRAN program BEST (*27*).

Cu(II) and Fe(III) Stability Constants. Prior to the determination of their stability constants, studies of the *o*,*p*-EDDHA/Fe^{3+} and *o*,*p*-EDDHA/Cu^{2+} absorption spectra were carried out to choose the wavelength adequate to run the photometric titrations. Twelve solutions

covering a pH range from 2 to 12 were prepared to be 1×10^{-4} M on iron chelate or 1×10^{-3} M on copper chelate, and the spectra between 350 and 800 nm were obtained in 10 nm intervals.

Stability constants for the Fe^{3+} and Cu^{2+} chelates were calculated from spectrophotometric data obtained after base titration (*14*). Four or six equivalents 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(III) and copper(II) chelates (1:1 metal/ligand ratio) were prepared under N_2 at 25.0 ± 0.5 °C, by slow addition of Fe(III) or Cu(II) standard solutions.

When the *o,p*-EDDHA/Fe^{3+} was formed, hydrochloric acid was added until the solution was colorless. The experimental solution was diluted to 500 mL with type I water (*22*) to be 1×10^{-4} M on iron chelate. Sixty milliliters was placed in a 150-mL thermostated jacketed reaction vessel and then 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(II) chelate, the pH was raised to 12 by the addition of NaOH. The experimental solution was diluted to 500 mL with type I water (*22*) to be 1×10^{-3}M on *o,p*-EDDHA/Cu^{2+}. Twenty-five milliliters of the experimental solution 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_{CuH2L}) were calculated from the data by an in-house program using Microsoft Excel Solver utilizing mass balance and known equilibrium constant constraints while minimizing the least-squares absorbance fit to the observed spectral curves (*14*).

pM Values. 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 *o,p*-EDDHA ligand 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 by considering only the proton affinities of the ligand and other chelate species such as protonated metal complexes, in accordance with the method of Bannochie (*29*). These values were computed for a pH range from 4.0 to 12.0, with ionic strength fixed at 0.100 M, using a 10% excess of ligand.

In the second model, pM values were calculated in a nutrient solution system to obtain the total sequestering ability of *o,p*-EDDHA/Fe^{3+} chelate in agronomic conditions. For this, both component and thermodynamic databases of the equilibrium speciation model MINTEQA2 program were modified to include the *o,p*-EDDHA ligand and its protonation and calcium, magnesium, copper(II), and iron(III) stability constants (*30*). *o,p*-EDDHA/Fe^{3+} chelate was introduced in the Hoagland nutrient solution (*21*), of which the composition was Fe^{3+} = ligand = 1×10^{-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, $Zn^{2+} = 1.54 \times 10^{-6}$ M, and $Mn^{2+} = 4.36 \times 10^{-6}$ M. pFe and pCu values were also calculated for *o,o*-EDDHA, *p,p*-EDDHA, and EDTA chelating agents using the two models. EDTA/Zn^{2+} and EDTA/Mn^{2+} chelates have been considered for pM and speciation distribution determinations. However, *o,o*-EDDHA/Zn^{2+} and *o,o*-EDDHA/Mn^{2+} chelates do not form in the presence of Fe^{3+} and do not affect pM and speciation distribution, so they were not included in either system (*30*).

Speciation Distribution. The *o,p*-EDDHA species distribution at pH range 4−13 were determined in three theoretical models considering their agronomic use: in solution, in hydroponic culture, and in soil conditions, respectively.

The first model is used to determine the behavior of *o,p*-EDDHA in solution conditions. Species distribution was made using the same methodology as that used to calculate pFe in agronomic conditions.

The second model is used to determine the behavior of *o,p*-EDDHA in hydroponic conditions. In this case Hoagland nutrient solution was also used but $Fe(OH)_3$(amorp) equilibrium was introduced in the system as a solubility controller.

The third model is used to determine the behavior of *o,p*-EDDHA in soil conditions. To predict the distribution of the *o,p*-EDDHA species in soils, it is necessary to use a theoretical model in which all soil components that could have some effect on *o,p*-EDDHA/Fe^{3+} stability were considered. Due to the competition observed between Fe^{3+} and Cu^{2+} for *o,p*-EDDHA, two soil types with unlimited and limited Cu^{2+} availability, respectively, were proposed to predict the stability of *o,p*-EDDHA/Fe^{3+} with high and low Cu^{2+} levels in soil, respectively.

Similarly, *o,o*-EDDHA, *p,p*-EDDHA, and EDTA species distributions were determined for the three models.

RESULTS AND DISCUSSION

Purity and Complexing Capacity. NMR spectra of the *o,p*-EDDHA sample show that it presents a high degree of purity. CH groups of the ortho system (4.24 ppm) are located very close to those corresponding to the *o,o*-EDDHA (4.14−4.24 ppm) (*14*). However, CH groups of the para system (3.5 ppm) cannot be compared with those of *p,p*-EDDHA because in *p,p*-EDDHA these are overlapped by the water signal. In the aromatic system the para and ortho systems are well separated. Anal. Calcd for $C_{18}H_{20}N_2O_6$: C, 59.99; H, 5.59; N, 7.77. Found: C, 59.76; H, 5.32; N, 7.56.

No significant differences were found when the titrimetric purity of the synthetic *o,p*-EDDHA, obtained by photometric titration with Fe^{3+} solution (95.4 ± 2.0%), was compared with the potentiometric titration with Cu^{2+} solution (94.1 ± 1.2%). The end points for each titration were determined using the two methods described under Materials and Methods in order to include their own analytical errors in the mean value (94.7 ± 1.1%). Because *o,p*-EDDHA is highly pure and the photometric and potentiometric plots are very clean, this product may be used in the HPLC method (*13*) as a standard compound to quantify the presence of this ligand in commercial brands.

