

Influence of pH, Iron Source, and Fe/Ligand Ratio on Iron Speciation in Lignosulfonate Complexes Studied Using Mössbauer Spectroscopy. Implications on Their Fertilizer Properties

Jaime Carrasco,[§] Krisztina Kovács,[#] Viktória Czech,[‡] Ferenc Fodor,[‡] Juan J. Lucena,^{*,§} Attila Vértes,^{#,†} and Lourdes Hernández-Apaolaza[§]

[§]Agricultural Chemistry Department, Universidad Autónoma de Madrid, Francisco Tomás y Valiente No. 7, 28049 Madrid, Spain

[#]Laboratory of Nuclear Chemistry, Institute of Chemistry, Eötvös Loránd University, Pázmány Péter lane 1/A, 1117 Budapest, Hungary

[‡]Department of Plant Physiology and Molecular Plant Biology, Eötvös Loránd University, Pázmány Péter lane 1/C, 1117 Budapest, Hungary

ABSTRACT: Iron chlorosis is a very common nutritional disorder in plants that can be treated using iron fertilizers. Synthetic chelates have been used to correct this problem, but nowadays environmental concerns have enforced the search for new, more environmentally friendly ligands, such as lignosulfonates. In this paper, Fe coordination environment and speciation in lignosulfonate (LS) complexes prepared under different experimental conditions were studied by ⁵⁷Fe Mössbauer spectroscopy in relation to the Fe-complexing capacities, chemical characteristics of the different products, and efficiency to provide iron in agronomic conditions. It has been observed that the complex formation between iron and lignosulfonates involves different coordination sites. When Fe²⁺ is used to prepare the iron–LS product, complexes form weak adducts and are sensitive to oxidation, especially at neutral or alkaline pH. However, when Fe³⁺ is used to form the complexes, both Fe²⁺ and Fe³⁺ are found. Reductive sugars, normally present in lignosulfonates, favor a relatively high content of Fe²⁺ even in those complexes prepared using Fe³⁺. The formation of amorphous ferrihydrite is also possible. With respect to the agronomical relevance of the Fe²⁺/Fe³⁺ speciation provided by the Mössbauer spectra, it seems that the strong Fe³⁺–LS complexes are preferred when they are applied to the leaf, whereas root uptake in hydroponics could be more related with the presence of weak bonding sites.

KEYWORDS: iron, lignosulfonates, complexes, Mössbauer spectroscopy

■ INTRODUCTION

Iron chlorosis is a common nutritional disorder of many susceptible crops growing on calcareous and/or alkaline soils covering >30% of the overland surface.¹ In such soils, calcium carbonate buffers soil solution pH in the range 7.5–8.5,² and it causes a high bicarbonate concentration,³ which means a decrease in the uptake and transport of iron to the plant.^{4,5} This agricultural problem is widespread in the Mediterranean basin; it is estimated that 20–50% of fruit crops are affected by iron deficiency,⁶ decreasing the development and productivity of plants, even prompting plant death under severe iron deficiency. Intervenal leaf yellowing in the young leaves, which is the most characteristic visual symptom, is due to the decrease of leaf photosynthetic pigment concentration, and it can be corrected through applying iron fertilizers.⁷

To cope with this problem, synthetic Fe chelates applied in soil or as foliar spray are the most effective solution,⁸ even though they are expensive and in most cases scarcely degradable. Nowadays, some new biodegradable chelates are under study.^{9–11} Natural Fe complexes, which are cheaper alternatives and environmentally friendlier than synthetic chelates, are being analyzed.^{10,12–17} It is well-known that their efficiency depends not only on their complexing capacity but also on other factors such as their foliar penetration capacity or their stability in soils.¹⁸ EU Directives 2003/2003 and 162/2007 include the authorized list of chelating agents for soil applications;

meanwhile, the one for the complexing agents is still pending elaboration, and only the incorporation of lignosulfonates (LSs) to the European legislation has been requested. Humic and fulvic acids, gluconic acids, amino acids, citric acid, heptagluconates, and LSs are recognized as ligands by Spanish legislation¹⁹ to cope with micronutrient deficiencies.

LSs are pulp and paper industrial byproducts obtained during the sulfite pulping process, in which lignin is broken down and fragments are made water-soluble by the incorporation of sulfonic groups in its structure. The exact structure of LSs is unknown. Due to their natural and polymeric origin from lignin adducts, they contain a large number of functional groups such as hydroxyphenolic, carboxylic, and sulfonic groups in their molecules, which can form coordinate bonds with metal ions. It is also remarkable that LSs usually have a variable quantity of reductive sugars in their composition.^{20,21} Although the use of lignosulfonates as Fe fertilizers is quite widespread, the parameters that affect their efficacy are not well-known. Moreover, different lignosulfonate/Fe³⁺ sources and application methods (root or foliar applications) provide different results on Fe uptake and distribution in plants.^{14,18} Also, the role of Fe²⁺

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Table 1. Physicochemical Characteristics of the Tested Lignosulfonates

	spruce LS ^a	eucalyptus LS ^a	std 1	std 2	std 3	std 4
pH	3.6	4.3	5.4	7.0	3.5	4.0
total S (g kg ⁻¹ DW ^b)	60	59	54	62	e	53
phenolic -OH (g kg ⁻¹ DW) ^c	19	19	21	18	e	15
COOH (g kg ⁻¹ DW)	26	35	32	46	e	41
DW (g kg ⁻¹)	580	510	988	920	e	991
LS content _{232.5 nm} (kg ⁻¹ DW)	830	587	976	817	633	479
av <i>M_w</i> (g mol ⁻¹) ^d	25732	6275	52000	52000	54000	18000
total sugars (g/kg DW)	27	218	nd ^f	40	200	160

^aBenedicto et al.;¹⁵ Rodríguez-Lucena et al.¹⁸ ^bDW, dry weight. ^cPrEN1603:2010(29). ^dFredheim et al.³⁰ ^eData not available. ^fnd: not determined.

complexed in LSs has arisen.¹⁸ As in chelates,⁸ the chemical binding between Fe and the lignosulfonate should play an important role in Fe uptake and translocation. Then, knowledge of the Fe surrounding in the LS-Fe³⁺ complex is a key point in the studies (1) on their efficacy as Fe fertilizers, (2) for the selection of the most appropriate lignosulfonate sources, and (3) on the modifications of lignosulfonates to obtain products of optimum efficacy.

Inasmuch as ⁵⁷Fe Mössbauer spectroscopy is a powerful, nondestructive method for analyzing iron-containing samples, giving detailed information about the immediate electronic and magnetic environment of the Fe nuclei; it has been applied in the structural studies of many thousands of iron complexes. The technique has also been used to determine the chemical speciation of iron in plants or in different plant-related compounds.^{23–28} Therefore, Mössbauer spectroscopy seems to have a great potential to evaluate the coordination sphere as well as the oxidation state of iron in these complexes and as a tool to evaluate the quality of the lignosulfonates complexes used as Fe fertilizers in agriculture.

