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Determination of chelating agents in fertilizers by ion chromatography

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Abstract

In agronomy, chelating agents are used to complex trace elements (Fe, Mn, Zn, Cu, Co) for fertilization. An EEC directive allows the use of six chelating agents (EDTA, HEEDTA, DTPA, EDDHA, EDDHMA and EDDCHA), and requires an effective stability of at least 80%. An ion chromatographic method was developed to identify and determine the total amount of chelating agents in fertilizers. Precolumn derivatization of the metal chelates to the corresponding Fe(III) chelates is followed by elution with HNO_3–NaOAc mixture, postcolumn reaction with $HClO_4$ and UV–Vis detection at 330 nm. The method offers a specific, sensitive technique for determining EDTA, HEEDTA and DTPA in fertilizers.

1. Introduction

The feature of controlling the concentration of the free form of metal ions is fundamental in the various applications of chelating agents. Best known is the example of EDTA, which, apart from being a common laboratory reagent, is widely used, *e.g.*, in the water treatment, cleaning, power, mining, textile, food, agricultural and pharmaceutical industries.

In agronomy, chelating agents are used for micronutrient fertilization in hydroculture and in foliar and soil application. The essential plant nutritive elements in mineral fertilizers can be divided into three groups: main elements (N, P, K), secondary elements (S, Ca, Mg, Na) and trace elements or micronutrients (Fe, Mn, Zn, B, Cu, Mo, Co). Trace elements deficiencies can be overcome by fertilization with salts, oxides, hydroxides, organomineral complexes and chelates of the trace elements. Chelates offer the highest efficiency, bringing the trace element into a plant-available form at relatively low doses from the soil to the root and into the plant cells. In Europe, EEC Directive 76/116 allows chelates of the elements Fe, Mn, Zn, Cu and Co to be used as such or incorporated in mixed fertilizers. An effective degree of chelation of at least 80% is required [1].

Six chelating agents are allowed to be used for this purpose. They all belong to the class of the aminocarboxylic acids, and are commonly abbreviated as EDTA, HEEDTA, DTPA, EDDHA, EDDHMA and EDDCHA. The full names and structures of the compounds considered in this study (EDTA, HEEDTA, DTPA, EDDHA, and two related compounds DCTA and NTA) are illustrated in Fig. 1.

The concentration of these chelating agents in commercial fertilizers can range from about 0.01% to more than 50%. At low levels, the identification and determination of these compounds constitute a difficult analytical problem, especially in the presence of a complex matrix

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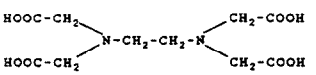
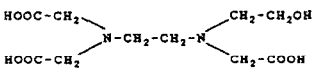
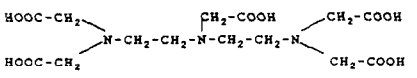
EDTA Ethylenediaminetetraacetic acid



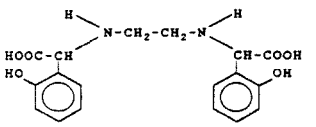
HEEDTA 2-Hydroxyethyl-ethylenediaminetriacetic acid



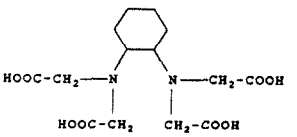
DTPA Diethylenetriaminepentaacetic acid



EDDHA Ethylenediamine-di-(2-hydroxyphenyl) acetic acid



DCTA 1,2-diamino-cyclohexanetetraacetic acid



NTA Nitrilotriacetic acid

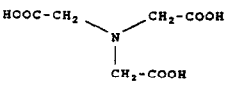


Fig. 1. Structures of some important chelating agents of the aminocarboxylic acid group [2].

with large amounts of other water-soluble substances.

Further, from an agronomic point of view, the determination of the stability of a chelate is considered to be a more important concern than the determination of the nature and the amount of a chelating agent. The lack of clear evidence about the efficiency of chelates under field conditions and the possible mobilization of heavy metals such as Cd, Ni and Pb call for a detailed study of the stability of a given chelate in fertilizer, soil and plant extracts.

