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Comparison of Two Analytical Methods for the Evaluation of the Complexed Metal in Fertilizers and the Complexing Capacity of Complexing Agents

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The aim of this research is to develop an analytical methodology for the determination of complexed element in fertilizers and, then, to obtain an adequate criteria for the inclusion of these products in European Regulations on Fertilizers. This paper compares the CEN method EN 13366:2001, based on the retention of the cations into a sulfonated resin, and an AOAC modified method, based on the precipitation of the inorganic forms at pH 9. A limited interlaboratory trial was carried out to demonstrate the applicability of the AOAC modified method and to study the effect of the removal of organic compounds and the addition of a matrix modifier solution before the element quantification. Then, a global interlaboratory trial was developed to evaluate the validation and quality parameters of the method. As a second objective, the AOAC modified method was applied to the determination of the complexing capacity of complexing agents based on lignosulfonates and amino acids. The AOAC modified method was the choice methodology because it is adequate for the determination of complexing capacity of micronutrients in fertilizer.

KEYWORDS: Metal complexes; complexing capacity; amino acid; lignosulfonate; humate; gluconate; micronutrient; fertilizer

INTRODUCTION

In Mediterranean agriculture, micronutrient deficiencies are usually corrected using synthetic chelates or either natural or synthetic complexes (*1*). Iron chlorosis is a problem in areas of calcareous and/or alkaline soils (*2*), and the lack of zinc (*3*) and manganese constitutes an important problem too, due to the low solubility of these elements in such as soils.

The use of synthetic chelates derived from polyamine−carboxylic acids is the most common and efficient agricultural practice to treat iron chlorosis and other micronutrient deficiencies (*4*), but it is an expensive practice used only in cash crops. However, complexes are cheaper (2−4 euros kg^{-1}) than synthetic chelates (6−12 euros kg^{-1}), so they can be used in a larger number of crops despite their lower efficacy.

Complexes are obtained by complexation of the micronutrients with natural substances or biosolids in order to increase their availability to plants. Complexing agents normally used are lignosulfonates, amino acids, organic acids, gluconates and humates, etc. Amino acid extracts are mainly obtained from acid or enzymatic partial or total hydrolysis of polypeptides from animal-processing industries. Lignosulfonates are byproducts of

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the paper industry, and gluconates are obtained by enzymatic oxidation (glucose oxidase and catalase) from glucose (*5*). Organic acids (mainly phenolic, acetic, and carboxilyc acids) are from different sources, mainly food industry residues such as potato juice.

The number of products containing synthetic chelates in the Spanish market was 553 in 2007 (*6*), whereas the number of products containing complexes is 299 (150% more than in 1990). In 2007, lignosulfonates and gluconates were the preferred complexing agents used to form complexes (42 and 21% of the total number of products marketed, respectively). The commercialization of products containing amino acids has slightly decreased over the years. Flavonoids represent only 0.3% of the total number of complexes marketed in 2007.

Despite the widespread use of complexes, and due to the lack of knowledge, the European regulations on fertilizers do not list the complexing agents allowed to complex micronutrients. Most of these products, mainly the natural ones, have a variable chemical structure, because the raw materials from which they are obtained may vary with time. Also, complexing agents are generally byproducts of plant- or animal-processing industries, so they are a complex mixture of compounds with similar functional groups but with variable complexing abilities. Therefore, classification is difficult. Moreover, despite the concentration of the complexed element being one of the quality indexes required by European and national regulations, there is no analytical methodology available to determine the amount of the element complexed that is present in commercial products. In recent years, a lot of effort has been directed to providing analytical methods for the quantification of synthetic chelates (*7*−*9*), but not on complexes. As a consequence the quality of the synthetic chelates has improved considerably in recent years (*10*). To identify and characterize different complexing agents, different methodologies can be used [HPLC for amino acids (*11*), infrared for lignosulfonates (*12*) and humates (*13*)]. As far as we know there are no specific methods for the quantification of the element complexed by amino acid extracts. For the quantification of metal chelated by humates, separation by coupled ion exchange column followed by detection by ICP-MS or cold vapor atomic fluorescence spectrometry (*14*) may be used. For the determination of the distribution of different elements among the molecular fractions of humic substances, size exclusion chromatography coupled on-line with UV−vis spectrophotometry and ICP-MS (*15*) or FAAS detection (*16*) methods are published. Also, gel permeation chromatography following the ICP-AES or FAAS determination has been used for lignosulfonates (*17*).

These methods apply to single complexing agents and are difficult to develop in a quality control of fertilizers program. However, for the determination of the complexed metal in a wide type of fertilizers, a sole and simple method that differentiates the complexed and the free forms of the metals should be used.

Therefore, the aim of our study is to present an experimental method for the determination of the complexed micronutrients in commercial fertilizers. We compare the CEN method (*18*) with a precipitation one that is a modification of an AOAC method (*19*). For the validation of the new methodology, a limited interlaboratory trial was carried out. In this test, the necessity of the removal of the organic compounds and the addition of the matrix modifier solution was studied. Once the conditions for the application of the AOAC modified method were set, a global interlaboratory trial was carried out to evaluate the quality of results obtained and to determine the validation parameters for the analytical method.

