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Nature of Impurities in Fertilizers Containing EDDHMA/Fe^{3+}, EDDHSA/Fe^{3+}, and EDDCHA/Fe^{3+} Chelates

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Iron chelates derived from ethylenediaminedi(*o*-hydroxyphenylacetic) acid (EDDHA), ethylenediaminedi(*o*-hydroxy-*p*-methylphenylacetic) acid (EDDHMA), ethylenediaminedi(2-hydroxy-5-sulfophenylacetic) acid (EDDHSA), and ethylenediaminedi(5-carboxy-2-hydroxyphenylacetic) acid (EDDCHA) are remarkably efficient in correcting iron chlorosis in plants growing in alkaline soils. This work reports the determination of impurities in commercial samples of fertilizers containing EDDHMA/Fe^{3+}, EDDHSA/Fe^{3+}, and EDDCHA/Fe^{3+}. The active components (EDDHMA/Fe^{3+}, EDDHSA/Fe^{3+}, and EDDCHA/Fe^{3+}) were separated easily from other compounds present in the fertilizers by HPLC. Comparison of the retention times and the UV−visible spectra of the peaks obtained from commercial EDDHSA/Fe^{3+} and EDDCHA/Fe^{3+} samples with those of standard solutions showed that unreacted starting materials (*p*-hydroxybenzenesulfonic acid and *p*-hydroxybenzoic acid, respectively) were always present in the commercial products. 1D and 2D NMR experiments showed that commercial fertilizers based on EDDHMA/Fe^{3+} contained impurities having structures tentatively assigned to iron chelates of two isomers of EDDHMA. These findings suggest that current production processes of iron chelates used in agriculture need to be improved.

KEYWORDS: EDDCHA; EDDHMA; EDDHSA; fertilizers; iron chelates

INTRODUCTION

Iron chlorosis is a nutritional disorder in plants that decreases the yield of many crops. Fertilization with synthetic iron chelates is the most common agricultural practice to relieve this problem (*1*). Ligands **1** having phenol groups in a polyamine−carboxylic acid backbone (Figure 1) are among the most efficient iron chelates because of their ability to form ferric complexes of high stability both in neutral and in alkaline solutions (*2*). The best known member of these compounds is ethylenediaminedi-(*o*-hydroxyphenylacetic) acid (Figure 1, structure **1a**), which is recognized by the initials EDDHA or EHPG.

Since these molecules were prepared for the first time (*3*), their uses have dramatically increased in fields so diverse as agricultural, medical, and analytical chemistry (*1*, *3*−*7*). Development of analytical methods for the quality control of these commercial chelates has focused on determining the content of chelated metal (*8*−*11*). However, an important aspect related to the quality of the commercial materials, namely, the presence of impurities, has been to the moment neglected. This is an especially dramatic point because EDDHA and its methyl congeners are commonly prepared from ethylenediamine, sodium glyoxylate, and an excess of phenol or cresol (*12*). This method is very sensitive to the reaction conditions, and it may produce mixtures of ortho−ortho, para−para, and ortho−para isomers in variable amounts. The lack of purity of commercial iron chelates of EDDHA has been recently reported by one of our research groups (*13*). Thereafter, we also demonstrated the presence of −OH positional isomers on the benzene ring of EDDHA in commercial EDDHA and EDDHA/Fe^{3+} samples by a combination of 1D and 2D NMR techniques (*14*).

Iron chelates derived from EDDHMA, EDDHSA, and EDDCHA (Figure 1, structures **1b**, **1c**, and **1d**, respectively) are also widely used for the treatment of iron chlorosis. These chemicals are prepared according to the method of Petree et al. (*12*) starting from *m*-cresol, *p*-hydroxybenzenesulfonic acid, or *p*-hydroxybenzoic acid, respectively. In addition to the isomers formed during EDDHA preparation, the synthesis of EDDHMA may produce also different −OH or $-CH_3$ positional isomers on the benzene ring (**2**−**7** in Figure 1). However, the preparation of EDDHSA or EDDCHA produces single regioisomers because the para position on the benzene ring is occupied by the sulfonic

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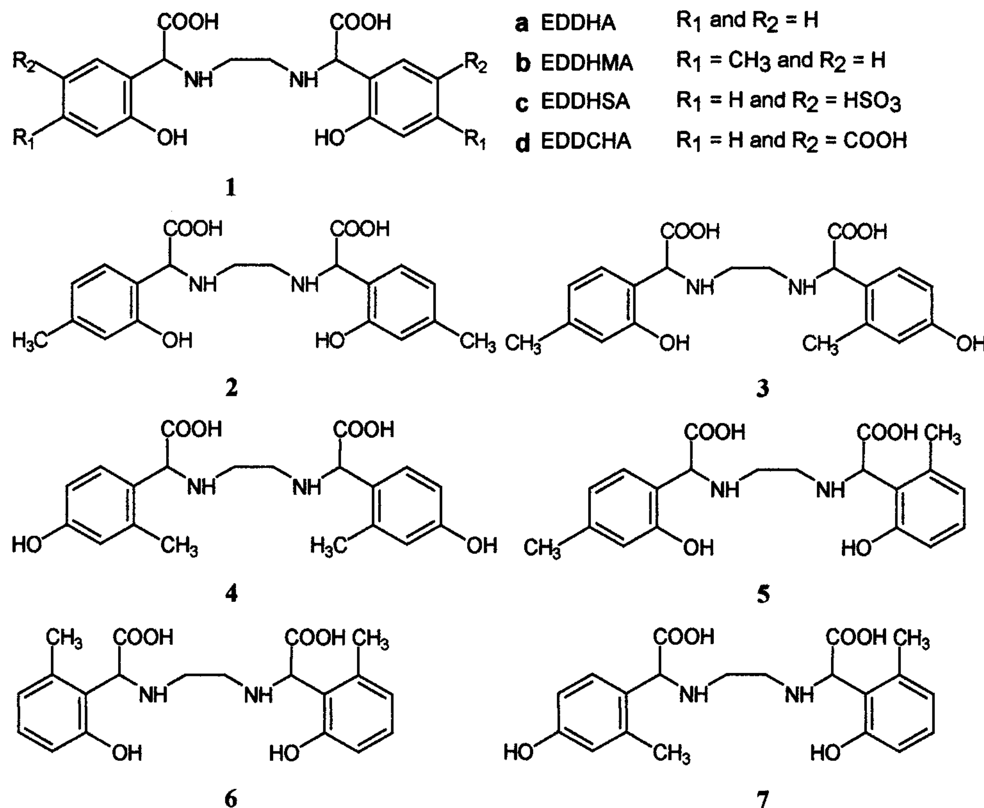