The aim of this work was to study the Fe³⁺/Fe²⁺ species formed in Fe-LS complexes, using ⁵⁷Fe Mössbauer spectroscopy. The effects of different parameters, such as iron source (Fe²⁺ or Fe³⁺), pH, and Fe/LS ratio, were also studied to improve the preparation of these complexes. This could increase the stability and quality of this type of fertilizer and presumably the amount of Fe provided to the plants when these products are applied. The relevance of the different chemical Fe species in the effectiveness of the fertilizer products will be discussed.

MATERIALS AND METHODS

Reagents. All reagents used were of recognized analytical grade, and solutions were prepared with type I grade water according to ISO 3696:1987,²⁹ free of organic contaminants (Millipore, Bedford, MA, USA). The standard LSs, solid formulations, were obtained from Sigma-Aldrich: lignosulfonic acid, sodium salt (ref 370975) (standard 1, std 1); lignosulfonic acid, sodium salt (ref 471038) (standard 2, std 2); lignosulfonic acid, sodium salt, sugared (ref 471011) (standard 3, std 3); and lignosulfonic acid, calcium salt (ref 471054) (standard 4, std 4). Their physicochemical characteristics (see methodologies below) are shown in Table 1.

Two commercial products, as liquid formulations, were also tested: a softwood spruce lignosulfonate (spruce LS) and a hardwood eucalyptus lignosulfonate (eucalyptus LS). All of the products were generously provided by LignoTech Iberica S.A. (Torrelavega, Cantabria, Spain), and their physicochemical characteristics, also shown in Table 1, were previously described.^{10,14,15}

Characterization of the Tested Products. The pH was determined in a 100 g standard or commercial product per liter of solution by using a pH-meter model Orion Research Expandable Ion Analyzer EA920. The dry weight content of products was evaluated by drying 0.2 g of sample in a stove at 60 °C until it reached constant

weight. The quantitative determination of LS content was carried out by UV-vis technique³⁰ employing a Shimadzu UV-160A spectrophotometer. In short, 0.15–0.20 g of the products was dissolved in deionized water; 4.0 g of a previously conditioned cation exchange resin Dowex 50WX8 and approximately 20 mL of deionized water were added with magnetic stirring for 20 min, allowing the ion-exchange process to take place. Then the solution was filtered into a 250 mL volumetric flask with cellulose membranes (Filter-Lab 1250) to remove the resin, and it was diluted to the mark with deionized water. A 10 mL aliquot was taken, the pH was adjusted between 4.0 and 5.0, and the solution was made up to 100 mL volume. Finally, the absorbance at 232.5 nm was measured. Sulfur, OH phenolic,³⁰ and carboxylic group contents and molecular weight³¹ were also determined.

The sugars content of the LSs can play an important role in iron speciation because of their reducing capacity and their ability to form weak bonds with iron. The sugars content of the standard and commercial products is shown in Table 1. These data were provided by Lignotech Iberica S.A.

Complexing Capacity with Fe²⁺ and Fe³⁺ of the Tested Products. For the determination of the Fe-complexing capacities, the titration method described by Villén et al. was employed.^{32,33} In short, increasing volumes of a 200 g L⁻¹ solution of FeSO₄·7H₂O for Fe²⁺ and FeCl₃·6H₂O for Fe³⁺ were added to 20 mL of the ligand solutions (100 g L⁻¹). After the addition of two drops of H₂O₂ (33% w/v), the pH was raised to 9.0 with NaOH. After 1 day of standing in dark, the pH was increased again to 9.0, and 2 h later solutions were transferred to a 100 mL volumetric flask and the volume was made up to 100 mL. Afterward, the solutions were centrifuged at 7500 rpm (at 4716g) at room temperature for 10 min, and the supernatants were filtered using 0.45 μm filters (Millipore). The complexed element was determined by AAS (AAAnalyst 800; Perkin-Elmer, Shelton, CT, USA) using 0.5% La, 0.2% Cs, and 5% HCl as matrix modifier and after removal of the organic compound in accordance with method 9.3 (EU Directive 2003/2003)³⁴ using H₂O₂ (33% w/v) and 0.5 M HCl for the digestion.

Preparation of the Fe-LS Complexes for Mössbauer Spectroscopy. Two types of sample preparation were done; the first one consisted of the freeze-drying of the Fe-LS formulations prepared with nonlabeled iron and the second one, prepared with ⁵⁷Fe isotope, applying a rapid-freezing (quenching) method.

The complexes for the freeze-drying measurements (solid samples) were prepared either with Fe²⁺ or Fe³⁺ salt to test the influence of the iron source on the complexing capacity of these compounds. Solutions of Fe-LS complexes were prepared by mixing an aliquot of a 200 g Fe L⁻¹ FeSO₄ or FeCl₃ solution with the suitable amount of product, calculated on the basis of their highest complexing ability. For the standards, the solid was previously dissolved in deionized water at pH 7.0 (adjusted with NaOH or HNO₃). The product/Fe ratio varied among 1.1:1, 1.5:1, and 2:1 according to the maximum complexing capacity data, where 1:1 product/Fe ratio is equal to the maximum capacity of binding. Then, the pH was set to 7.0 or 4.0, and the solutions were allowed to stand overnight. Finally, the pH was readjusted to 7.0 or 4.0 and diluted to final volume with Milli-Q water. The final concentration of the solutions was 10 g Fe L⁻¹. The samples were freeze-dried using a Thermo Scientific freeze-dryer model Heto PowerDry LL3000.

The samples obtained by the application of the rapid-freezing (quenching) method (liquid samples) were prepared according to the same procedure described above applying ^{57}Fe -enriched (Isoflex, 95.70% isotopic enrichment), with either $^{57}\text{FeSO}_4$ or $^{57}\text{Fe}(\text{NO}_3)_3$ salt from 0.1 M stock solutions. The final concentration of ^{57}Fe in the solutions was set to 0.015 M with deionized water. Any precipitates formed were removed by centrifuging the solution at 7500 rpm (4716g) at room temperature for 10 min. Four hundred microliters of the resulting supernatant was measured by Mössbauer spectroscopy after application of the rapid-freezing (quenching) method.³⁵

Mössbauer Spectroscopy. Mössbauer measurements were performed at $T = 80\text{ K}$ with a ^{57}Co (Rh) source of $\sim 10^9\text{ Bq}$ activity. The spectrometer (WissEl, Germany) was calibrated with α -iron at room temperature. The spectrum evaluation was carried out with the assumption of Lorentzian line shape using Mosswin 4.0 software,³⁶ and the parameters calculated for the spectral components were isomer shift (δ , mm s^{-1}), quadrupole splitting (Δ , mm s^{-1}), line width (full width at half-maximum, Γ , mm s^{-1}), and partial resonant absorption areas (S_p , %). These principal parameters obtained from the spectrum provide information on the electron densities at the Mössbauer nuclei (indicating the valence state), on the magnitude of any electric field gradients (providing valuable information about the coordination number of the resonant atom). The quantitative analytical information for the species can be obtained from the relative spectral areas.²²