Also, as a second objective, the proposed method was tested for its application in the quantification of the complexing capacity of ligands (lignosulfonates and amino acids) in order to provide an adequate criterion for the inclusion of these products as complexing agents in European regulations on fertilizers.

Table 1. Chemical Composition of the Lignosulfonate Products NaLS1 and NaLS2

	NaLS1	NaLS2
water content[a] (g kg^{-1})	75	37
lignosulfonates[b] (g kg^{-1})	677	348
C[c] (g kg^{-1})	450	305
H (g kg^{-1})	46.3	30.2
N (g kg^{-1})	1.4	0.6
S (g kg^{-1})	57.5	101.1
Na[d] (g kg^{-1})	69.8	180.8
K (g kg^{-1})	21.1	19.7
Ca (g kg^{-1})	1.1	0.09
Mg (g kg^{-1})	0.05	0.06
Fe (mg kg^{-1})	316	170
Cu (mg kg^{-1})	2.78	1.91
Mn (mg kg^{-1})	5.41	5.42
Zn (mg kg^{-1})	2.15	3.30

[a] Determined by weight loss at 105 °C. [b] Joyce and Kleinert (*23*). [c] Elemental analysis. Elemental Analyzer LECO CHNS-932. [d] Dissolution, filtration, mineralization, and FAAS determination.

Table 2. Chemical Characterization of Amino Acid Extracts (AA1–AA4)

	AA1	AA2	AA3	AA4
free amino acids[a] (g kg^{-1})	191	57	69	47
total amino acids[a] (g kg^{-1})	267	248	114	57
hydrolyzis degree[a] (%)	71.8	23.0	60.3	81.8
total N[a] (g kg^{-1})	56	42	14	11
α-aminic N[a] (g kg^{-1})	27.6	13.4	11.2	7.7
ammonium N[a] (g kg^{-1})	4.29	2.29	0.86	0.95
total P[a] (g kg^{-1})	1.57	0.47	0.69	0.10

[a] *Official Methods of Analysis* (*24*).

MATERIALS AND METHODS

Chemicals and Reagents. All reagents (in particular $FeSO_4 \cdot 7H_2O$, $ZnSO_4 \cdot H_2O$, $CuSO_4 \cdot 5H_2O$, and $MnSO_4 \cdot H_2O$ used to form the complexes and titrate the complexing agents; HNO_3, HCl, NaOH, and H_2O_2 used in the extraction of the soluble or complexed elements or to remove the organic compounds; and HCl, CsCl, and $LaNO_3$ in the matrix modifier solution) and standards ($EDTANa_2$, Titriplex III, Merck, and FAAS standards) were of recognized analytical grade. All water used for the preparation of reagent, standard, or fertilizer solutions conforms to EN ISO 3696 (*20*), grade I, free of organic contaminants.

Fertilizers. Complexing agents used were mainly lignosulfonates (LS) and amino acids (AA). Solid NaLS1 (sodium lignosulfonate from fir, Germany and Norway) and NaLS2 (American modified sodium lignosulfonate) were provided by BASF-CURTEX (Tarragona, Spain), and their characteristics are shown in the **Table 1**. The amino acid extracts (AA) were provided by Bioibérica (Barcelona, Spain), and their characteristics are shown in the **Table 2**. To establish their complexing capacity, 100 g L^{-1} lignosulfonate solutions were prepared. Amino acid extracts were directly used.

Fe(II)LS1, Fe(II)LS2, Zn(II)LS1, Zn(II)LS2, Cu(II)LS1, Cu(II)LS2, Fe(II)AA1, Fe(II)AA2, Zn(II)AA1, and Zn(II)AA2 complexes, used for the comparison of the AOAC modified and CEN methods, were prepared from the lignosulfonates (NaLS1, NaLS2) and amino acid extracts (AA1 and AA2) after the addition of the appropriate amount of metal solution (200 g L^{-1} $FeSO_4 \cdot 7H_2O$ or 100 g L^{-1} $ZnSO_4 \cdot H_2O$ or

Table 3. Characteristics of Products Used in the Limited Interlaboratory Trial

sample	complexing agent	element	soluble element concn (g kg^{-1})
FeAA3	amino acid	Fe	53 ± 3
ZnAA3	amino acid	Zn	58 ± 10
AA multi	amino acid	Fe	4.1 ± 0.5
		Zn	5.0 ± 1.2
		Mn	5.0 ± 1.0
		Cu	4.1 ± 0.7
GA multi	gluconate	Fe	12 ± 2
		Zn	17 ± 2
		Mn	11 ± 1
FeGA	gluconate	Fe	88 ± 10
FeLS3	lignosulfonate	Fe	120 ± 2
ZnLS3	lignosulfonate	Zn	120 ± 13
Fe–CuH	humate	Fe	3.1 ± 0.4
		Cu	2.9 ± 0.0

Table 4. Characteristics of Products Used in the Global Interlaboratory Trial

sample	complexing agent	element	soluble element concn (g kg^{-1})
ZnOA	organic acid	Zn	47± 2
FeLS4	lignosulfonate	Fe	54 ± 2
ZnLS4	lignosulfonate	Zn	76 ± 4
FeGA2	gluconate	Fe	62 ± 6
MnGA	gluconate	Mn	78 ± 4

$CuSO_4 \cdot 5H_2O$) to lignosulfonate solutions (100 g L^{-1}) or amino acid extracts.

