



Green synthesis and evaluation of metabolic activity of starch mediated nanoceria

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Received 13 July 2013; received in revised form 20 July 2013; accepted 20 July 2013

Available online 31 July 2013

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 CeO₂. 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.

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Keywords: A. Sol–gel processes; B. Electron microscopy; D. CeO₂

1. Introduction

Nanoceria (CeO₂), 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 nanocerium. Therefore, starch was chosen as a new delivery medium for the synthesis of water-soluble nanocerium. 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 nanocerium. The nanocerium 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 [$\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$, 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 $\mu\text{g}/\text{ml}$, and penicillin at 100 U/ml. All cells were incubated at 37 °C in a humidified 5% CO_2 atmosphere.

2.2. Synthesis of nanocerium powders

To prepare the nanocerium powders, 0.2 g of soluble starch powder 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 nanocerium 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 $\mu\text{g}/\text{ml}$ 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 \pm SD of triplicates.

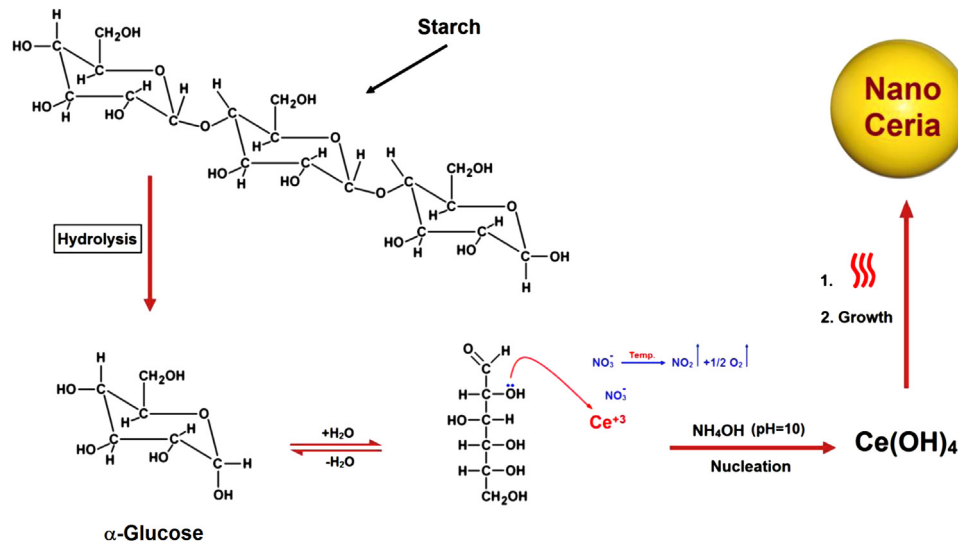
2.4. Characterization of nanocerium powders

The prepared nanocerium 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. Nanocerium formation mechanism

Based on this experiment, a tentative responsible mechanism for the formation of nanocerium 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 nanocerium powders involves several complicated reactions [27], controlling the nucleation of initial precipitate $\text{Ce}(\text{OH})_3$, however, will mainly determine the properties of the final nanocerium. As the NH_4OH was added into the precursor, $\text{Ce}(\text{OH})_3$ precipitate was formed immediately due to extreme low-solubility constant ($K_{\text{sp}} = 6.3 \times 10^{-24}$) [28]. Under such a basic condition, high-alkaline environment favored the oxidation of $\text{Ce}(\text{OH})_3$ to hydrated $\text{Ce}(\text{IV})$ 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 $\text{Ce}(\text{OH})_4$ and precipitation. Hydroxyl ions play an important role in this process and strongly affect the super saturation degree of initial precipitate and oxidation of $\text{Ce}(\text{III})$ to $\text{Ce}(\text{IV})$ [29]. The subsequent sol–gel procedure



Scheme 1. Schematic plan of sol-gel formation mechanism of nanoceria.