The use of spectrophotometric and potentiometric titrations as complementary tools to determine of the purity of the phenolic chelating agents has been already profusely discussed in a earlier research work (*14*). We concluded that this analytical determination is adequate to quantify the complexing capacity independently of the presence of small amounts of impurities produced in the synthesis pathways which did not form colored complexes.

The molar absorptivity (ϵ) of *o,p*-EDDHA/Fe^{3+} can be easily determined from the photometric plot (see 2.1.A in Figure 2S, Supporting Information) at 480 nm. This molar absorptivity (2129 ± 53) is very close to that published recently corresponding another *o,p*-EDDHA/Fe^{3+} chelate (2130) (*17*). These values are almost half of the ϵ obtained for the *o,o*-EDDHA/Fe^{3+} chelate at the same wavelength (4721) (*17*) and using the same methodology. At 480 nm, the light is being absorbed by the iron−phenolate bond, and there are two of these bonds in the structure of the *o,o*-EDDHA/Fe^{3+} complex (**1** in **Figure 1**). The value of ϵ obtained for the *o,p*-EDDHA/Fe^{3+} is consistent with a structure in which only one iron−phenolate is present. This explanation is very useful in understanding why no *p,p*-EDDHA/Fe^{3+} is formed.

Protonation and Ca(II) and Mg(II) Stability Constants. The absorption band maximum at 291 nm has been considered to be adequate for the determination of the first two phenolate protonations, K_1^H and K_2^H, of *o,p*-EDDHA. The wavelength maxima is chosen using the spectroscopic equilibrium curves (see Figure 3S, Supporting Information). Due to the low value obtained for K_2^H, this constant was also refined with the other protonation constants calculated by potentiometric method (see Figure 4S, Supporting Information) using BEST.

The protonation constants obtained are shown in **Table 1**. These values are compared with those of *o,o*-EDDHA and *p,p*-EDDHA (**1** and **3** in **Figure 1**) obtained in an earlier work using the same methodology (*14*). Although the three regioisomers

Table 1. log Protonation and log Ca^{2+} and Mg^{2+} Stability Constants[a] with *o,o*-EDDHA, *o,p*-EDDHA, and *p,p*-EDDHA

quotient	*o,p*-EDDHA	*o,o*-EDDHA[b]	*p,p*-EDDHA[b]
$[LH^{3-}]/[H^+][L^{4-}] \rightleftharpoons K_1^H$	11.18	11.94	9.94 ± 0.04
$[LH_2^{2-}]/[H^+][LH^{3-}] \rightleftharpoons K_2^H$	10.18 ± 0.04	10.73	9.07 ± 0.02
$[LH_3^-]/[H^+][LH_2^{2-}] \rightleftharpoons K_3^H$	8.65 ± 0.05	8.66 ± 0.04	6.85 ± 0.06
$[LH_4]/[H^+][LH_3^-] \rightleftharpoons K_4^H$	6.19 ± 0.02	6.18 ± 0.06	4.36 ± 0.07
$[LH_5^+]/[H^+][LH_4] \rightleftharpoons K_5^H$	2.57 ± 0.02		
$[LH_6^{2+}]/[H^+][LH_5^+] \rightleftharpoons K_6^H$	1.49 ± 0.07		
$[CaL^{2-}]/[Ca^{2+}][L^{4-}]$	4.12 ± 0.10	7.29 ± 0.30	3.54 ± 0.52
$[CaLH^-]/[Ca^{2+}][H^+][L^{4-}]$	14.27 ± 0.16	16.77 ± 0.33	12.93 ± 0.58
$[CaLH_2]/[Ca^{2+}][H^+]^2[L^{4-}]$	23.23 ± 0.32	25.95 ± 0.50	21.21 ± 0.65
$[MgL^{2-}]/[Mg^{2+}][L^{4-}]$	5.64 ± 0.16	9.76 ± 0.05	3.74 ± 0.57
$[MgLH^-]/[Mg^{2+}][H^+][L^{4-}]$	15.55 ± 0.03	18.18 ± 0.15	12.89 ± 0.39
$[MgLH_2]/[Mg^{2+}][H^+]^2[L^{4-}]$	23.83 ± 0.32	25.36 ± 0.24	20.79 ± 0.57

[a] μ = 0.1 M (NaCl); t = 25 °C. [b] Reference *14*.

have the same functional groups, the relative positions of the phenolic groups produce significant differences between their highest protonation constants. Therefore, two intramolecular interactions in *o*-hydroxyphenylglycine (in *o,o*-EDDHA) involve the formation of two hydrogen bonds between the basic nitrogens and the phenolic groups. No intramolecular interactions occur when both functional groups are too apart to bind (*p*-hydroxyphenylglycine in *p,p*-EDDHA) and, therefore, their protonation constants are very close to that of phenol (pK_a = 10.00) (*14*). On the other hand, the molar absorptivity of [*o,p*-EDDHA−H]$^{3-}$ (ϵ_{max} = 3567 M^{-1} cm^{-1} at 291 nm) is close to that obtained for the L^{3-} species of the ligand [*N*-(2-hydroxybenzyl)-*N*′-benzylethylenediamine-*N,N*′-diacetic acid] (named L3 by the authors) (ϵ_{max} = 3500 M^{-1} cm^{-1} at 292 nm), corresponding also to the phenolic deprotonation (*32*). In the case of *o,p*-EDDHA, only one intramolecular interaction in *o*-hydroxyphenylglycine occurs, corresponding to the highest protonation constant. However, as no intramolecular interactions occur in the second protonation constants, its value is very close to that of *p,p*-EDDHA. No differences are found when protonation constants of the amine groups of *o,o*-EDDHA and *o,p*-EDDHA are compared because the intramolecular interactions affect the other protonation constants. No dissociation constants of carboxylic groups from *o,o*-EDDHA and *p,p*-EDDHA can be determined because the chelating agent below pH 5 precipitated (*33*). However, as no precipitation is observed in *o,p*-EDDHA, the two lowest protonation constants can be determined, although the species formed are predominant at very low pH and almost negligible at agronomic pH values.

