SYNTHESIS, CHARACTERIZATION AND ACUTE AQUATIC TOXICITY OF CERIUM OXIDE NANOPARTICLES TO FRESHWATER GREEN ALGAE

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Abstract

The article describes the preparation of cerium oxide nanoparticles (nanoCeO_2) via precipitation method from the selected default salt $Ce(NO_3)_3 \cdot 6H_2O$ and its acute aquatic toxicity. The resulting nanoparticles were characterized by X-ray powder diffraction analysis, which showed the cerium oxide nanoparticles to be with the crystallite size of 58 nm. Morphology of the cerium oxide nanoparticles was examined by scanning electron microscopy, where agglomerates of spherical shaped nanoparticles were observed. The elemental composition of the product was confirmed by EDX analysis. Freshwater green algae (*Desmodesmus subspicatus*) served as a model organism for evaluation of acute aquatic toxicity. Effective concentration of toxicity EC_{50} was determined for the concentration 0.49 $g \cdot dm^{-3}$ of cerium oxide nanoparticles to freshwater green algae *Desmodesmus subspicatus*.

Keywords:

Acute aquatic toxicity, algae *Desmodesmus subspicatus*, cerium oxide nanoparticles.

1. INTRODUCTION

Nanomaterials are generally defined as materials whose dimensions are in the range of 1-100 nm in at least one direction [1] and because nanomaterials have different physical, chemical and electrical properties in comparison with traditional bulk materials, nanomaterials may be used for new products and applications, and may also be incorporated into various industrial processes [2].

Currently, the increasing production and usage of nanoparticles for various industrial applications raised questions and concerns about their impact on human health and environment [3]. The cerium oxide nanoparticles are currently under a great interest in view of their possible use in many applications, such as sunscreens, outdoor coating materials, products for wood care or cerium oxide nanoparticles are being used as fuel additives in the automotive industry to reduce emissions of particulate exhaust emissions [4, 5, 6]. Further, the cerium oxide nanoparticles are used as ultraviolet absorption agents, gas sensors, oxygen pumps, in solar cells, and also in the metallurgical and glass/ceramic applications [7].

A number of recent publications has shown that exposure to nanoparticles of cerium oxide may have an negative effect on health due to the formation of reactive oxygen species that lead to oxidative stress, inflammation, and may lead to the stress-induced cell death eventually. Contrary to these studies some other studies showed that cerium oxide nanoparticles exhibited antioxidant properties, which support cell survival in terms of oxidative stress [7] and therefore cerium oxide nanoparticles can be also used as antioxidants for blocking the enzymatic activity [8].

Consequently, the aim of the work was preparation and characterization of cerium oxide nanoparticles and evaluation its acute aquatic toxicity to freshwater green algae *Desmodesmus subspicatus*.

2. EXPERIMENTAL

2.1 Synthesis of cerium oxide nanoparticles

Cerium oxide nanoparticles were prepared by the precipitation. Aqueous solution of $Ce(NO_3)_3 \cdot 6H_2O$ (Lachema) with the concentration of 0.1 mol·dm^{-3} was heated to 25 °C and subsequently precipitated with ammonium hydroxide (Sigma Aldrich) with the concentration of 0.04 mol·dm^{-3}. The reaction proceeded with stirring at temperature of 25 °C for 20 ± 4 hours. The resulting product was centrifuged, dried at 150 °C and calcined at 600 °C for 4 hours.

2.2 Characterization of cerium oxide nanoparticles

For the study of the phase composition of the product was used powder X-ray diffraction analysis (XRD - Bruker D8 Advance), for the diffraction patterns evaluation software DIFFRAC plus BASIC (Bruker AXS) was used and for the identification of phases the PDF 2 Release 2004 (International Centre for Diffraction Data) was used.

Crystallite size was determined by the Scherrer equation [9]

$$L_c = \frac{K \cdot \lambda}{\beta \cdot \cos\theta} \quad (1)$$

, where K is a factor of the microstructure, λ is the wavelength of the radiation of the lamp (Co-lamp), β is the half width of the diffraction of the sample, and θ is the diffraction peak position of the profile.

Scanning electron microscope Quanta FEG 450 (FEI) with EDX analysis APOLLO X (EDAX) was used as a microscopic tool for characterization of morphology and elemental composition of the studied samples.