RESULTS AND DISCUSSION

Characterization of the Tested Products. The LS content for the tested products is presented in Table 1. Std 1 has the highest LS content, whereas std 4, the LS–calcium salt, is the one with the lowest LS content. Moreover, sulfur and $-\text{COOH}$ contents are higher in the case of std 2, whereas std 1 is the one with the highest amount of hydroxyphenolic groups. The high proportion of total sugars in the eucalyptus LS, std 3, and std 4 is also remarkable, which can increase the reductive abilities of this type of product. For the commercial LS products (spruce and eucalyptus LS) the above data have already been published.^{15,18}

Although the structure of LSs is not clearly known, due to their polymeric nature as lignin derivate products, they have a wide variety of functional groups such as aliphatic, aromatic, sulfonic, carboxylic, and hydroxyl groups, which are able to form complexes with metal ions, for example, Zn^{2+} , Fe^{2+} , and Fe^{3+} . For example, Figure 1 shows the titration curves obtained for the calculation of the Fe-complexing capacity of one of the LSs tested (std 1) with either Fe^{2+} or Fe^{3+} . The iron that remains in solution at pH 9.0, that is, complexed, is represented versus the Fe added. In all cases, the graphs present a rising segment, which would correspond to the Fe-complexing process by the LSs. This segment was followed by a decreasing one, probably due to the coagulation of material by an excess of metal.³² The intersection of the two segments represents the highest complexing capacity point.

Table 2 shows the amount of Fe complexed by each LS at the maximum complexing capacity (MCC) calculated from the titration curves and the LS DW contents shown in Table 1. For all of the products, the MCC with regard to Fe^{3+} was higher than that for Fe^{2+} . This could be related to a higher stability of the Fe^{3+} –LS complex compared to Fe^{2+} –LS. However, a certain amount of the Fe^{2+} added to the complexing agent may be oxidized in air, which could lead to the formation of iron oxides and hydroxides that decrease the soluble amount of the metal. An oxygen-free atmosphere may be required to complete a successful complexation process. No significant differences among the standard LSs could be observed due to their pH,

functional group content, sugar content, or molecular weight (Table 1).

Mössbauer Characterization of the Complexes. *Effect of the Iron Source and LS Products Used To Form the Complex.* The LS complexes were formed by the addition of either Fe^{2+} or Fe^{3+} inorganic salts to the lignosulfonate product, at pH 4.0 or 7.0 and at different LS/Fe ratios. Mössbauer spectra of solid Fe^{2+} –LS complexes prepared at pH 4.0 and 1.1:1 LS/Fe ratio are shown in Figure 2. The spectra can be evaluated by assuming three or four doublets (according to their Mössbauer parameters, one Fe^{3+} and two or three Fe^{2+} components, respectively) with Mössbauer parameters listed in Table 3. The Mössbauer parameters of the components show a wide variety depending on the chemical composition of the LS products; however, no direct correlation could be found between the iron components present in the product according to the Mössbauer spectra and the physicochemical characteristics (or MCC value) of the different LS products tested. The Mössbauer parameters of Fe^{3+} components fall into a range of $\delta = 0.46\text{--}0.55\text{ mm s}^{-1}$ and $\Delta = 0.45\text{--}0.78\text{ mm s}^{-1}$, whereas the Fe^{2+} components have $\delta = 1.15\text{--}1.35\text{ mm s}^{-1}$ and $\Delta = 2.20\text{--}3.40\text{ mm s}^{-1}$.

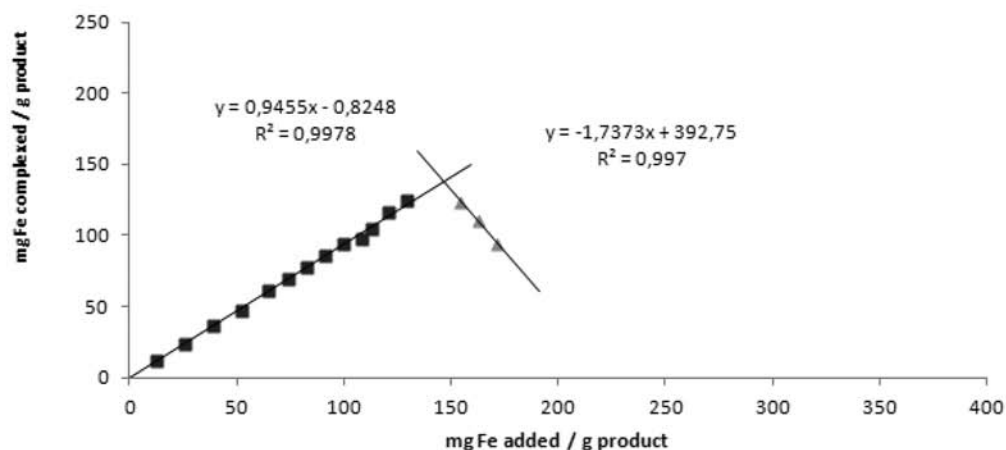
The Mössbauer parameters of the Fe^{2+} components are in good agreement with those of high-spin Fe^{2+} in octahedral O_6 coordination, which may indicate complex formation between the Fe^{2+} ions and $-\text{COO}^-/-\text{OH}/-\text{SO}_3^-$ groups of the LSs. However, one of the Fe^{2+} species with parameters of $\delta \sim 1.3\text{ mm s}^{-1}$ and $\Delta \sim 3.4\text{ mm s}^{-1}$ could be assigned to $[\text{Fe}(\text{H}_2\text{O})_x]^{2+}$ type complexes,³⁵ which suggests that a minor part of Fe^{2+} in the solid Fe^{2+} –LS complexes is only partly coordinated to the functional groups of the LS compounds and has H_2O groups in the first coordination sphere. This can represent very weakly bound $\text{Fe}^{2+}_y-(\text{H}_2\text{O})_x$ –LS adducts and confirms that the coordination of divalent iron to LS compounds is not really favored, as was also suggested by the MCC data.

Besides the Fe^{2+} components, a high amount of Fe^{3+} can be observed even at pH 4.0 which shows that some part of the Fe^{2+} is oxidized to Fe^{3+} possibly due to the oxygen content of air during preparation. This finding confirms that part of the divalent iron attached to the LS ligands does not form a strong Fe^{2+} –LS complex and, thus, it is sensitive to oxidation.

Although the relative abundance of the Fe^{2+} components depends on the structure of the LS product applied (Figure 3), further significant changes can be found when different conditions were applied during the preparation (pH, Fe/ligand ratio, see below). By comparison of the total relative amount of Fe^{2+} found in the different Fe^{2+} –LS samples, it can be seen that the highest Fe^{2+} content could be observed in the case of Fe^{2+} –std 3 LS complex, whereas the lowest Fe^{2+} content was found in the case of Fe^{2+} –std 1 LS complex. The Fe^{2+} content may be influenced by the reductive components present in the LS products, for example, the total sugar content (Table 1). However, other structural units also play an important role in the redox reactions $\text{Fe}^{2+}/\text{Fe}^{3+}$ because the Fe^{2+} –eucalyptus LS has a much lower Fe^{2+} amount, whereas Fe^{2+} –std 2 and spruce LS contain much higher Fe^{2+} than expected according to their total sugar content. These results indicate that the redox properties of the LS products are influenced by several factors; besides the reductive sugar content, the complex formation with other functional groups (e.g., phenolic $-\text{OH}$, $-\text{COOH}$ contents) can also modify it.

