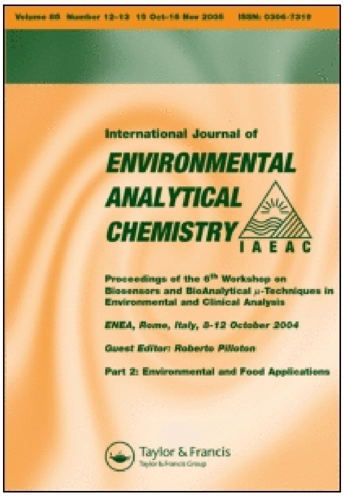

**International Journal of Environmental Analytical Chemistry**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

# The determination of the degree of zinc complexation by chelating agents with differential pulse voltammetry

Magdalena Borowiec[a]; Krystyna Hoffmann[a]; Józef Hoffmann[a]
[a] Institute of Inorganic Technology and Mineral Fertilizers, Wroclaw University of Technology, 50-372 Wrocław, Poland

**To cite this Article** Borowiec, Magdalena , Hoffmann, Krystyna and Hoffmann, Józef(2009) 'The determination of the degree of zinc complexation by chelating agents with differential pulse voltammetry', International Journal of Environmental Analytical Chemistry, 89: 8, 717 — 725

**To link to this Article: DOI:** 10.1080/03067310802691672

**URL:** http://dx.doi.org/10.1080/03067310802691672

*Intern. J. Environ. Anal. Chem.*
Vol. 89, Nos. 8–12, 15 July–15 October 2009, 717–725

Taylor & Francis
Taylor & Francis Group

# The determination of the degree of zinc complexation by chelating agents with differential pulse voltammetry

Magdalena Borowiec, Krystyna Hoffmann and Józef Hoffmann*

*Institute of Inorganic Technology and Mineral Fertilizers, Wroclaw University of Technology, ul. Smoluchowskiego 25, 50-372 Wrocław, Poland*

(*Received 31 July 2008; final version received 12 December 2008*)

The degree of zinc complexation was related to the type of the chelating agent and to the pH, whereas the stability of the zinc complexes was examined according to the chelating agents chosen from the group of aminopolycarboxylates. The degree of zinc complexation by the chelating compounds of the aminopolycarboxylates [ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), N-(hydroxyethyl)ethylenediaminetriacetic acid (HEEDTA), nitrilotriacetic acid (NTA), S,S-ethylenediaminedisuccinic acid (S,S-EDDS), methylglycinediacetic acid (MGDA), glutamic acid diacetic acid (GLDA)] in the environment of the supporting electrolyte was determined by differential pulse voltammetry using a mercury electrode operating in the SMDE mode. Reference electrode was silver-chloride electrode and auxiliary electrode was glassy carbon electrode. Voltammetric researches were carried out on the AUTOLAB PGSTAT 12 (Eco Chemie – The Netherlands) with GPES software with the use of mercury electrode 663 VA Stand (Metrohm – Swiss). The degree of zinc complexation increased with the increase in the concentration of the chelating agent and with the rise in the pH. Zinc complexes with EDTA, HEEDTA, DTPA and S,S-EDDS preserved their stability with time elapsed.

**Keywords:** zinc; voltammetry; degree of complexation; stability of chelate complexes

## 1. Introduction

Plants are not able to complete their developmental cycles when they lack nutrients that are indispensable for their physiology. The indispensable nutrient participates in the nutrition of the plant and cannot be replaced by any other nutrient. Besides carbon, hydrogen and oxygen (the major constituents of organic substances), there are also 14 mineral nutrients regarded as essential for the growth and reproduction of higher green plants [1]. According to their concentration in the plant, they are classified into two groups: macroelements (C, H, O, N, P, S, K, Ca and Mg) and microelements (Fe, Mn, Zn, Cu, B, Mo, Cl and Ni) [1,2]. A microelement participates in the nutrition of the plant either as a metabolite component or as an element essential for the functioning of enzymatic systems. Microelements perform a number of useful functions, which exclude their replacement with other elements [1,2].