2.3 Acute aquatic toxicity bioassay

Acute aquatic toxicity test was done according to the ČSN EN ISO 8692 Standard [10] and OECD Guideline 201 [11]. The aim of the test was to determine the median inhibition concentration EC_{50}, i.e. the concentration of a toxicant which causes 50 % inhibition of algal cells growth in comparison with control [12]. As detection organism for the evaluation of toxicity, *Desmodesmus subspicatus* (Institute of Botany of the Academy of Sciences of the Czech Republic in Třeboň), freshwater green algae, was used.

Toxicity tests were conducted with 3 days old algae culture. Before the test, number of cells was counted using light microscope and counting Bürker chamber (**Fig. 1**) and the volume of culture which was added at the beginning of the toxicity test to the suspension of nutrient medium and nanoparticles of cerium oxides was calculated. The prepared solution of cerium oxide nanoparticles with concentration of 1 g·dm^{-3} was placed into the ultrasonic bath for 6 hours in order to form a suspension with better dispersion of the particles in the entire volume of suspension. In this manner, the prepared suspension was diluted to the selected concentrations: 1 g·dm^{-3}, 0.8 g·dm^{-3}, 0.6 g·dm^{-3}, 0.4 g·dm^{-3}, 0.2 g·dm^{-3}. The samples prepared for the toxicity tests were placed in the conditioned box with temperature (23 ± 2) °C, with 24 hours exposure to daylight on a horizontal shaker with 125 ± 25 rpm. Toxicity tests were performed over a period of 72 ± 2 hours. At the end of the test, the cells were counted (April 14, 2014), from which was determined by growth inhibition (EC_{50}) [10, 11].

Fig. 1: A) Image of the counting Bürker chamber. B) Light microscopy image of cells of green algae *Desmodesmus subspicatus* in counting Bürker chamber.

3. RESULTS AND DISCUSSION

3.1 Characterization of the prepared material

The precipitation method of the selected default cerium salt produced yellow powder of cerium oxide nanoparticles (see **Fig. 2**).

Fig. 2: The illustration image of the product of the precipitation synthesis.

XRD analysis (**Fig. 3**) revealed the presence of cerium oxide as the only phase in the sample according to the presence of the basal diffraction. According to the Scherrer equation the crystallite size was calculated equal to 58 nm.

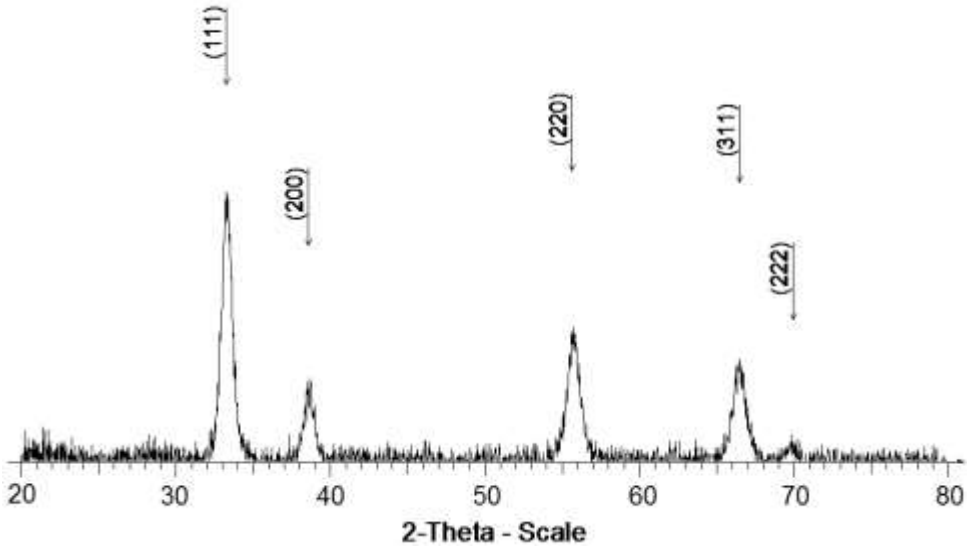

Fig. 3: X-ray powder diffraction pattern of the prepared sample.