Figure 1. Structures of the molecules noted in the text.

or the carboxylic group, respectively. Thus, the impurities present in these compounds should be markedly different from those contained in EDDHMA or EDDHA. EDDHMA is separated from the excess of *m*-cresol used in its synthesis by a liquid−liquid extraction with water and organic solvent (*12*), a method that is analogous to the one used for EDDHA. This method is ineffective in purifying EDDHSA and EDDCHA because *p*-hydroxybenzenesulfonic acid and *p*-hydroxybenzoic acid are both water soluble. It is thus reasonable to assume that they will be present in the commercial formulations containing EDDHSA and EDDCHA. The presence of impurities in these iron fertilizers raises doubts about their environmental hazard after application to soil.

As a part of our ongoing work directed toward the determination of the nature and the quantity of the impurities present in commercial iron chelates, we report here our results for EDDHMA, EDDHSA, and EDDCHA commercial iron chelates obtained by using a combination of HPLC and 1D and 2D NMR techniques.

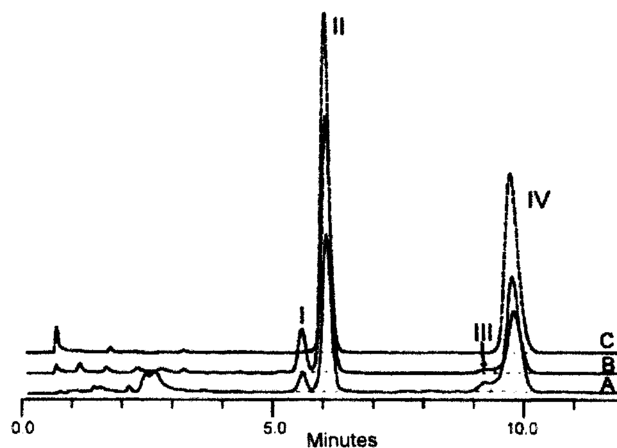

Figure 2. Chromatogram at 280 nm of a commercial fertilizer sample containing EDDHMA/Fe^{3+} (A), of the Fe complex formed by EDDHMA-x (B), and of the standard of EDDHMA /Fe^{3+} (C).

MATERIALS AND METHODS

The ion-pair high-performance liquid chromatographic method developed by Lucena et al. (*11*) was applied to 8 EDDHMA/Fe^{3+}, 22 EDDHSA/Fe^{3+}, and 2 EDDCHA/Fe^{3+} samples of commercial chelates. A Waters Symmetry C_{18}, 150 × 3.9 mm column, and an HPLC with a Waters 2690 separation module (Alliance), a Waters 996 photodiode array detector, and a Millenium 2010 chromatography data system were used. Solutions of the commercial products were prepared by dissolving the formulations in deionized water. The solutions were left to stand overnight, filtered, and made up to volume. To identify the HPLC peaks, their retention times and their absorption spectra (200−600 nm) were compared with those of standard solutions of EDDHMA/Fe^{3+}, EDDHSA/Fe^{3+}, EDDCHA/Fe^{3+}, the iron chelate of a product that is EDDHMA prepared according to the method of Petree et al. (*12*) (labeled EDDHMA-x), sodium *p*-hydroxybenzenelsulfonate (Aldrich), and sodium *p*-hydroxybenzoate (Sigma). Pure EDDHMA and EDDCHA used as standards were synthesized by using the new synthesis pathway developed by us (*15*). An assay of the ligand EDDHMA by Fe(III) HPLC titration analysis showed that it was 92.3 ± 0.5% pure. NAC Química S.A. (Spain) provided the sodium salt of EDDHSA. EDDHMA-x was obtained from the reaction of 433 mmol of *m*-cresol, 16.7 mmol of ethylenediamine, and 33.3 mmol of glyoxylic acid carried out in the conditions described in ref *12* and which are normally used in the industrial synthesis of EDDHMA.

