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Characterization of Commercial Iron Chelates and Their Behavior in an Alkaline and Calcareous Soil

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Iron deficiency is a common problem for many plants grown in alkaline and calcareous soils. To correct this problem, iron is supplied to plants as chelates. Several iron chelates are sold under diverse trademarks with different characteristics. This work evaluated 18 commercial products containing the most representative chelated iron sources used in agricultural practice in Spain when the study was done, namely the ferric chelates of EDDHA, EDDHMA, EDDCHA, EDDHSA, EDTA, and DTPA. The chelates were comprehensively characterized and quantitated by several techniques, including several chromatographic methods. Iron and chelate dynamics in soil were also studied in a model alkaline and calcareous soil. Results indicate that, in this model soil, among the different iron compounds studied only FeEDDHA and analogues have the capacity to maintain soluble iron in soil solution over time. These results are in agreement with general experience under field conditions. Furthermore, among the different ortho−ortho isomers of FeEDDHA's, FeEDDHSA and FeEDDCHA showed greater capacity than FeEDDHA and FeEDDHMA to maintain the chelated iron in soil solution over time.

KEYWORDS: Alkaline and calcareous soils; chelate stability; iron chelates; iron chlorosis; soil incubation

INTRODUCTION

Iron deficiency is a common problem for many plants grown in alkaline and calcareous soils, causing the symptom known as iron chlorosis. It is a well-established fact that the performance of iron sources used to correct chlorosis depends on soil type and the chemical properties and/or purity of these products. It has also long been known that competing cations such as Ca^{2+} and Mg^{2+} may displace Fe^{3+} from some chelates, rendering it unavailable because of subsequent precipitation in calcareous soil. Ferrous sulfate also precipitates because of alkaline pH. For these reasons, in most alkaline and calcareous soils ferrous sulfate, FeEDTA, or FeDTPA applications result in meager or zero results (*1*).

FeEDDHA chelates, on the other hand, have been successfully used as Fe sources for chlorosis remediation in alkaline and calcareous soils. Most authors agree that the ortho−ortho isomer is the most active or even the sole active iron source of this chelate. These chelates are quite expensive, and many questions have been raised about the relative merits of commercial brands. For instance, several studies have revealed that only about half the iron is present as *o*,*o*-FeEDDHA chelate (*2*). The composition and speciation of the rest of the iron remains largely unknown but has been putatively ascribed to FeEDDHA positional isomers different from the ortho−ortho isomer (*3*). Along with FeEDDHA, several other structurally analogous substances are present on the market, namely FeEDDHMA, FeEDDHSA, and FeEDDCHA.

A number of studies have dealt with the physicochemical characterization of chelates, analysis of commercial formulations (*2*, *4*), soil chemistry (*5*, *6*), and agronomic performance (*7*, *8*).

In this paper, we studied several physicochemical, analytical, and soil behavior characteristics of ferric chelates, with special emphasis on their practical, applied side. First, we set up several methods for chelate quantification in formulations and soils. Second, we proposed a soil incubation methodology that predicts chelate performance. In the literature there are several approaches to chelate testing in soils (*6*, *9*, *10*). We have followed a method similar to that of references *6* and *9* that to our understanding better reflects usual field conditions. We have therefore studied both iron and chelate kinetics for 50 days in field capacity moist soil and chelate concentrations similar to those found in fertirrigation wet bulbs.

The lack of commercial standards for FeEDDHMA, FeEDDCHA, and FeEDDHSA when this work was done hampered their complete characterization. However, we circumvented this problem through chromatographic purification, fraction collection, and AAS and HPLC analysis.

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MATERIALS AND METHODS

Iron Products. Commercial chelate samples were chosen among the most representative brands and obtained through manufacturers or local retailers between 1995 and 1999. They were coded according to their composition as HA2 to HA9 for FeEDDHAs, SA1 to SA5 for FeEDDHSAs, MA1 for FeEDDHMA, CA1 for FeEDDCHA, EDTA1 for FeEDTA, DTPA1 for FeDTPA, and MIX for a sample labeled as FeEDDHA but also containing FeEDTA, as revealed by HPLC. Ferrous sulfate, reagent grade, was also used as a nonchelated iron source. Products SA1 and SA3 were liquid formulations.

Sample HA1 corresponds to laboratory-made FeEDDHA. It was prepared by dissolving acid EDDHA (Sigma, ref. E-4135, lot. 117F50221) in distilled water with the addition of diluted NaOH until complete ligand dissolution. Fe was added as nitrate in a 5% excess of the stoichiometric ratio 1:1. This solution was alkalinized to pH 7 and allowed to stand overnight; then it was filtered through 8 μm paper filter (Papelera del Besós, Barcelona, Spain) and evaporated in the dark at room temperature.

Iron Analysis. Iron content in samples was determined by AAS against carefully matched standards, with a detection limit of 0.1 mg L^{-1}. Samples and standards were acidified with analytical-grade HCl to a final concentration of approximately 1 M (10 mL of solution + 1 mL of 11 M HCl).