EDX (**Fig. 4**) confirmed the presence of cerium and oxygen. Carbon present in EDX pattern is caused by carbon tape on which the sample was placed and then sputter-coated with Au/Pd thin layer.

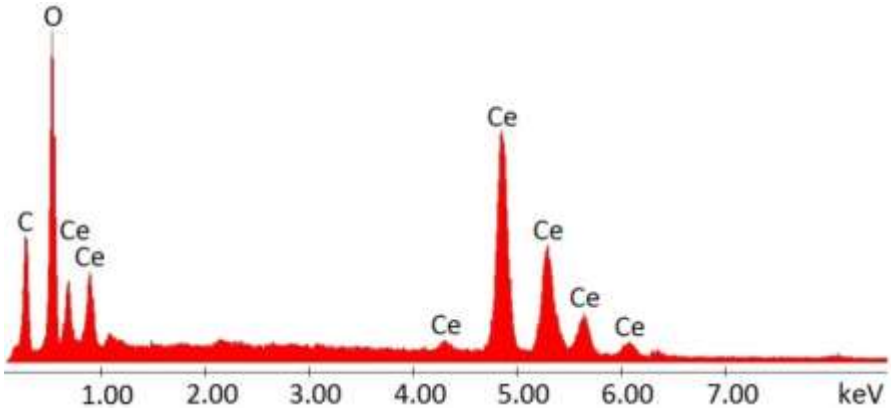

Fig. 4: EDX spectrum of the sample.

The SEM images were taken in the secondary electrons mode (**Fig. 5**). It is evident that during the preparation agglomeration of cerium oxide nanoparticles occurred.

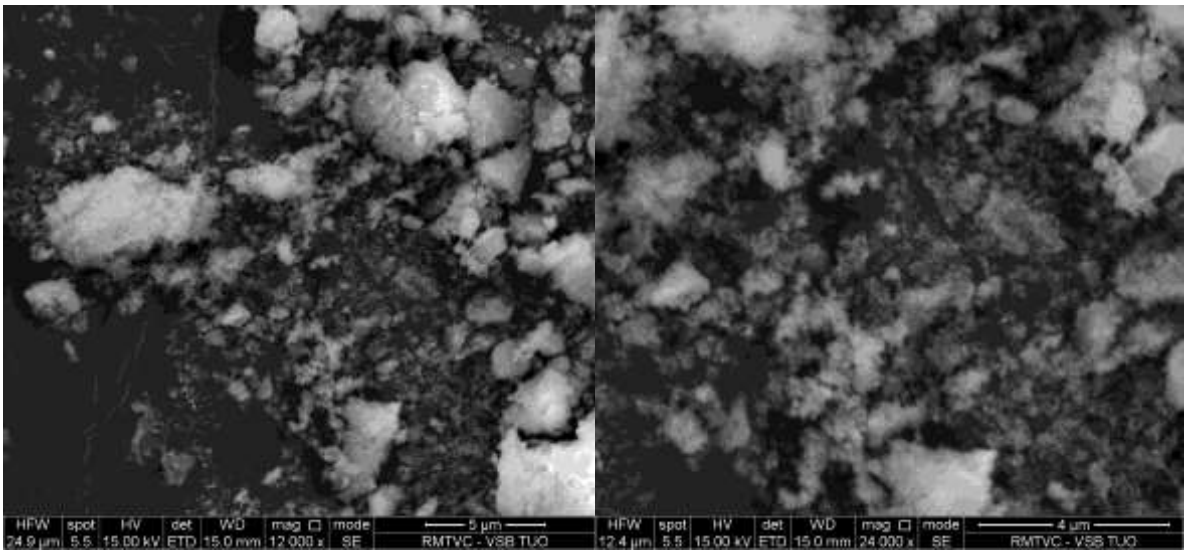

Fig. 5: SEM images of the sample at different magnifications.

3.2 Acute aquatic toxicity

Toxicity tests were evaluated after exposure of 72 ± 2 hours. Effective concentration of toxicity (EC_{50}) was determined from the acquired experimental data (**Table 1**). EC_{50} was subtracted from the biomass growth curve inhibition in % to a concentration of the sample in $g \cdot dm^{-3}$ (**Fig. 6**). The parameter EC_{50} was determined for the concentration 0.49 $g \cdot dm^{-3}$ of cerium oxide nanoparticles to freshwater green algae *Desmodesmus subspicatus*.