---

*Corresponding author. Email: jozef.hoffmann@pwr.wroc.pl

ISSN 0306–7319 print/ISSN 1029–0397 online

DOI: 10.1080/03067310802691672
http://www.informaworld.com

### 1.1 *Significance of zinc in the nutrition of plants*

Zinc enters the plant in the form of $Zn^{2+}$, when taken up through the roots. But the plant also obtains zinc through its leaves, and then the micronutrient is taken up both in ionic form and in the form of chelates [1–3]. The microelement moves mainly in xylem, slight mobility being also observed in phloem. A major physiological function performed by zinc is the formation of enzyme–substrate bonds [1]. Furthermore, being a component of RNA polymerase, zinc participates in the nitrogen metabolism of the plant and in the synthesis of protein [1–3]. Because of its occurrence at the second stage of oxidation only, zinc does not take part in red-ox processes [2].

Optimal zinc content varies from 20 to 100 mg in 1 kg of dry matter, the concentration of 200 $mg\,kg^{-1}$ being toxic to the majority of plant species [1,2].

Deficiency of zinc manifests in the intervenular chlorosis of young leaves and in their dwarfing, in the light green chromatosis of older leaves and in the widespread, white stripes on both sides of the main leaf vein [1–3].

When zinc occurs in excess (over 100 $mg\,kg^{-1}$ dry matter), it exerts a toxic effect on the plant; both roots and leaves grow at a slower rate, and thus contribute to the development of chlorosis [2].

### 1.2 *Micronutrient fertilizers*

The microelement fertilizers that occur in soils are not easily available to the growing plants, and that is why the deficiency of a certain nutrient must be compensated by the application of fertilizers [1].

Micronutrient fertilizers are mineral or organic-mineral compounds containing one or more microelements [1,2]. They are divided into the following groups: fritted glass products (frits), liquid fertilizers with nutrients, micro-fertilizer chelates and special micro-fertilizers [2].

### 1.3 *Micronutrient chelates*

Microelements have the tendency to form sparingly soluble, ineffective precipitates with some of the components of the fertilizers applied. That is why a microelement must be complexed by another component in order to become bioavailable. For this purpose use is made of natural or synthetic organic compounds, and the complexes that are formed with microelements are defined as chelates. From the chelates, the nutrients are gradually released to the relevant ionic form, since the dissociation of these compounds proceeds at a slow rate, despite their high water-solubility [1,3].

### 1.4 *Chelating compounds*

The metal cation should be permanently keep in solution by chelating compounds, regardless of the other components of the fertilizer and the range of the pH [4,5]. The microelements must be made available to the plant within a time span that corresponds with their degradability time, and the degree of nutrient complexation should amount to at least 80% [6,7].

From the synthetic chelating agents for the production of micronutrient chelates, the following ones have found the widest acceptance: ethylenediaminetetraacetic acid

(EDTA), diethylenetriaminepentaacetic acid (DTPA), N-(hydroxyethyl)ethylenediaminetriacetic acid (HEEDTA) and their salts [7]. Because of their detrimental effect on the environment, much attention has been given to such complexing agents that could replace them in the production of liquid fertilizers, namely to methylglycinediacetic acid (MGDA), glutamic acid diacetic acid (GLDA), S,S-ethylenediaminedisuccinic acid (S,S-EDDS) and their salts [4,8].

The structure and applications of EDTA, DTPA and HEEDTA are similar. These chelating substances are widely used in the production of detergents, fertilizers and cosmetics, as well as in the photographic and paper-making industries [4,5]. However, it is essential to note that EDTA, DTPA and HEEDTA are not readily biodegradable [9]. EDTA is believed to produce environmental hazards by causing water eutrophication, and is being blamed for long-lasting accumulation in the environment and heavy metal remobilisation in natural conditions [5]. High concentrations of DTPA may cause toxic effects on microorganisms and inhibit their growth [10,11]. Nitrilotriacetic acid (NTA) was found to be totally biodegradable; nevertheless, it could cause the carcinogenic effects [12,13].