Iron content can be considered from different viewpoints. First, total iron was measured in commercial products by dissolving them in 1 M HCl. This procedure will dissolve virtually any iron, even that present as oxides. We also measured soluble iron by dissolving samples in water. As a measure of complexed iron, we analyzed iron content of samples dissolved in a pH 8.75 buffer. At this pH any uncomplexed iron must precipitate readily or remain insoluble. These parameters were measured by stirring samples of about 60 mg L^{-1} Fe for 1 h. Then, samples were filtered through 8 μm paper filter, diluted 10 times with distilled water, and acidified as above. Iron content in commercial products was named according to the following operative definitions: total iron, iron soluble in 1 M HCl; soluble iron, iron soluble in distilled water; complexed iron, iron soluble in pH 8.75 buffer (0.54 M NH_4-Cl/0.1 M NH_4OH); chelated iron, chelated iron measured by HPLC; chelation degree, proportion of chelated iron with respect to total iron; solution pH, pH of water-soluble iron solution. Iron sources used in chelate manufacture (chloride or sulfate) were determined qualitatively by precipitation with silver nitrate and barium chloride solutions, respectively.

HPLC. HPLC separation and analysis were carried out in a Waters chromatographic system, with a 616 pump, 717 Plus autosampler, 996 photodiode array detector, Fraction Collector II, and Millennium chromatographic software V. 3.05.01.

For FeEDDHA and FeEDDHMA: column Lichrospher 60 RP-select B (5 μm) (Merck, ref 1.50984.0001), 250 mm × 4 mm, flow 1 mL/min, oven temperature 40 °C, detection wavelength 282 nm, injection volume 10 μL. Gradient mobile phase: solvent A ammonium acetate 0.05 M, pH 7; solvent B methanol. Time 0 min: 95% A, 5% B. Time 3 min: 95% A, 5% B. Time 6 min: 75% A, 25% B. Time 8 min: 75% A, 25% B. Time 15 min: 65% A, 35% B. Time 18 min: 95% A, 5% B. Time 23 min: 95% A, 5% B. Retention times (min) of ferric chelates: *rac*-FeEDDHA, 5.7; *meso*-FeEDDHA, 11.9; *isomer I*-FeEDDHMA, 11.9; *isomer II*-FeEDDHMA, 18.4.

For FeEDTA, FeDTPA, FeEDDHSA, and FeEDDCHA: column Hypersil ODS 3 μm, 10 mm × 4.6 mm (Tracer, ref. TR-013093), flow 1 mL/min, oven temperature 40 °C, detection wavelength 282 nm, injection volume 10 μL. Isocratic mobile phase: solvent A tetra-*n*-butylammonium hydroxide 0.05 M, pH 6.5, 62%; solvent B methanol 38%. Retention times (min) of ferric chelates: FeEDTA, 2.2; FeDTPA, 3.1; FeEDDHSA, 16.6; FeEDDCHA, 12.1. FeEDDHSA and FeEDDCHA appear as two fused peaks corresponding to meso and racemic isomers; we were not able to resolve them. For this reason, they were considered as a single peak.

Peak identification as meso or racemic isomers in FeEDDHA and isomers I and II in FeEDDHMA was tentatively made according to UV−vis spectra in the bibliography (*2*, *11*). Quantification was carried out by the external standard method.

Fraction collection of *meso*- and *rac*-FeEDDHA: injection volume 15 μL. Isocratic mobile phase: solvent A ammonium acetate 0.05 M, pH 7, 83%; solvent B methanol, 17%. Rest of conditions as in the method for FeEDDHA and FeEDDHMA. Retention times (min) of ferric chelates: *rac*-FeEDDHA, 3.8; *meso*-FeEDDHA, 7.8. Iron in collected fractions was measured by AAS. Isomers in collected fractions were measured by HPLC following the above-described isocratic and gradient methods.

Fraction collection of isomers I and II FeEDDHMA: injection volume 15 μL. Isocratic mobile phase: solvent A ammonium acetate 0.05 M, pH 7, 70%; solvent B methanol, 30%. Rest of conditions as in the method for FeEDDHA and FeEDDHMA. Retention times (min) of ferric chelates: *isomer I*-FeEDDHMA, 4.5; *isomer II*-FeEDDHMA, 9.1. Iron in collected fractions was measured by AAS. Isomers in collected fractions were measured by HPLC following the above-described isocratic and gradient methods.

Fraction collection of *meso*- and *rac*-FeEDDHSA: injection volume 30 μL. Isocratic mobile phase: solvent A tetra-*n*-butylammonium hydroxide 0.05 M, pH 6.5, 59%; solvent B methanol 41%. Rest of conditions as in the method for FeEDDHSA. Retention times (min) of *rac*- and *meso*-FeEDDHSA, 11.3. Iron in collected fractions was measured by AAS. Isomers in collected fraction were measured by HPLC following the above-described isocratic method.

Fraction collection of *meso*- and *rac*-FeEDDCHA: injection volume 20 μL. Isocratic mobile phase: solvent A tetra-*n*-butylammonium hydroxide 0.05 M, pH 6.5, 63%; solvent B methanol 37%. Rest of conditions as in the method for FeEDDCHA. Retention times (min) of *rac*- and *meso*-FeEDDCHA, 12.6. Iron in collected fractions was measured by AAS. Isomers in collected fractions were measured by HPLC following the above-described isocratic method.

HPLC/MS. LC/MSD Hewlett-Packard 1100 series, pump G1311A, autosampler G1313A, diode array detector G1315A, and mass detector G1946A. Chromatographic software HP ChemStation Rev. A.06.03.

MS detection mode: API-ES negative, scan mass range 100−600 amu, fragmenter voltage 30 V, capillary voltage 2500 V. Selected ions (*m*/*z*): FeEDTA, 344; FeDTPA, 445; FeEDDHA, 412; FeEDDHMA, 440; FeEDDHSA, 572; FeEDDCHA, 500.

