PREPARATION AND CHARACTERIZATION OF ZINC COMPLEXES AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITY

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Abstract: Zinc chelates with diethylenetriaminepentaacetic acid (DTPA), ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA) and iminodiacetic acid (IDA) have been prepared and conditions for Zn^{2+} release have been studied. The prepared Zn^{2+} chelate complexes are of following compositions: $ZnCl_2$(EDTA), $Zn(ClO_4)_2 \cdot 6H_2O$ (EDTA), $ZnCl_2$(NTA), $ZnCl_2(NTA)_3$(btc), $Zn(ClO_4)_2 \cdot 6H_2O$ (NTA), $ZnCl_2$ (NDA), $Zn(ClO_4)_2 \cdot 6H_2O$ (NDA), $ZnCl_2(NDA)_3$(btc). (H_3btc = 1,3,5-benzenetricarboxylic acid). All variants of Zn^{2+} complexes were diluted to the concentration range 0–1420 µM and the absorbance spectra in the range of 230–330 nm were measured. The antimicrobial properties of Zn^{2+} complexes were studied by the method of the growth curves of the bacteria cultures *Staphyloccocus aureus*. The 50% inhibitory concentration was determined to 500 µM of each Zn^{2+} complex. It has been found that the Zn^{2+} complexes showed increased antimicrobial effect on *Staphyloccocus aureus*.

Key Words: Zinc, EDTA, nitriloacetic acid, spectrophotometry, antimicrobial activity

INTRODUCTION

Zinc is an essential element in living organisms. It plays a key role in variety metabolic pathways, cell differentiation, eliminating of oxidative stress, apoptosis and proteins stability (Kambe et al. 2015). The deficiency of zinc is usually due to insufficient dietary intake, but it could be associated with various diseases, such as diabetes, burns, Down´s syndrome, chronic liver disease, chronic renal disease, sickle cell disease or malignancy (Miller et al. 2015). The two main factors affect the zinc absorption from meal: content of inositol hexakisphosphate or phytic acid in the meal (Lazarte et al. 2015). These two compounds are known as a principal storage form of phosphorus in many plant tissues. Phytic acid has a strong binding affinity to important minerals, such as calcium, iron, and zinc (Iwai et al., 2012). In the diet of livestock predominates grains, such as maize, legumes, and soybeans, which are rich in phytic acid. Considering this fact, the Zn^{2+} deficiency could be caused by inappropriate feeding. Zinc deficiency in livestock is manifested by reduced growth rate, reduced fertility, para keratosis (thickening and scaling of skin cells), loss of hair, dermatitis (inflammation of the skin), and an increased susceptibility to foot rot and other foot infections (Rincker et al. 2005). While clinical cases of zinc deficiency are rare, sub-clinical deficiencies can be more accurately assessed with a feed analysis that will help determine a potential deficiency and possible solution. The zinc could be added in the diet by supplements or included in trace mineralized salts or chelated mineral supplements, which may be useful in availability difficulties of mineral due to interference of absorption (Bertinato et al. 2012). In our study, we focused on four chelating agents ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA) and iminodiacetic acid (NDA). EDTA usually binds a metal cation through its two amines and four carboxylates. In contrast to EDTA, NTA is easily biodegradable and is almost completely removed during wastewater treatment. The iminodiacetate anion can act as a tridentate ligand to form a metal complex with two, fused, five membered chelate rings, in addition forms stronger complexes than the bidentate ligand glycine and weaker complexes than the tetradentate ligand nitrilotriacetic acid (Martorelli et al. 2015).

The aim of our study was to prepare zinc chelate complexes with EDTA, NTA and NDA chelating agents. The complexes were characterized spectrophotometrically and the antimicrobial activity was determined.

MATERIAL AND METHODS

Preparation of Zn complexes

Zn EDTA-1

Solution of $ZnCl_2$ (0.136 g) was stirred with EDTA (0.292) on magnetic stirrer. The pH was adjusted to 7 by addition of NaOH and the volume of sample was diluted to 100 mL · 50 mL of solution was left crystalization.

Zn EDTA-2

Solution of $Zn(ClO_4)_2 \cdot 6H_2O$ (0.366 g) was stirred with EDTA (0.292 g) on a magnetic stirrer. The pH was adjusted to 7 by addition of NaOH and the volume of sample was diluted to 100 mL.

