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Synthesis of Stable Aqueous Ceria Sols and Study of Their Toxicity

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Cerium dioxide is a unique material which is promising for biomedical applications. The properties of ceria are definitely determined by synthesis procedure and further treatment conditions. The vast majority of currently existing methods of synthesis lead to formation of CeO_2 in the form of nanopowders, but in some cases (e.g., in biological applications), such powders can not be used because they do not quite satisfy practical requirements. So, in this work we succeed in synthesis of ceria-stable sols with controlled small particle size (2-4 nm) using an inexpensive and facile method and investigated the effect of changing the concentrations and the molar ratio of initial reagents on particle size of CeO_2. In this paper we also propose a method of evaluation of toxicity of ceria sols using bioluminescent microorganism Vibrio fischeri. According to the results obtained CeO_2 sols of different concentrations ($6.3*10^{-4}$ – 0,02M) are not toxic to Vibrio fischeri.

Keywords: Ceria, Colloid solutions, Biological activity, Vibrio fischeri.

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1. INTRODUCTION

Cerium dioxide and ceria-based materials are widely used in industrial applications, i.e. in manufacturing of fuel cells, gas sensors, UV filters, three way catalysts, etc. In the last decade, cerium dioxide attracts the attention of researchers as a potentional inorganic antioxidant. Numerous biomedical applications of ceria-based preparations call for nanoceria in the form suitable for dosing, i.e., as aggregatively stable CeO_2 sols stabilized by biocompatible ligands. According to the data of ISI Web of Science, the publications on nanobiotechnology double every year. Nevertheless, until now the biological activity (including biotoxicity) of nanomaterials was studied only in isolated cases [1]. Therefore, in the present paper, we report a method of synthesis of stable colloid solutions of CeO_2 nanoparticles with controlled particle size. We have also performed an investigation of the toxiticy of ceria sols using Vibrio fischeri luminescent microorganism.

2. EXPERIMENTAL

To prepare initial solutions, $Ce(NO_3)_3 \cdot 6H_2O$ (reagent grade), citric acid (reagent grade), and aqueous ammonia (pure grade) were used. A CeO_2 sol was obtained by dissolving calculated amount of reagents in 25 mL of distilled water (molar ratios of $Ce(NO_3)_3 \cdot 6H_2O$ and citric acid were 1:1, 1:2, 1:4, 2:1). Concentrations of the reagents were 0.01, 0.025, 0.05, 0.1, 0.2M. The resulting solution was rapidly poured under stirring into 100 mL of a 3M ammonia solution and allowed to stand for 3 h.

The size and shape of nanoceria particles were determined by transmission electron microscopy on a Leo 912 AB Omega electron microscope (100 kV accelerating voltage). The optical absorption spectra were recorded on an Ocean Optics QE-65000 spectrophotometer in the wavelength range of 275-900 nm. The size of CeO_2 particles was measured by the dynamic light scattering method on a Malvern Zetasizer Nano ZS analyzer. Before measurements, the sols were diluted with distilled water. The X-ray powder diffraction of the solid products of the synthesis was performed on a Rigaku D/MAX 2500 diffractometer (Cu$K\alpha$ radiation). The size of the coherent scattering domains (CSD) was determined by the Scherrer formula.

The toxicity of sols was assayed by the luminescent bacteria test based on the inhibition of their luminescence with different chemical compounds. Standard lyophilized bacteria Vibrio fischeri were used as a biosensor. The intensity of bacterial luminescence (I) was evaluated using a Kikkoman Lumitester luminometer. For investigation of toxicity, we took two ceria sols with initial concentrations of 0.005 and 0.02M and molar ratio of cerium to citric acid 1:1, and then made a series of dilutions. Final concentrations were $6.3*10^{-4}$;$1.3*10^{-3}$ $2.5*10^{-3}$, $5*10^{-3}$, 0.01, 0.02M.

To the resulting ceria sol a calculated amount of NaCl was added, so that it concentration was 2%. Rehydration of lyophilized Vibrio fischeri bacteria was performed by 2% NaCl solution at 4 °C for 30 min further at 15 °C for 30 min. The control sample was a 2% solution of NaCl. The bioluminescence intensity was measured immediately after mixing of bacteria with CeO_2 sols and then every 20 min for 2-2.5 h. The duration of each measurement was 10 s.

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3. RESULTS AND DISCUSSIONS

Ceria forms immediately after mixing initial solutions of cerium(III) nitrate, citric acid, and ammonia, which is reflected by the appearance of an absorption band with an edge at ~400 nm in the UV-Vis spectrum (see Fig. 1). The absorption intensity increases as the reaction proceeds because of the increase in the concentration and size of CeO_2 nanoparticles in the sol. Our data of UV-Vis spectra indicate that, under the conditions used, the formation of the CeO_2 phase was completed in 1.5–2 h. The band gap (E_g) for the resulting sols is 3.4-3.5 eV, which noticeably exceeds the E_g value for macrocrystalline CeO_2 (~3.2 eV) and is evidence of small size of CeO_2 particles. E_g and the size of particles were reanalyzed again after a month. It should be noted that the results changed only slightly, indicating a good stability of sols.