Column Lichrospher 60 RP-select B (5 μm) (Merck, ref 1.50984.0001), 250 mm × 4 mm, flow 0.5 mL/min, oven temperature 40 °C, injection volume 5 μL. Gradient mobile phase: solvent A ammonium acetate 0.001 M, pH 7; solvent B methanol. Time 0 min: 95% A, 5% B. Time 3 min: 95% A, 5% B. Time 6 min: 75% A, 25% B. Time 12 min: 75% A, 25% B. Time 14 min: 95% A, 5% B. Time 19 min: 95% A, 5% B. Retention times (min): *rac*-FeEDDHA, 4.5; *meso*-FeEDDHA, 9.1; *isomer I*-FeEDDHMA, 9.1; *isomer II*-FeEDDHMA, 14.3; FeEDTA, 2.0; FeDTPA, 1.7; FeEDDHSA, 1.5; FeEDDCHA, 1.6.

TLC. Thin-layer chromatography was performed according to Bannochie (*11*) using silica gel 60 sheets (Merck ref 1.05748), mobile phase 10:2:1 *n*-butanol/water/acetic acid, running time 5 h. 2 μL samples were applied with the aid of a chromatographic syringe as water solutions (5% w/w).

FeEDDHA TLC showed two main spots: racemic (red, R_f 0.28) and meso (violet, R_f 0.34). The other chelates appeared in a similar fashion: FeEDDHMA R_f isomer I, 0.39; isomer II, 0.45; FeEDDCHA R_f, 0.34 (two unresolved spots). FeEDDHSA, FeEDTA, and FeDTPA did not elute (R_f 0) under the experimental conditions. Spots of meso (violet) and racemic (red) isomers were scraped together, stirred with 3 mL of 1 M HCl, centrifuged, analyzed by AAS, and named TLC iron. Iron in samples (soluble iron) was likewise determined with 2 μL of sample.

Soil Characteristics. An alkaline *Typic Calcixerept* soil was taken from a commercial lemon tree orchard with ferric chlorosis problems in Santomera (Murcia, Spain). This was used as the incubation substrate once air-dried and sieved. This is a typical alkaline and calcareous soil from the Mediterranean coast of Spain. **Table 1** shows the analytical characteristics of this soil.

Soil Incubation. 25 g soil samples were weighed in 60 mL covered containers and wetted to field capacity with 10 mL chelate solutions, tightly covered, and stored in the dark. These solutions were prepared to provide approximately 7 mg kg^{-1} Fe in field-capacity moistened soil (10 mg kg^{-1} Fe on dry soil basis). These solutions correspond to

Table 1. Physicochemical Characteristics of Testing Soil

parameter	analytical method	units	value
organic carbon	oxidation	g kg^{-1}	6.0
pH	saturation extract		8.2
EC	saturation extract	dS m^{-1}	1.02
$CaCO_3$, total	gasometry	g kg^{-1}	420
active lime	oxalate	g kg^{-1}	123
texture	hydrometer		silt loam
sand	hydrometer	%	30.9
silt	hydrometer	%	49.5
loam	hydrometer	%	19.6
P	Olsen	mg kg^{-1}	60
K	ammonium acetate ext	cmolc kg^{-1}	0.91
Na	ammonium acetate ext	cmolc kg^{-1}	3.65
Ca	ammonium acetate ext	cmolc kg^{-1}	22.3
Mg	ammonium acetate ext	cmolc kg^{-1}	8.7
cation exchange capacity	ammonium acetate ext	cmolc kg^{-1}	35.6
Fe	DTPA extractable	mg kg^{-1}	2.9
Cu	DTPA extractable	mg kg^{-1}	0.8
Zn	DTPA extractable	mg kg^{-1}	2.2
Mn	DTPA extractable	mg kg^{-1}	1.7

time 0. After 1, 5, 12, 22, and 50 days, samples received 20 mL of distilled water and were tumble-mixed for 2 h and filtered through 8 μm paper filter. Fe was quantitated by AAS after sample acidification (5 mL sample + 0.5 mL of 11 M HCl). Three containers were prepared and measured for each product and time tested, and the results are presented as averages, with RSD values below 5% in most cases (RSD maximum 8%). Iron measured by AAS in the extracts described above is called water-extractable iron to distinguish it from soluble iron.

RESULTS AND DISCUSSION

Chemical Characterization. Table 2 shows iron content measured as indicated, and pH of water solution. Total iron content of HA's was very close to 6%, except for HA5. This product had the lowest percentage of complexed with regard to total iron, but due to its higher total iron the final percentage of complexed iron is even higher than that in the other products.

SA's total iron contents were slightly below the 3.5% label content for liquid formulations and above the declared content for the others. Total iron content in MA1 was above 6.5%. Total iron in CA1, DTPA1, EDTA1, and MIX was very close to the declared values.

We found that differences between total, soluble, and complexed iron were negligible for most chelates. Only HA5 and HA8 showed some differences, with values of complexed/total iron of 77.8 and 87.8%, respectively. We may conclude that for practical purposes all iron in the products studied is complexed. As was predictable, no iron remained soluble at pH 8.75 in iron sulfate solutions.

Commercial chelates tested are prepared from iron chloride or sulfate. No conclusions can be drawn regarding the influence, if any, of iron salt sources and chelate chemical properties studied.