Also, complexes of a humate with Fe(II), Fe(III), Cu(II), Co(II), and Zn(II) were used for the comparison of the methods. EDTA complexed at various percentages (25, 50, 75, or 100%) with Fe(III), Cu(II), Co(II), and Zn(II) were prepared as reference.

The humic system used in the preparation of the complexes was a humic acid extracted from leonardite using the International Humic Substances Society (IHSS) methodology (*21*). The main composition of the purified humic acid was as follows: 585 g kg^{-1} C, 14.6 g kg^{-1} N, 26.9 g kg^{-1} H, 258 g kg^{-1} O, 0.1 g kg^{-1} P, 9.9% S, 10.2 g kg^{-1} Fe, and 9.5 g kg^{-1} Al. The contents of C, H, and N were obtained using elemental analysis (LECO CHN 2000), whereas the contents of P, S, Fe, and Al were obtained by ICP-OES spectrometry (Thermo Elemental Co., Iris Intrepid II XDL). The content of O was calculated by difference. The main acidic functional group concentration, obtained using potentiometric analysis as described below, was 1.98 mmol g^{-1} of humic acid of carboxylic groups and 1.18 mmol g^{-1} of humic acid of phenolic groups.

In the limited interlaboratory trial the commercial complexes used were lignosulfonates (FeLS3 and ZnLS3), amino acids (FeAA3, ZnAA3, and AA multicomponent), gluconates (FeGA and GA multicomponent), and one humate (Fe−CuH). Their compositions are given in **Table 3**.

In the global interlaboratory trial the products used were organic acid (ZnOA), lignosulfonates (FeLS4 and ZnLS4), and gluconate (FeGA2 and MnGA). Their characteristics are shown in **Table 4**.

Soluble Metal Analysis. Determination of soluble element was made in all of the complexes using the European Official method for fertilizers (method 9.2 EC 2003/2003 Regulation). In brief, 5 g of each product was shaken with 400 mL of type I water during 30 min, and then the volume was made to 500 mL. Solutions were filtered. The soluble element in the fertilizers was determined after removal of organic compound (method 9.3 EC 2003/2003 Regulation) to allow the assessment of the element by FAAS without interference.

Complexed Metal Analysis. *CEN Method.* The CEN method was applied in accordance with EN 13366. Five grams of each of the commercial products was extracted for 30 min with water and then the volume made up to 500 mL with water and filtered. The conductivity was measured with a Crison micro CM 2200 conductivity meter. When the conductivity was >1.5 dS m^{-1}, the samples were diluted. Fifteen milliliters of sample was placed into a 100 mL beaker and adjusted to pH 7.0 with 0.1 M HNO_3 or 0.1 M NaOH. The solution was transferred to a 100 mL volumetric flask and diluted to the mark with type I water. An aliquot of 25 mL was placed with an amount of resin corresponding to 2.5/CEC of wet sodic resin (Amberlite IR-120 Plus, Sigma) in a polyethylene recipient, protected with aluminum foil to avoid light exposure, and was shaken at 30−40 s^{-1} for 4 h. Samples were filtered and transferred to a 100 mL volumetric flask and made to volume with type I water. The complexed element content was determined by FAAS after removal of the organic compounds (except for EDTA references and humate samples).

AOAC Modified Method. This method is based on AOAC Official Method 983.03 (1983). In brief, 5 g of each of the complexes was dissolved in type I water and the volume made up to 500 mL. Two drops of H_2O_2 (33%, P.A.) were added to 20 mL of sample solution, and the pH increased to 9.0 with 0.5 M NaOH (pH 10 in the EDTA and the humate complexes). The pH was increased again to 9.0 after 30 min and the beaker stopped with Parafilm. The solution was allowed to stand for 1 day in the dark. Afterward, the pH was readjusted to 9.0 and the samples were transferred to a 100 mL volumetric flask and diluted to the mark with type I water. These solutions were filtered through a 0.45 μm Millipore filter. If precipitation was observed, then samples were centrifuged at 7500 min $^{-1}$ at 15−25 °C for 10 min before filtration.

The complexed element in the fertilizers was determined after removal of organic compound (except for EDTA references and humate samples) to allow the assessment of the element by FAAS.

Effect of Organic Matter Removal and Matrix Modifier Addition on the AOAC Modified Method. A limited interlaboratory trial with four laboratories was carried out to demonstrate the applicability of the AOAC modified method. Commercial samples (**Table 3**) were sent to the participating laboratories for the determination of the soluble and complexed (AOAC modified method) elements, and they were instructed to analyze two replicates. Each laboratory performed the final quantification following their own analytical procedures. Because variability was high, the effect of the removal of the organic compounds before the quantification and the addition of a matrix modifier for the quantification by FAAS were studied for all samples considered using the AOAC modified method. Data were statistically evaluated using analysis of variance ($\alpha = 0.05$) to find significant differences among methods. The parameters RSDr and RSDR for each method were obtained, too.