Potentiometric titration curves for *o,p*-EDDHA and its [1:1] and [10:1] metal/ligand pH profiles with Ca^{2+} and Mg^{2+} are shown in Figure 4S (Supporting Information). These data were analyzed using the FORTRAN program BEST (*27*) to obtain the *o,p*-EDDHA/Ca and *o,p*-EDDHA/Mg stability constants that are presented in **Table 1**. These values are compared with those of *o,o*-EDDHA and *p,p*-EDDHA. From the Ca and Mg potentiometric curves, the presence of at least three species of the metal chelates MH_2L, MHL^-, and ML^{2-} (see Figure 4S) may be assumed. If the most stable ML^{2-} species are compared, *o,p*-EDDHA/Mg stability constants are higher than those of *o,p*-EDDHA/Ca. This fact is also observed for *o,o*-EDDHA and *p,p*-EDDHA, and it is in strong agreement with other phenolic ligands analogous to *o,o*-EDDHA, where the presence of phenolate groups favors the binding to small metals (e.g., Mg^{2+}) (*14*). *p,p*-EDDHA and *o,p*-EDDHA form weaker Ca^{2+} and Mg^{2+} chelates than *o,o*-EDDHA. [*o,o*-EDDHA−metal]$^{2-}$ species involve coordination to the ethylenediamine nitrogens and the phenolate groups. However, when [*o,o*-EDDHAH$_2$−metal]^0 is formed, the metal is bonded to the ethylenediamine nitrogens and the carboxylate oxygens. [*o,o*-EDDHAH−metal]$^-$ involves the coordination to the ethylenediamine nitrogens, one phenolate group, and one carboxylate oxygen. In *o,p*-EDDHA, the ML^{2-} species is less stable than in *o,o*-EDDHA due to the para-position of one hydroxyphenyl, which cannot bind metal effectively (**2** in **Figure 1**). To prove this, if Ca^{2+} and Mg^{2+} stability constants are compared as a function of their species ($[MLH]^-/[M]^{2+}[HL]^{3-}$) and by taking into account the protonation constant of the species involved, the differences between MLH^- and ML^{2-} are lower for *o,p*-EDDHA (1.03 for Ca^{2+}; 1.27 for Mg^{2+}) and *p,p*-EDDHA (0.55 for Ca^{2+}; 0.79 for Mg^{2+}) than for *o,o*-EDDHA (2.46 for Ca^{2+}; 3.52 for Mg^{2+}). This implies that the MLH^- species is more important for *o,p*-EDDHA and *p,p*-EDDHA than for *o,o*-EDDHA for Ca^{2+} and Mg^{2+} systems.

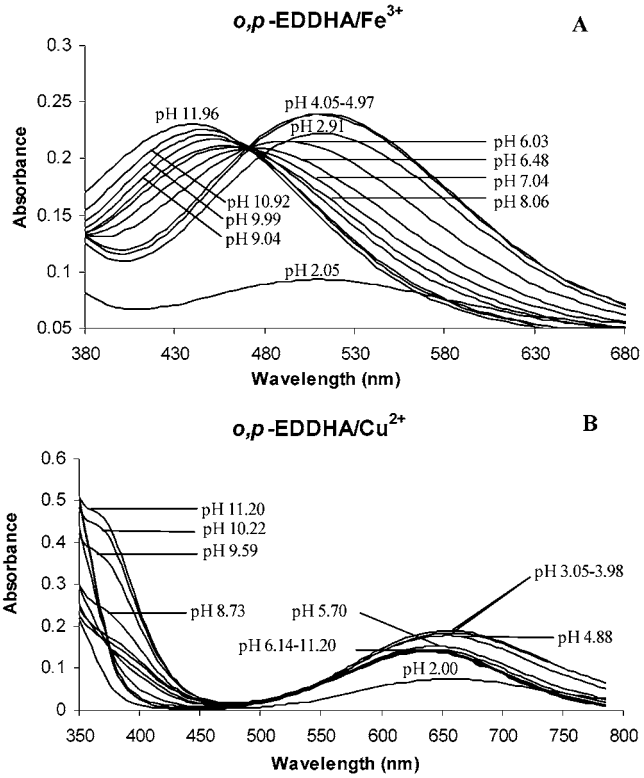

Figure 2. Absorption spectra of (A) *o,p*-EDDHA/Fe^{3+} and (B) *o,p*-EDDHA/Cu^{2+} as a function of pH. [*o,p*-EDDHA/Fe^{3+}] = 1 × 10^{-4} M and [*o,p*-EDDHA/Cu^{2+}] = 1 × 10^{-3} M. t = 25 °C; μ = 0.100 M (NaCl).