Chromatographic Characterization. Table 3 shows iron content as chelate quantified by HPLC, and its percentage relative to complexed iron. Iron in EDTA1 and DTPA1 was practically 100% chelated as FeEDTA and FeDTPA, respectively, and is very close to the theoretical values.

FeEDDHA's, on the other hand, contained only approximately 40−50% iron as *o*,*o*-FeEDDHA chelate, with the exception of laboratory-made HA1. These results reflect that effective chelation degree is remarkably similar among the different manufacturers of HA's used in our experiments.

FeEDDHSA's contained 10−30% iron as FeEDDHSA, MA1 60% as FeEDDHMA, and CA1 27% as FeEDDCHA. The rest of the iron was somehow complexed, as it was soluble and did not precipitate at pH values as alkaline as 8.75. Our chromatographic methods (diode-array and MS) give no clues as to the nature of these unidentified complexes. It is necessary to point out that nowadays there are chelates on the market with a higher chelation degree.

Chelate quantification by HPLC UV−vis detection has usually been carried out in the literature presuming that *meso*- and *rac*-FeEDDHA display the same molar extinction at the analytical wavelength and mobile phase composition used, and peak areas can be thus added up as if belonging to a single chemical entity. This question, to our knowledge, has not been substantiated in the literature. In an attempt to resolve this matter, we collected fractions of pure isomers of FeEDDHA and of FeEDDHMA. We measured iron content in these fractions by AAS and chromatographic areas at several wavelengths using the isocratic and gradient analytical methods described above. Wavelengths chosen corresponded to UV and visible maxima. Results are shown in **Table 4**. Chromatographic response of the first eluting isomer is slightly higher than that for the second eluting isomer, both for FeEDDHA and FeEDDHMA, but differences were of small importance from the

Table 2. Chemical Characterization of Products with Amounts Given as Percentages

product	label iron content	total iron	soluble iron	soluble/total iron	complexed iron	complexed/total iron	solution pH	iron salt source
HA1		5.79	5.79	100.0	5.79	100.0	7.11	NO_3^-
HA2	6.0	5.75	5.72	99.5	5.70	99.1	7.45	Cl^-
HA3	6.0	6.03	5.89	97.7	5.44	90.2	7.64	SO_4^{2-}
HA4	6.0	6.51	6.23	95.7	6.00	92.2	7.53	SO_4^{2-}
HA5	6.0	8.48	8.01	94.5	6.60	77.8	7.27	Cl^-
HA6	6.0	6.09	6.06	99.5	5.72	93.9	7.17	SO_4^{2-}
HA7	6.0	6.49	6.42	98.9	6.34	97.7	6.80	SO_4^{2-}
HA8	6.0	6.17	5.87	95.1	5.42	87.8	7.40	SO_4^{2-}
HA9	6.0	5.65	5.44	96.3	5.47	96.8	8.02	SO_4^{2-}
MIX	7.0	6.66	6.49	97.5	6.24	93.7	8.36	SO_4^{2-}
SA1	3.5	2.90	2.85	98.3	2.89	99.7	6.68	Cl^-
SA2	6.5	7.65	7.13	93.2	7.13	93.2	6.39	SO_4^{2-}
SA3	3.5	3.09	3.07	99.4	3.06	99.0	6.88	Cl^-
SA4	5.6	5.65	5.30	93.8	5.32	94.2	6.04	SO_4^{2-}
SA5	6.0	6.17	5.92	96.0	5.72	92.7	6.88	Cl^-
MA1	6.5	6.97	6.96	99.9	6.93	99.4	7.90	Cl^-
CA1	6.0	5.94	5.89	99.1	5.96	100.3	6.37	SO_4^{2-}
DTPA1	11.0	11.85	11.64	98.2	12.06	101.8	3.49	
EDTA1	13.9	14.10	14.17	100.5	13.70	97.2	5.26	
sulfate		20.09	19.36	96.4	0.0	0.0	5.15	SO_4^{2-}

Table 3. Chromatographic Characterization of Iron Products

product	% chelated iron	% chelated/ complexed iron	% unidentified/ complexed iron
HA1	5.86	101.2	−1.20
HA2	2.95	51.8	48.2
HA3	2.59	47.6	52.4
HA4	2.90	48.3	51.7
HA5	3.35	50.7	49.3
HA6	2.35	41.0	59.0
HA7	2.91	45.9	54.1
HA8	2.35	43.3	56.7
HA9	2.73	49.9	50.1
MIX	0.89[a]	14.3	
	3.66[b]	58.7	
	4.55[c]	73.0	27.0
SA1	0.65	22.5	77.5
SA2	0.93	13.0	87.0
SA3	0.52	17.0	83.0
SA4	1.25	23.5	76.5
SA5	1.73	30.3	69.7
MA1	4.16	60.1	39.9
CA1	1.63	27.3	72.7
DTPA1	11.95	99.1	0.9
EDTA1	13.16	96.1	3.9

[a] As *o,o*-FeEDDHA. [b] As FeEDTA. [c] Total.

practical point of view at 282 nm in isocratic or gradient conditions. Differences were somewhat higher at visible wavelengths, where spectral differences were evident. We can therefore conclude that isomer areas can be added up as if belonging to a single chemical entity without significant error under the analytical conditions we used.