Removal of Organic Compounds, Matrix Modifier, and Element Quantification. Removal of the organic compound was made in accordance with method 9.3 (EC 2003/2003 Regulation) using H_2O_2 (33% w/v) and 0.5 M HCl for the digestion of the samples and 0.5% La as $La(NO_3)_3$, 0.2% Cs as CsCl, and 5% HCl as matrix modifier.

Micronutrients were quantified by FAAS using a Perkin-Elmer Analyst 800 spectrophotometer with a hollow cathode lamp, with wavelengths and slit widths as recommended, and a spoiler.

Global Interlaboratory Trial. Once the conditions for the application of the AOAC modified method were set, an interlaboratory test organized by the Grupo de Trabajo Sectorial de Fertilizantes (Spanish Ministry of Agriculture, Fisheries and Food) with seven participating laboratories was carried out to evaluate the quality of results obtained in previous experiments and to determine the validation parameters for the analytical methods used. Two replicates of soluble and complexed elements were carried out by each participating laboratory. The soluble and complexed elements were measured by FAAS after the removal of organic compounds and the addition of the matrix modifier solution. The characteristics of the commercial products sent to the laboratories are shown in **Table 4**. Statistical analysis was performed following ISO 5725-2:1994 (*22*).

Metal Complexing Capacity Analysis. The complexing capacity of different ligands was studied using a titration technique and the AOAC modified method. Increasing volumes (from 1 to 25 mL) of a 200 g L^{-1} solution of $FeSO_4 \cdot 7H_2O$ and 100 g L^{-1} solutions of $ZnSO_4 \cdot$

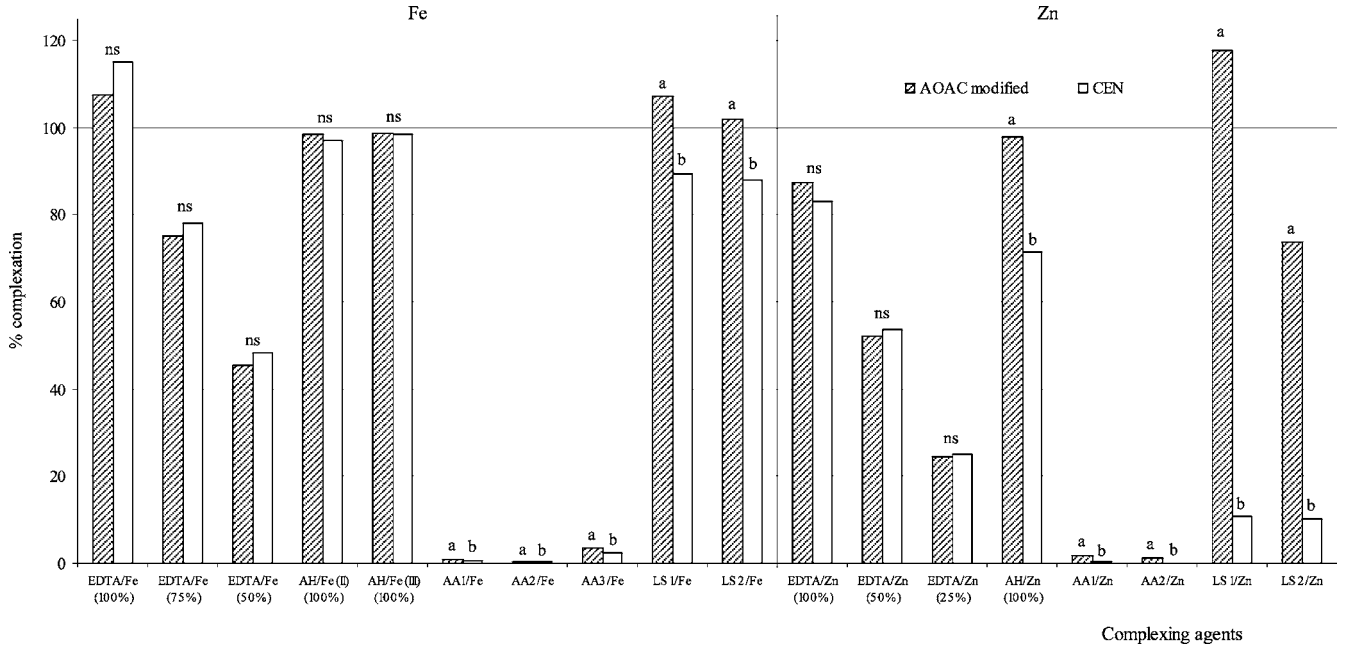

Figure 1. Complexed iron and Zn determined using CEN and AOAC modified methods in EDTA, humate, amino acid, and lignosulfonate products. Different letters denote significant differences among the treatments according to Duncan's multiple-range test ($\alpha = 0.05$). ns = not significant.