The chemistry involved in the formation and stability of a chelate in complex media is characterized by a number of chemical equilibria, which can be characterized by their physical constants [3,4]. The most important equilibria, illustrated for Mn–EDTA as an example, are as follows:

(i) complexation of the metal ion by the chelating agent (stability constant K_c):

$$Mn^{2+} + EDTA^{4-} \rightleftharpoons Mn\text{–}EDTA^{2-}$$

(ii) protonation of the chelating agent (dissociation constant K_A):

$$H_4EDTA \rightleftharpoons H_3EDTA^- \rightleftharpoons H_2EDTA^{2-} \rightleftharpoons HEDTA^{3-} \rightleftharpoons EDTA^{4-}$$

(iii) oxidation–reduction of the metal ion (reduction potential E_0):

$$Mn^{2+} \rightleftharpoons Mn^{3+} \rightleftharpoons Mn^{4+}$$

(iv) hydroxylation of the metal ion:

$$Mn^{2+} \rightleftharpoons Mn(OH)^+ \rightleftharpoons Mn(OH)_2 \rightleftharpoons Mn(OH)_3^-$$

(v) precipitation of the metal ion (solubility product K_s):

$$MnO_2;\ MnCO_3;\ Mn_3(PO_4)_2;\ Mn(OH)_2$$

It is clear that the entire chelation process is considerably influenced by (1) the pH, affecting all of the above equilibria, (2) the nature of the chelating agent and the metal ion and (3) the presence of other competing metal ions and complexing or chelating agents.

As these chelates tend to form highly water-soluble ionic species, the choice of ion-exchange chromatography as an analytical method seems to be justified. Although the retention mechanism is governed by ion exchange, the complexation behaviour plays an important role, both thermodynamically and kinetically. Further, non-ionic interactions of the analytes with the stationary phase can be expected, *e.g.*, the interactions of the phenolic groups of some chelating agents with a polystyrene–divinylbenzene-based substrate.

The final objective, the separation of a complex mixture of chelates, formed by reaction of different chelating agents with different metal ions, has not been reported. Because of the complexity of the system, subdivision of the problem should be considered: the separation of different chelating agents associated with a single

metal ion; and the separation of different metal ions associated with a single chelating agent. Existing methods were considered in this respect. A standard application, developed for polyphosphates and other polyvalent complexing agents, using acidic elution and postcolumn derivatization with UV–Vis detection, offered good prospects for the separation of different chelating agents [5,6].

2. Experimental

2.1. Reagents and solutions

Eluents were prepared by dissolving or diluting analytical-reagent grade products (nitric acid, sodium acetate and potassium hydrogentartrate, all from Merck, Darmstadt, Germany) in water purified with an Elgastat UHQ system (Elga, UK) and degassed with helium. Eluents containing potassium hydrogentartrate became cloudy after a few days and were therefore replaced daily. Stock standard solutions (1 m*M*) of metal ions such as Fe(III), Mn(II), Zn(II), Cu(II) and Co(II) were prepared by dissolving high-purity salts [$Fe(NO_3)_3 \cdot 9H_2O$, $MnSO_4 \cdot H_2O$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, and $CoSO_4 \cdot 7H_2O$, all from Merck] in water. The Fe(III) solution was prepared immediately before use.

Stock standard solutions (1 m*M*) of the chelating agents EDTA, HEEDTA, DTPA, EDDHA, DCTA and NTA were prepared by dissolving the corresponding sodium salts in water or the acids in 5 m*M* NaOH using: $Na_2EDTA \cdot 2H_2O$ (Merck), $Na_3HEEDTA$ (Fluka, Buchs, Switzerland), H_5DTPA (Merck), H_4EDDHA (Sigma, St. Louis, MO, USA), $H_4DCTA \cdot H_2O$ (UCB, Brussels, Belgium) and H_3NTA (Merck).