The assignment of FeEDDHMA isomers is not straightforward. To our knowledge there is no study reporting the isolation and structural characterization by single-crystal X-ray diffraction of the FeEDDHMA isomers. This has been done for the FeEDDHA isomers (*12*, *13*). As a means to assign HPLC peaks, we considered the UV−vis spectra of peaks. FeEDDHA and FeEDDHMA are very similar molecules, differing just in one single *p*-methyl group and thus showing very similar UV−vis spectra. This supports the identification of the first eluting FeEDDHMA isomer as the racemic one. This is in disagreement with other published results (*4*, *14*). For this reason they will be named henceforth as isomer I and II. In any case, other studies in progress may resolve this controversy (Lucena, personal communication).

Fraction collection also allowed the separation and purification of standards for the quantification of FeEDDHMA, FeEDDHSA, and FeEDDCHA in commercial products, as is shown in **Table 3**.

Table 5 shows the results of soluble and TLC iron in samples, TLC iron relative to soluble iron, and the comparison with HPLC results for HA's, MA1, and CA1. Results from TLC scraped samples show a reasonable agreement with HPLC results. These results point to TLC as an easy, semiquantitative approach for testing commercial chelates. This can be done by direct observation of spots by or scraping and analysis by AAS or UV−vis spectroscopy.

Along with the main spots, TLC showed several other violet and red spots. These spots probably belonged to positional isomers other than the ortho−ortho isomer that were not detected under our HPLC analytical conditions.

Soil Incubation. Table 6 shows the evolution of water-extractable iron as measured by AAS. Time 0 shows iron concentration in milligrams per kilogram on a field capacity moistened soil basis; the rest of the values are expressed as

Table 4. Differences in Chromatographic Responses of FeEDDHA and FeEDDHMA Isomers at Selected Wavelengths (Chromatographic Areas Expressed as mV·s)

FeEDDHA, Isocratic

	chromatographic areas		area equivalent to 1 mg L^{-1} Fe		
wavelength	racemic	meso	racemic	meso	% diff
282	488	501	384	355	7.6
478	218	212	172	150	12.4
487	217	216	171	153	10.3
mg L^{-1} Fe (AAS)	1.27	1.41			

FeEDDHMA, Isocratic

	chromatographic areas		area equivalent to 1 mg L^{-1} Fe		
wavelength	isomer I	isomer II	isomer I	isomer II	% diff
282	476	458	445	420	5.5
285	491	468	459	429	6.4
485	201	184	188	169	10.1
496	198	186	185	171	7.8
mg L^{-1} Fe (AAS)	1.07	1.09			

FeEDDHA, Gradient

	chromatographic areas		area equivalent to 1 mg L^{-1} Fe		
wavelength	racemic	meso	racemic	meso	% diff
282	491	514	387	365	5.7
478	221	218	174	155	11.2
487	217	222	171	157	7.9
mg L^{-1} Fe (AAS)	1.27	1.41			

FeEDDHMA, Gradient

	chromatographic areas		area equivalent to 1 mg L^{-1} Fe		
wavelength	isomer I	isomer II	isomer I	isomer II	% diff
282	480	468	449	429	4.3
285	491	479	459	439	4.2
485	201	189	188	173	7.7
496	197	190	184	174	5.3
mg L^{-1} Fe (AAS)	1.07	1.09			

Table 5. TLC Characterization of Iron Products

product	soluble iron (mg L^{-1})	TLC iron (mg L^{-1})	TLC iron relative to soluble (%)	chelated relative to soluble iron (HPLC, %)
HA2	2.47	1.49	60.3	51.6
HA3	2.43	1.19	49.0	44.0
HA4	2.75	1.50	54.5	46.5
HA5	3.27	1.50	45.9	41.8
HA6	2.60	1.17	45.0	38.7
HA7	2.70	1.43	53.0	45.3
HA8	2.47	1.22	49.4	40.0
HA9	2.15	1.07	49.8	50.2
MIX	2.80	0.52	18.6	14.3
MA1	2.95	2.19	74.2	59.8
CA1	1.97	0.59	29.9	27.6

percentage with respect to time 0, which was considered 100%. Results from triplicate incubations gave very similar results, with RSD below 5% in most cases (RSD maximum 8%). Results were consequently expressed as averages

It is necessary to stress that data presented here derive from an aqueous soil extract. There are several causes than can account for nonextracted iron, such as chelate adsorption on soil components, chelate destruction by microorganisms, and

Table 6. Evolution of Water-Extractable Fe from Soil Incubation by AAS Analysis (nq = nonquantifiable)