The Mössbauer parameters of Fe^{3+} compounds formed during the preparation of Fe^{2+} –LS complexes (Table 3) are very typical of high-spin Fe^{3+} in distorted octahedral O_6 coordination.

Standard 1 (Fe(II))



Standard 1 (Fe(III))

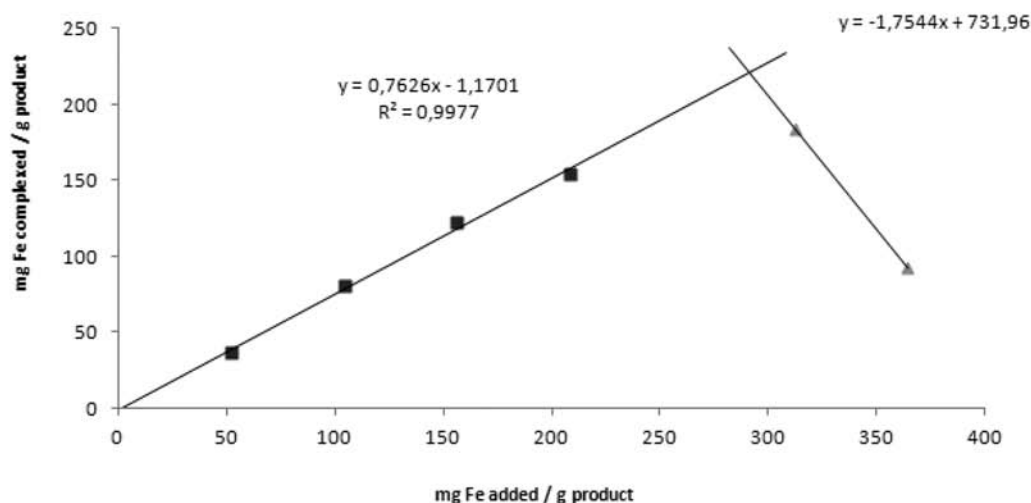


Figure 1. Titration curve for the determination of the maximum complexing capacity (MCC), as the iron needed to reach the intersection point, of standard 1 with Fe^{3+} and Fe^{2+} .

Table 2. Maximum Fe-Complexing Capacity (MCC) of Tested Products Obtained by Titration with Fe^{3+} or Fe^{2+} Salts³²

product	mg Fe^{2+} g ⁻¹ LS DW ^a	mg Fe^{3+} g ⁻¹ LS DW
standard 1	143 ± 12	229 ± 4
standard 2	183 ± 6	302 ± 3
standard 3 ^b	175 ± 21	324 ± 3
standard 4	188 ± 43	254 ± 20
spruce LS	178 ± 26	243 ± 9
eucalyptus LS	180 ± 11	287 ± 2

^aDW, dry weight. ^bAn average value of 976 g kg⁻¹ DW was used.

It is not possible to determine unequivocally from the measurements done only at $T = 80$ K if the Fe^{3+} is coordinated to O-donor groups of LS ligands or is partly forming amorphous hydrous ferric oxides (e.g., ferrihydrite) with various stoichiometries. The

relatively large line width ($\Gamma > 0.55$ mm s⁻¹) of the Fe^{3+} component suggests the presence of both a Fe^{3+} -LS complex (where $-\text{COO}^-/-\text{OH}/-\text{SO}_3^-$ groups of the LS can all take part in the coordination) and an amorphous ferrihydrite ($\text{Fe}(\text{OH})_x(\text{H}_2\text{O})_y$) compound.

For the LSs prepared by the addition of a Fe^{3+} salt, the Mössbauer spectra taken at $T = 80$ K of the solid Fe^{3+} -eucalyptus LS product are shown in Figure 4, and the corresponding parameters calculated are depicted in Table 4. The evaluation of the Mössbauer spectra shows again the presence of both Fe^{3+} and Fe^{2+} compounds. The occurrence of Fe^{2+} components can be well understood by taking into account the reductive effect of the LS products. The highest relative amount of the reduced Fe^{2+} component can be observed in the case of eucalyptus LS, std 3, and std 4 (39, 36, and 36%, respectively) with high sugar content (218, 200, and 160 g sugar kg⁻¹ DW, respectively). This finding suggests that the total sugar content