Standard solutions of metal chelates were prepared by mixing appropriate volumes of metal ion solutions with those of chelating agents to obtain stoichiometrically balanced combinations of metal ions and chelating agents.

Samples of fertilizers were ground, sieved to 0.1 mm, extracted with water at room temperature and filtered through folded Whatman 2V filter-paper. Before injection, solutions were, if necessary, filtered again through a 0.2-μm membrane filter. Unless stated otherwise, all metal chelates were injected in concentrations of 0.01–0.1 m*M*.

2.2. Instrumentation

A Dionex Series 2003i ion chromatograph was used with a 50-μl injection loop, an isocratic pump and a UV–Vis detector with a 330-nm filter. For postcolumn derivatization an unheated reagent delivery module was used. Different 4-mm columns were used, all from Dionex. An Ion Pac AS7 separator in combination with an Ion Pac AG7 or NG1 guard column was used. The Ion Pac AS7 separator contains a 10-μm poly(styrene–divinylbenzene) (PS–DVB)-based substrate agglomerated with an aminated ion-exchange latex. Ion Pac NG1 is a PS–DVB-based reversed-phase guard column without any functional groups. The flow-rate was 0.5 ml/min. Chromatograms were recorded with a Shimadzu Series C-R5A Chromatopac integrator. For atomic absorption spectrometry, a Perkin-Elmer Series 2380 spectrometer with deuterium-arc background correction was used.

3. Results and discussion

The standard application for polyvalent complexing agents utilizes 30–70 m*M* HNO_3 as the eluent (isocratic), postcolumn derivatization with 1 g/l $Fe(NO_3)_3 \cdot 9H_2O$ in 2% $HClO_4$ and UV–Vis detection at 330 nm. The separation column used features a high capacity (80 μequiv. per column) and a relatively high hydrophobic nature, having both anion- and cation-exchange capacity [7]. With a sample containing only free chelating agents, *e.g.*, H_4EDTA, or the corresponding Na salts, the forms eluting on the column under acidic conditions ($pH \approx 1.3$) are the free, fully protonated forms [8].

Fig. 2 illustrates the separation of a mixture of the sodium salts of NTA, EDTA, HEEDTA, DTPA and EDDHA. However, when the sample contains stronger chelates such as Fe(III)–

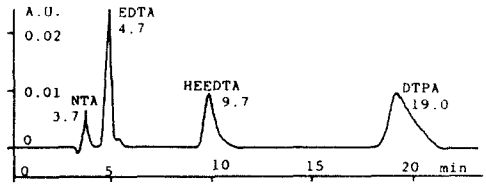


Fig. 2. Separation of the sodium salts of NTA, EDTA, HEEDTA and DTPA. Eluent, 70 m*M* HNO_3; columns, Ion Pac AS7 + guard; detection, postcolumn reaction with Fe(III)–$HClO_4$ with subsequent UV spectrophotometry at 330 nm EDDHA was not detected.

EDTA, some of which only partially dissociate, an influence of the associating metal ion on the retention time of the corresponding chelating agent can be observed, as illustrated in Fig. 3.

Table 1 shows the stability constants of some metal chelates of EDTA, HEEDTA and DTPA [2]. In an acidic environment, one would expect the sodium salt and the relatively weak EDTA complexes (Mn, Zn) to dissociate and the acid form of EDTA to elute (Fig. 3a–c). Only Fe(III) would remain complexed and elute as the less strongly retained Fe–EDTA (Fig. 3d). Cu–EDTA creates an intermediate situation, with the chelate partially dissociating during the run, giving a Cu–EDTA peak moving ahead of a

