ZINC PHOSPHATE NANOPARTICLES PREPARATION AND THEIR ANTIMICROBIAL ACTIVITY

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Abstract

Zinc phosphate particles were prepared by reaction of zinc nitrate with hydrogen phosphate, diphosphate and triphosphate, and characterized by Dynamic Light scattering (DLS) and Scanning Electron Microscopy (SEM). Antibacterial effect of ZnO nanoparticles and zinc phosphates ones were compared. Bacterial strains of (*Escherichia coli* NCTC 13216, *Staphylococcus aureus* NCTC 8511, and Methicillin-resistant *S. aureus* CCM 7110 were used for the testing. Oxidative stress was determined the GSH/GSSG ratio by using High-pressure liquid chromatography with twelve-channel CoulArray electrochemical detector (HPLC-ED). It has been demonstrated the zinc phosphate particles shown better antibacterial properties and smaller toxicity, than the conventional ZnO.

Keywords: Zinc phosphate, Zinc oxide, Antimicrobial properties

1. INTRODUCTION

Zinc is major element, which a responsible for physiological function in the body of animals or human, such as reproduction [1], replication and transcription [2], immune system, protection from bacterial endotoxins and antibody production [3]. On the other hand, Zn treatment of antibacterial activity have positive results in the concentration (2000 - 2500 mg/kg) during 14 days and these high doses, however, carry a high risk of large amounts of zinc in the food chain [4]. Alternative forms of Zn, such as ZnO, Zn phosphate or ZnS have a best potential and widely used in agriculture in the form of animal feed supplement [5], biomedical applications, due to their low toxicity and biodegradability [6]. Nowadays, Zn nanoparticles may be used how alternative as antibiotics for animals, due as their antibacterial properties [7]. Aim of our study was to prepared of Zn NPs and their applications on *Escherichia coli* NCTC 13216, *Staphylococcus aureus* NCTC 8511, and *Methicillin-resistant S. aureus* CCM 7110. Morphology of Zn nanoparticles were characterized by scanning electron microscope.

2. METHODOLOGICAL BASES AND EXPERIMENTAL PART

2.1. Chemicals

Zinc nitrate. $(NH_4)_2HPO_4$, Na_2HPO_4, $Na_4P_2O_7$, $Na_5P_3O_{10}$, GSH, GSSG, ZnO, methanol, trifluoroacetic acid were obtained from Sigma Aldrich (St. Louis, Missouri, USA).

2.2. Determination of reduced and oxidised glutathione and selenium

Reduced and oxidised glutathiones were determined using HPLC-ED. Experimental conditions were adopted from [8].

2.3. Dynamic light scattering and Scanning electron microscopic (SEM) characterization of Zn NPs

The average particle size and size distribution were determined by quasielastic laser light scattering with a Malvern Zetasizer (NANO-ZS, Malvern Instruments Ltd., Worcestershire, UK). Structures of particles were characterized by electron microscope MIRA 3 XMU (Tescan, a.s., Brno, Czech Republic). The SEM was fitted with Everhart-Thronley type of SE detector, high speed YAG scintillator based BSE detector, panchromatic CL Detector. For automated acquisition of selected areas, a TESCAN proprietary software tool called Image Snapper was used.

2.4. Powder X-ray analysis

Powder X-ray analysis of Zn NPs was carried out on D8 Advance ECO (Bruker, AXS GmbH, Karlsruhe, Germany). Bragg-Brentano geometry, CuKα radiation (λ = 1.54178 Å), the range of 2θ = 4-60° and room temperature were used.

2.5. Preparation of microparticles

Zn-A. Zinc nitrate dissolved in water was heated to 60 °C and $(NH_4)_2HPO_4$ was added with stirring. White precipitate was formed.

Zn-B. Zinc phosphate was prepared similarly, only Na_2HPO_4 was used instead of ammonium salt.

Zn-C. Solution of $Na_4P_2O_7$ was added to stirred solution of zinc nitrate. White precipitate was obtained, filtered and dried.

Zn-D. Zinc nitrate was mixed with $Na_5P_3O_{10}$ solution. White precipitate was immediately formed. The suspension was filtered and product dried.

Zn-AC. Zinc nitrate dissolved in water was heated to 60 °C and solution of citric acid and $(NH_4)_2HPO_4$ was added with stirring. White precipitate was formed.