Cu(II) and Fe(III) Stability Constants. Optical absorption spectra for both *o,p*-EDDHA/Cu^{2+} and *o,p*-EDDHA/Fe^{3+} are shown in **Figure 2**. Whereas the absorbance maximum of the *o,o*-EDDHA/Fe^{3+} is constant at 480 nm throughout the pH range (*33*), the *o,p*-EDDHA/Fe^{3+} complex reveals a shift from 520 nm at pH 4.05 to 440 nm at pH 11.96. In addition, when the pH increased from 6.5 to 11.96, the spectra showed an isosbestic point at 470 nm, indicating that there are only two species with different absorptions in this pH range (see **Figure 2A**). The same tendency is observed when the UV−vis spectrum was made for L3 (ligand analogue to *o,p*-EDDHA) (*32*), which showed a maximum at 525 nm that is attributed to a phenolate-to-Fe^{3+} charge-transfer transition at pH 1.8.

However, the UV−vis spectra for *o,p*-EDDHA/Cu^{2+} present the same trend as for *o,o*-EDDHA/Cu^{2+} (*6*, *33*) and *o,o*-EDDHSA/Cu^{2+} systems (*14*). At pH <6 the *o,p*-EDDHA/Cu^{2+} has the typical deep blue color normally associated with Cu^{2+}−

Table 2. log Stability Constants[a] for Cu^{2+} and Fe^{3+} Chelates

quotient	*o,p*-EDDHA[a]	*o,o*-EDDHA[b]	*p,p*-EDDHA[b]
$[FeL^-]/[Fe^{3+}][L^{4-}]$	28.72 ± 0.05	35.09 ± 0.28	
$[FeHL]/[Fe^{3+}][L^{4-}][H^+]$	35.02 ± 0.05	36.89 ± 0.21	
$[FeH_2L^+]/[Fe^{3+}][L^{4-}][H^+]^2$	37.35 ± 0.10		
$[FeOHL^{2-}]/[Fe^{3+}][L^{4-}][OH^-]$	19.45 ± 0.19	23.66 ± 0.27	
$[CuL^{2-}]/[Cu^{2+}][L^{4-}]$	21.74 ± 0.38	25.13 ± 0.00	14.74 ± 0.06
$[CuHL^-]/[Cu^{2+}][L^{4-}][H^+]$	30.96 ± 0.09	32.61 ± 0.01	22.39 ± 0.06
$[CuH_2L]/[Cu^{2+}][L^{4-}][H^+]^2$	36.17 ± 0.12	37.31 ± 0.01	28.50 ± 0.04
$[CuH_3L^+]/[Cu^{2+}][L^{4-}][H^+]^3$	38.14 ± 0.07		31.09 ± 0.04

[a] μ = 0.1 M (NaCl); t = 25 °C. [b] Reference *14*.

amino acid chelates, characterized by a broad absorption band at ~645 nm. As the pH is increased above 6, a second and much stronger band appears at 375 nm, the intensity of which increases with increasing pH (see **Figure 2B**). It seems that this new band must be due to a resonating system involving the metal−phenolate linkages. Because only one copper−phenolate bond is formed for *o,p*-EDDHA/Cu^{2+}, no absorption maxima is found at 375 nm.

Fe^{3+} stability constants were determined using the absorption bands at 480 nm. The curves were also determined at 530 and 450 nm, as absorption maxima of *o,p*-EDDHA/Fe^{3+} at acid and alkaline pH values, respectively, to detect more clearly the change of the predominant species (see A, B, and C in Figure 6S, Supporting Information). Cu^{2+} stability constants were determined using photometric curves at 650 nm (see D in Figure 6S) (*14*). The Fe^{3+} and Cu^{2+} stability constants are shown in **Table 2**. These stability constants were compared with those of *o,o*-EDDHA and *p,p*-EDDHA. Because Fe^{3+} is octahedrally coordinated, *o,o*-EDDHA has six donor groups able to occupy the six positions. The [*o,o*-EDDHA/Fe]$^-$ species is the predominant one in a wide pH range (1.80−11.43).

However, in *o,p*-EDDHA the position corresponding to the *p*-hydroxy phenolate does not bind the Fe^{3+}, and a water molecule occupies this vacant position. The *p*-hydroxy phenolate takes a proton to form $FeHL{\cdot}H_2O$ (**5** in **Figure 3**) over an important pH range (up to 6.30). Above this pH a proton from the water can be released, forming the FeL^- species (**6** in **Figure 3**) that is in fact $Fe(LH)(OH)^-$. At pH 9.27, the phenol dissociates to give the species $FeLOH^{2-}$ (**7** in **Figure 3**). This pH value could be compared, although the Fe(III) binding can produce a larger acidity over the phenolate protonation, with the second protonation constant of *o,p*-EDDHA or with the two first protonation constants of *p,p*-EDDHA. The stronger binding of Fe^{3+} in *o,o*-EDDHA produces higher Fe^{3+} stability constants than *o,p*-EDDHA (**Table 2**). However, a higher Fe^{3+} ion affinity is observed for *o,p*-EDDHA than for EDTA (log K = 25.10) and their analogues (*31*), despite the fact that they have the six octahedral positions occupied with the ligand. This is due to the high affinity between Fe^{3+} and the phenolate donor. Morever, the [*o,p*-EDDHA/Fe]$^-$ stability constant agrees with that of the L3 ligand (log FeL = 27.00) (*32*). This chelating agent is similar to *o,p*-EDDHA lacking the hydroxy group in the para-position. Therefore, on the basis of the stability constants of the chelates, *o,p*-EDDHA/Fe^{3+} could be used as a ferric chelate better than EDTA/Fe^{3+} for agronomic purposes.