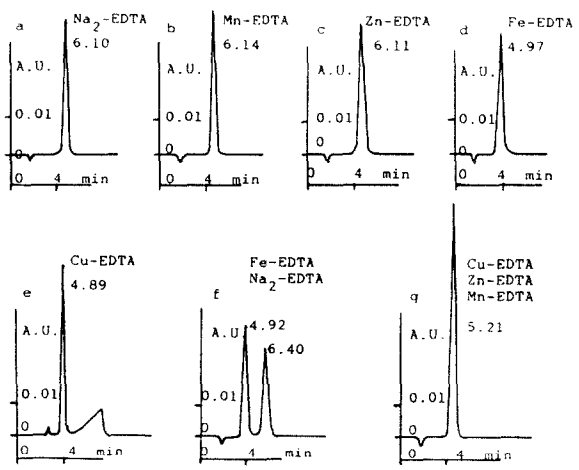


Fig. 3. Influence of the associating metal ion on the retention time of EDTA. Eluent, 50 m*M* HNO_3; columns and detection, as in Fig. 2. Injection of (a) Na_2EDTA, (b) Mn–EDTA, (c) Zn–EDTA, (d) Fe–EDTA, (e) Cu–EDTA, (f) Na_2EDTA + Fe–EDTA and (g) Cu–EDTA + Zn–EDTA + Mn–EDTA; solute concentrations 0.04 m*M*.

Table 1
Stability constants (log K_c) of different metal chelates of EDTA, HEEDTA and DTPA [3]

Metal ion	Log K_c		
	EDTA	HEEDTA	DTPA
Al^{3+}	16.3	14.3	18.6
Ba^{2+}	7.86	6.3	8.87
Ca^{2+}	10.69	8.3	10.83
Co^{2+}	16.31	14.6	19.27
Cu^{2+}	18.80	17.6	21.55
Fe^{2+}	14.32	12.3	16.5
Fe^{3+}	25.1	19.8	28.0
Mg^{2+}	8.79	7.0	9.3
Mn^{2+}	13.87	10.9	15.6
Ni^{2+}	18.62	17.3	20.32
Pb^{2+}	18.04	15.7	18.80
Sr^{2+}	8.73	6.9	9.77
Zn^{2+}	16.50	14.7	18.4

fronting peak of free EDTA (Fig. 3e). Injection of a mixture of Na_2EDTA and Fe–EDTA results in two peaks, corresponding to free EDTA and Fe–EDTA (Fig. 3f). Mixtures of Mn–EDTA, Zn–EDTA, and Cu–EDTA elute as a single peak with an average retention time, shifted toward the retention time of the most stable complex (Fig. 3g).

Subsequently, the method was modified by including a precolumn treatment of the sample with Fe(III) in an acidic medium, replacing the chelated metal ion present in the sample with Fe(III). This simple procedure permits only Fe chelates to be eluted and a single retention time to be obtained for a given chelating agent, independent of the associated metal ion. The precolumn treatment is carried out by mixing four volumes of the sample solution (concentration of chelating agents between 0.01 and 0.1 m*M*) with 1 volume of a solution of 5 g/l $Fe(NO_3)_3 \cdot 9H_2O$ in 0.15 *M* HNO_3, allowing it to react for 5 min at room temperature. The postcolumn reaction is carried out with 2% $HClO_4$, stabilizing the Fe chelates and increasing the absorbance at 330 nm.

As illustrated in Fig. 4, the influence of the eluent composition on retention, resolution and

detection was studied with mixtures of sodium salts of NTA, EDTA, HEEDTA, DTPA and EDDHA. In comparison with Fig. 2, it is clear from Fig. 4a that Fe chelates elute much faster than the corresponding free chelating agents. As generally expected, Fig. 4a–c show for DTPA as an example that with increasing pH the negative charge of the chelate also increases, causing a longer retention. The pH of all eluents was kept acidic to prevent the influence of matrix components in the fertilizers. The presence of acetate and tartrate in the eluent positively affects the selectivity and permits better resolution of EDTA, HEEDTA and DTPA (Fig. 4b–d).