2.6. Determination of antibacterial properties by method of growth curves

The method is based on measuring of the absorbance using the apparatus Multiskan EX (Thermo Fisher Scientific, Germany) and subsequent analysis in the form of growth curves. Bacteria were cultivated in GTY medium for 24 h with shaking and were diluted with GTY medium using Specord spectrophotometer 210 (Analytik, Jena, Germany) at a wavelength of 600 nm to absorbance 0.1. On the microplate, these cultures were mixed with various concentrations of NPs and a control.

3. RESULTS AND DISCUSSION

Aim of our study was preparation of zinc phosphates under different reaction conditions and their physico-chemical study. For preparation of zinc phosphate sodium as well as ammonium salt were used. From powder XRD it follows that using ammonium salt only hopeite $Zn_3(PO_4)_2{\cdot}4H_2O$ was obtained (see **Figure 1**). When sodium salt was used under the same conditions than 90 % of product is hopeite but 10 % is zinc phosphate dehydrate. For Zn-C preparation diphosphate was applied and product was identified as $Zn_2P_2O_7{\cdot}5H_2O$. More complicated situation is for Zn-D. XRD analysis shows on a mixture of three products. The most abundant component (60 %) was as expected zinc triphosphate $Zn_2HP_3O_{10}{\cdot}6H_2O$ but there is 30 % of $Zn_2P_2O_7{\cdot}5H_2O$ and 10 % of $ZnHPO_4{\cdot}3H_2O$. We have also tried addition of citric acid to the reaction mixtures. For the product Zn-AC $Zn_3(PO_4)_2{\cdot}4H_2O$, $Zn_3(PO_4)_2{\cdot}4H_2O$ and $ZnHPO_4{\cdot}H_2O$ (89, 8 and 3 %) were obtained. Addition of citric acid (Zn-DC) to reaction mixture containing triphosphate leads to nearly complete decomposition of triphosphate and $Zn_2P_2O_7{\cdot}5H_2O$ (97 %) with only minor part of $Zn_2HP_3O_{10}{\cdot}6H_2O$ resulted.

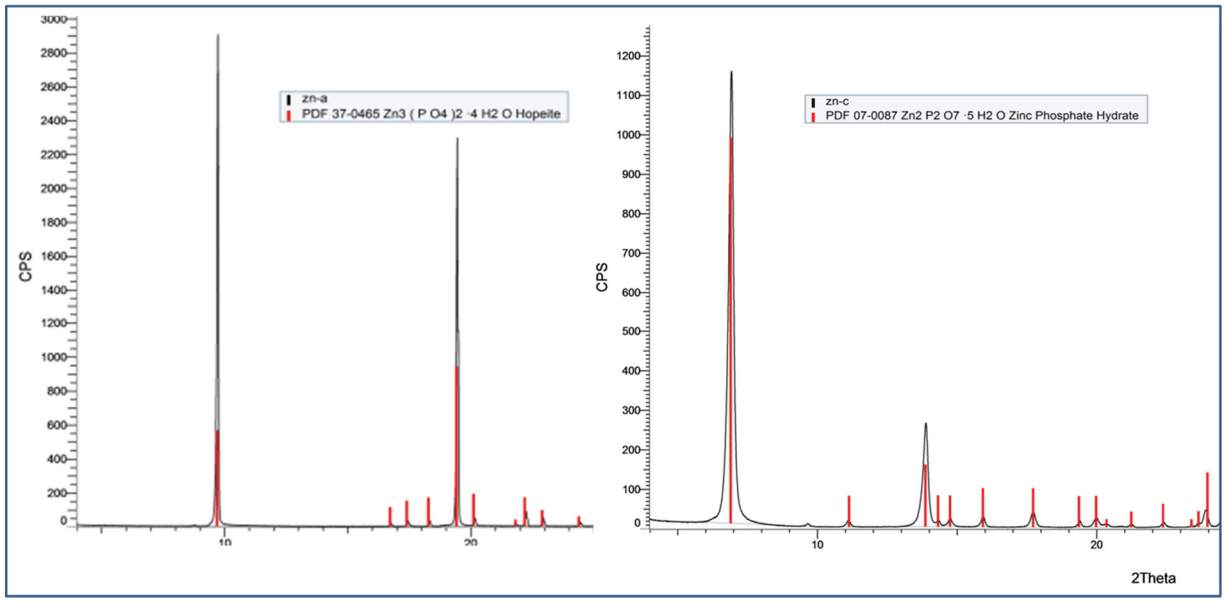

Figure 1 XRD spectra of Zn-A (upper part) and Zn-C. (CPS = counts per second)