Zn NTA-1

Solution of $ZnCl_2$ (0.136 g) was stirred with sodium salt of NTA (0.257 g) on the magnetic stirrer. The pH was adjusted to 7 and the volume of sample was diluted to 100 mL.

Zn NTA-2

Solution of $ZnCl_2$ (0.136 g) was stirred with sodium salt of NTA (0.257 g) and H_3btc (0.092 g) on the magnetic stirrer. The pH was adjusted to 7 by addition of NaOH and the volume of sample was diluted to 100 mL.

Zn NTA-3

Solution of $Zn(ClO_4)_2 \cdot 6H_2O$ (0.366 g) was stirred with NTA (0.257 g) and H_3btc (0.092 g) on the magnetic stirrer. The pH was adjusted to 7 and the volume of sample was diluted to 100 mL.

Zn NDA-1

Solution of $ZnCl_2$ (0.136 g) was stirred with IDA (0.133 g) on the magnetic stirrer. The pH was adjusted to 7 by addition of NaOH and the volume of sample was diluted to 100 mL.

Zn NDA-2

Soution of $ZnCl_2$ (0.136 g) was stirred with IDA (0.133 g) and H_3btc (0.092 g) on the magnetic stirrer. The pH was adjusted to 7 and the volume of sample was diluted to 100 mL.

Zn NDA-3

Solution of $Zn(ClO_4)_2 \cdot 6H_2O$ (0.366 g) was stirred with NTA (0.257 g) and H_3btc (0.092 g) on the magnetic stirrer. The pH was adjusted to 7 and the volume of sample was diluted to 100 mL.

Spectrophotometric determination of Zn^{2+} complexes

Absorption spectra were acquired by multifunctional microplate reader Tecan Infinite 200 PRO (TECAN, Switzerland). Absorbance spectra were measured within the range from 230– 850 nm per 2-nm steps. The samples were placed in UV-transparent 96 well microplate with flat bottom by CoStar (Corning, USA). To each well was placed 100 µL of sample. All measurements were performed at 30°C controlled by Tecan Infinite 200 PRO (TECAN, Switzerland).

Determination of antimicrobial activity

S. aureus (NCTC 8511) was obtained from the Czech Collection of Microorganisms, Faculty of Science, Masaryk University, Brno, Czech Republic. Cultivation media (LB = Luria Bertani) were inoculated with bacterial culture and were cultivated for 24 hours on a shaker at 40 g and 37°C. Bacterial culture was diluted by cultivation medium to OD600 = 0.1 for the following experiments. Growth curves were used to test the antibacterial properties. The antimicrobial effect of tested compounds was determined by measuring the absorbance using an apparatus Multiskan EX (Thermo Fisher Scientific, Germany). In a microtitration plate, *S. aureus* cultures were mixed with Zn^{2+} complexes. The total volume in the microtitration plate wells was always 300 µL.

Descriptive Statistics

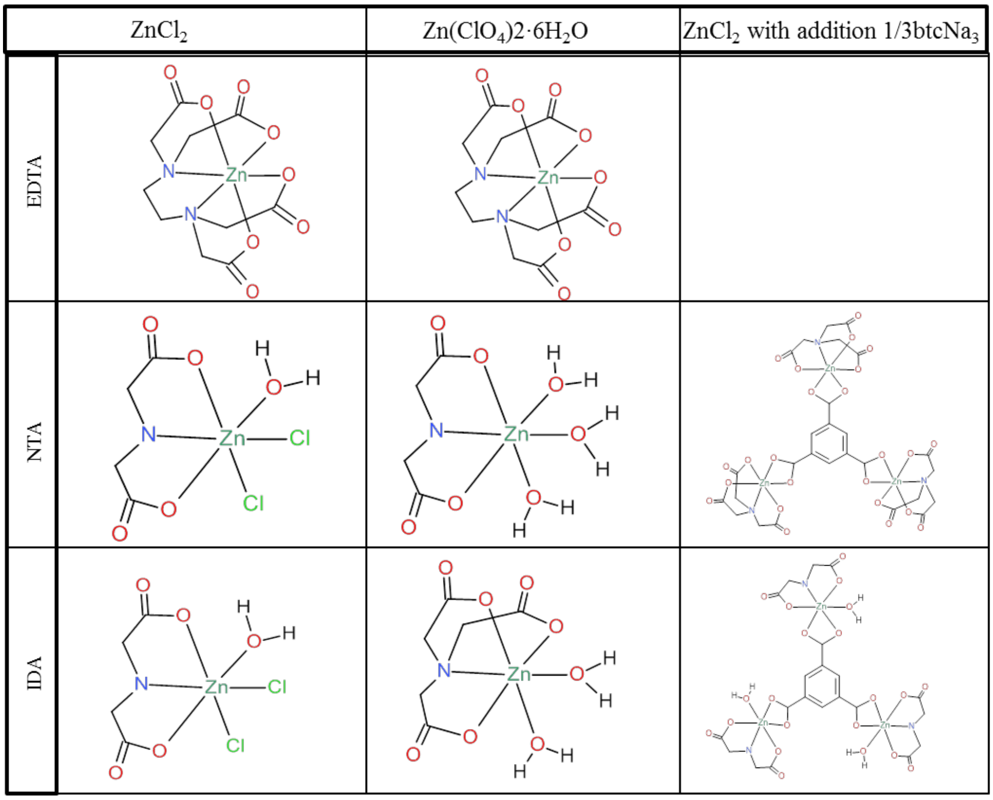
Data were processed using MICROSOFT EXCEL® (Microsoft, Albuquerque, New Mexico Manufacturers, USA) with the pair assay for comparison between control sample and treated samples. The results are expressed as mean ± standard deviation (S.D.) unless noted otherwise (EXCEL®).