For the reason of potential environmental hazards resulting from the use of non-biodegradable compounds, much effort has been reported into finding such chelating agents that would be biodegradable and efficient, and could safely replace the ones applied so far. In the literature it is showed that S,S-EDDS, MGDA and GLDA are non-toxic compounds of a high biodegradability [9,13–15,17]. S,S-Ethylenediaminedisuccinic acid could form stable complexes with metals and it is a powerful chelating agent for transition metal ions [8,16]. Furthermore, the metal-complexing power of MGDA is stronger than that of citrates, and the complexes formed by MGDA display a high stability over a wide range of both pH and temperature [14,15,17].

### 1.5 *Aim of work*

The aim of the study was to determine the degree of zinc complexation with different compounds of the aminopolycarboxylic group in the aquatic environment, in the presence of the supporting electrolyte, and at a varying pH. The stability of the complexes of zinc with the metal-complexing compounds mentioned was analysed as a function of time.

According to the Regulation (EC) No 2003/2003 of the European Parliament and of the Council of 13 October 2003 relating to fertilizers, at least 80% of the declared microelement content must be in chelated form [6]. For this reason, our study also addressed the problem of determining the time and conditions required for complexing at least 80% of the micronutrient.

## 2. Experimental

### 2.1 *Chemicals*

The microelement solution was prepared by dissolving suitable amount of inorganic salt in distilled water ($ZnSO_4 \cdot 7H_2O$). The concentration of microelement in samples was $0.001\,mol\,L^{-1}$. All chelating agents solutions were prepared by dissolving organic acids or their salts in distilled water (ethylenediaminetetraacetic acid disodium salt – EDTA, diethylenetriaminepentaacetic acid – DTPA, N-(hydroxyethyl)ethylenediaminetriacetic acid – HEEDTA, nitrilotriacetic acid – NTA, S,S-ethylenediaminedisuccinic acid trisodium salt – S,S-EDDS, glutamic acid diacetic acid tetrasodium salt – GLDA, methylglycinediacetic acid trisodium salt – MGDA). Methylglycinediacetic acid is produced by BASF as TRILON®M LIQUID (concentration of MGDA is 40%). Glutamic acid diacetic acid is produced by Akzo Nobel as Dissolvine® GL (concentration of GLDA is 38%).

The experiments were conducted in room temperature and in an aquatic environment: in the solution of the supporting electrolyte ($0.1\,\mathrm{mol\,L^{-1}}$ KCl) with a 0.01% gelatin solution, and at a varying pH, because the metal-complexing capacity of the chelating agents was pH dependent. The pH of the experimental media was determined with $0.1\,\mathrm{mol\,L^{-1}}$ HCl and $0.1\,\mathrm{mol\,L^{-1}}$ NaOH. The oxygen was removed by running nitrogen (99.999%) through the sample.

In the course of the experiments the concentrations of the chelating compounds were varied and concentration of the microelement was kept constant.

### 2.2 *Experimental conditions*

The degree of zinc complexation with particular chelating agents (EDTA, S,S-EDDS, HEEDTA, DTPA, NTA, MGDA and GLDA) was determined by differential pulse voltammetry using a mercury electrode operating in the SMDE mode. The method consisted in the measurement of current intensity during flow through the solution (containing the substance being analysed) in the course of electrolysis, according to the linearly changing potential. The electrode operating throughout the experiments was a static mercury drop electrode, the reference and auxiliary being a silver-chloride electrode and a glassy carbon electrode, respectively. Voltammetry was performed by means of the Eco Chemie (Netherlands) apparatus AUTOLAB PGSTAT 12 with GPES software, using a 663 VA Stand dropping mercury electrode (Metrohm, Switzerland).

The degree of zinc complexation was determined from the height of the current intensity peak which is directly proportional to the concentration of the free metal ion in solution. The concentration of the free metal ion and the degree of metal complexation were established from the difference in the heights of the peaks for the metal cation alone and for the metal cation with the chelating agent added.

The experiments had a duration of 0, 8, 24, 72, 120, 168 and 336 h.