DLS analysis of obtained materials show on particles in the range of 50 - 520 nm as well as some particles are much bigger probably due to the formation of aggregates. The size and shape were confirmed by SEM analysis (see **Figure 2**). For comparison zinc oxide nanoparticles and Zn-D are presented. ZnA and ZnB particles had spherical shape with the average diameter 477 and 520 nm, whereas ZnC formed small aggregates with average diameter 452 nm. Zn-D nanoparticles are comparable with those of ZnO nanoparticles with diameters from 50 to 100 nm. It can be clearly seen that nanoparticles were formed as well as their crystalline nature that was also proved by XRD analysis.

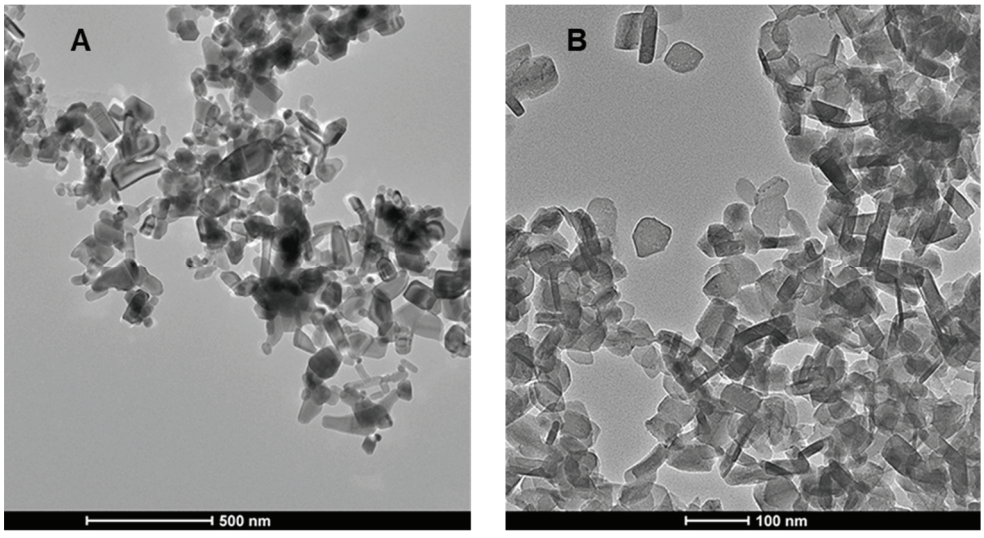

Figure 2 SEM micrographs of ZnO-N (A) and Zn-D (B)

The antibacterial activity of ZnNPs, ZnO and ZnO-N after 24 h was confirmed by the method of the growth curves. For the comparison ZnO particles and nanoparticles were used. From the growth curves inhibition curves (**Figure 3**) were obtained showing on either similar or better antibacterial properties of prepared zinc phosphates. From the literature, it is known that mostly higher antibacterial properties can be expected for lower sized nanoparticles and the activity depends on shape and solubility of nanoparticles. Interestingly, it was found for *E. coli* that with higher concentrations of nanoparticles inhibition of bacteria is lower. It can be

explained by recovery of bacteria or by aggregation of nanoparticles in solution leading to lower active surface for interaction with microbial cell. Thus, better activity against *E. coli* show zinc phosphates Zn-A and Zn-B.