	time evolution (days) of water-extractable Fe					
product	0	1	5	12	22	50
HA1	8.02	102.2	102.1	103.6	98.3	87.4
HA2	7.48	72.1	68.3	67.0	63.3	57.6
HA3	7.32	61.0	58.5	55.9	50.2	41.3
HA4	7.89	58.7	57.3	55.0	52.1	45.4
HA5	7.49	51.1	50.3	47.6	44.2	37.0
HA6	7.92	57.3	52.8	51.5	46.8	39.9
HA7	7.76	59.3	55.5	56.2	51.1	44.2
HA8	7.75	55.8	51.0	49.8	46.1	36.4
HA9	8.08	67.9	63.9	60.6	56.1	44.5
avg		60.4	57.2	55.5	51.2	43.3
std dev		6.7	6.3	6.2	6.2	6.7
RSD (%)		11.1	11.0	11.2	12.0	15.5
MIX	7.60	51.2	34.9	22.9	19.9	16.1
SA1	7.25	39.6	38.8	38.3	38.9	36.0
SA2	7.53	28.6	27.4	27.0	26.0	24.4
SA3	7.51	33.7	31.6	31.8	29.6	27.8
SA4	5.43	45.8	44.2	43.0	42.4	40.5
SA5	5.26	58.4	55.6	53.5	54.8	49.1
avg		41.2	39.5	38.7	38.3	35.6
std dev		11.6	11.1	10.3	11.4	9.9
RSD (%)		28.0	28.0	26.5	29.6	27.9
MA1	7.27	91.3	85.8	88.6	78.1	65.3
CA1	5.63	65.0	60.1	56.8	51.0	45.3
DTPA1	8.04	40.2	26.5	20.7	20.8	28.9
EDTA1	7.87	53.4	26.2	7.1	2.3	nq
sulfate	5.64	nq	nq	nq	nq	nq

Table 7. Evolution of Chelated Iron from Soil Incubation by HPLC Analysis

	time evolution (days) of chelated Fe					
product	0	1	5	12	22	50
HA1	8.12	101.5	98.9	98.1	92.3	81.8
HA2	3.86	100.8	98.6	95.7	91.3	77.9
HA3	3.22	100.2	97.5	96.4	90.7	74.3
HA4	3.67	100.5	99.5	96.6	91.5	77.9
HA5	3.13	97.1	97.1	94.7	90.0	73.6
HA6	3.06	99.3	97.3	96.7	91.4	76.8
HA7	3.51	99.9	96.9	96.4	89.3	76.4
HA8	3.10	101.8	97.9	95.4	90.6	73.1
HA9	4.06	99.2	94.3	90.0	82.4	68.8
MIX	1.05	98.6	96.9	93.0	86.3	68.1
avg		99.9	97.5	95.3	89.6	74.9
std dev		1.4	1.4	2.3	3.0	4.2
RSD (%)		1.4	1.5	2.4	3.4	5.6
SA1	1.65	100.6	98.0	98.4	103.2	101.2
SA2	0.98	104.9	102.3	100.2	99.2	94.3
SA3	1.26	96.4	92.4	92.3	87.8	85.1
SA4	1.28	104.5	98.7	100.9	98.4	95.5
SA5	1.54	102.4	100.3	96.4	97.8	95.8
avg		101.8	98.3	97.6	97.3	94.4
std dev		3.5	3.7	3.5	5.7	5.8
RSD (%)		3.4	3.8	3.5	5.9	6.2
MA1	4.35	99.0	95.5	89.6	82.2	61.1
CA1	1.55	99.0	106.0	98.5	91.8	88.9
DTPA1	8.25	32.4	18.7	9.9	5.0	1.4
EDTA1	7.31	51.4	24.3	7.4	2.8	1.8
MIX	4.29	42.9	21.3	5.3	3.4	2.3
avg		47.2	22.8	6.4	3.1	2.1

iron displacement from the chelate and subsequent precipitation of iron as hydroxides.

Water-extractable iron from ferrous sulfate dropped to zero immediately. This is in agreement with the well-known inefficiency of this iron source in alkaline and calcareous soils. Water-extractable iron declined quickly with FeEDTA and FeDTPA. This is in agreement with agronomic experience, where these products have limited or no usefulness (*1*). We know of no explanation for the increment in water-extractable iron of FeDTPA on day 50; in any case, this amount of iron was small in relation to those for other chelates, except FeEDTA. Goos and Germain (*6*) found a similar anomalous behavior of FeDTPA in two kinds of soils, one Ulen sandy loam (*Aeric Calciaquolls*) and a silty loam of unknown classification. This behavior has been explained by rapid FeDTPA soil adsorption followed by slow desorption (*9*) or the decomposition of DTPA into ligand products (*5*).

HA's displayed similar kinetic behavior, with quite small relative standard deviations. There was a steep fall in water extractable iron at time 1 day, but afterward this fall was slower. This may reflect that there was a considerable amount of complexed iron, about 40% that precipitated or was retained quickly under our experimental conditions. This portion of iron would not be useful as a plant iron source in alkaline and calcareous soils such as the one studied. Laboratory made chelate HA1 showed no initial drop in water-extractable iron. This confirms that HA1 purity is near 100%. CA1 kinetics were very similar to those for HA's, but the CA1 FeEDDCHA chelation degree was lower than that in HA's (**Table 3**).

There were important differences among SA's at time 1. This supports the idea that chelation degree differs depending on manufacturer, as revealed by HPLC and AAS (**Table 3**) with values smaller than those for HA's. Later, water-extractable iron kinetics were similar, with a shallow slope.

The initial drop between days 0 and 1 was very small for MA1. After time 1, the water-extractable iron decline was similar to those for HA's and CA1. Water-extractable iron was greater with MA1 at any time.

Table 7 shows the evolution of chelated iron from soil incubation as measured by HPLC. Time 0 shows iron concentration in milligrams per kilogram, and this value was set as 100%; the rest of the values are expressed as percentages with respect to time 0.

The FeEDTA decay of EDTA1 and MIX as measured by HPLC is similar to that displayed by water-extractable iron of EDTA1 measured by AAS. FeDTPA kinetics of DTPA1 differed from those of water-extractable iron measured by AAS, mainly at times 12, 22, and 50. It declines to practically zero levels in the later stages.