RESULTS AND DISCUSSION

Preparation of Zn^{2+} complexes

In the experiment, three groups of Zn^{2+} complexes were prepared differing in applied chelating agent, EDTA, nitrilotriacetic acid, iminodiacetic acid and in combination with 1,3,5-benzenetricarboxylic acid. As a source of zinc were used zinc chloride and zinc perchlorate. Proposed structures of the complexes are depicted in Figure 1.

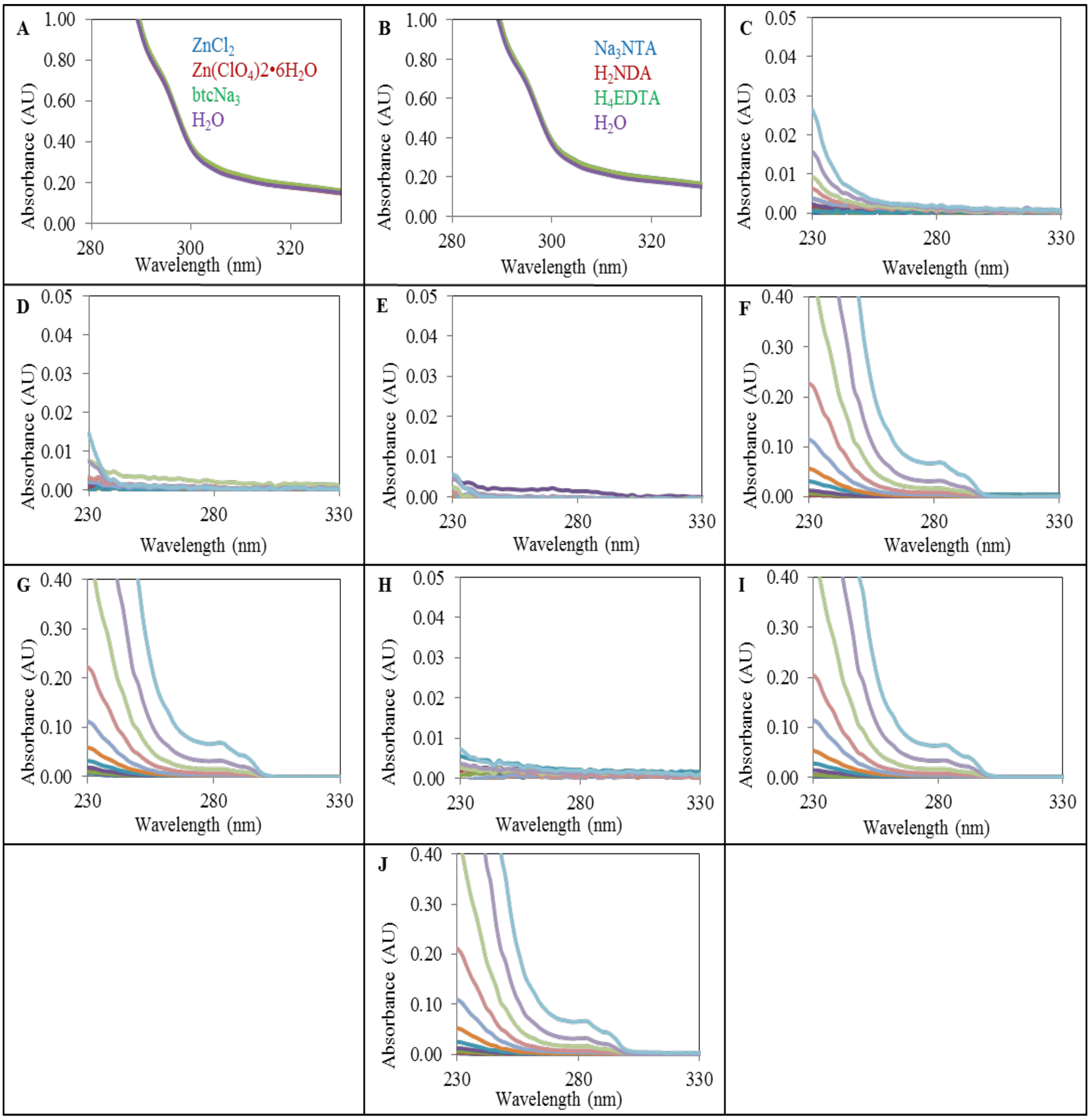
Figure 1 Proposed structures of Zn^{2+} complexes. Lines indicate the used chelating agents, columns stand for Zn salts