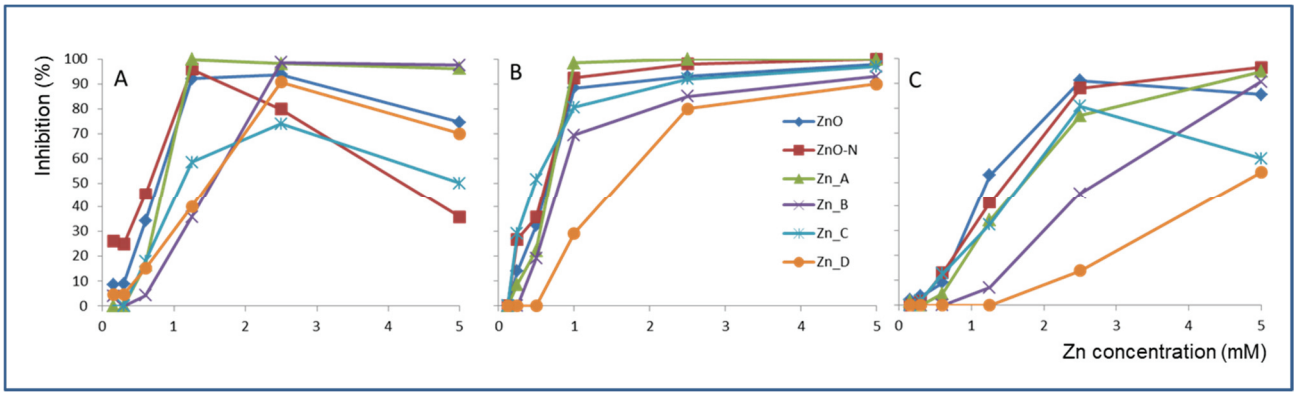

Figure 3 Inhibition curves obtained for (A) E. coli, (B) S. aureus and (C) MRSA obtained under different concentrations of zinc phosphates (Zn-A, Zn-B, Zn-C, Zn-D) and zinc oxides (ZnO, ZnO-N)

Levels of GSH were obtained by analysis of blood, kidney and liver samples of Laboratory male rats of the outbreed strain *Wistar albino* were selected as model animals. The rats were divided into four groups of ten rats each by phosphates or zinc oxides. Four groups of rats were fed with phosphate-based zinc nanoparticles (ZnA, ZnB, ZnC, ZnD) in the dose of 2000 mg Zn/kg diet. All groups of rats had monodiet including 2.7 mg/kg of zinc. The experiment duration was 28 days. The animals had access to feed and drinking water ad *libitum*. From animals were putted to death the blood, kidney and liver samples were collected for subsequent analyses of GSH and GSSG using HPLC-ED. The levels were related to total protein concentration. From the data it was apparent that GSH concentration significantly increases up to concentration 13 ±2 nmol GSH/g protein after zinc treatment in liver. The applied zinc nanoparticles have different effects on the GSH levels in the blood and kidneys and values of GSH are usually slightly higher in comparison with control. In the case of GSSG no significant difference between the different types of zinc phosphate nanoparticles was observed.

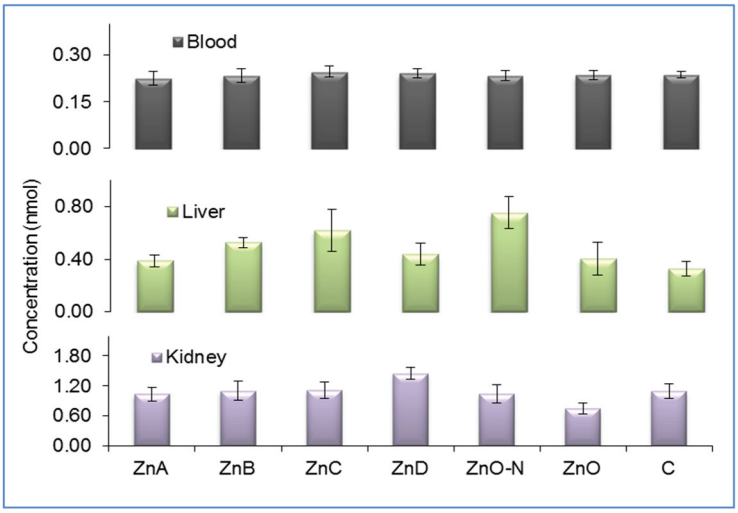

Figure 4 Levels of GSH on blood, liver and kidney. Results are related to the total protein content.

3. CONCLUSION

In this study, zinc phosphate particles were prepared and characterized by SEM and powder X-ray analysis. The particles were tested for their antibacterial properties and it was found that they inhibit grow of bacteria

better than zinc oxide which is usually used in veterinary practice. Lowering of zinc content is promising for application in agriculture.

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