Table 1: The values of biomass growth inhibition for the chosen concentrations of cerium oxide nanoparticles.

Dose (concentration of nano CeO_2) [$g \cdot dm^{-3}$]	Inhibition [%]
1	95.41
0.8	84.48
0.6	56.24
0.4	46.80
0.2	38.45

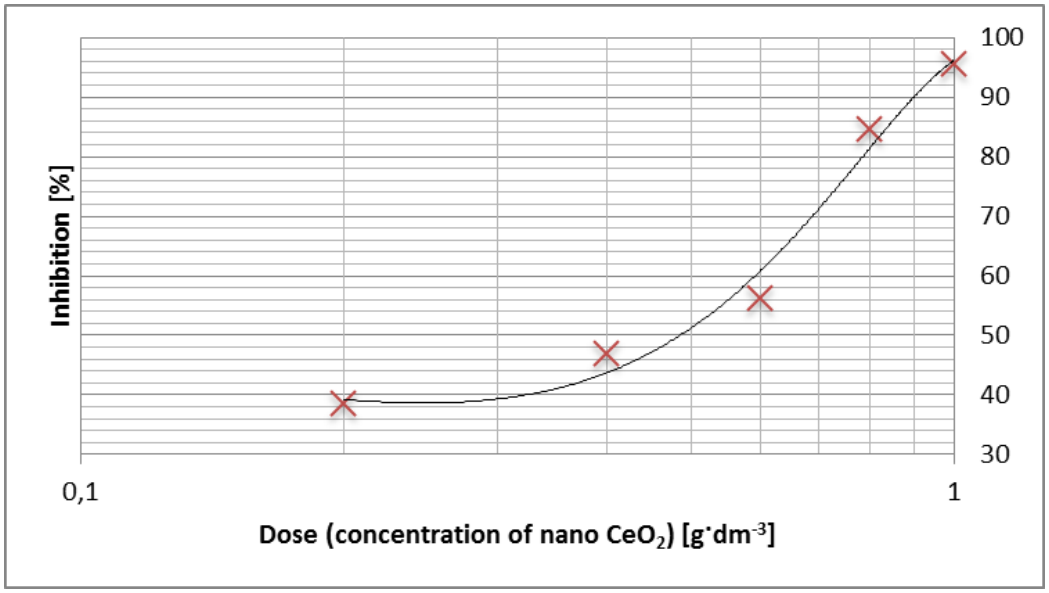

Fig. 6: Biomass growth inhibition curve of cerium oxide nanoparticles.

4. CONCLUSIONS

Cerium oxide nanoparticles with crystallinity size of 58 nm were prepared by the precipitation method from the selected default salt $Ce(NO_3)_3 \cdot 6H_2O$. Consequently, cerium oxide nanoparticles prepared by this approach were evaluated for acute aquatic toxicity to freshwater green algae (*Desmodesmus subspicatus*). It was found that 50 % inhibition of algal growth in culture is caused by the concentration of 0.49 g of cerium oxide nanoparticles per liter.

From the published studies the increase of toxicological studies of cerium oxide nanoparticles is evident, however there is heterogeneity in the obtained results. Especially there is a lack of toxicity data for cerium oxide nanoparticles impact to *Desmodesmus subspicatus* algae. Toxicity of cerium oxide nanoparticles on the algae culture *Desmodesmus subspicatus* was already studied in the other work dealing with the ecotoxicity of nanomaterials [13], but without clear results. The study did not determine EC_{50} for cerium oxide nanoparticles of size 47.7 nm. Only the EC_{50} for 2.1 nm sized nanoparticles was determined equal to 0.081 $g \cdot dm^{-3}$. Because of the different particle sizes it is not possible to compare the results obtained in this study with the results in the presented work absolutely.

It is evident that an inseparable part of the evaluation of the toxicity of nanomaterials is the characterization of their physicochemical properties, because this knowledge is important for comparability between studies. Hereinafter, for the future it is important to evaluate of toxicity with other detection organisms than the green algae, because each organism reacts differently to the same toxicant. Finally, it is important to effort to create proper methodology for evaluation of the toxicity of nanomaterials, which are not currently sufficient in comparison with conventional methods for assessing the toxicity of materials.

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