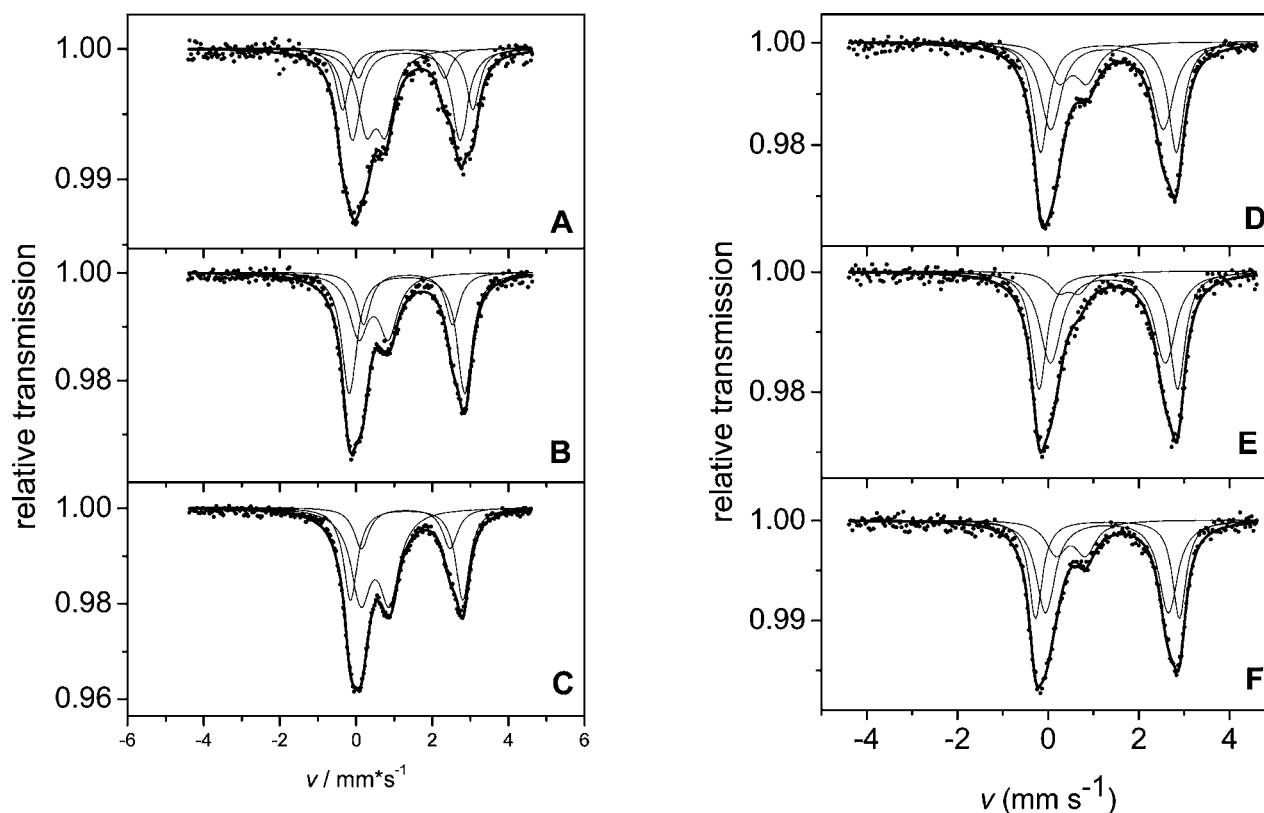


Figure 2. Mössbauer spectra taken at $T = 80$ K of solid Fe^{2+} –LS product samples prepared with (A) eucalyptus LS, (B) spruce LS, (C) std 1, (D) std 2, (E) std 3, and (F) std 4 at 1.1:1 LS/Fe ratio and at pH 4.0.

Table 3. Mössbauer Parameters ($T = 80$ K) of Solid Fe^{2+} –Product Samples Prepared with Eucalyptus LS, Spruce LS, Std 1, Std 2, Std 3, and Std 4 at 1.1:1 Product/Fe Ratio and at pH 4.0^a

	eucalyptus LS	spruce LS	std 1	std 2	std 3	std 4
Fe^{3+}						
S_r	32	31	48	18	9	17
δ	0.53(1)	0.46(1)	0.50(1)	0.55(2)	0.46(4)	0.50(2)
Δ	0.50(2)	0.78(3)	0.75(2)	0.61(2)	0.45(1)	0.65(2)
Γ	0.56(3)	0.59(2)	0.63(1)	0.57(4)	0.51(7)	0.54(4)
Fe^{2+}						
S_r	10	20	17	42	48	
δ	1.19(4)	1.37(2)	1.31(2)	1.30(1)	1.32(1)	
Δ	2.26(7)	2.34(3)	2.33(4)	2.48(3)	2.53(5)	
Γ	0.4(1)	0.44(3)	0.48(3)	0.55(2)	0.55(3)	
Fe^{2+}						
S_r	21	49	35	40	43	36
δ	1.36(1)	1.34(1)	1.33(1)	1.33(1)	1.33(1)	1.31(1)
Δ	3.42(4)	3.03(2)	2.95(2)	2.99(2)	3.05(3)	3.17(2)
Γ	0.42(4)	0.47(1)	0.44(1)	0.41(2)	0.41(2)	0.36(2)
Fe^{2+}						
S_r	37					47
δ	1.32(1)					1.30(1)
Δ	2.82(4)					2.71(4)
Γ	0.50(5)					0.50(2)

^a S_r in percentage, partial resonant absorption areas; δ in mm s^{-1} , isomer shift; Δ in mm s^{-1} , quadrupole splitting; Γ in mm s^{-1} , line width. Errors in the last digits are given in parentheses; for S_r (%) relative error is $\leq 5\%$.

of the LS products plays an important role in the reduction of Fe^{3+} . The Mössbauer parameters of the reduced Fe^{2+} component are in the same range as found for Fe^{2+} complexes, indicating a complex formation between the reduced Fe^{2+} and the LS product.

Mössbauer parameters of the Fe^{3+} compound also suggest a similar coordination sphere of Fe^{3+} as found in the case of Fe^{2+} –LS. They can be assigned to both Fe^{3+} –LS and $\text{Fe}^{3+}(\text{OH})_x(\text{H}_2\text{O})_y$ species. Of course, the relative occurrence of the Fe^{3+} is higher than in the Fe^{2+} –LS complexes.

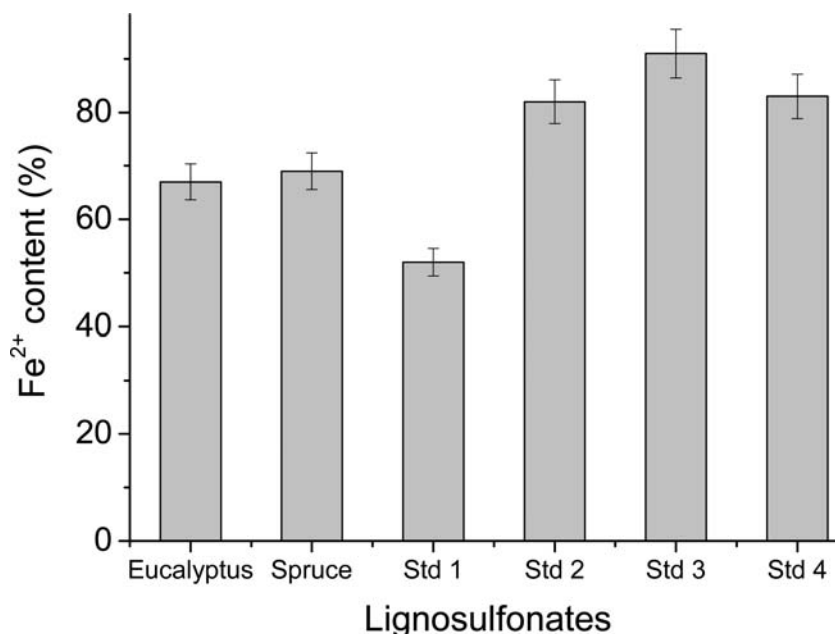


Figure 3. Relative content of Fe²⁺ species in the case of different Fe²⁺–LS product compounds (prepared with 1.1:1 product/Fe ratio and at pH 4.0) according to their Mössbauer spectra taken at $T = 80$ K. Relative errors are $\leq 5\%$.

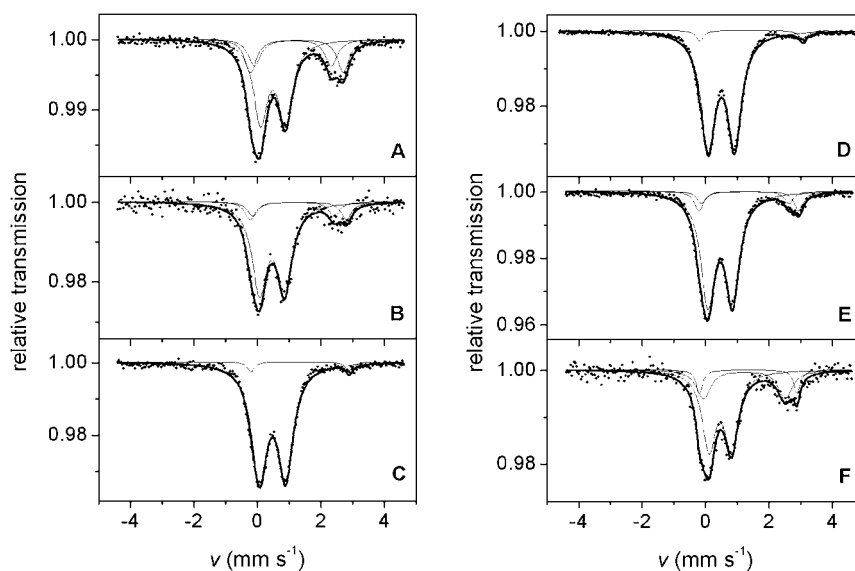


Figure 4. Mössbauer spectra taken at $T = 80$ K of solid Fe³⁺–LS product samples prepared with (A) eucalyptus LS, (B) spruce LS, (C) std 1, (D) std 2, (E) std 3, and (F) std 4 at 1.1:1 product/Fe ratio and at pH 4.0.