The importance of non-ionic interactions of the analyte with the stationary phase was demonstrated for EDDHA and DCTA. EDDHA does not elute when both guard and separation columns are used. However, using the much shorter guard column AG7 and an eluent consisting of 50 m*M* HNO_3–50 m*M* NaOAc, Fe–EDDHA elutes as a single peak, indicating that the

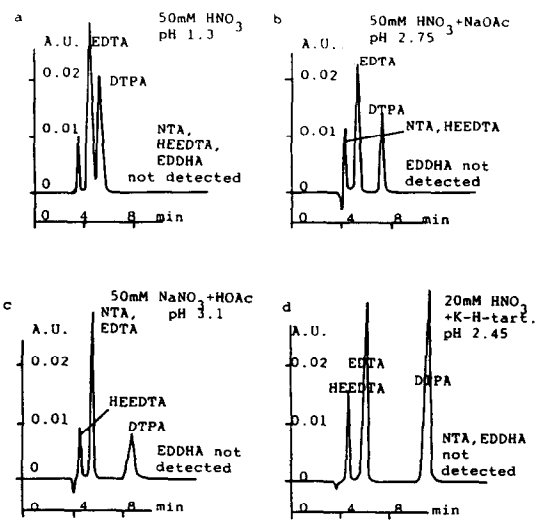


Fig. 4. Influence of eluent composition on retention, resolution and detection. Columns, as in Fig. 2, precolumn reaction of the sodium salts of NTA, EDTA, HEEDTA, DTPA and EDDHA with Fe(III)–HNO_3; postcolumn reaction with $HClO_4$ with subsequent UV spectrophotometry at 330 nm. Eluents: (a) 50 m*M* HNO_3 (pH 1.3); (b) 50 m*M* HNO_3–50 m*M* NaOAc (pH 2.75); (c) 50 m*M* $NaNO_3$–50 m*M* HOAc (pH 3.1); (d) 20 m*M* HNO_3–20 m*M* potassium hydrogentartrate (pH 2.45).

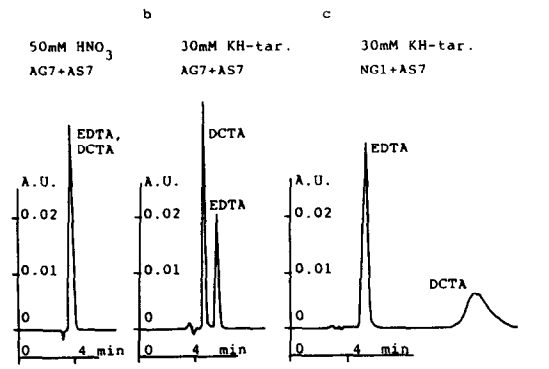


Fig. 5. Resolution of EDTA and DCTA. Precolumn reaction of the sodium salts of EDTA and DCTA with Fe(III)–HNO_3; detection, postcolumn reaction with $HClO_4$ and subsequent UV spectrophotometry at 330 nm, (a) 50 m*M* HNO_3 as the eluent and Ion Pac AS7 + guard columns as the separator; (b) 30 m*M* potassium hydrogentartrate as the eluent and Ion Pac AS7 + guard columns as the separator; (c) 30 m*M* potassium hydrogentartrate as the eluent and Ion Pac AS7 + NGl guard columns as the separator.

compound adsorbs on the substrate material due to its phenolic groups. As illustrated in Fig. 5, non-ionic interactions also determine the retention behaviour of DCTA.

The above-described method using an AS7 separator in combination with an NG1 guard column, 50 m*M* HNO_3–50 m*M* NaOAc as the eluent, precolumn treatment with Fe(III)–HNO_3, postcolumn reaction with $HClO_4$ and UV detection at 330 nm was tested to check its analytical performance. Standard solutions of EDTA, HEEDTA and DTPA with concentrations between 0.01 and 0.2 m*M* were injected and peak heights measured. Sensitivities, detection limits and correlation coefficients were derived from three calibration graphs and are given in Table 2. Table 2 also shows that, after fifteen injections of standard solutions (0.01–0.2 m*M*), the retention times do not shift significantly.