For preparing primary standard of the iron chelate, the chelating agent was dissolved in NaOH (1:3 molar ratio). Then an amount of $Fe(NO_3)_3$ that was calculated to be 5% in excess of the molar amount of ligand was added, the pH was adjusted to 7.0 with NaOH, and the solution was left to stand overnight to allow excess Fe to precipitate as oxides. The final solution, with an Fe concentration of 100 mg/L, was filtered and made to volume with water. Standard solutions of sodium *p*-hydroxybenzoate (2.0, 1.0, 0.6, and 0.1 mM) and sodium *p*-hydroxybenzenosulfonate (2.0, 0.8, 0.4, and 0.2 mM) were also

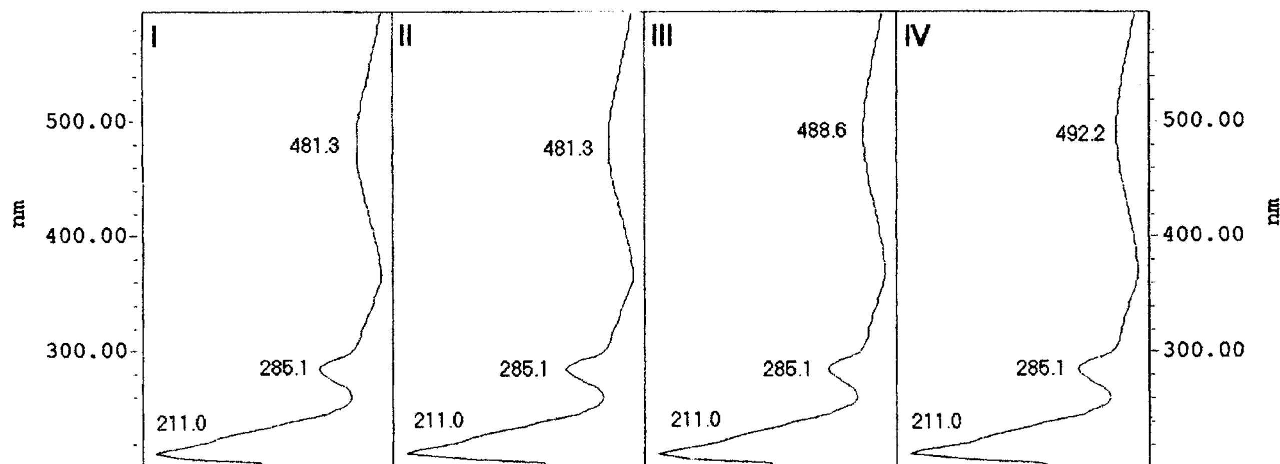

Figure 3. UV–visible normalized spectra (200–600 nm) of peaks I–IV of Figure 2.

prepared to identify and quantify the amount of these compounds in the fertilizers samples.

1D and 2D NMR spectra of EDDHMA-x were recorded at 200 and 400 MHz with Bruker AC 200 using simultaneous (SIM) mode and Varian Mercury VX 400 instruments and processed with the program VNMR-SGI by Varian. Bruker free induction decays (FIDs) were first transferred to an SGI workstation using the equipment described in ref *16*, then converted to the VNMR format with the macro "convertbru", and subsequently processed by backward linear prediction of the first three points prior to complex FT, so as to obtain very flat baselines. Library NMR sequences were always used, except for nuclear Overhauser enhancement (NOE) experiments that were implemented according to the method of ref *17*. Samples for NMR were prepared by dissolving 10−20 mg of EDDHMA-x or the iron-free EDDHMA extracted from a commercial fertilizer in 650 μL of D_2O at pH ∼0. All of the chemical shifts are referenced to the methene signal of EDDHMA (^1H = 5.18 ppm; ^{13}C = 60.0 ppm); in turn, proton and carbon chemical shifts of EDDHMA were measured once for all by comparison with an internal standard of acetone. Iron-free EDDHMA for NMR analysis was extracted from a commercial fertilizer containing the EDDHMA/Fe^{3+} complex following the procedure developed by us (*14*).

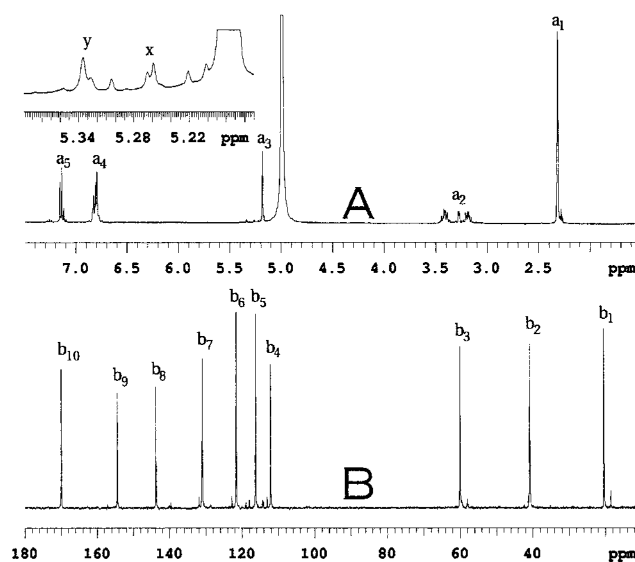

Figure 4. NMR spectra of a sample of EDDHMA-x in D_2O at 298 K (400 MHz): (A) ^1H spectrum; (B) ^{13}C spectrum.

RESULTS AND DISCUSSION

Commercial Fertilizers Containing EDDHMA/Fe^{3+}. Chromatographic separation of the EDDHMA/Fe^{3+}standard yields two peaks (II and IV in Figure 2C) corresponding, respectively, to the meso and racemic diastereomers of the complex described previously by us (*11*). Figure 2A shows a typical chromatogram obtained for a commercial fertilizer containing EDDHMA/Fe^{3+}, whereas Figure 2B shows the chromatogram recorded for the Fe complex formed with EDDHMA-x. All of the products tested display two additional small peaks (I and III, Figure 2A) having retention times close to the ones of the meso (II, Figure 2) and racemic (IV, Figure 2) EDDHMA/Fe^{3+} peaks. Their UV−vis absorbance spectra (Figure 3) are similar to those obtained for the meso and racemic EDDHMA/Fe^{3+} peaks. Interestingly, the broad band of absorption at ∼480 nm attributable to iron−phenol binding (*8*) is also present in these peaks. As stated above, the commercial synthesis of EDDHMA may, in principle, produce compounds **2**−**7** (Figure 1). It is reasonable to assume that the additional peaks may be byproducts of the synthesis. This hypothesis can be confirmed by comparing chromatogram A (Figure 2) and the chromatogram of the iron complex synthesized from the ligand EDDHMA-x (*12*; Figure 2B). Because these chromatograms are identical at retention times from 5 to 11 min, peaks I and III should be assigned to iron complexes formed from the impurities generated during the synthesis of the ligand.