The researches were made with the smallest size of mercury drope ($\sim 0.25\,\mathrm{mm^2}$). The step potential was 0.00495 V, modulation amplitude was 0.00255 V and modulation time was 0.05 s. Equilibrium time was 5 s and number of scans were 2.

Figure 1 shows the voltamogram of free zinc ions in supporting electrolyte ($0.1\,\mathrm{mol\,L^{-1}}$ KCl) in pH 5, in $t = 0$ [(1) $C_{\mathrm{Zn}} = 10^{-6}\,\mathrm{mol\,L^{-1}}$, (2) $C_{\mathrm{Zn}} = 10^{-5}\,\mathrm{mol\,L^{-1}}$, (3) $C_{\mathrm{Zn}} = 10^{-4}\,\mathrm{mol\,L^{-1}}$, (4) $C_{\mathrm{Zn}} = 5 \times 10^{-4}\,\mathrm{mol\,L^{-1}}$, (5) $C_{\mathrm{Zn}} = 10^{-3}\,\mathrm{mol\,L^{-1}}$].

## 3. Results and discussion

Table 1 summarises the values of the degree of zinc complexation in the chelates with the compounds chosen from the group of aminopolycarboxylates, which were obtained at $t = 0$, pH $= 3$ and a varying molar ratio of the chelating agent to zinc.

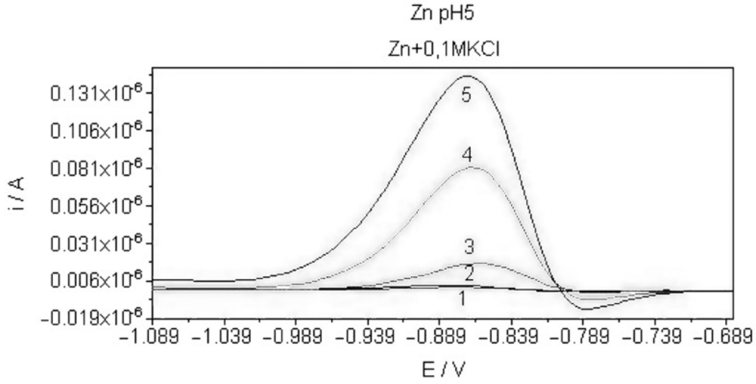

Figure 1. Voltamogram of free zinc ions in supporting electrolyte (0.1 mol $L^{-1}$ KCl) in pH 5, in $t = 0$ (1. $C_{Zn} = 10^{-6}$ mol $L^{-1}$, 2. $C_{Zn} = 10^{-5}$ mol $L^{-1}$, 3. $C_{Zn} = 10^{-4}$ mol $L^{-1}$, 4. $C_{Zn} = 5 \times 10^{-4}$ mol $L^{-1}$, 5. $C_{Zn} = 10^{-3}$ mol $L^{-1}$).

Table 1. Degree of zinc complexation (%) with different chelating agents at pH = 3, $t = 0$.

| Molar ratio $C_{chel}/C_{Zn}$ | EDTA | HEEDTA | NTA | DTPA | S,S-EDDS | GLDA | MGDA |
|---|---|---|---|---|---|---|---|
| 0.25 | 35.85 | 43.45 | 47.80 | 37.30 | 21.85 | 30.45 | 36.20 |
| 0.5 | 58.79 | 58.18 | 51.81 | 59.71 | 22.95 | 42.90 | 42.50 |
| 0.75 | 76.90 | 80.06 | 54.33 | 77.95 | 31.05 | 52.31 | 49.25 |
| 1 | 97.27 | 91.79 | 56.40 | 98.20 | 33.85 | 59.63 | 57.96 |
| 1.5 | 99.79 | 96.93 | 70.34 | 99.77 | 38.45 | 73.65 | 66.25 |
| 2 | 99.87 | 98.54 | 76.18 | 99.87 | 38.50 | 80.31 | 70.85 |
| 2.5 | 99.91 | 98.60 | 83.07 | 99.89 | 41.30 | 79.70 | 70.26 |
| 5 | 100 | 99.07 | 91.89 | 99.94 | 54.97 | 87.66 | 82.84 |
| 8 | 100 | 99.53 | 94.48 | 99.93 | 77.03 | 91.59 | 89.06 |
| 10 | 100 | 99.61 | 94.76 | 100 | 83.06 | 93.03 | 90.54 |

The degree of zinc complexation exceeded 80% at the following molar ratios: EDTA to zinc = 1; HEEDTA to zinc = 0.75; NTA to zinc = 2.5; DTPA to zinc = 1; S,S-EDDS to zinc = 10; GLDA to zinc = 2; and MGDA to zinc = 5.

