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Green synthesis and evaluation of metabolic activity of starch mediated nanoceria

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Abstract

Crystalline nanoceria powders have been synthesized by green-mediated sol–gel method in aqueous solution. The cerium nitrate hexahydrate as precursor and starch were taken in water and precipitated with ammonium hydroxide at pH 10. To terminate the growth of nanoceria particles and also to stabilize them, long-chain starch molecules were utilized. The sample was then calcined for 2 h in a temperature range of 120–600 °C and characterized by the number of techniques, including powder X-ray diffraction (PXRD), UV–vis spectrophotometry, and high-magnification transmission electron microscopy (TEM). The particle size obtained from TEM was in the range of 6 nm with uniform shape and narrow particle size distribution. The diffraction pattern completely indexed with the cubic fluorite structure of CeO_2. The calcined nanoceria powders showed strong UV absorption. *In vitro* cytotoxicity studies on Neuro2A cells showed a dose dependent toxicity with non-toxic effect of concentration up to 175 μg/mL. The results show that starch is an interesting material that can be used as a stabilizer in the sol–gel processes for preparing small nanoceria particles.

Keywords: A. Sol–gel processes; B. Electron microscopy; D. CeO_2

1. Introduction

Nanoceria (CeO_2), an important rare-earth oxide nanoscale material, has attracted enormous interest in recent years due to its unique physical and chemical properties that are significantly different from those of bulk materials [1]. Therefore, it has been widely considered in various areas, such as catalysis [2], gas sensors [3], fuel cells [4], hydrogen storage materials [5], optical devices [6], ultraviolet absorbers [7], polishing materials [8], and biomedical science fields [9]. Several preparation methods have been used to obtain dispersed nanoceria, including conventional hydrothermal [10], sol–gel [11], co-precipitation [12], polymeric precursor [13], pyrolysis [14], organometallic decomposition [15], microwave-assisted heating [16], sonochemical [17], W/O microemulsions [18], and mechanochemical [19] methods. However, some of these routes (e.g., sol–gel and precipitation) have been used in colloidal media (e.g., emulsions or polymers) in order to improve or control the chemical or physical properties (e.g., control the particle growth and surface area of the nanoceria) [20]. In fact, different types of natural polymers can also be used as bio-templates in the green synthesis of nanoceria. Polymers are a category of macromolecules. When polymers are used as a capping agent, the diameter of metal oxide in nanoparticles can be logically controlled [21]. Starch which is a polymer of hexacarbon monosaccharide-D-glucose as a single helix structure [22] can cover the surface of

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the nanoparticles by hydroxyl groups as a capping agent. The ability of starch as a suitable dispersant or stabilizer for the preparation of different nanoparticles has been demonstrated by various studies [23–25]. However, this ability has not been tested for preparation of nanoceria. Therefore, starch was chosen as a new delivery medium for the synthesis of water-soluble nanoceria. Starch is easily available, eco-friendly and economical as compared to other stabilizers available on the market. In this work, a facile, homogeneous, and modified sol–gel route was applied for preparation of nanoceria. The nanoceria powders were first synthesized with cerium nitrate hexahydrate and starch was used as starting material at different calcination temperatures.

2. Materials and methods

2.1. Materials and reagents

Chemicals and reagents which were used in this work were analytical grade and used as received without further purification. Cerium(III) nitrate hexahydrate [$Ce(NO_3)_3 \cdot 6H_2O$, Merck], starch (Amylose molecular form, soluble, Aldrich), and ammonium hydroxide solution (NH_4OH, 25 vol%; Merck) were used as starting materials. For the evaluation of metabolic activity, Neuro2A murine neuroblastoma cells (ATCC CCL-131, Manassas, VA, USA) were grown in Dulbecco's modified Eagle's medium (1 g/L glucose, 2 mM glutamine), supplemented with 10% FBS, streptomycin at 100 μg/ml, and penicillin at 100 U/ml. All cells were incubated at 37 °C in a humidified 5% CO_2 atmosphere.