Spectrophotometric measurement of Zn^{2+} complexes

These complexes were characterized spectrophotometrically. The absorbance spectra are shown on the Figure 2 A, B. All variants of Zn^{2+} complexes were diluted to the concentration range 0-1420 µM and the absorbance spectra in the range 230 – 330 nm were measured. All the spectra of Zn^{2+} complexes are similar and there is the characteristic absorbance signal. It is obvious; the absorbance spectra is not dependent on the method of preparation. The complexes absorb the light in the wavelengths maxima 284 nm, which corresponds to absorbance maximum at 292 nm. For the remaining chelated Zn complexes, the absorbance spectra were not estimated. In the case of Zn^{2+} complex chelated by EDTA, the absorbance spectra differ in their spectrum. The fluorescence properties of the compounds have not been observed.

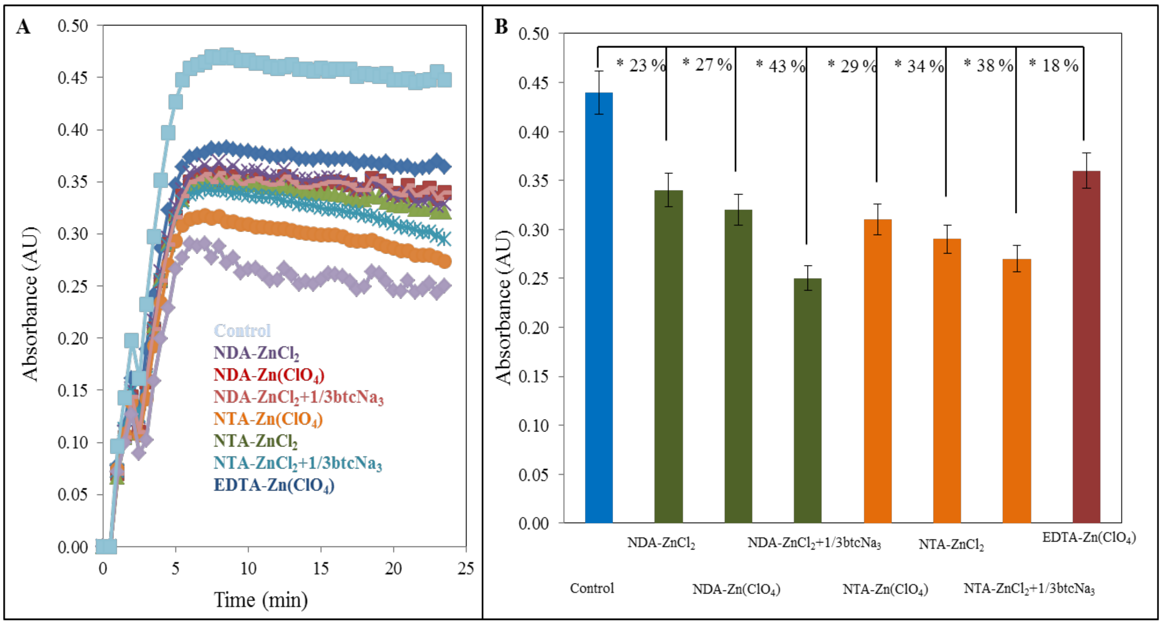
*Figure 2 Absorbance spectra **A)** Zn salts and btcNa$_3$, **B)** chelatation agents and Zn-complexes (1.4–1420 µM) **C)** $ZnCl_2$(EDTA), **D)** $Zn(ClO_4)_2{\cdot}6H_2O$ (EDTA), **E)** $ZnCl_2$(NTA), **F)** $ZnCl_2(NTA)_3$(btc), **G)** $Zn(ClO_4)_2{\cdot}6H_2O$ (NTA), **H)** $ZnCl_2$ (IDA), **I)** $Zn(ClO_4)_2{\cdot}6H_2O$ (IDA), **J)** $ZnCl_2(IDA)_3$(btc)*