UV spectra of the peaks in the chromatograms in Figure 2 can be used to identify the impurities. According to Lucena et al. (*13*), the relative height of the maximum in the visible spectrum of *o*,*p*-EDDHA/Fe^{3+} (one of the impurities observed in commercial samples of EDDHA) was *half* that of the racemic and meso *o*,*o*-EDDHA/Fe^{3+} peaks. This is due to the bonding of a single phenol group to the iron center in the *o*,*p*-EDDHA compared with two phenol−iron bonds in racemic and meso *o*,*o*-EDDHA/Fe^{3+}. By analogy, because the relative height of the broad absorption band at ∼480 nm of peaks I and III is *equal* to that of peaks II and IV in commercial EDDHMA/Fe^{3+} fertilizers (Figure 3), we conclude that the minor impurities have *two* iron−phenolate bonds. These coordinations exclude ligands **3**, **4**, and **7** (Figure 1), having at least a hydroxy group at the para position. This structure makes hexacoordination by the ligand impossible. Therefore, structures **5** and **6** should be the only ones considered for these impurities.

Assuming that both iron chelates of **5** and **6** are present in the commercial formulations, the presence of only two additional chromatographic peaks (instead of four, i.e., two diastereomers per compound) may indicate either that they elute simultaneously or that one of the two complexes is not present in a

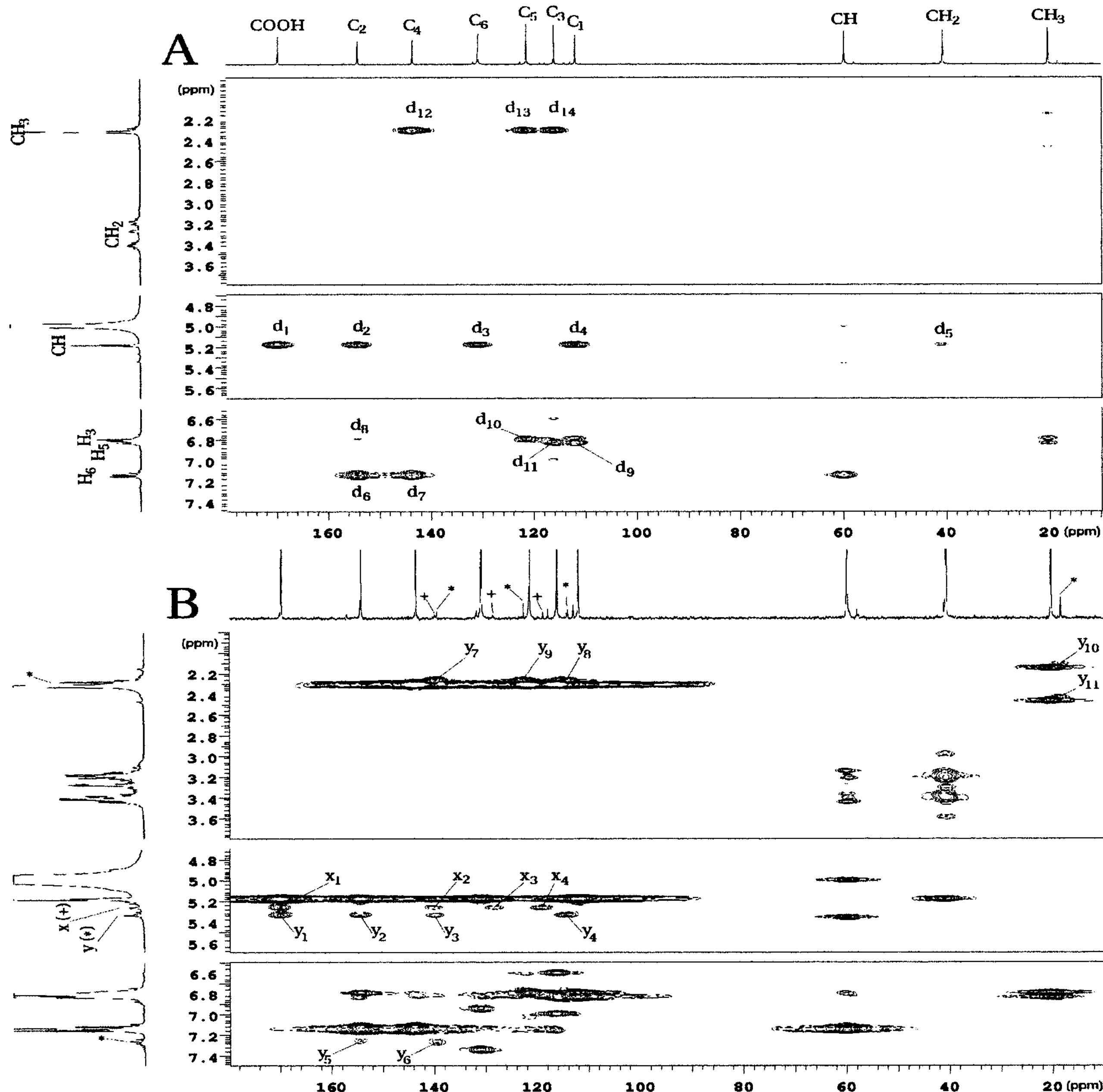

Figure 5. HMBC 2D spectrum of a sample of EDDHMA-x recorded in D_2O at 400 MHz and 298 K: (A) greater threshold; (B) lesser threshold. In (B) side and top spectra are plotted out of scale to evidence the assigned peaks belonging to **5** (labeled '*') and **3** (or **4** or **7**, labeled '+').