Of the chelating agents chosen, EDTA was found to be the most powerful and S,S-EDDS the least powerful complexing compound at pH = 3. At an equimolar ratio of the chelating compound to zinc, a 90% complexation was achieved with EDTA, HEEDTA and DTPA as the chelating agents. However, with the application of NTA, S,S-EDDS, GLDA or MGDA, the degree of zinc complexation failed to reach 60%. Despite the high excess of the chelating compound, the complexation of zinc was incomplete when use was made of HEEDTA, NTA, S,S-EDDS, GLDA or MGDA. There were only two molar ratios at which no traces of zinc were detected: that of EDTA to zinc = 5 and that of DTPA to zinc = 10.

Table 2 shows the values of the degree of zinc complexation in the chelates with the chosen complexing compounds, which were determined at $t = 0$, pH = 5 and a varying molar ratio of the chelating agent to zinc.

Table 2. Degree of zinc complexation (%) with different chelating agents at pH = 5, $t = 0$.

| Molar ratio $C_{\mathrm{chel}}/C_{\mathrm{Zn}}$ | EDTA | HEEDTA | NTA | DTPA | S,S-EDDS | GLDA | MGDA |
|---|---|---|---|---|---|---|---|
| 0.25 | 30.10 | 39.50 | 71.08 | 38.55 | 38.15 | 33.50 | 35.95 |
| 0.5 | 52.86 | 57.27 | 78.82 | 68.32 | 64.79 | 54.50 | 54.60 |
| 0.75 | 72.21 | 79.78 | 80.80 | 81.38 | 81.78 | 73.80 | 75.32 |
| 1 | 98.68 | 99.88 | 89.93 | 99.69 | 97.93 | 92.79 | 94.82 |
| 1.5 | 100 | 100 | 98.21 | 99.92 | 99.27 | 97.41 | 98.15 |
| 2 | 100 | 100 | 98.62 | 100 | 99.39 | 98.08 | 98.42 |
| 2.5 | 100 | 100 | 99.32 | 100 | 99.52 | 98.55 | 98.61 |
| 5 | 100 | 100 | 99.24 | 100 | 99.73 | 99.04 | 99.13 |
| 8 | 100 | 100 | 99.73 | 100 | 99.80 | 99.26 | 99.38 |
| 10 | 100 | 100 | 99.72 | 100 | 100 | 99.43 | 99.39 |

Table 3. Degree of zinc complexation (%) with different chelating agents at pH = 7, $t = 0$.

| Molar ratio $C_{\mathrm{chel}}/C_{\mathrm{Zn}}$ | EDTA | HEEDTA | NTA | DTPA | S,S-EDDS | GLDA | MGDA |
|---|---|---|---|---|---|---|---|
| 0.25 | 30.90 | 40.05 | 75.65 | 41.90 | 39.65 | 36.20 | 39.35 |
| 0.5 | 53.80 | 56.69 | 80.02 | 70.07 | 66.03 | 62.43 | 58.13 |
| 0.75 | 74.21 | 79.86 | 85.20 | 82.57 | 83.84 | 83.70 | 80.43 |
| 1 | 98.84 | 100 | 94.06 | 99.78 | 99.90 | 98.37 | 99.18 |
| 1.5 | 99.97 | 100 | 99.71 | 100 | 99.92 | 99.78 | 99.78 |
| 2 | 100 | 100 | 99.73 | 100 | 100 | 99.88 | 99.83 |
| 2.5 | 100 | 100 | 99.91 | 100 | 100 | 99.87 | 99.88 |
| 5 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 8 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 10 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

The degree of zinc complexation exceeded 80% at the following molar ratios: EDTA to zinc = 1; HEEDTA to zinc = 1; NTA to zinc = 0.75; DTPA to zinc = 0.75; S,S-EDDS to zinc = 0.75; GLDA to zinc = 1; and MGDA to zinc = 1.