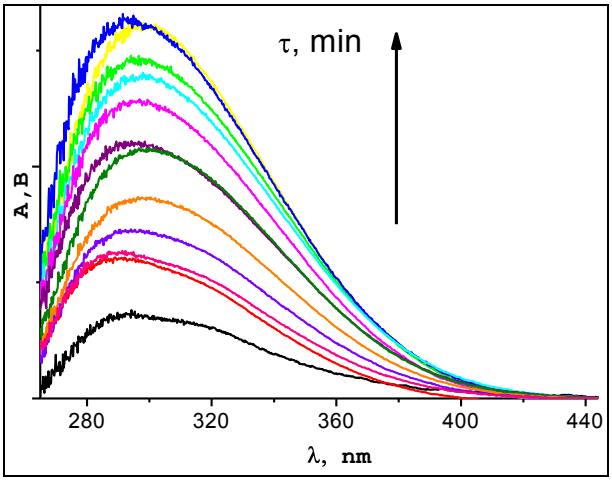

Fig. 1 – UV-visible absorption spectra of colloidal ceria solution formed by mixing aqueous ammonia solutions with $Ce(NO_3)_3$ (0.025M) and citric acid (0.025M).

According to the X-ray powder diffraction data, all samples synthesized were identified as cerium dioxide with a fluorite structure and with the size of the coherent scattering domain (CSD), calculated from the broadening of the diffraction maximum (111), lying in a range of 2.8–3.5 nm. The transmission electron microscopy (TEM) data are in a good agreement with the X-ray powder diffraction data. According to TEM (see Fig. 2), CeO_2 particles are 2.0–3.6 nm in size. Analysis of TEM data indicates that increasing the concentration of initial reagents does not lead to significant change in particle size.

Dynamic light scattering (DLS) data indicate that the hydrodynamic diameter of CeO2 particles for different samples varies from 3 to 6.7 nm. It should be noted that the relatively small values of the hydrodynamic diameters generally indicate a weak aggregation of ceria nanoparticles. In addition, according to the DLS it can be seen that upon increasing the concentrations of cerium nitrate (III) and citric acid the particle size decreases from 6.7 to 3.3 nm. Similarly, upon increasing the molar excess of citric acid from 1:1 to 1:2 and 1:4, the particle size also decreases from 4.5 to 3 nm.

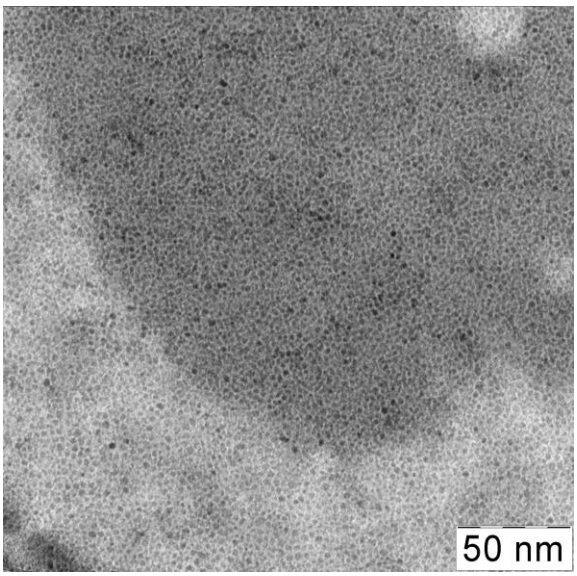

Fig. 2 – TEM images of CeO2 nanoparticles in citrate sol.

It was found that CeO_2 sol, obtained from mixed $Ce(NO_3)_3$ (0.05M) and citric acid (0.1M) solution is characterized by the smallest particle size and the narrowest particle size distribution.

Toxicity tests reveal that the luminescence intensity of the biosensor placed for 20 min in the NaCl solution (2%) is not more than 60-70% of the initial value due to inhibition of enzymatic activity of bacteria. According to the results obtained on ceria sols, CeO_2 nanoparticles do not induce any further decrease in the enzymatic activity of Vibrio fischeri, as opposed to ammonium citrate stabilizer solution (EC50 = 0.2M).

4. CONCLUSIONS

In this paper, we have proposed a method of synthesis of cerium dioxide sols stabilized by ammonium citrate with controlled particle size. Detailed physicochemical attestation of the sols was performed. It was established that ceria sols have no toxic effect on Vibrio fischeri bacteria.

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