HA's showed a remarkable similarity in their kinetics, with relative standard deviation lower than 6% at every time. The FeEDDHA decline was slow and dropped to only about 75% at time 50. These results reflect the great similarity in FeEDDHA chelate soil behavior contained in HA's, as was expected.

SA's also showed a remarkable similarity in their kinetics, with relative standard deviations of less than 6% at every testing time. FeEDDHSA decay was even slower than that of FeEDDHA and dropped to only about 95% at time 50. These results reflect the great similarity in FeEDDHSA chelate soil behavior contained in SA's. However, chelation degree was substantially different among products. Thus, the percents complexed iron will not be sufficient to discriminate among SA products. In any case, water-extractable iron from studied SA's in soil tests was lower than that for HA's.

The FeEDDHMA decline was faster than that of FeEDDHA. Its chelation degree was, in contrast, higher. FeEDDCHA

Table 8. Evolution of Chelated Fe versus Water-Extractable Fe from Soil Incubation Expressed as Percent of Chelated Fe/Water-Extractable Fe (nq = nonquantifiable)

	time (days)					
product	0	1	5	12	22	50
HA1	101.2	100.6	98.0	95.9	95.2	94.7
HA2	51.6	72.0	74.4	73.7	74.4	69.6
HA3	44.0	72.0	73.4	75.8	79.6	78.9
HA4	46.5	79.7	80.8	81.6	81.8	79.9
HA5	41.8	79.6	80.6	83.4	85.2	83.4
HA6	38.6	67.0	71.3	72.5	75.5	74.4
HA7	45.2	76.1	78.9	77.8	79.1	78.1
HA8	40.0	72.7	76.7	76.4	78.7	80.1
HA9	50.2	73.4	74.1	74.6	73.7	77.5
avg	44.8	74.1	76.3	77.0	78.5	77.7
std dev	4.6	4.3	3.5	3.8	3.9	4.2
RSD (%)	10.3	5.8	4.6	4.9	4.9	5.4
SA1	22.8	57.8	57.7	58.3	60.3	64.0
SA2	13.0	47.7	48.5	48.3	49.5	50.0
SA3	16.8	47.8	48.7	48.5	50.0	51.2
SA4	23.5	53.8	52.5	55.4	54.8	55.5
SA5	29.3	51.5	53.1	52.7	52.4	57.0
avg	21.1	51.7	52.1	52.6	53.4	55.5
std dev	6.3	4.3	3.8	4.3	4.4	5.6
RSD (%)	30.0	8.3	7.2	8.3	8.2	10.0
MA	59.8	64.9	66.7	60.6	63.0	56.0
CA	27.6	41.8	48.5	47.8	49.5	54.1
MIX	70.3	73.8	73.2	69.0	68.9	65.9
DTPA1	102.6	82.7	72.3	49.4	24.6	5.2
EDTA1	92.9	89.5	86.4	96.6	110.5	nq

kinetics were intermediate between those of FeEDDHA and FeEDDHSA.

Table 8 shows the evolution of identified chelated Fe versus water-extractable Fe from soil incubations. After a significant initial increase of identified FeEDDHA chelate, its level in regard to water-extractable iron remained quite constant until the end of the experiment, at about 78%. This suggests that there is an amount of unidentified complexed iron with a kinetic behavior similar to that of *o,o*-FeEDDHA. The nature of this or these putative complexes remains unknown to us, but their behavior was comparable to that of *o,o*-FeEDDHA in our soil. SA's showed a similar behavior.

There was a small percentage of unidentified complexed iron in EDTA1. This percentage increased considerably at times 22 and 50. We think this is due to analytical errors, because iron concentrations in samples were near or even below the AAS detection limit. We were not able to explain the decline in percent FeDTPA/water-extractable iron values.

Table 9 shows the results of time evolution of FeEDDHA and FeEDDHMA isomers during soil incubation. *meso*-FeEDDHA was less stable than racemic (67.9 vs 82.3% remaining at time 50). The small relative standard deviations for each testing time and each product reflect the great similarity of the FeEDDHA chelated portion of all the products. FeEDDHMA isomers showed the same pattern. Isomer I FeEDDHMA was more stable than isomer II (67.5 vs 55.4% remaining at time 50).

On the basis of these data, it is possible to classify the products that we had studied according to several parameters. If we take in consideration chelation degree (the percentage of iron present in the form of label-declared chelate, viz. FeEDTA, FeDTPA, and the *o,o*-isomers of FeEDDHA, FeEDDHMA, FeEDDHSA, and FeEDDCHA), the order might be