The highest complexation effects were achieved with the most powerful chelating agents, EDTA and HEEDTA; the lowest were those obtained with MGDA. At an equimolar ratio of the chelating compound to zinc, a 90% complexation was achieved when use was made of EDTA, HEEDTA, DTPA, S,S-EDDS, GLDA and MGDA as the chelating agents. However, with the application of NTA, the degree of zinc complexation failed to reach 90%. Despite the high excess of the chelating compound, the complexation of zinc was incomplete when NTA, GLDA and MGDA were used. There were four molar ratios at which no traces of zinc were detected: EDTA to zinc = 1.5; HEEDTA to zinc = 1.5; DTPA to zinc = 2, and S,S-EDDS to zinc = 10.

Table 3 includes the values of the degree of zinc complexation in the chelates examined, which were obtained at $t = 0$, pH = 7 and a varying molar ratio of the chelating agent to zinc.

The degree of zinc complexation exceeded 80% at the following molar ratios: EDTA to zinc = 1; HEEDTA to zinc = 1; NTA to zinc = 0.5; DTPA to zinc = 0.75; S,S-EDDS to zinc = 0.75; GLDA to zinc = 0.75; and MGDA to zinc = 0.75.

N-(Hydroxyethyl)ethylenediaminetriacetic acid (HEEDTA) and GLDA were found to be the most and the least powerful chelating agents for zinc, respectively. At an equimolar ratio of the chelating substance to metal, a 90% complexation of zinc was achieved with all the chelating agents chosen. No traces of zinc were detected upon application of the following molar ratios: EDTA to zinc = 2; HEEDTA to zinc = 1; NTA to zinc = 5; DTPA to zinc = 1.5; S,S-EDDS to zinc = 2 and MGDA to zinc = 5.

The stability of the chelates was examined with the same method and under the same conditions as in the tests on zinc complexation.

Figures 2–4 show how the degree of zinc complexation in the chelates with the chosen complexing compounds varied with time, at an equimolar ratio of the chelating agent to zinc, and at the pH of 3, 5 and 7.

With time elapsed, at pH = 3, the degree of zinc complexation with MGDA decreased by approximately 20%. When use was made of GLDA, DTPA, EDTA, NTA or HEEDTA as the chelating agent, the decrease was insignificant. The use of S,S-EDDS

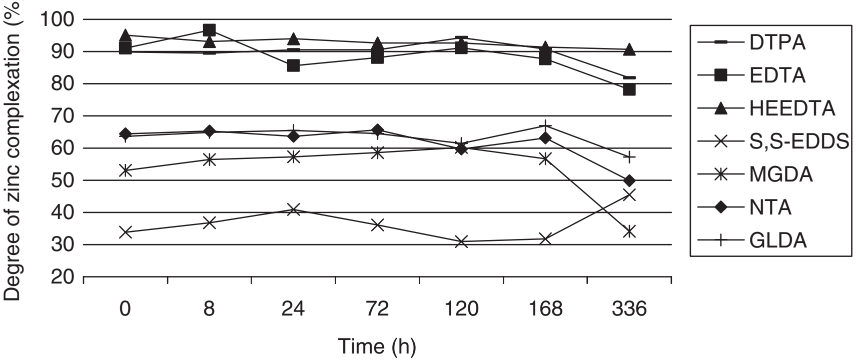

Figure 2. Time-related variations in the degree of zinc complexation with different chelating compounds, at pH = 3 and an equimolar ratio of the chelating agent to zinc.