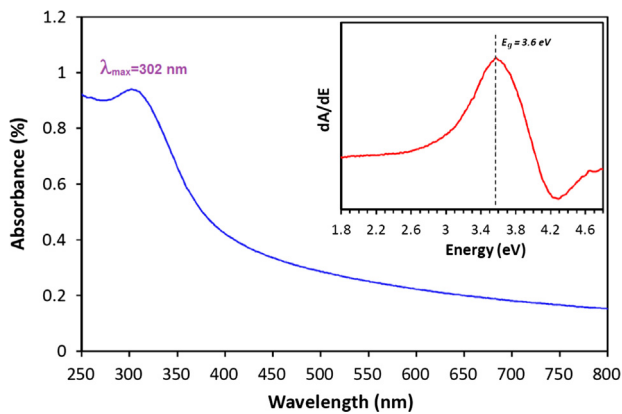


Fig. 1. UV-vis spectrum and band gap estimation (inset) of C3.

and heat treatment afforded enough energy for the complete conversion of the $\text{Ce}(\text{OH})_4$ nuclei into CeO_2 nuclei via dehydration and the subsequent growth of highly crystallized nanoceria, which were stabilized by starch molecules.

3.2. Optical properties of calcined nanoceria

Typical UV-vis absorption spectrum of C3 is shown in Fig. 1. The nanoceria powders were dispersed in water, and the suspension was then used to perform the UV-vis measurement in the wavelength range of 250–800 nm. The spectrum revealed a characteristic absorption peak at wavelength of 302 nm for C3, which can be assigned to the intrinsic band-gap absorption of nanoceria powder due to the electron transitions from the valence band to the conduction band. In other words, the absorption of the charge transfer transition from O 2p to Ce 4f in CeO_2 produces the band at approximately 300 nm [30,31]. As shown in Fig. 1, the maximum peak in the absorbance spectrum does not correspond to the true optical band gap of the nanoceria. A common way to obtain the band

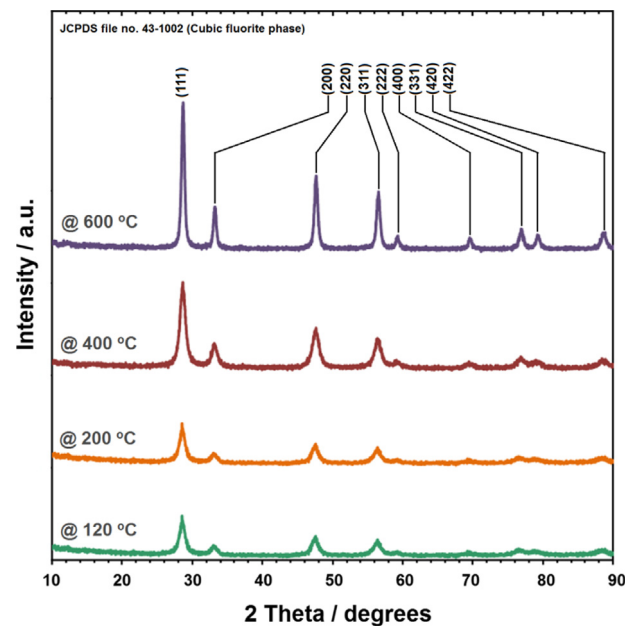


Fig. 2. PXRD patterns of synthesized nanoceria powders at different temperatures.

gap of the materials with a direct band gap from the absorbance spectra is to get the first derivative of the absorbance with respect to the photon energies. The band gap can be estimated from the maximum in the derivative spectrum at the lower energy sides [32,23]. The derivative of the absorbance of the nanoceria shown in the inset of Fig. 1 indicates a band gap of 3.6 eV for the sample.

3.3. PXRD analysis

Fig. 2 shows PXRD patterns of as-dried and calcined nanoceria powders. The same crystalline structure for all conditions was observed. All of the detectable Bragg peaks

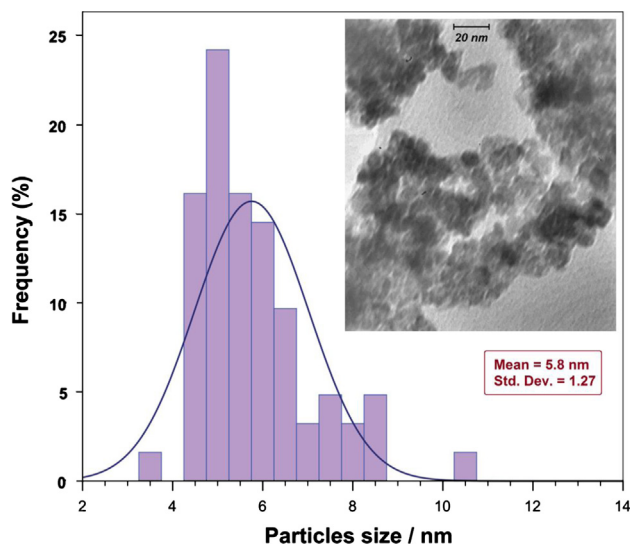


Fig. 3. Typical TEM image and corresponding size distribution of C3.