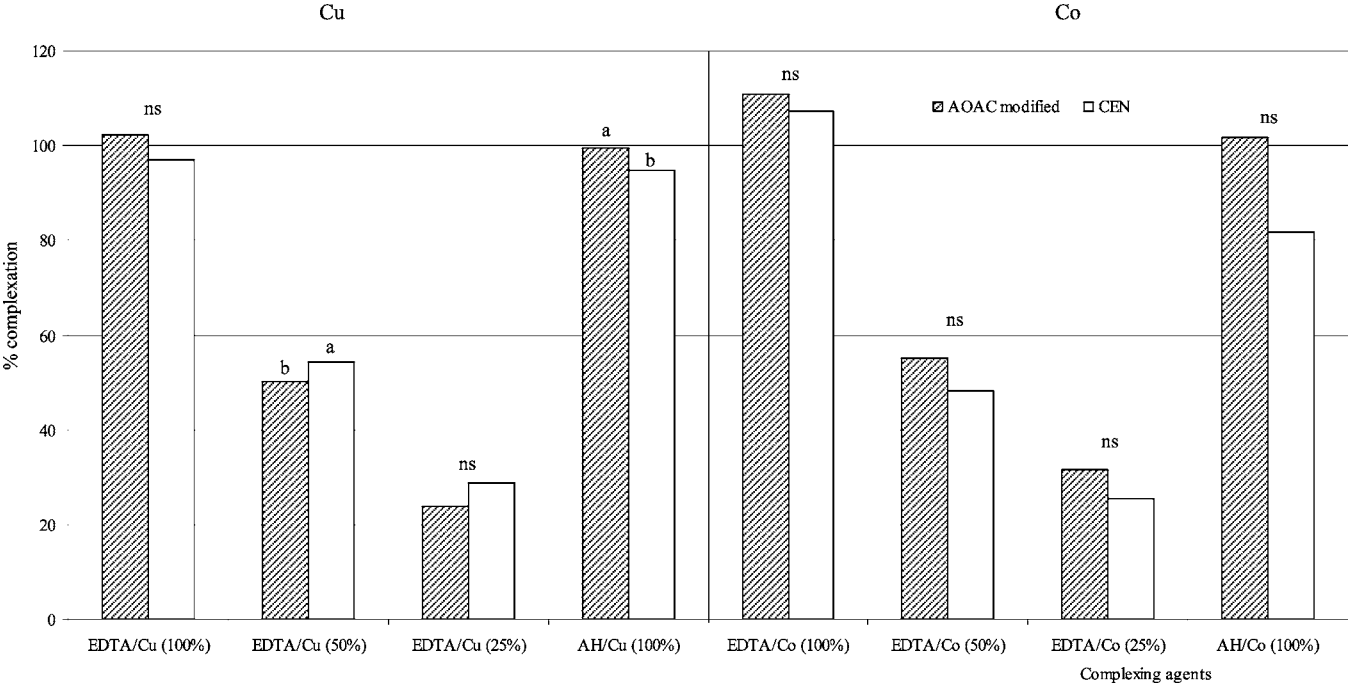

Figure 2. Complexed copper and cobalt determined using CEN and AOAC modified methods in EDTA and humate products. Different letters denote significant differences among the treatments according to Duncan's multiple-range test ($\alpha = 0.05$). ns = not significant.

H_2O, $MnSO_4 \cdot H_2O$, and $CuSO_4 \cdot 5H_2O$ were added over 20 mL of complexing agent solutions (100 g L^{-1} NaLS1 and NaLS2 and directly commercial products AA1, AA2, AA3, and AA4), and the complexed elements determined by the AOAC modified method were assessed by FAAS after the removal of the organic compounds by digestion and addition of the matrix modifier.

RESULTS AND DISCUSSION

Comparison of Two Methods for Complexed Metal Determination. The percentage of complexed element with respect to the soluble element determined by both methods is presented in **Figure 1** for Fe and Zn and in **Figure 2** for Cu and Co. Results obtained for EDTA reference samples are similar in the AOAC modified and CEN methods for all elements studied except Cu, for which higher amounts of complexed element were obtained with the CEN method, and correspond adequately with the calculated percentage of complexation of the reference samples. These results were as expected because both methods are described for synthetic chelates. For humates, the results obtained using both methods were similar, too, except for Zn and Co (**Figures 1** and **2**), for which 70% of the soluble Zn and 80% of the soluble Co are considered to be complexed according the CEN method.

Only Fe and Zn were evaluated as soluble and complexed elements for lignosulfonates and amino acids. For lignosulfonates, the CEN method gives a slightly lower percentage of

complexed Fe than the AOAC modified method, but, in the case of Zn complexation, differences between methods are higher. For amino acids the results showed a high difference between both methods. Assayed amino acid extracts are weak complexing agents for Fe and Zn, but always the CEN method provided lower values.