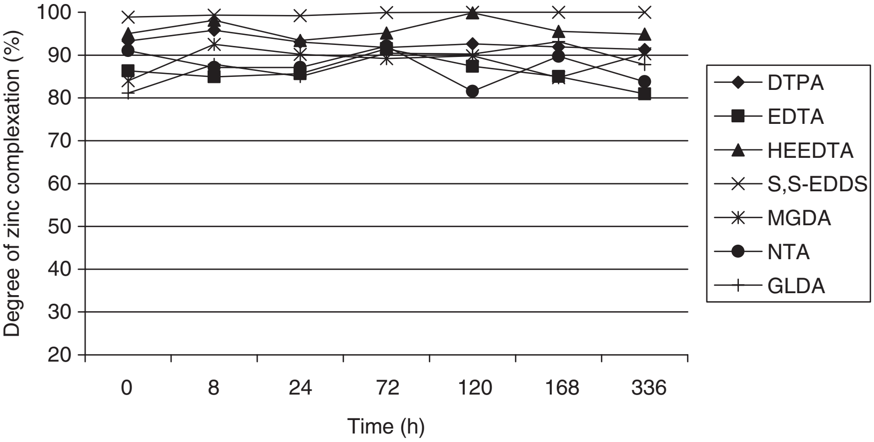

Figure 3. Time-related variations in the degree of zinc complexation with different chelating compounds, at pH = 5 and an equimolar ratio of the chelating agent to zinc.

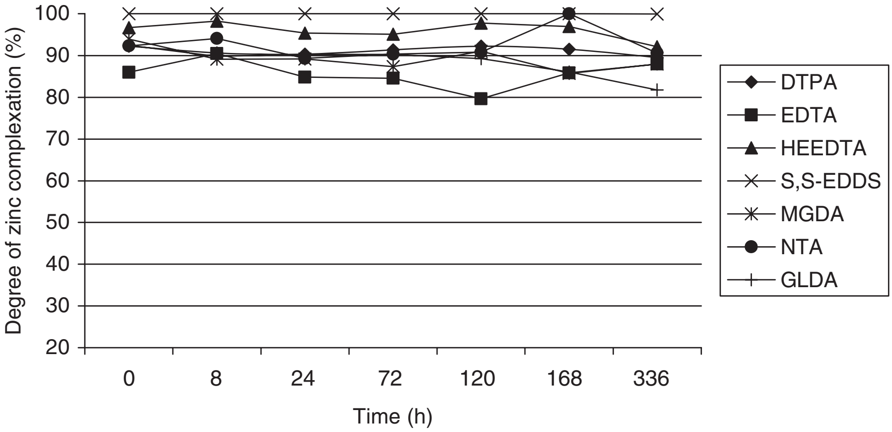

Figure 4. Time-related variations in the degree of zinc complexation with different chelating compounds, at pH = 7 and an equimolar ratio of the chelating agent to zinc.

brought about a 10% increase in zinc complexation. The experiments have shown that the complexes formed by DTPA, EDTA, HEEDTA, S,S-EDDS, NTA and GLDA are stable.

With time, at pH = 5, the degree of zinc complexation in the chelate with NTA decreased by about 8%, but with DTPA, EDTA or HEEDTA as the complexing compound the decrease was negligible. A rise in the degree of complexation was observed when S,S-EDDS, MGDA or GLDA formed complexes with zinc. As can be inferred from the analysis of the results obtained, the zinc–aminopolycarboxylates complexes formed by DTPA, EDTA, HEEDTA, S,S-EDDS, MGDA and GLDA are stable.

When GLDA and MGDA were used as a zinc complexing compounds the degree of complexation at pH = 7 decreased with time by about 9 and 6%, respectively, whereas with DTPA, NTA, S,S-EDDS and HEEDTA used as chelating agents, this decrease was found to be insignificant. When EDTA was used as the metal-complexing compound, the degree of zinc complexation increased. The experimental results provide evidence that the zinc–aminopolycarboxylates complexes formed by DTPA, EDTA, HEEDTA, S,S-EDDS and NTA are stable.