For Cu^{2+}, the tendency is similar to that of Ca^{2+} and Mg^{2+} described above. In these chelates, Cu^{2+} has four main coordination positions in a square arrangement. *o,o*-EDDHA has the highest Cu^{2+} stability constant because it is able to complex copper with the two phenolate and two amine groups. However, in the [*o,p*-EDDHA/Cu]$^-$ species, the metal is bonded

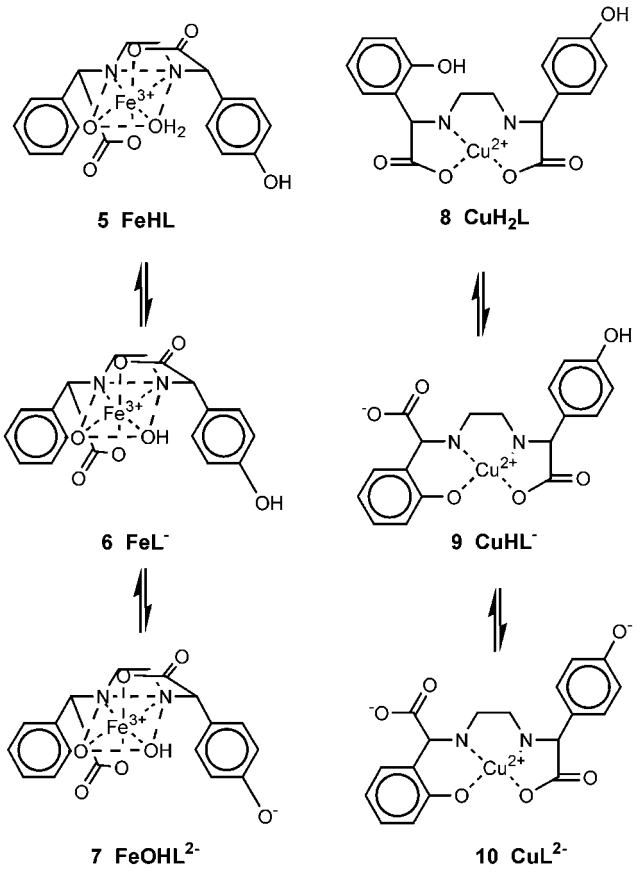

Figure 3. *o,p*-EDDHA/Fe^{3+} and *o,p*-EDDHA/Cu^{2+} species formed.

to one carboxylate and one phenolate (see **10** in **Figure 3**). This is consistent with the relatively low absorption at 375 nm at high pH values for *o,p*-EDDHA/Cu^{2+} (**Figure 2**), with respect to that of *o,o*-EDDHA/Cu^{2+} (*14*) which presents a maximum at this wavelength corresponding with the two Cu^{2+}−phenolate bonds. The protonated and diprotonated species of *o,p*-EDDHA/Cu^{2+} are similar to those of *o,o*-EDDHA/Cu^{2+} described elsewhere (*14*) because the copper involves the coordination with two amino groups and one or two carboxylate groups (see **9** and **8** in **Figure 3**, respectively).

pM Values. The stability constants taken by themselves do not provide a comparable basis or measurement of the relative effectiveness of *o,p*-EDDHA in agronomic conditions. The different degrees of hydrogen ion and competing metals with the ferric and cupric ions for the ligands provide different metal affinities at a given pH. In fact, high affinities of strongly basic donor groups for metal ions are partially reversed by high affinities for hydrogen or competing metal ions. **Figure 4** presents both pFe and pCu computed values from the first and second models described under Materials and Methods. pFe−3pH or pCu−2pH values instead of pFe or pCu are shown in order to have a better visualization of the comparison among chelates. For the first model, *o,o*-EDDHA is the most effective ligand to bind Fe^{3+} and Cu^{2+} at pH values above 6 and 7.5, respectively (see **Figure 4A**,**B**). *o,p*-EDDHA has a higher affinity for Fe^{3+} and Cu^{2+} than does the EDTA ligand at pH above 9.5 and 7.5, respectively; this is due to the presence of phenolate groups that provide a larger complexing ability at alkaline pH values. Due to the presence of two chiral carbons, *o,p*-EDDHA takes several geometric isomer forms (*RR′*, *RS′*, *SR′*, and *SS′*), similarly to *o,o*-EDDHA (racemic mixture and meso isomer). The pFe determined for *o,p*-EDDHA is then an average for the isomer forms. This has already been pointed