The CEN method and the AOAC modified method should offer equivalent results because in both methods the element that is not present in a neutral or negative complex is retained or precipitated. However, the chemical reaction in both methods can be more or less displaced toward the element retention or precipitation depending on the conditions. In the CEN method the adsorption process is unspecific, but the sulfonic groups that are present in elevated concentrations in the resin can also act as complexing agents for metals (surface complexation) (reaction 1), favoring the release of the metal from the complex (reaction 2) and producing a partial destruction of the complex:

$$M^{n+} + \text{resin}-SO_3^- \rightarrow [\text{resin}-SO_3-M^{(n-1)+}] \quad (1)$$

$$ML^{(n-y)} \rightarrow M^{n+} + L^{y-} \quad (2)$$

This displacement should be nil in the case of strong complexes such as the EDTA ones, but of importance for weak complexes.

Something similar should happen in the AOAC modified method: the precipitation (reaction 3) can favor the displacement of the complex (reaction 2):

$$M^{n+} + nOH^- \rightarrow M(OH)_n \quad (3)$$

Because the displacement in the case of the CEN method is larger, the retention reaction (reaction 1) should be more displaced to the formation of products than in the case of the precipitation (reaction 3), indicating a higher affinity of the metals by the resin than for the precipitation reaction. Weak complexes are used in agriculture in foliar applications and other purposes, so the CEN method seems to be not adequate for this type of complex. Moreover, if the formed complexes are not neutral or negative, they can be retained by the resin and then not quantified as complexed elements by the CEN method. Hence, the CEN method may not evaluate the total amount of complexed element, so it is considered to be less adequate than the AOAC modified.

The AOAC modified method can also produce slight displacement of the metals, mainly because the pH is fixed at 9, where the precipitation is favored. Nevertheless, it seems more suitable for the determination of the complexed element, because the complex must be able to maintain the elements in adverse conditions and not only to the pH of products. Moreover, it is an index that does not depend on the ionic form of the complex, but on the stability of the complex at pH 9 and, therefore, may be applied to determine the complexed element and to compare different complexing agents.

It has to be noted that the obtained value for the complexing capacity is an index, because metal and ligand exchange reactions (involving other ligands, metal complexes, and other metals of the fertilizer) may occur during the analysis, and therefore the total amount of complexed metal determined may differ from the original one. However, high-pH conditions could well represent the situation that the complexes have to endure when they are applied in agronomic conditions.

Effect of Organic Matter Removal and Matrix Modifier Addition on the AOAC Modified Method. The results of the limited interlaboratory trial are shown in **Table 5**. In general, the relative standard deviation in repetitivity conditions (RSDr) and the relative standard deviation in reproducibility conditions (RSDR) are quite high (average of RSDR = 30%). However, it is important to note that for samples that comply with the Spanish Regulation RD 825/2005 (>50% complexed element) the variability is not so high for most of the samples (average of RSDR = 16%).

Table 5. Interlaboratory Trial Results

sample	element	complexed element (g kg^{-1})	% complexed element with respect to soluble element	RSDr[a] (%)	RSDR[b] (%)
FeAA3	Fe	0.2	0.4	7.9	123
ZnAA3	Zn	12.3	21	13	28
AA multi	Fe	0.7	18	10	80
	Zn	3.1	62	9.4	20
	Mn	0.7	13	13	31
	Cu	4.6	112	0.7	4.7
GA multi	Fe	11.1	94	1.8	21
	Zn	14.4	83	1.4	36
	Mn	11.4	104	1.6	8.7
FeGA	Fe	78.6	89	1.5	5.3
FeLS3	Fe	119	98	4.9	5.5
ZnLS3	Zn	97.7	82	2.3	20
Fe–CuH	Fe	2.7	88	5.8	22
	Cu	2.6	90	2.3	16

[a] RSDr is the relative standard deviation in repetitivity conditions. [b] RSDR is the relative standard deviation in reproducibility conditions.

Table 6. Global Interlaboratory Trial Results of Complexed Element

product	complexed element (g kg^{-1})	% complexed element with respect to soluble element	RSDr[a] (%)	RSDR[b] (%)
ZnOA	44.7	96	0.6	2.5
FeLS4	44.6	83	6.4	15.3
ZnLS4	74.2	98	1.9	1.9
FeGA2	3.50	6	23.6	36.7
MnGA	70.9	91	4.5	17.4

[a] RSDr is the relative standard deviation in repetitivity conditions. [b] RSDR is the relative standard deviation in reproducibility conditions.

Regarding the observed percentage of complexation (**Table 5**), the amino acids have a low complexing capacity for iron and manganese (0.4−18%), whereas gluconates have a good complexing capacity for Fe, Zn, and Mn and lignosulfonates for Fe and Zn. The last two complexing agents present >50% of metal chelated, the minimum required for complexes in the Spanish regulation. Copper and iron in the humate present low concentration of soluble element, but the complexing capacity was satisfactory, too.