Comparing the different standard and commercial LS compounds, the Mössbauer spectra are very similar and no correlation can be found between the Fe³⁺ species and the structural differences of the LS ligands.

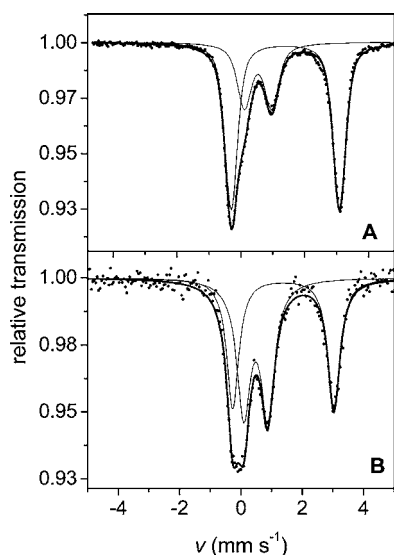
To get information about Fe speciation in the solution, Mössbauer spectra of the frozen solutions (after removal of the precipitate formed during preparation) of Fe²⁺ and Fe³⁺–eucalyptus LS samples were also taken (Figure 5). The corresponding Mössbauer parameters are depicted in Table 5. The components found in the spectra of the solutions are different from those of the solid samples. Namely, these spectra can be evaluated suggesting only two iron components, a Fe²⁺ and a Fe³⁺, respectively. The Fe²⁺ components present in the Fe²⁺ complex solutions prepared at different conditions have almost the same isomer shifts and quadruple splittings ($\delta \sim 1.4$ mm s⁻¹,

$\Delta \sim 3.4$ mm s⁻¹). They are in good agreement with those calculated for the [Fe(H₂O)₆]²⁺ complex present in ⁵⁷FeSO₄ solutions³⁵ and with the parameters measured for the original ⁵⁷FeSO₄ solution used in the preparations (spectrum not shown). This indicates that Fe²⁺ is not directly coordinated to the LS ligands in water solution and also explains the weak coordination of Fe²⁺ in the solid Fe²⁺–LS complexes discussed previously. The parameters of the Fe³⁺ components are similar to those obtained in the case of the solid Fe³⁺ complexes, so a similar coordination of Fe³⁺ in water solution compared to the solid Fe³⁺ complexes can be suggested. This is in good agreement with the preferred complex formation between Fe³⁺ and LS ligands indicated before, compared to Fe²⁺. The occurrence of the Fe³⁺ doublet in the frozen solution Mössbauer spectra also indicates that Fe³⁺–LS complexes have dimeric/oligomeric

Table 4. Mössbauer Parameters ($T = 80$ K) of Solid Fe^{3+} –Product Samples Prepared with Eucalyptus LS, Spruce LS, Std 1, Std 2, Std 3, and Std 4 at 1.1:1 Product/Fe Ratio and at pH 4.0^a

	eucalyptus LS	spruce LS	std 1	std 2	std 3	std 4
Fe^{3+}						
S_r	61	79	96	95	64	64
δ	0.49(1)	0.46(1)	0.48(2)	0.50(1)	0.49(1)	0.46(1)
Δ	0.78(1)	0.79(1)	0.82(1)	0.83(1)	0.77(1)	0.71(1)
Γ	0.54(1)	0.56(2)	0.58(1)	0.53(1)	0.51(1)	0.52(2)
Fe^{2+}						
S_r	17	9			6	26
δ	1.12(2)	1.14(6)			1.16(2)	1.22(2)
Δ	2.41(3)	2.6(2)			2.44(7)	2.58(5)
Γ	0.45(2) ^b	0.40(6) ^b			0.34(7)	0.54(7)
Fe^{2+}						
S_r	22	12	4	5	30	10
δ	1.25(1)	1.30(5)	1.35(2)	1.44(1)	1.30(1)	1.34(1)
Δ	2.91(3)	3.0(1)	3.10(3)	3.26(1)	3.02(1)	3.07(3)
Γ	0.45(2) ^b	0.40(6) ^b	0.26(3)	0.28(2)	0.44(2)	0.24(7)

^a S_r in percentage, partial resonant absorption areas; δ in mm s^{-1} , isomer shift; Δ in mm s^{-1} , quadrupole splitting; Γ in mm s^{-1} , line width. Errors in the last digits are given in parentheses; for S_r (%) relative error is $\leq 5\%$. ^bLine widths are constrained to be the same.

**Figure 5.** Mössbauer spectra of the frozen solutions of (A) $^{57}\text{Fe}^{2+}$ –eucalyptus LS and (B) $^{57}\text{Fe}^{3+}$ –eucalyptus LS at 1.1:1 eucalyptus LS/Fe ratio and at pH 4.0. The total ^{57}Fe concentration was 0.015 M; measurements were taken at $T = 80$ K.

structure, and no monomeric Fe^{3+} species can be found in them.³⁶ This hints at a significant difference compared to Fe^{3+} chelates formed with EDTA or analogous ligands in the same concentration and pH range.^{37,38} Taking into account literature data on the dimerization of Fe^{3+} species in aqueous solutions,^{37,38} one can assume the presence of $-\text{OH}/\text{O}$ -bridged $\mu-(\text{OH}/\text{O})_x\text{Fe}_y\text{-LS}$ type complexes.

Even though Mössbauer spectroscopy is a powerful tool attending the microenvironment of the iron, because of its sensitivity, sometimes there is a problem in clarifying differences among the same bounds (geometry, strength, variations in the chemical groups involved). Other techniques (FTIR, XRD) could complete the coordination site information, although the complexity of our sample matrix does not allow us to ensure a good result.