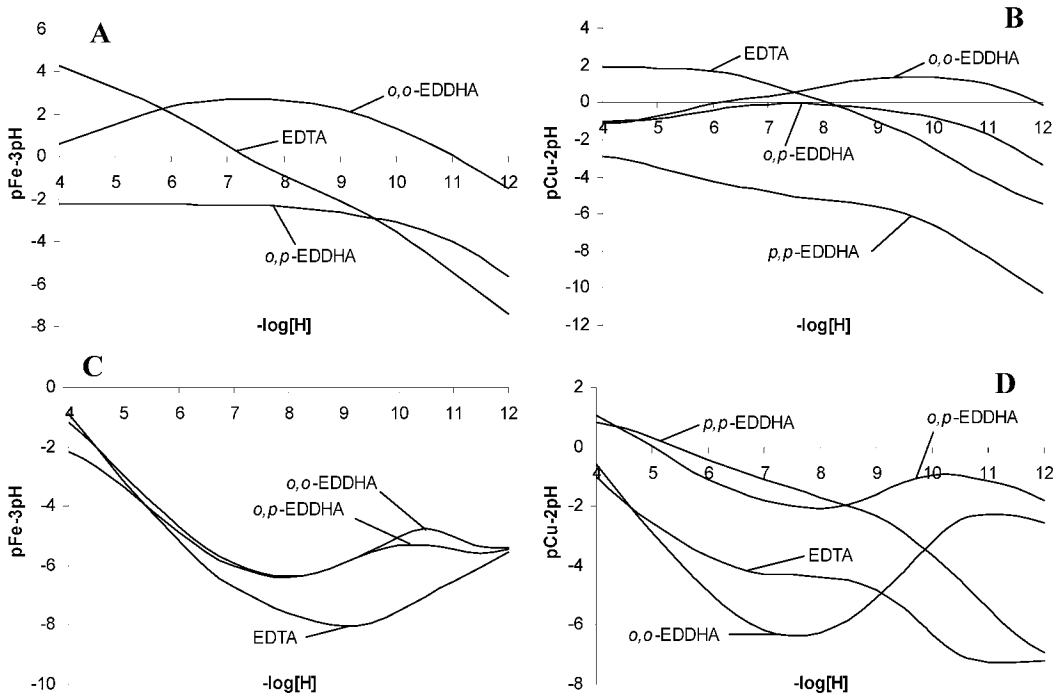

Figure 4. pFe (A and C) and pCu (B and D) values of several ligands against pH. In panels A and B, pFe and pCu have been determined according to the first model [considering only the proton affinities of the ligand and other chelate species such as protonated metal complexes (*29*); pH range from 4.0 to 12.0, ionic strength fixed at 0.100 M, and using a 10% excess of ligand]. In panels C and D, pFe and pCu have been determined using the second model [iron chelates (10^{-4} M) were introduced in the Hoagland nutrient solution composition (*21*) in the speciation model MINTEQA2 program (*30*)].

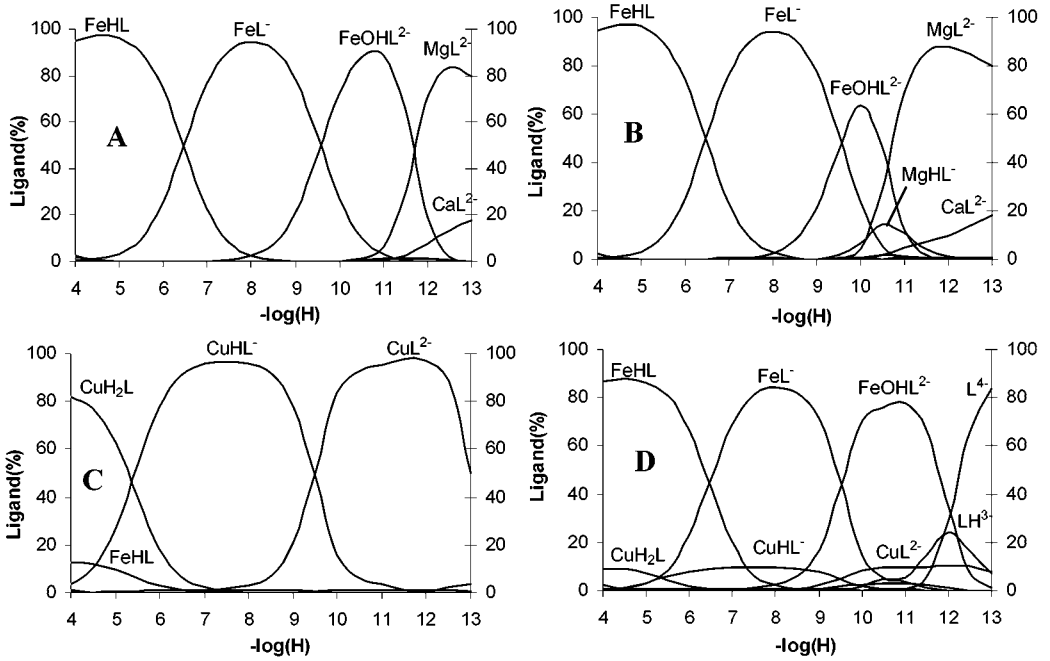

Figure 5. Distribution diagrams of the system *o,p*-EDDHA in (A) solution, (B) nutrient solution, (C) soil with unlimited Cu^{2+} availability, and (D) limited Cu^{2+} availability.

out for several analogue ligands [EDDHA (*29*), EDDH4MA (*4*), and TMPHPG (*29*)]. At an agronomic pH of 7.5, the pFe value of *o,p*-EDDHA (20.2) is comparable to those of isomers of TMPHPG ligand (*rac*-TMPHPG = 22.0; *meso*-TMPHPG = 21.6) (*29*).

Additionally, to compare the effectiveness of a ligand, the pFe/pCu ratio is a more reliable parameter than their values such as (*31*). When this parameter is applied to *o,o*-EDDHA and *o,p*-EDDHA, it is predicted that Cu^{2+} would be more effective in *o,p*-EDDHA than in *o,o*-EDDHA.

pFe and pCu are very useful in comparing the effectiveness of these ligands when they are applied in solution containing only one metal. However, in agronomic uses, the iron chelates are used in systems in which several other metals (i.e., Cu^{2+}, Ca^{2+}, Mg^{2+}, and so on) are present. The presence of those metals can modify the relative effectiveness of its iron chelate and, therefore, the pFe could vary. For this reason, a second model has been used in determining pFe and pCu values (see **Figure 4C**,**D**). pFe values obtained for *o,o*-EDDHA and *o,p*-EDDHA are of the same magnitude and higher than those of EDTA