2.2. Synthesis of nanoceria powders

To prepare the nanoceria powders, 0.2 g of soluble starch powders was dissolved in 20 ml of distilled water and stirred for 10 min at 60 °C to achieve a clear starch solution. Meanwhile, the required amount of 0.5 M cerium nitrate solution was added to the starch solution slowly under vigorous stirring. The resulting solution was stirred for 30 min, and an excess amount of 1 M of ammonia solution was added in a drop-wise manner until the solution pH reached 10. At the start, the solution color changed to light yellow; as the ammonia concentration increased, it turned to yellow. The solution was allowed to stir for one more hour. The yellow-colored final precipitate was centrifuged and washed several times with acetone and water to make it free from nitrate, ammonia, and organic impurities and subsequently dried at 80 °C for 12 h. The sample was stored in a vacuum desiccator for further studies. The obtained sample was then divided into 5 parts that were heat treated at 120 °C (C1), 200 °C (C2), 400 °C (C3), and 600 °C (C4) for 2 h each and characterized.

2.3. Evaluation of metabolic activity

The metabolic activity of nanoceria powders was evaluated by the method using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [26]. Briefly, Neuro2A cells were seeded at a density of 1×10^4 cells per well in 96-well plates and incubated for 24 h. Thereafter, the cells were treated with various concentrations of nanopowders in the presence of 10% FBS. The sample of C3 was suspended in a stock solution at 5 μg/ml in a solution of dimethyl sulfoxide (DMSO)/double distilled water. After 24 h of incubation, 20 μl of 5 mg/ml MTT in the PBS buffer was added to each well, and the cells were further incubated for 4 h at 37 °C. The medium containing unreacted dye was discarded, and 100 μl of DMSO were added to dissolve the formazan crystal formed by live cells. Optical absorbance was measured at 590 nm (reference wavelength 630 nm) using a microplate reader (Statfax-2100, Awareness Technology, USA), and cell viability was expressed as a percent relative to untreated control cells. Values of metabolic activity are presented as means ± SD of triplicates.

2.4. Characterization of nanoceria powders

The prepared nanoceria powders were characterized by using X-ray diffraction (XRD, Philips, X'pert, Cu K_α), ultraviolet–visible spectroscopy (UV–vis, Evolution 300® Thermo Fisher Scientific, Germany), and transmission electron microscopy (TEM, Hitachi H-7100®, Japan). The particle size distributions of nanopowders were determined using the UTHSCSA Image Tool® Version 3.00 program and SPSS software Version 18.

3. Results and discussion

3.1. Nanoceria formation mechanism

Based on this experiment, a tentative responsible mechanism for the formation of nanoceria powders has been proposed and illustrated in Scheme 1. Starch becomes soluble in water when the temperature of the cloudy solution reaches 75 °C and the semi-crystalline structure is lost. After adding the cerium nitrate solution to starch solution, the metal cations are attracted by oxygen of the OH branches. By continuing the heating process to decrease the amount of water, the smaller amylose molecules start forming a network that holds water, resulting in increase of the mixture's viscosity. This process is called starch gelatinization. The nitrate decomposed to nitrogen dioxide and oxygen during the heating process, and will be removed from the compounds [23]. Although the formation of nanoceria powders involves several complicated reactions [27], controlling the nucleation of initial precipitate $Ce(OH)_3$, however, will mainly determine the properties of the final nanoceria. As the NH_4OH was added into the precursor, $Ce(OH)_3$ precipitate was formed immediately due to extreme low-solubility constant ($K_{sp} = 6.3 \times 10^{-24}$) [28]. Under such a basic condition, high-alkaline environment favored the oxidation of $Ce(OH)_3$ to hydrated Ce(IV) and the color of the initial solution changed from colorless to light yellow. Oxidation of Ce^{3+} to Ce^{4+} in solution takes place at high pH with subsequent hydrolysis to $Ce(OH)_4$ and precipitation. Hydroxyl ions play an important role in this process and strongly affect the super saturation degree of initial precipitate and oxidation of Ce(III) to Ce(IV) [29]. The subsequent sol–gel procedure