The metal complexed as determined by the AOAC modified method could be an adequate index of the complexed metal micronutrients Fe, Mn, Cu, and Zn, but due to the large RSDr and RSDR values the method needed further standardization. Then the elimination of the organic matter and the addition of the matrix modifier solution before the determination by FAAS were studied. An ANOVA indicated that samples analyzed without the organic matter removal and without the addition of the matrix modifier presented significantly ($p < 0.05$) lower values than those when both sample preparation techniques were used. These differences were observed using a global

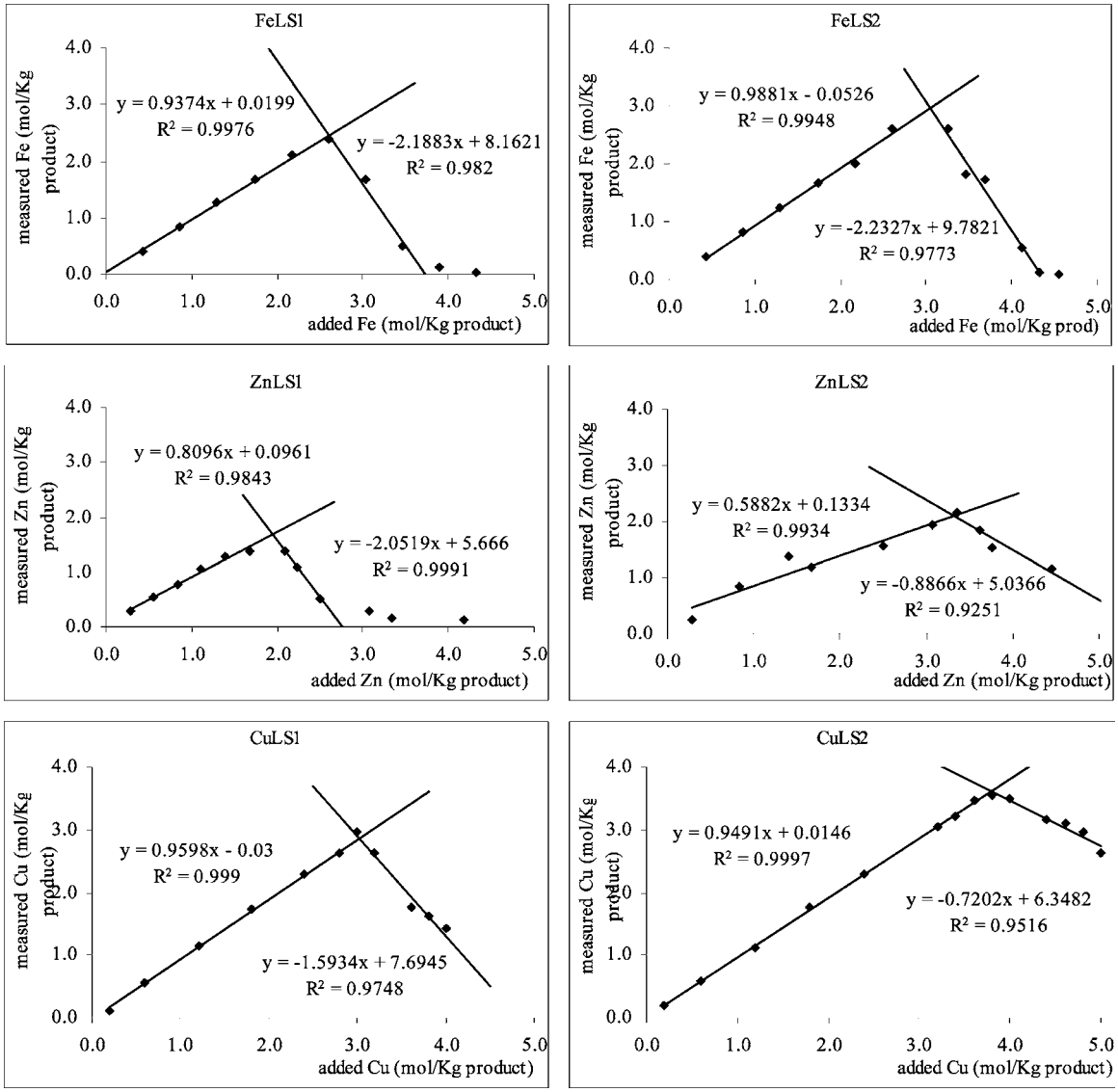

Figure 3. Complexing capacity for lignosulfonates with Fe, Zn, and Cu.

statistical analysis (including all of the elements and samples), but they were especially important for Zn analysis. Comparison between the addition or not of the matrix modifier in samples from which the organic matter was removed by digestion did not yield statistical differences. However, and due to the recommendation of the EC 2003/2003 Regulation (method 9.4: determination of micronutrients in fertilizer extracts by atomic absorption spectrometry) to use it, we decided to propose the use of the matrix modifier solution in the modified AOAC method.

Global Interlaboratory Trial. Results obtained in the global interlaboratory trial using the AOAC modified method are shown in **Table 6**. For complexed Zn determination, the repetitivity and reproducibility of the method are good.

The quality parameters obtained for the determination of the complexed iron are questionable. For FeGA2 the method is valid because none of the laboratories detected appreciable amounts of complexed iron in the sample. The high values of RSDr and RSDR are consequences of the low value of complexed element that is near the quantification limit (0.7, 0.06, and 0.35 g kg^{-1} for Fe, Mn, and Zn, respectively).

Results of complexed manganese present quite high reproducibility due to the disagreement among laboratories.