## 4. Conclusions

From the analysis of the experimental results the following can be inferred:

- When the molar ratio of the chelating agent to the microelement increases, so does the degree of zinc complexation.
- The rise in the pH is parallelled by the rise in the degree of zinc complexation.
- At pH = 3, the use of EDTA as the chelating compound accounted for the strongest and the use of S,S-EDDS for the poorest complexation of zinc. Eighty percent complexation was achieved at different molar ratios, with all of the chelating agents used. The complexes are stable, the only exception being the chelate compound formed by MGDA, where the degree of zinc complexations decreased by 20%.

- At pH = 5, the highest values of zinc complexation were obtained with EDTA and HEEDTA, while those achieved with MGDA were the lowest. Eighty percent complexation was attained at the molar ratio of 0.75 or 1, with all the chelating agents tested. The complexes are stable; it was only in the chelate formed by NTA that the degree of zinc complexation decreased by 8%.
- At pH = 7, the highest and the lowest degree of zinc complexation was obtained with HEEDTA and GLDA, respectively. Each of the chelating compounds tested accounted for an 80% complexation at various molar ratios. The complexes are stable; only in those formed by GLDA and MGDA the degree of complexation decreased by 9% and 6%, respectively.
- The new chelating agents (S,S-EDDS, MGDA, GLDA), chosen for the reason of their biodegradability, were characterised by weaker complexing properties than popular used compounds as EDTA, DTPA and HEEDTA. S,S-Ethylenediaminedisuccinic acid (S,S-EDDS) was the best compound from the new chelating agents group for zinc complexation in pH 5 and 7.
- The best complexing properties, from group of the new biodegradable compounds, had S,S-EDDS. This compound is a structural isomer of EDTA and this structure has an influence on the good complexing properties.
- MGDA and GLDA, which structure is similar to NTA, were characterised by weaker zinc complexing properties than for example EDTA in all tested values of pH.
- All zinc complexes with the new chelating agents were stable in time.

## References

[1] E. Gorlach and T. Mazur, *Chemia rolna, Podstawy żywienia i zasady nawożenia roślin* (Wydawnictwo Naukowe PWN, Warszawa, 2001).

[2] S. Mercik, *Chemia rolna, Podstawy teoretyczne i praktyczne* (Wydawnictwo SGGW, Warszawa, 2002).

[3] M. Fotyma and S. Mercik, *Chemia rolna* (Wydawnictwo Naukowe PWN, Warszawa, 1995).

[4] EDTA na cenzurowanym, Przem Chem. **7**, 279 (1998).

[5] C. Oviedo and J. Rodriguez, Quim. Nova **6**, 901 (2003).

[6] Regulation (EC) No 2003/2003 of the European Parliament and of the Council of 13 October 2003 relating to fertilizers (Text with EEA relevance).

[7] J. Hoffmann and H. Górecki, Zesz. Probl. Post. Nauk Rol. **471**, 637 (2000).

[8] P.W. Jones and D.R. Williams, Appl. Radi. Isot. **54**, 587 (2001).

[9] T.P. Knepper, Trends Anal. Chem. **10**, 708 (2003).

[10] S. Metsärinne, P. Rantanen, R. Aksela, and T. Tunkanen, Chemosphere **55**, 379 (2004).

[11] J.B. Rodriguez, A. Mutis, M.C. Yeber, J. Freer, J. Baeza, and H.D. Mansilla, Wat. Sci. Tech. **11-12**, 267 (1999).

[12] A.T.M. van der Steen and M. Heus, Safety Data Sheet Dissolvine GL-38, According to EC-Directive 2001/58/EC (2005).

[13] T. Egli, J. Biosci. Bioeng. **2**, 89 (2001).

[14] Technical Bulletin, Methylglycinediacetic acid, Environ. Sci. Technol. **38**, 937 (2004).

[15] M. Bucheli-Witschel and T. Egli, FEMS Microbiol. Rev. **25**, 69 (2001).

[16] Chelating agents, Question and Answers, NTA (1990). <http://www.cefic.org/Templates/shwAssocDetails.asp?NID=473&HID=27&ID=53> (accessed 29 July 2008).

[17] Trilon® M-liquid reagent, Technical Bulletin, BASF The Chemical Company (2003).