Table 9. Evolution of FeEDDHA and FeEDDHMA Isomers during Soil Incubation

	time evolution (days)					
product	0	1	5	12	22	50
	rac-o,o-FeEDDHA Isomer (%)					
HA1	100	102.3	100.4	100.6	95.8	89.2
HA2	100	104.9	102.8	100.7	97.4	87.9
HA3	100	102.9	100.6	100.0	95.0	82.7
HA4	100	102.8	102.3	100.3	95.9	85.6
HA5	100	97.8	98.6	97.0	92.9	80.4
HA6	100	99.2	98.7	98.9	94.8	84.3
HA7	100	99.5	98.0	98.0	91.9	83.5
HA8	100	103.1	99.3	96.2	92.1	78.8
HA9	100	98.2	92.7	87.4	79.6	67.6
MIX	100	107.6	105.2	103.5	97.7	82.6
avg	100	101.8	99.9	98.3	93.3	82.3
std dev		3.1	3.4	4.4	5.2	6.0
RSD (%)		3.1	3.4	4.4	5.6	7.3
	meso-o,o-FeEDDHA Isomer (%)					
HA1	100	100.7	97.5	95.7	88.8	74.3
HA2	100	97.3	94.9	91.5	85.9	69.1
HA3	100	97.5	94.6	92.8	86.4	66.0
HA4	100	98.3	96.9	93.0	87.4	70.5
HA5	100	96.5	95.7	92.6	87.4	67.6
HA6	100	99.4	96.0	94.6	87.9	69.3
HA7	100	100.2	95.9	95.0	87.0	70.1
HA8	100	100.4	96.5	94.5	89.0	67.1
HA9	100	100.5	96.2	93.1	85.9	70.3
MIX	100	90.3	89.2	83.2	75.7	54.6
avg	100	98.1	95.3	92.6	86.1	67.9
std dev		3.1	2.3	3.5	3.8	5.2
RSD (%)		3.2	2.4	3.8	4.4	7.7
	I and II *o,o*-FeEDDHMA Isomers (%)					
isomer I	100	99.5	95.9	91.4	85.5	67.5
isomer II	100	98.6	95.2	87.9	79.3	55.4

$$\text{DTPA1} > \text{EDTA1} > \text{MA1} > \text{HA's} > \text{SA's} \approx \text{CA1}$$

Regarding soil water-extractable iron as measured by AAS, the order of the products might be the following

$$\text{MA1} > \text{HA's} \approx \text{CA1} > \text{SA's} > \text{DTPA1} > \text{EDTA1}$$

Evidently, both these classifications will vary as a function of the quality of the manufacturing process.

Finally, on the basis of the capacity of label-declared chelate to withstand soil conditions, the order would be

$$\text{FeEDDHSA} > \text{FeEDDCHA} > \text{FeEDDHA} > \text{FeEDDHMA} > \text{FeEDTA} \approx \text{FeDTPA}$$

This last classification has general validity, since it does not depend on the chelation degree but on the chemical nature of each chelate and soil characteristics.

This result indicates that SA's and CA's might be good or even better alternatives to HA's and MA's were manufacturers able to supply commercial products with the same or higher chelation degree as that of commercial HA's and MA's. It is necessary, however, to stress that only one commercial product has been tested for FeEDDHMA, FeEDDCHA, and FeDTPA.

Other factors that have not been addressed in this work, such as plant iron bioavailability from different sources or price, along with the above considerations, could be used as guidelines for the selection of the best chelate. In any case, these results show the suitability of this soil incubation methodology. The study of water-extractable iron and iron chelates over time (1 to 50 days) in soil at field capacity is a useful tool for evaluating the

potential of iron products to supply iron to plants under adverse soil conditions.

At present the lack of easy analytical tests has made the evaluation of the true quality of HA's and HA analogue chelates difficult. We suggest that TLC could be a useful and cheap safeguard against gross frauds and also a means for semiquantitative comparisons of HA's, MA's, and CA's.

The precise identification and quantification of iron chelates can only be accomplished at present by HPLC (*2*, *4*, *15*). This technique, however, is not always available at agronomic laboratories. In addition, satisfactory standards were not commercially available when this work was completed. FeEDDHA and FeEDDHMA standards can be laboratory prepared from commercial EDDHA and EDDHMA, but this may add some degree of uncertainty regarding standard purity and concentration, as these parameters rely on supplier quality and laboratory ability to accurately measure iron. Moreover, to our knowledge, there are no available standards of EDDHSA and EDDCHA. Regulatory bodies, manufacturers, and researchers need these standards as a requisite for any kind of quantitative work.

A possible way to circumvent this problem might be the commercialization of ferric chelate standards made by subsampling commercial iron chelate batches analyzed and certified with HPLC coupled to UV and ICP/MS detection. These samples would have a definite amount of iron associated to each chromatographic peak, even those of the optical isomers. These standards could be used to greater advantage than the laboratory-made standards in terms of reliability for HPLC chelate analysis.

ABBREVIATIONS

AAS, atomic absorption spectroscopy (flame); HPLC, high performance liquid chromatography; MS, mass spectrometry; TLC, thin-layer chromatography; EDDHA, *N*,*N*′-ethylenediamine-di-(*o*-hydroxyphenylacetic acid); EDDHSA, *N*,*N*′-ethylenediamine-di-(*o*-hydroxy-p-sulfoxyphenylacetic acid); EDDHMA, *N*,*N*′-ethylenediamine-di-(*o*-hydroxy-*p*-methylphenylacetic acid); EDDCHA, *N*,*N*′-ethylenediamine-di-(5-carboxy-2- hydroxyphenylacetic acid); EDTA, ethylenediaminetetraacetic acid; DTPA, diethylenetriaminepentaacetic acid; FeEDDHA, ferric EDDHA chelate; FeEDDHSA, ferric EDDHSA chelate; FeEDDHMA, ferric EDDHMA chelate; FeEDDCHA, ferric EDDCHA chelate; FeEDTA, ferric EDTA chelate; FeDTPA, ferric DTPA chelate; HA, commercial product containing FeEDDHA; SA, commercial product containing FeEDDHSA; MA, commercial product containing FeEDDHMA; CA, commercial product containing FeEDDCHA.

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