In summary, the AOAC modified method has been revealed to be more reproducible than the CEN method for the determination of complexed Zn in products based on complexing agents. However, this method is not completely satisfactory for the determination of complexed Fe and Mn due to the high values of RSDr and RSDR obtained in the global interlaboratory trial. Moreover, the AOAC modified method gives better results than the CEN method. We propose the use of the AOAC modified method until a better method is developed.

Complexing Capacity. Because the AOAC modified method seems to be a suitable methodology for the determination of the complexed fraction in commercial products, this methodology, combined with a method of successive additions, has been proposed to evaluate the complexing capacity of lignosulfonates and amino acids.

Lignosulfonates. **Figure 3** shows the measured element versus added element. The type of curve obtained corresponds to that presented in **Figure 4a** having a rising segment, which corresponds with the complexing process, followed by another decreasing that implies the coagulation of the material by the excess of metal. Also, in **Table 7** the complexing capacity obtained from the intersection point of the two obtained lines is presented. In general, a large amount of elements can be

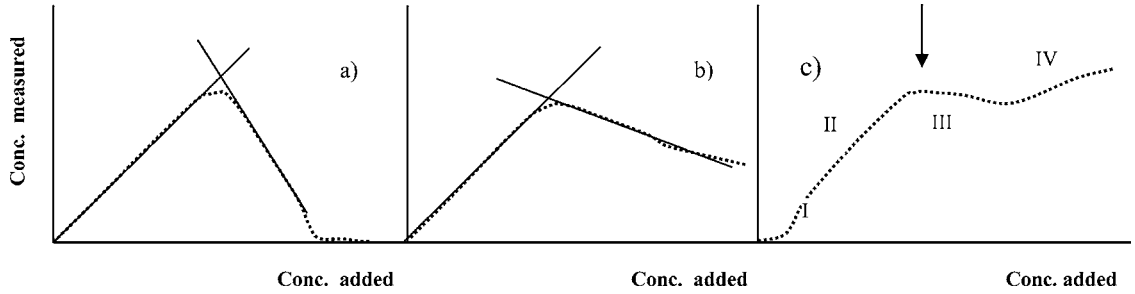

Figure 4. Types of titration curves observed for complexing agents with different metals: (**a**, **b**) typical; (**c**) detected for some amino acid titrations with Fe.

Table 7. Complexing Capacity of Lignosulfonates with Fe, Zn, and Cu[a]

	Fe		
	mol of Fe kg^{-1} of product	mol of Fe kg^{-1} of lignosulfonate	% FeL/ soluble Fe
LS1	2.46	3.63	95
LS2	2.96	8.51	97
	Zn		
	mol of Zn kg^{-1} of product	mol of Zn kg^{-1} of lignosulfonate	% ZnL/ soluble Zn
LS1	1.59	2.35	80
LS2	2.12	6.69	64
	Cu		
	mol of Cu kg^{-1} of product	mol of Cu kg^{-1} of lignosulfonate	% CuL/ soluble Cu
LS1	2.87	4.24	95
LS2	3.69	10.6	95

[a] Data are referred to the amount of product or to the amount of lignosulfonate in the product.

complexed by the lignosulfonates and the degree of complexation (percentage complexed respect soluble element) is high.

Amino Acids. In the titration of the amino acid extracts two types of curves were observed. For Zn the one described in **Figure 4b** was the most common, indicating a normal saturation curve with a slight coagulation effect. For Fe another type of curve (**Figure 4c**) with four phases has been normally observed: initially (I), at low Fe/AA ratio, exits a low of complexation of the metal. It seems that the strongest complexing places for Fe in the amino acid extract produce coagulation or precipitation of the polypeptide. With an increase in the Fe concentrations (segment II) the normal complexing process has occurred, forming soluble complexes and Fe concentration measured increased. In the third phase (segment III) an increase in the addition of the metal does not imply more complexation, but the flocculation phenomenon is not important. Finally, an excess of iron created new complexing places, but they are weaker (low slope of the curve). It is necessary to emphasize that this second type of curve is the one that has motivated the election of maximum criterion for the quantification of the complexing capacity (value with the maximum complexation in the phase III) that is somewhat arbitrary, instead of the one of the intersection of the lines. However, this is not a handicap for the application of the method, because in all cases, when this type of curve appears the amount of element complexed or the degree of complexation is lower than that expected for the EC and Spanish Regulations, so they cannot be considered complexes to be commercialized.

The AOAC modified method combined with the addition of metal solutions may provide a tool for the determination of complexing capacity in lignosulfonates and amino acids. Lignosulfonates present a good complexing capacity, higher than that required for the Spanish Regulation, but in amino acids the capacity of complexing metal is too low.

ABBREVIATIONS USED

AOAC, Association of Official Analytical Chemists; CEN, European Committee for Standardization, EC: European Community; EDTA, ethylenediaminetetraacetic acid; CEC, cationic exchange capacity; HPLC, high-performance liquid chromatography; ICP-AES, inductively coupled plasma atomic emission; ICP-MS, inductively coupled plasma mass spectrometry; ICP-OES, inductively coupled plasma optical emission spectrometry; FAAS, flame atomic absorption spectrometry.

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