Effect of pH on the Formation of the Fe–LS Complex. When the pH was raised from 4.0 to 7.0, a significant decrease

Table 5. Mössbauer Parameters ($T = 80$ K) of the Frozen Solutions of $^{57}\text{Fe}^{2+}$ –Eucalyptus LS and of $^{57}\text{Fe}^{3+}$ –Eucalyptus LS at 1.1:1 Product/Fe Ratio and at pH 4.0^a

	$^{57}\text{Fe}^{2+}$ –eucalyptus LS	$^{57}\text{Fe}^{3+}$ –eucalyptus LS
Fe^{3+}	29	53
S_r		
δ	0.53(1)	0.48(1)
Δ	0.88(2)	0.78(1)
Γ	0.51(1)	0.53(1)
Fe^{2+}		
S_r	71	47
δ	1.40(1)	1.39(1)
Δ	3.39(2)	3.30(1)
Γ	0.44(1)	0.47(1)

^a S_r in percentage, partial resonant absorption areas; δ in mm s^{-1} , isomer shift; Δ in mm s^{-1} , quadrupole splitting; Γ in mm s^{-1} , line width. Errors in the last digits are given in parentheses; for S_r (%) relative error is $\leq 5\%$.

of the $\text{Fe}^{2+}/\text{Fe}^{3+}$ ratio could be observed in the case of Fe^{2+} –eucalyptus LS complex, as shown in Figures 2A and 6A and Tables 3 and 6. This indicates a higher oxidation rate at alkaline pH values, which is well-known for Fe^{2+} in water solutions.³⁹ The Mössbauer parameters of both Fe^{2+} and Fe^{3+} are also slightly different at pH 7.0 and pH 4.0, which can be explained by different coordination spheres of Fe^{2+} probably due to the modified protonation of the ionic groups at different pH values. This is in good agreement with previous studies demonstrating the pH effect on the molecular dimensions of LS, namely, the expansion or shrinkage of the molecules due to change of the molecularly charged status.¹⁸

In the case of Fe^{3+} complexes, the reductive effect of eucalyptus LS is much lower at pH 7.0 than at slightly acidic conditions (Figures 4A and 6B and Table 6). This would suggest that the Fe^{3+} complex should be prepared at neutral pH; however, one should take into account that at neutral or alkaline pH, the formation of amorphous ferrihydrite may have also occurred.

Effect of the LS/Fe Ratio. Increasing the product/Fe ratio, an increase of the Fe^{3+} compound can be observed in the case of Fe^{2+} –eucalyptus LS complex, as shown in Figures 2A, 6A,

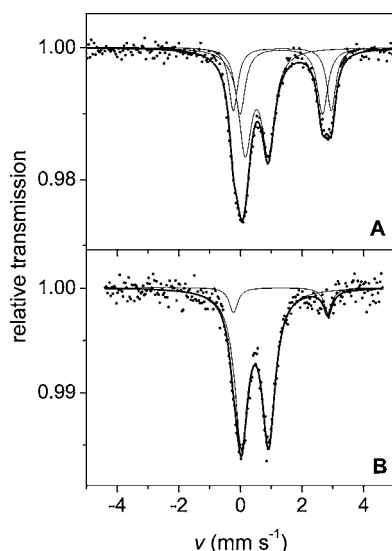


Figure 6. Mössbauer spectra taken at $T = 80$ K of the solid (A) Fe^{2+} -eucalyptus LS and (B) Fe^{3+} -eucalyptus LS samples prepared with 1.1:1 eucalyptus LS/Fe ratio and at pH 7.0.

Table 6. Mössbauer Parameters ($T = 80$ K) of the Solid Fe^{2+} -Eucalyptus LS and Fe^{3+} -Eucalyptus LS Samples Prepared with 1.1:1 Product/Fe Ratio and at pH 7.0^a

	Fe^{2+} -eucalyptus LS	Fe^{3+} -eucalyptus LS
Fe^{3+}		
S_r	51	90
δ	0.53(1)	0.47(1)
Δ	0.75(1)	0.89(1)
Γ	0.50(1)	0.52(1)
Fe^{2+}		
S_r	22	10
δ	1.36(1)	1.31(2)
Δ	3.18(4)	3.07(6)
Γ	0.35(3)	0.34(8)
Fe^{2+}		
S_r	27	
δ	1.33(1)	
Δ	2.68(4)	
Γ	0.41(3)	

^a S_r in percentage, partial resonant absorption areas; δ in mm s^{-1} , isomer shift; Δ in mm s^{-1} , quadrupole splitting; Γ in mm s^{-1} , line width. Errors in the last digits are given in parentheses; for S_r (%) relative error is $\leq 5\%$.

and 7 and Tables 3, 6, and 7. This effect can be observed at both pH 4.0 and pH 7.0 and suggests that the high ligand content favors the formation of the Fe^{3+} compound. However, in the case of Fe^{3+} complexes, the total amount of the reduced Fe^{2+} component shows no correlation with the product/Fe ratios (Figures 4A, 6B, and 8 and Tables 4, 6, and 8). This indicates again that the reduction/oxidation of $\text{Fe}^{3+}/\text{Fe}^{2+}$ is a very complicated reaction; it may be influenced by the complex formation with both Fe^{3+} and Fe^{2+} , by the composition and reductive compounds of the LS products, and also by the possible formation of amorphous ferrihydrite.

The quadrupole splitting values of $\text{Fe}^{2+}/\text{Fe}^{3+}$ components found in the Mössbauer spectra of $\text{Fe}^{2+}/\text{Fe}^{3+}$ -eucalyptus LS samples are slightly different in each case, even if only the LS/Fe ratio is changed (Tables 7 and 8). This can be explained

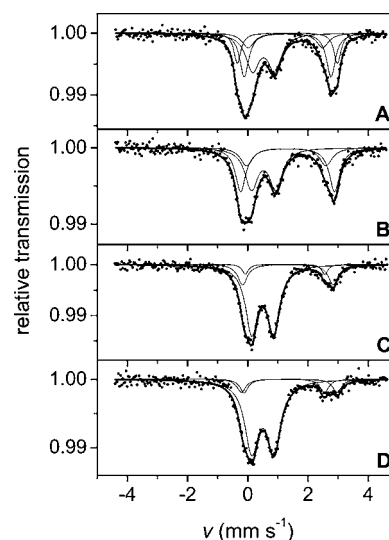


Figure 7. Mössbauer spectra taken at $T = 80$ K of solid Fe^{2+} -eucalyptus LS samples prepared with (A) 1.5:1 or (B) 2:1 LS/Fe ratio at pH 4.0 and (C) 1.5:1 or (D) 2:1 eucalyptus LS/Fe ratio at pH 7.0.

Table 7. Mössbauer Parameters ($T = 80$ K) of the Solid Fe^{2+} -Eucalyptus LS Samples Prepared with 1.5:1 or 2:1 Product/Fe Ratio at pH 4.0 and 1.5:1 or 2:1 Product/Fe Ratio at pH 7.0^a

	1.5:1, pH 4.0	2:1, pH 4.0	1.5:1, pH 7.0	2:1, pH 7.0
Fe^{3+}				
S_r	38	42	75	80
δ	0.53(1)	0.53(1)	0.49(1)	0.50(1)
Δ	0.74(2)	0.81(2)	0.73(1)	0.74(1)
Γ	0.54(3)	0.52(3)	0.49(1)	0.55(1)
Fe^{2+}				
S_r	14	19	6	10
δ	1.26(4)	1.28(3)	1.24(2)	1.21(3)
Δ	2.44(9)	2.6(1)	2.64(5)	2.67(6)
Γ	0.47(8)	0.5(1)	0.24(9)	0.3(1)
Fe^{2+}				
S_r	22	39	19	10
δ	1.32(1)	1.32(1)	1.33(1)	1.38(4)
Δ	3.29(4)	3.11(3)	3.01(4)	3.17(7)
Γ	0.31(4)	0.43(4)	0.39(4)	0.36(7)
Fe^{2+}				
S_r	26			
δ	1.32(1)			
Δ	2.85(4)			
Γ	0.33(7)			

^a S_r in percentage, partial resonant absorption areas; δ in mm s^{-1} , isomer shift; Δ in mm s^{-1} , quadrupole splitting; Γ in mm s^{-1} , line width. Errors in the last digits are given in parentheses; for S_r (%) relative error is $\leq 5\%$.

by the presence of different coordination sites of LS to bind Fe because the ratio of LS to Fe can also determine the functional groups of the large LS molecule, which will be involved in the complex formation.