detectable amount. The second hypothesis seems more likely as formation of **6** should be hampered by steric hindrance. In fact, the approach of the iminium salt derived from glyoxalate and ethylenediamine to the aromatic ring of *m*-cresol has to occur in a position flanked by the methyl and the phenol group. Accordingly, an estimate of the **5**/**6** ratio is obtained by assuming that the reaction proceeds randomly to yield **2**, **5**, and **6** (Figure 1), and peaks I and III (Figure 2) belong to the two diastereomers of the iron complex of **5** (Figure 1). The average ratio between the Fe complexes of **2** and **5** in the eight commercial products tested is 87.1:12.9 (with small differences among the samples, the standard deviation of the ratio being 2.3); hence, we can estimate equilibrium percentages for the iron complexes of **2**, **5**, and **6** as 86.7, 12.8, and 0.5%, respectively.

A further analysis indicates that **5** and **6** are not the only impurities that may be present in commercial fertilizers containing the EDDHMA/Fe^{3+} chelate. The ^{1}H NMR spectrum of the ligand (EDDHMA-x), obtained through the method of Petree et al. (*12*), shows in addition to the signals corresponding to methyl, ethylene, methene, and aromatic groups of EDDHMA (respectively, peaks a_1, a_2, a_3, and $a_4 + a_5$, Figure 4A) a number of minor peaks. Most of the resonances in the range of 5.2−5.4 ppm may be attributed to the CH signals of EDDHMA isomers (Figure 4A, inset). This is also confirmed by inspection of the ^{13}C NMR spectrum (Figure 4B), where many small peaks flank the methyl, methylene, methene, and aromatic signals of the major compound (respectively, peaks b_1, b_2, b_3, and $b_4 - b_9$), thus indicating a chemical resemblance between major and minor compounds.

Complete assignment of ^{1}H and ^{13}C signals of EDDHMA has been carried out by means of the gradient HMBC (*18*) technique analogously to what has been recently reported for EDDHA (*14*). Starting from the almost overlapped ^{1}H signal pair due to the methene signals of the two diastereomers of EDDHMA at 5.18 ppm, five cross-peaks (cp's) are encountered (Figure 5A) corresponding to long-range couplings to the

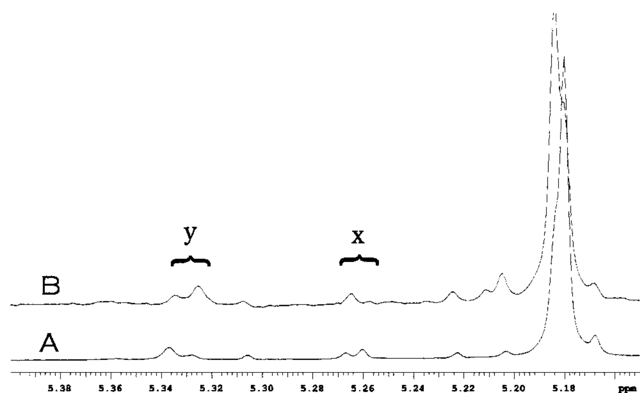

Figure 6. Comparison between the CH region of the ^{1}H NMR spectra of (A) EDDHMA-x and (B) a sample of iron-free EDDHMA extracted from a commercial fertilizer.

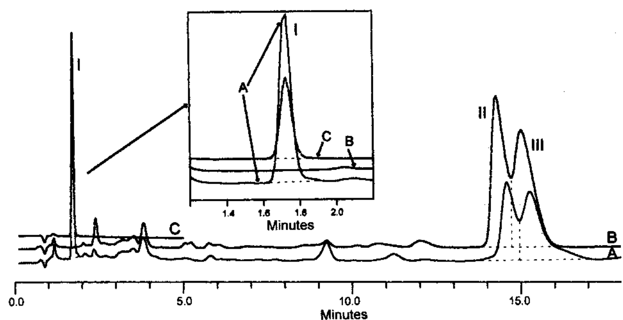

Figure 7. Chromatogram at 280 nm of a commercial fertilizer sample containing EDDHSA/Fe^{3+} (A), the standard of EDDHSA/Fe^{3+} (B), and the *p*-phenolsulfonate standard (C). Expansion at the retention time range from 1.2 to 2.2 min of the three chromatograms is presented.

carboxylic carbon (cp d_1, 169.9 ppm), aromatic C_2, C_6, and C_1 (cp's d_2, d_3, and d_4 at 154.4, 131.0, and 112.1 ppm, respectively), and the methylene carbon (cp d_5, 40.8 ppm). Moving vertically at the C_2 frequency, H_6 is recognized at 7.14 ppm. Its assignment is justified by the known difference in magnitude between $^{3}J_{CH}$ (~7 Hz) and $^{2}J_{CH}$ (~1 Hz) in substituted aromatics (*18*). The same reasoning aids locating C_4 from H_6 (cp d_7, 143.7 ppm). H_3 is assigned at 6.79 ppm because of the presence of a small cp (due to $^{2}J_{CH}$) at the C_2 frequency (cp d_8). By exclusion, H_5 is found at 6.82 ppm because of its cp with C_1 via $^{3}J_{CH}$ (cp d_9). C_5 and C_3 are detected at 121.6 and 116.3 ppm, respectively, once more via $^{3}J_{CH}$ (cp's d_{10} and d_{11}). Finally, C_4, C_5, and C_3 are long-range-coupled to the methyl protons at 2.30 ppm (cp's d_{12}, d_{13}, and d_{14}, respectively).