Antimicrobial activity of Zn^{2+} complexes

In the next part, the antimicrobial properties of studied Zn^{2+} complexes were confirmed by the method of the growth curves of the bacteria cultures *Staphyloccocus aureus*. The applied concentration of each chelated Zn^{2+} complex was 1 mM. From obtained results (see Figure 3A) is evident, the slight antimicrobial effect of Zn^{2+} complexes in the comparison with control. The statistical evaluation of results is shown on Figure 3B. From the picture is evident the NDA-$ZnCl_2$+1/3btcNa$_3$ complex has a strongest antimicrobial activity in comparison with control sample.

*Figure 3 Growth curves after application of Zn(EDTA), $Zn(NTA)Cl_2$, $Zn(NTA)(H_2O)_2$, $Zn_3(NTA)_3(btc)$, $Zn(NTA)(H_2O)_2$, $Zn(NDA)Cl_2$, $Zn(NDA)(H_2O)_3$ and $Zn_3(NDA)_3(btc)$. All data represent mean ± S.D. NS, not significant, * $p < 0.05$*



CONCLUSION

Zinc chelate complexes were prepared and characterized by spectrophotometry. The characteristic absorbance spectra were estimated in the cases of all zinc complexes. These complexes show slight antimicrobial activity against S. aureus.

ACKNOWLEDGEMENT

The research was financially supported by the MENDELU IGA/TP01/2015

REFERENCES

Bertinato J., Sherrard L., Plouffe L. J. 2012. EDTA disodium zinc has superior bioavailability compared to common inorganic or chelated zinc compounds in rats fed a high phytic acid diet. *Journal of Trace Elements in Medicine and Biology*, 26(4): 227–233.

Iwai T., Takahashi M., Oda K., Terada Y., Yoshida K. T. 2012. Dynamic Changes in the Distribution of Minerals in Relation to Phytic Acid Accumulation during Rice Seed Development. *Plant Physiology*, 160(4): 2007–2014.

Kambe T., Tsuji T., Hashimoto A., Itsumura N. 2015. The Physiological, Biochemical, and Molecular Roles of Zinc Transporters in Zinc Homeostasis and Metabolism. *Physiological reviews*, 95(3): 749–84.

Lazarte C. E., Carlsson N.-G., Almgren A., Sandberg A.-S., Granfeldt Y. 2015. Phytate, zinc, iron and calcium content of common Bolivian food, and implications for mineral bioavailability. *Journal of Food Composition and Analysis*, 39(111–119.

Martorelli E., Antonucci A., Luciano A., Rossi E., Raboni M., Mancini G., Viotti P. 2015. EDTA Chelating Process for Lead Removal: Evaluation of Approaches by Means of a Reactive Transport Model. *Water Air and Soil Pollution*, 226(4).

Miller L. V., Hambidge K. M., Krebs N. F. 2015. Zinc Absorption Is Not Related to Dietary Phytate Intake in Infants and Young Children Based on Modeling Combined Data from Multiple Studies. *Journal of Nutrition*, 145(8): 1763–1769.

Rincker M. J., Hill G. M., Link J. E., Meyer A. M., Rowntree J. E. 2005. Effects of dietary zinc and iron supplementation on mineral excretion, body composition, and mineral status of nursery pigs. *Journal of Animal Science*, 83(12): 2762–2774.