Table 3. Composition of Theoretical Soil Model with Unlimited Cu^{2+} Availability Used To Predict the Stability of *o,p*-EDDHA/Fe^{3+} in Soil Conditions[a]

component	equilibrium	log K^0
soil – Ca*	soil – Ca ⟺ Ca^{2+}	–2.50
soil – Mg*	soil – Mg ⟺ Mg^{2+}	–3.00
soil – Cu	soil – Cu + $2H^+$ ⟺ Cu^{2+}	2.80
soil – Fe	Fe_{soil} + $3H^+$ ⟺ Fe^{3+} + $3H_2O$	2.70
soil – Zn	soil – Zn + $2H^+$ ⟺ Zn^{2+}	5.80
*These equilibria were replaced at pH 7.5–13 by the following:		
calcite	$CaCO_3$ ⟺ Ca^{2+} + CO_3^{2-}	–8.41
dolomite	$CaMg(CO_3)_2$ ⟺ Mg^{2+} + Ca^{2+} + $2CO_3^{2-}$	–3.00

[a] *o,p*-EDDHA, *o,o*-EDDHA, and EDTA ligands are introduced with a total concentration of 100 μM. CO_2 is present at 0.0003 atm.

ligand (see **Figure 4C**). This is due to the greater competition of Ca^{2+} and Mg^{2+} for EDTA that produces the displacement of Fe^{3+} from the chelate and then the decrease of pFe. When pCu of *o,o*-EDDHA and *o,p*-EDDHA are compared, the tendency is similar to that found for pFe. *o,p*-EDDHA presents a higher effectiveness to bind Cu(II) than *o,o*-EDDHA, but the *p,p*-EDDHA presents the highest value around pH 8.0 (see **Figure 4D**). This can be very important in the case of commercial ferric chelates with large amounts of *o,p*-EDDHA and *p,p*-EDDHA, because as no *p,p*-EDDHA/Fe^{3+} is formed, the Cu^{2+} present in the soil solution can be complexed by *p,p*-EDDHA and, therefore, Fe^{3+} can be complexed more effectively by *o,p*-EDDHA and *o,o*-EDDHA.

Species Distribution in Agronomic Conditions. The first model is shown in **Figure 5A**; the distribution of the different species of *o,p*-EDDHA is in accordance with their stability constants. The species of *o,p*-EDDHAH_x/Fe^{3+} are the predominant species at agronomic pH. Only at pH >11.5 are the *o,p*-EDDHA/Mg^{2+} and *o,p*-EDDHA/Ca^{2+} species important. When the second model is considered (nutrient solution; see **Figure 5B**), the same tendency is found. Because precipitation of Fe(OH)$_3$(amorp) is allowed in this medium, the presence of the FeOHL species is reduced at high pH values, although the stability is enough to maintain all of the iron in solution at pH <8.5, which is a normal limit for agronomic purposes. The *o,p*-EDDHA/Cu^{2+} is very low when compared with *o,p*-EDDHA/Fe^{3+} (see **Figure 5A**,**B**) because the Cu^{2+} molar concentration (3.15×10^{-7}) is lower than that of Fe(III) (1.00×10^{-4}) in Hoagland nutrient solution. However, if the levels of chelated Cu^{2+} among the three chelating agents (*o,p*-EDDHA, *o,o*-EDDHA, and EDTA) are compared, *o,p*-EDDHA/Cu^{2+} is the most stable because almost 100% of the soluble Cu^{2+} remains chelated at all pH ranges (data not shown).

The soil model used with unlimited Cu^{2+} availability is described in **Table 3**. In this theoretical model the main components that can affect the iron chelate stability are included. Ca and Mg equilibria are included in the soil model because these elements are considered to be the main competitors in calcareous conditions for weak chelating agents, such as EDTA (*31*). Depending on the pH zone, the predominant solid phases controlling Ca^{2+} and Mg^{2+} solubilities are different (Ca−soil and Mg−soil equilibria at pH <7.5 and calcite and dolimite equilibria at pH >7.5). Cu^{2+} equilibrium has been included in the soil model to determine the ability of this metal to replace Fe^{3+} from *o,p*-EDDHA/Fe^{3+}.

In this model, *o,p*-EDDHA/Cu^{2+} is the chelate mainly formed instead of *o,p*-EDDHA/Fe^{3+} (see **Figure 5C**) despite the fact that the Fe^{3+} chelate formation constants are higher than the corresponding Cu^{2+} ones (see **Table 2**). This is due to the greater

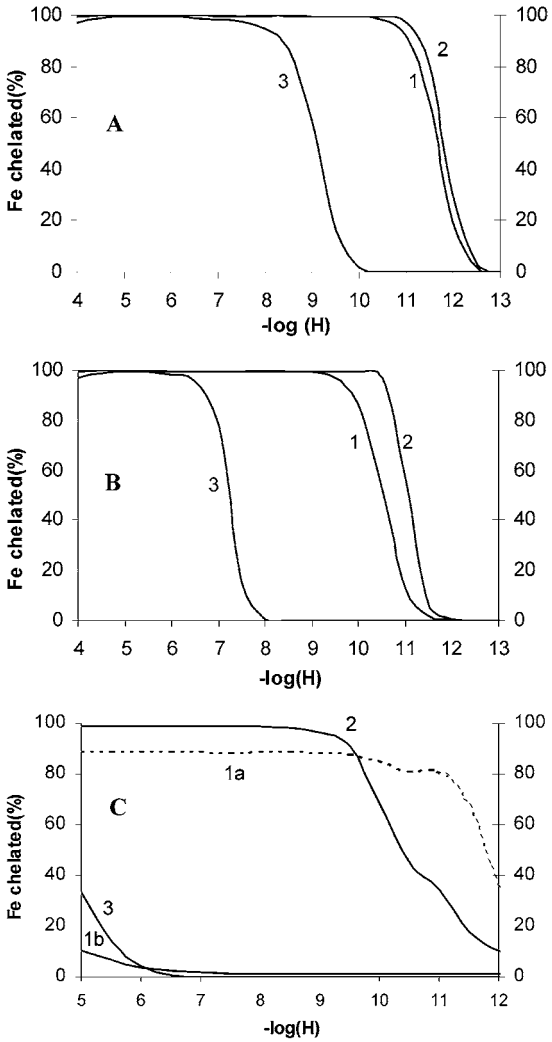

Figure 6. Percentage of chelating agents in solution that is binding Fe(III): comparison of the behavior of (1) *o,p*-EDDHA/Fe^{3+} [(1a) limited Cu^{2+} and (1b) unlimited Cu^{2+}], (2) *o,o*-EDDHA/Fe^{3+}, and (3) EDTA/Fe^{3+} in (A) solution, (B) nutrient solution, and (C) soil conditions.