Table 1. ^{1}H and ^{13}C NMR Signal Assignments for Compounds **2**, **5**, and **3** (or **4** or **7**) at Acidic pH[a]

atom		2		5[b]		3 (or 4 or 7)[c]	
CH_3	(CH_3)	2.30	(20.4)	2.27[d]	(18.2)	2.28[d]	(nd)[f]
CH_2	(CH_2)	3.30	(40.8)	3.28[d]	(nd)	3.34[d]	(nd)
CH	(CH)	5.18[e]	(60.0)	5.33[e]	(nd)	5.26[e]	(nd)
	(COOH)		(169.9)		(170.0)		(170.0)
	(C_1)		(112.1)		(114.4)		(119.3)[g]
H_2	(C_2)		(154.4)		(154.4)		(140.2)
H_3	(C_3)	6.79	(116.3)	nd	(nd)	nd	(nd)
H_4	(C_4)		(143.7)	7.26	(nd)		(nd)
H_5	(C_5)	6.82	(121.6)	nd	(122.9)	nd	(nd)
H_6	(C_6)	7.14	(131.0)		(139.8)	7.18[d]	(128.5)[g]

[a] Measured in D_2O at 298 K and referenced to acetone. In units of ppm at 9.4 T (^{1}H, 400 MHz; ^{13}C, 100.59 MHz) unless otherwise stated. [b] Moiety containing the 2-hydroxy-6-methylphenyl group. [c] Moiety containing the 4-hydroxy-2-methylphenyl group. [d] From NOE experiments at 200 MHz. [e] Center of the asymmetric doublet corresponding to meso/racemic diastereomers. [f] nd, not detected. [g] Assignment interchangeable.

When the same HMBC spectrum is plotted with a lesser threshold, a number of small cross-peaks stemming from the supposedly benzylic proton signals of the impurities become apparent (Figure 5B). Each of the proton signal pairs x and y centered at 5.26 and 5.33 ppm (most probably due to couples of diastereomers) display four well-resolved cp's at 170.0, 140.2, 128.5, and 119.3 ppm (cp's x_1, x_2, x_3, and x_4, respectively) and at 170.0, 154.4, 139.8, and 114.4 ppm (cp's y_1, y_2, y_3, and y_4, respectively). According to the chemical shifts, the latter group of ^{13}C signals may correspond to COOH, C_2, C_6, and C_1 carbons of a compound containing the ethylenediamine(2-hydroxy-6-methylphenylacetic) acid moiety (such as **5** and **6**). The presence of the ethylenediamine bridge and that of the *o*-methyl is confirmed by an NOE difference experiment (not shown) in which the y peaks are saturated and two signals are detected at 2.27 ppm (sharp, 6-methyl) and 3.28 ppm (multiplet, $-NCH_2-$). Further connections due to $^{3}J_{CH}$ in aromatics (*18*) are found between both C_2 and C_6 and H_4 at 7.26 ppm (cp's y_5 and y_6) and between C_6 and C_1 and the previously found methyl at 2.27 ppm (cp's y_7 and y_8). The methyl is also coupled to C_5 at 122.9 ppm (cp y_9). Methyl carbon frequency is at 18.2 ppm, as found by exploiting the two HMQC cp's y_{10} and y_{11} due to $^{1}J_{CH}$ and not perfectly suppressed by the gHMBC sequence.

An NOE difference experiment performed by saturating signal pair x (not shown) yields three signals that can be assigned to a methyl (sharp signal at 2.28 ppm), a deshielded methylene (multiplet at 3.34 ppm), and an aromatic proton (broad doublet

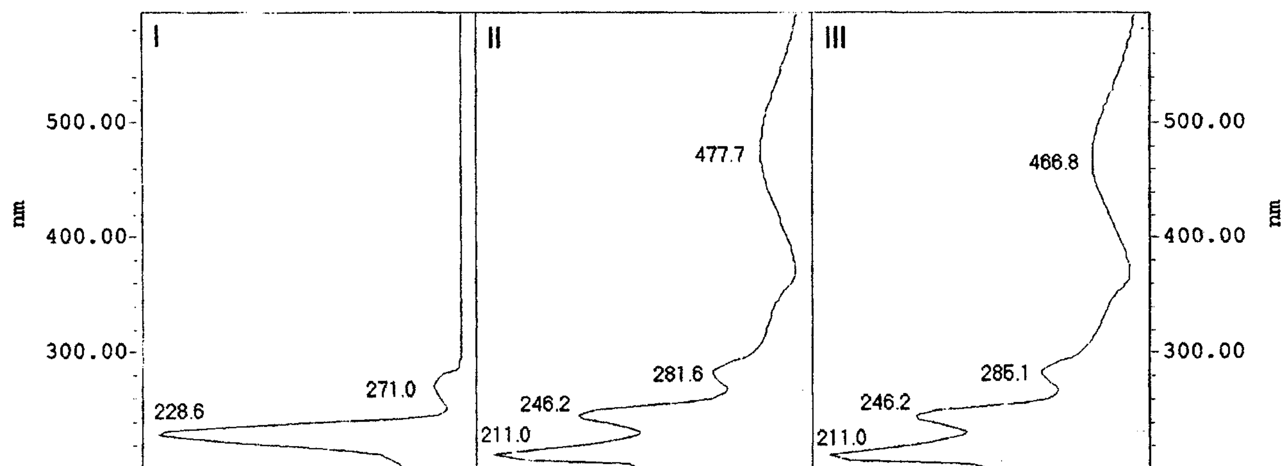

Figure 8. UV–visible normalized spectra (200–600 nm) of peaks I–III of Figure 7.