A recovery study was carried out with three commercial NPK fertilizers not containing chelated trace elements. Before extraction, the samples were spiked with three levels of EDTA, HEEDTA and DTPA, corresponding to amounts of 0.01, 0.1 and 1% (w/w), and an excess of 10% (mol Cu/mol chelating agent) of Cu(II) was added. Two fertilizers were only

Table 2
Sensitivities, detection limits, correlation coefficients and retention times of EDTA, HEEDTA and DTPA (0.01–0.2 m*M*)

Parameter	EDTA	HEEDTA	DTPA
Sensitivity (absorbance/m*M*)	0.312	0.208	0.364
Detection limit (m*M*)	0.00578	0.00659	0.00717
Correlation coefficient	0.99989	0.99986	0.99984
Retention time (min)	4.539 ± 0.109	3.977 ± 0.073	6.399 ± 0.155

Precolumn treatment with Fe(III)–HNO_3; eluent, 50 m*M* HNO_3–50 m*M* NaOAc; AS7 separation + NG1 guard columns; postcolumn reaction with $HClO_4$; UV detection at 330 nm.

spiked with EDTA and the third fertilizer was spiked with EDTA, HEEDTA and DTPA. The results are given in Table 3. Matrix components from fertilizers interfere only slightly by increasing the void volume signal, as illustrated in Fig. 6.

The method proved to be satisfactory for the determination of the total amount of EDTA, HEEDTA and DTPA in mixed fertilizers at levels down to 0.1%. In fact, the system is completely insensitive even to large amounts of chloride, nitrate, carbonate, sulphate and orthophosphate. It must be emphasized that, after repeated injections of solutions treated with excess of Fe(III), the characteristics of the separation column are irreversibly altered. Residual iron on the column leads to a decrease in sensitivity for sulphate and orthophosphate. Solutions of Mn–EDTA, Zn–EDTA, Co–EDTA and Cu–EDTA were injected onto the "contaminated" column, without precolumn treatment with Fe(III)–HNO_3. After postcolumn reaction with Fe(III)–$HClO_4$ and UV detection at 330 nm, the eluate was fractionated and the

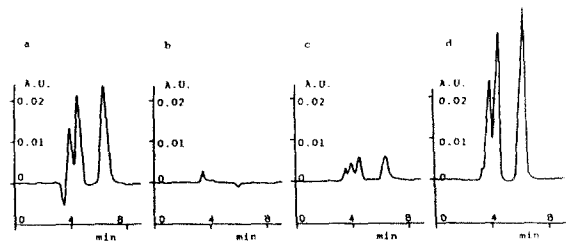


Fig. 6. Chromatograms for (a) a standard solution of HEEDTA, EDTA and DTPA (each 0.08 m*M*), (b) NPK 15.5.15 fertilizer, and the same sample spiked with (c) 0.1% (w/w) and (d) 1% (w/w) of HEEDTA, EDTA and DTPA, in the presence of an excess of Cu(II). Method: see Table 2.

Table 3
Recoveries of EDTA, HEEDTA and DTPA when added at levels of 0.01, 0.1 and 1% (w/w) to commercial fertilizers, in the presence of an excess of Cu(II)

Sample declaration	Concentration added (%, w/w)	Recovery (%)		
		EDTA	HEEDTA	DTPA
NPK 12.8.16 + 10 SO_3	0.01	97	–[a]	–[a]
	0.1	94	–[a]	–[a]
	1	114	–[a]	–[a]
NPK 9.8.18 + 15 SO_3	0.01	107	–[a]	–[a]
	0.1	95	–[a]	–[a]
	1	96	–[a]	–[a]
NPK 15.5.15	0.01	65	–[b]	137
	0.1	103	114	94
	1	103	98	109

Method: see Table 2.
[a] No spike added.
[b] Below determination limit.

collected EDTA fraction analysed by atomic absorption spectrometry. In all instances, except with Cu–EDTA, the only metal present in the collected fraction was iron. When Mn–EDTA, Zn–EDTA or Co–EDTA was injected, these metals were not recovered in the EDTA fraction, but in the void volume fraction. In spite of the acidic eluent, residual iron remains on the column, replacing manganese, zinc and cobalt from their weak complexes, letting the free metal ions run rapidly through the column.

Acknowledgement

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