solubility of Cu^{2+} from the solid phase. Normally calcareous soils, where iron chelates are frequently used, also present low Cu^{2+} availability. Cu^{2+} in solution (10^{-4} M) reached in the first model soil is very high and unusual in soils (only in soils contaminated by the addition of pesticide). Then, in the second model soil the total Cu^{2+} concentration in solution is limited at 1×10^{-5}M. In this case, *o,p*-EDDHA/Fe^{3+} chelate is the main chelate, although all Cu^{2+} is as *o,p*-EDDHA/Cu^{2+} (see **Figure 5D**).

These results are in good agreement with those obtained by Álvarez-Fernández et al. (*34*). They reported that the *o,p*-EDDHA/Fe^{3+} fraction, from commercial EDDHA/Fe^{3+} products, was partially replaced by Cu^{2+} after the interaction between commercial EDDHA/Fe^{3+} chelates and a soil with high Cu^{2+} availability. However, no replacement occurred when the same test was used with a soil with low Cu^{2+} availability.

Comparisons between *o,p*-EDDHA/Fe^{3+}, *o,o*-EDDHA/Fe^{3+}, and EDTA/Fe^{3+} in solution, nutrient solution, and soil conditions are shown in panels A, B, and C, respectively, of in **Figure 6**. The behavior of *o,p*-EDDHA/Fe^{3+} is close to that of *o,o*-EDDHA/Fe^{3+} in solution, hydroponic conditions, and soil with low Cu^{2+} level (see **Figure 6A**−**C**) because ferric chelate is the main component in agronomic conditions. EDTA maintains

soluble Fe^{3+} in solution conditions and in hydroponic conditions at pH <7.

In summary, the following can be deduced from our research: *o*,*p*-EDDHA provided by Syngenta is highly pure and is useful when used as standard to identify *o*,*p*-EDDHA/Fe^{3+} from commercial products. Due to the para-position of one phenol group in *o*,*p*-EDDHA, the protonation constants and Ca and Mg stability constants have different values from those of *o*,*o*-EDDHA and *p*,*p*-EDDHA regioisomers. Although *o*,*p*-EDDHA has only five functional groups that are able to complex Fe^{3+} ion, its *o*,*p*-EDDHA/Fe^{3+} stability constants are higher than those of EDTA/Fe^{3+} but lower than those of *o*,*o*-EDDHA/Fe^{3+}. Stability order, measured through pFe value in solution using the MINTEQA2 speciation program, at all normal agronomic pH values, was as follows: *o*,*o*-EDDHA/Fe^{3+} ≥ *o*,*p*-EDDHA/Fe^{3+} > EDTA/Fe^{3+}. In hydroponic conditions, *o*,*p*-EDDHA/Fe^{3+} can be used as iron chelate because it is completely formed at normal agronomic pH. In soil conditions with limited Cu^{2+} availability *o*,*p*-EDDHA/Fe^{3+} is stable, but when the soil presents high availability of Cu^{2+}, it can displace the Fe^{3+} from the chelate.

ABBREVIATIONS USED

o,*o*-EDDHA, ethylendiamine-*N*,*N*′-bis(*o*-hydroxyphenylacetic) acid; *o*,*p*-EDDHA, ethylendiamine-*N*(*o*-hydroxyphenylacetic)-*N*′(*p*-hydroxyphenylacetic) acid; *p*,*p*-EDDHA, ethylendiamine-*N*,*N*′-bis(*p*-hydroxyphenylacetic) acid; EDTA, ethylenediaminetetraacetic acid; DTPA, diethylenetriaminepentaacetic acid; HEDTA, *N*-(hydroxyethyl)ethylenediamine-*N*,*N*′-triacetic acid; EDDH4MA, ethylendiamine-*N*,*N*′-bis(*o*-hydroxy-4-methylphenylacetic) acid; EDDCHA, ethylendiamine-*N*,*N*′-bis(*o*-hydroxy-5-carboxyphenylacetic) acid; EDDHSA, ethylendiamine-*N*,*N*′-bis(*o*-hydroxy-5-sulfonylphenylacetic) acid; TMPHPG, *N*,*N*′-trimethylenebis[2-(2-hydroxy-3,5-dimethylphenyl)glycine]; L3, *N*-(2-hydroxybenzyl)-*N*′-benzylethylenediamine-*N*,*N*′-diacetic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid.

ACKNOWLEDGMENT

We thank Miguel Angel Sierra for NMR analysis.

Supporting Information Available: Photometric and potentiometric plots used to determine purity, protonations, and Ca^{2+}, Mg^{2+}, Cu^{2+}, and Fe^{3+} stability constants shown in Figures 2S, 3S, 4S, and 6S. This material is available free of charge via the Internet at http://www.pubs.acs.org.

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Received for review March 27, 2003. Revised manuscript received June 18, 2003. Accepted June 18, 2003. This research was carried out with the financial support Syngenta Crop Protection.

JF034304R