Agronomic Relevance. In a previous work¹⁴ it has been observed that ^{59}Fe uptake by tomato plants from the nutrient solution and its translocation was more efficient when $^{59}\text{Fe}^{3+}$ -eucalyptus LS was used than when Fe was applied as $^{59}\text{Fe}^{3+}$ -spruce LS. On the contrary, when both lignosulfonates were foliar applied to tomato plants,¹⁴ $^{59}\text{Fe}^{3+}$ -spruce LS provided a

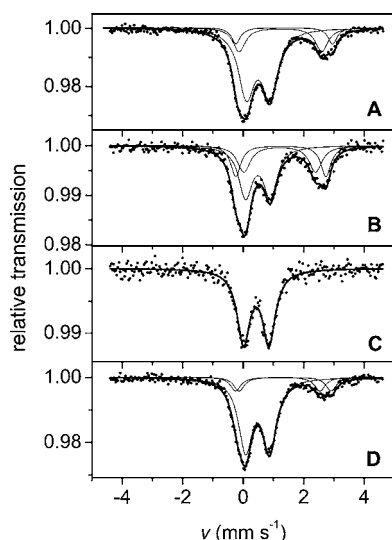


Figure 8. Mössbauer spectra taken at $T = 80$ K of solid Fe^{3+} -eucalyptus LS samples prepared with (A) 1.5:1 or (B) 2:1 LS/Fe ratio at pH 4.0 and (C) 1.5:1 or (D) 2:1 eucalyptus LS/Fe ratio at pH 7.0.

Table 8. Mössbauer Parameters ($T = 80$ K) of the Solid Fe^{3+} -Eucalyptus LS Samples Prepared with 1.5:1 or 2:1 Product/Fe Ratio at pH 4.0 and 1.5:1 or 2:1 Product/Fe Ratio at pH 7.0^a

	1.5:1, pH 4.0	2:1, pH 4.0	1.5:1, pH 7.0	2:1, pH 7.0
Fe^{3+}				
S_r	70	50	100	77
δ	0.50(1)	0.49(2)	0.43(1)	0.47(1)
Δ	0.77(1)	0.82(3)	0.84(1)	0.80(1)
Γ	0.64(1)	0.54(2)	0.51(1)	0.56(1)
Fe^{2+}				
S_r	20	26		12
δ	1.23(1)	1.21(3)		1.20(2)
Δ	2.73(3)	2.37(3)		2.67(5)
Γ	0.50(3)	0.50(3)		0.41(3) ^b
Fe^{2+}				
S_r	10	24		11
δ	1.36(1)	1.25(1)		1.33(2)
Δ	3.19(4)	2.98(4)		3.16(4)
Γ	0.36(5)	0.40(4)		0.41(3) ^b

^a S_r , in percentage, partial resonant absorption areas; δ in mm s^{-1} , isomer shift; Δ in mm s^{-1} , quadrupole splitting; Γ in mm s^{-1} , line width. Errors in the last digits are given in parentheses; for S_r (%) relative error is $\leq 5\%$. ^bLine widths are constrained to be the same.

better uptake and translocation. Also in cucumber plants,^{14,18} Fe translocation was better achieved using $^{59}\text{Fe}^{3+}$ -spruce LS in a short experiment or $^{57}\text{Fe}^{3+}$ -spruce LS in a long experiment. However, in those papers, no correlation between plant uptake or distribution and different chemical properties of the complexes could be observed, but the implication of the $\text{Fe}^{2+}/\text{Fe}^{3+}$ speciation in their efficacy was hypothesized. In the present work a higher affinity of the spruce LS for the Fe^{3+} in comparison with the eucalyptus LS (Table 4) was observed. Therefore, when these products are applied to leaves, Fe^{3+} -spruce LS reaches the leaf surface in a more stable form than Fe^{3+} -eucalyptus LS, providing a better use of the applied Fe. As shown by the Mössbauer data most of the Fe^{2+} is present in weak $\text{Fe}^{2+}_y-(\text{H}_2\text{O})_x$ -LS adducts. Possibly this Fe^{2+} will lead to Fe precipitation on the leaf top, as when FeSO_4 is used,

reducing its activity as Fe fertilizer. When applied in nutrient solution, the weaker complex formed with the eucalyptus LS allows a faster release of the iron to the roots.¹⁴ This is in good agreement with the findings of Lucena and Chaney,⁴⁰ which suggest that the most efficient products in hydroponics are those of lowest stability, as long as they are stable enough in solution. However, the physiological role of the weak Fe^{2+} complexes during Fe uptake requires further study.

General Conclusions. The MCC and Mössbauer results for both Fe^{2+} -LS and Fe^{3+} -LS show no correlation with the LS content of the products, indicating that the complex formation between Fe and LS also involves different coordination sites. All of the products tested have a lower maximum complexing capacity value for Fe^{2+} than for Fe^{3+} , which suggests that these fertilizers could be prepared more effectively by adding an iron(III) inorganic salt to the complexing agent solution.

Moreover, Fe-LS complexes prepared by the addition of an Fe^{2+} salt showed weak adducts between Fe^{2+} and the LS ligands, which are sensitive to oxidation especially at neutral (or alkaline) pH; thus, they are suggested to be prepared at slightly acidic pH. The Fe^{2+} content of the different Fe^{2+} complexes depends on several factors, including the different characteristic structural units and reductive sugars present in the LS products. Std 3 was shown to be the most effective to form the Fe^{2+} -LS complex. In the case of Fe-LS complexes prepared by adding Fe^{3+} , the formation of both Fe^{2+} and Fe^{3+} components can be observed. To achieve high Fe^{3+} content, std 1, std 2, and spruce LS can be used. Neutral pH decreases the reduction of Fe^{3+} to Fe^{2+} during preparation; thus, Fe^{3+} -LS complexes are suggested to be prepared at pH 7.0.

With respect to the agronomical relevance of the $\text{Fe}^{2+}/\text{Fe}^{3+}$ speciation provided by the Mössbauer spectra, it seems that the strong Fe^{3+} -LS complexes are preferred when they are applied to the leaf, whereas the root uptake in hydroponics could be more related with the presence of weak bonding sites.

AUTHOR INFORMATION

Corresponding Author

*Phone: + 34 91 497 39 68. Fax: +34 91 497 38 25. E-mail: juanjose.lucena@uam.es.

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DEDICATION

[†]In memoriam.

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