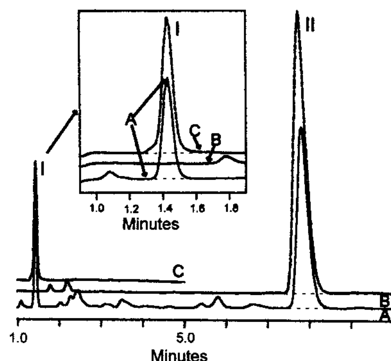

Figure 9. Chromatogram at 280 nm of a commercial fertilizer sample containing EDDCHA/Fe^{3+} (A), the standard of EDDCHA/Fe^{3+} (B), and the *p*-hydroxybenzoate standard (C). Expansion at the retention time range from 0.9 to 1.9 min of the three chromatograms is presented.

Table 2. Percentage of *p*-Phenolsulfonic Acid in Commercial EDDHSA/Fe^{3+} Chelates[a]

R	% *p*-phenol-sulfonic acid	R	% *p*-phenol-sulfonic acid
44-a (6%)	8.39 ± 0.38	**53** (6%)	10.41 ± 0.31
44-b (6%)	10.74 ± 0.29	**54** (6%)	12.36 ± 0.09
45-a (6%)	12.36 ± 0.83	**55** (6%)	11.42 ± 0.05
45-b (6%)	12.55 ± 0.83	**56** (6%)	8.92 ± 0.18
46 (6%)	12.31 ± 0.44	**57** (2.4%)	3.35 ± 0.00
47 (6%)	10.70 ± 0.80	**58** (2.4%)	3.54 ± 0.39
48 (6%)	11.15 ± 0.35	**59** (2.4%)	3.42 ± 0.14
49 (6%)	11.12 ± 0.56	**60-a** (2%)	4.18 ± 0.09
50 (6%)	10.73 ± 0.06	**60-b** (2%)	4.48 ± 0.28
51 (6%)	12.03 ± 0.80	**61** (2%)	1.34 ± 0.08
52 (6%)	12.60 ± 0.65	**62** (3.5%)	4.99 ± 0.33

[a] Data are the mean ±SD (n = 3) per product. R = lab reference. Numbers in parentheses next to the reference indicate the percentage of Fe chelated declared by the manufacturer.

at 7.18 ppm). This is indicative of a compound containing the ethylenediamine(4-hydroxy-2-methyl)phenylacetic acid moiety (such as **3**, **4**, or **7**), which is the last isomer of (2-hydroxy-4-methyl)phenylacetic acid (contained in EDDHMA-x) expected to be formed during the reaction of *m*-cresol, ethylenediamine, and glyoxylic acid (*12*) on the basis of simple organic chemistry reasoning. Its structure is also supported by the gHMBC spectrum, which shows long-range couplings appearing between x and COOH (cp x_1), C_2 (cp x_2), and aromatic carbons C_1 and C_6 (cp's x_3 and x_4, not assignable because of the lack of other cp's). All of the NMR assignments are summarized in Table 1.

Unfortunately, none of the other minor peaks detected in the CH region of the EDDHMA-x spectrum could be identified by NMR. Nevertheless, they are due to other unknown ligand impurities and can be exploited, together with x and y, for assessing the quality of the ligand fraction extracted from an EDDHMA/Fe^{3+}-containing fertilizer. An example is shown in Figure 6B. Besides some differences in the relative amount of the two diastereomers of EDDHMA and in the relative height of the peaks forming the x and y pairs, spectrum B is very similar to the one of EDDHMA-x. The conclusion is that the chelate contained in the commercial preparation was synthesized from a not sufficiently purified ligand.

Finally, it is worth noting that the assignment of HPLC peaks I and III (Figure 2A,B) to compound **5** discussed above is confirmed by NMR. Recalling that the chromatographic product ratio **5**/**2** (Figure 1) is 87.1:12.9, we can calculate the ratio of the (2-hydroxy-4-methyl)phenylacetic acid with respect to the (2-hydroxy-6-methyl)phenylacetic acid moieties as 93.6:6.4. As the ratio among the NMR peaks y, x, and CH of EDDHMA in Figure 6B is 6.9:1.9:91.6, we compute the NMR ratio between the above moieties either as 93.0:7.0 (x being assigned to **4**), 92.9:7.1 (x being assigned to **3**), or 94.7:5.3 (x being assigned to **7**) in very good agreement with HPLC.

Commercial Fertilizers Containing EDDHSA/Fe^{3+}. For the first time a chromatographic technique has been applied to EDDHSA/Fe^{3+} and EDDCHA/Fe^{3+} chelates. The chromatographic separation of EDDHSA/Fe^{3+} standard showed two peaks (II and III, Figure 7) having UV−visible spectra (II and III, Figure 8) typical for iron chelates derived of molecules such as **1** in Figure 1 (*8*). These peaks are attributable to the diastereoisomers of EDDHSA/Fe^{3+}. Both diastereoisomers of EDDHSA/Fe^{3+} have very similar absorbance spectra in the UV and visible ranges (II and III. Figure 8), with peaks at 211.0 (aromatic ring), 246.2 (−HSO_3 groups), and 281.6 nm (−OH groups) and a broad band of absorption in the visible range (iron−phenol binding), peaking at ~480 nm. The difference in the placement of the peak between the two isomers is ~11 nm similar to that found for the stereoisomers of EDDHA/Fe^{3+} (*8*). All chromatograms obtained for the fertilizer samples containing EDDHSA/Fe^{3+} presented an additional peak labeled I in Figure 7, having a retention time and UV−visible spectrum (200−600 nm) (I, Figure 7) matching those of *p*-hydroxybenzenesulfonate anion. This fact was proved by comparing Figure 7A with Figure 7C. The amount of *p*-hydroxybenzenesulfonic acid in the commercial iron−EDDHSA products was calculated, and the data obtained are presented in Table 2. These data showed

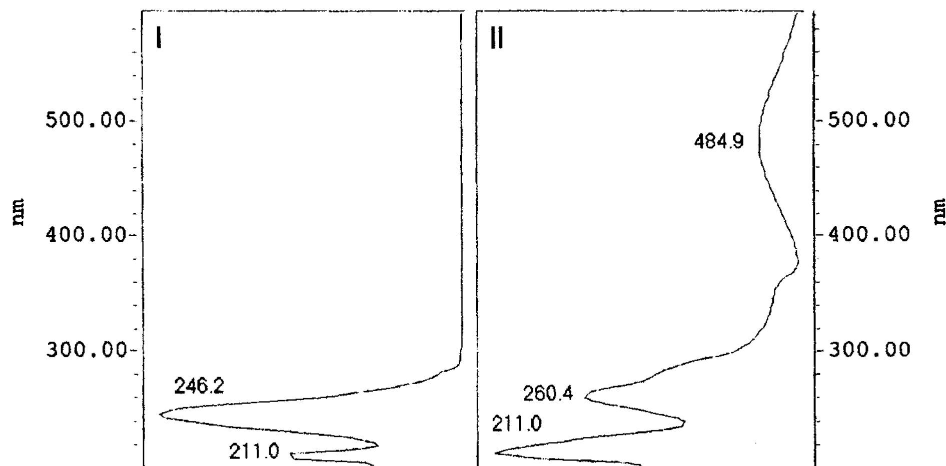

Figure 10. UV−visible normalized spectra (200−600 nm) of peaks I and II of Figure 9.

that the larger the content of chelated iron that is declared, the larger amount of *p*-hydroxybenzenesulfonic acid is present. Therefore, we can conclude that the amount and presence of this substance are intrinsic to the method of synthesis of the EDDHSA and therefore unavoidable as was stated above.

Commercial Fertilizers Containing EDDCHA/Fe^{3+}. The chromatographic separation of the EDDCHA/Fe^{3+} standard generated the chromatogram reported in Figure 9A that showed only one peak (II) attributable to the iron-chelated species. In this case, and under the conditions we used, the two stereoisomers of EDDCHA/Fe^{3+} coeluted in a single peak at room temperature at 7.7 min (Figure 9). This peak had a UV−visible spectrum (200−600 nm) typical of this kind of iron chelate complexes. This spectrum is reported in Figure 10 (II) and presented three peaks at 211.0 (aromatic ring), 260.4 (−COOH substitution on the benzene ring), and 484.9 nm (iron−phenol binding). The peak at 260.4 nm had a shoulder peaking at ∼280 nm due to the −OH substitution on the benzene ring. Figure 9A shows an additional peak (I) having a UV−visible spectrum and a retention time matching those of *p*-hydroxybenzoate anion (see Figure 9C). The two EDDCHA/Fe^{3+} commercial iron chelates tested presented at least 8% of *p*-hydroxybenzoic acid.

p-Hydroxybenzoate and *p*-hydroxybenzenosulfonate anions do not have any value as Fe fertilizers. Moreover, their environmental behavior after application to soil is unknown. This is in clear contrast with the behavior of the impurities seen in EDDHMA. In this case, one of the two different −OH and $-CH_3$ positional isomers on the benzene ring (Figure 1, structure **5**) should be as capable in chelating Fe as EDDHMA, whereas the other may be inefficient. Both are, however, not allowed by EU regulations (98/3/EC directive).

The presence of these impurities could explain the low percentages of iron chelated in the commercial fertilizers analyzed. For the products that declared 6% of iron chelated (all of the tested products except seven EDDHSA/Fe^{3+} products) the values found ranged from 2.54 to 4.08% of Fe as EDDHMA/Fe^{3+}, from 0.80 to 1.54% of Fe as EDDHSA/Fe^{3+}, and from 1.60 to 1.90% of Fe as EDDCHA/Fe^{3+}. Nevertheless, it should be noted that for EDDHSA/Fe^{3+} and EDDCHA/Fe^{3+} products, only the iron chelated by the complex 1:1 (Fe/ligand) was determined, but in these cases other iron complexes with different ratios may exist (*19*).

In conclusion, through this work the presence and nature of impurities in three of the most used iron chelates have been disclosed. From these results and those obtained for EDDHA (*14*), we feel that it is time for manufacturers to improve the decades-old process for the production of iron chelates used in agriculture. By keeping production procedures in their current status, many tons of potentially polluting compounds, the agronomical value of which is in the best case unknown and in the worse nil, will continue to be delivered to soil every year.

ABBREVIATIONS USED

cp, cross-peak; EDDHA, ethylenediaminedi(*o*-hydroxyphenylacetic) acid; EDDCHA, ethylenediaminedi(5-carboxy-2-hydroxyphenylacetic) acid; EDDHMA, ethylenediaminedi(*o*-hydroxy-*p*-methylphenylacetic) acid; EDDHSA, ethylenediaminedi-(2-hydroxy-5-sulfophenylacetic) acid; HPLC, high-performance liquid chromatography; NMR, nuclear magnetic resonance; HMBC, heteronuclear multiple bond correlation; HMQC, heteronuclear multiple quantum correlation; NOE, nuclear Overhauser enhancement.

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