with Miller indices (111), (200), (220), (311), (222), (400), (331), (420), and (422) can be indexed to a pure cubic fluorite structure of nanoceria (space group: $Fm\bar{3}m$), which is in agreement with the JCPDS file for CeO_2 (JCPDS 043-1002). It is worth noting that the overwhelmingly intensive diffraction peak is located at $2\theta=28.51^\circ$, which is from the [111] lattice plane of fcc CeO_2 . The broadening of the peaks indicates that the crystallite sizes are below 10 nm, according to the literature [33]. This result indicates that the size of the obtained samples is small, as confirmed by the TEM image and its corresponding size distributions of C3 (Fig. 3). After the as-prepared nanoceria powders were calcined at different temperatures (i.e., 200, 400, and 600 °C for 2 h), PXRD peaks became sharper with increasing temperature and FWHM decreased, indicating that the crystallinity of nanoceria powders is accelerated by the calcination process (Fig. 2). Nanoceria powders prepared under all experimental conditions lead to the formation of particle agglomerates due to van der Waal's forces responsible for the formation of ultra-fine nanoceria particles.

3.4. TEM analysis

Fig. 3 illustrates the TEM image and particles size distribution of C3 at 100 kV. It is evident from the picture that the particles were small in size and uniform in shape. The estimated particle size was to be around 5.8 ± 1.27 nm with a narrow size distribution.

3.5. Evaluation of metabolic activity of nanoceria

The results of in vitro cytotoxicity studies after 24 h of incubation with different concentrations of nanoparticles, ranging from 0 to 175 $\mu\text{g}/\text{mL}$, are shown in Fig. 4. The samples demonstrated no significant toxicity even in concentrations up to 175 $\mu\text{g}/\text{mL}$ in the MTT assay meaning that the prepared nanoceria is well tolerated by Neuro2A cells. Few studies have investigated the toxicity induced by nanoceria and

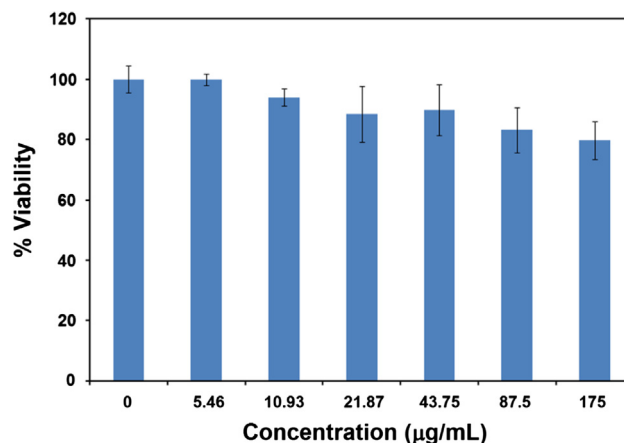


Fig. 4. Cell viability of Neuro2A cells measured by the MTT assay. Cells were incubated for 24 h with the indicated concentrations of the nanoparticles.

the results of this study can be useful for potential biological applications of nanoceria such as drug delivery studies [34–36].

4. Conclusions

Nanoceria powders with narrow size distribution were prepared by starch-mediated sol–gel method in aqueous solutions. This method is found to be simple, green, and economical for large scale preparation of cerium oxide in nanoscale with required characteristics. The obtained results suggest that the mean diameter of nanoceria powders was found to be about 6 nm, with cubic fluoride structure. The nanoceria particles showed a strong UV–vis absorption below 400 nm with a well-defined absorption peak at 302 nm, the direct band gap was found to be 3.6 eV. It is expected that this material can find other potential applications in several fields as catalysts, storage hydrogen devices, and optical or electrical materials. In the present study nanoceria powders exhibited very low cytotoxic effect on Neuro2A cell lines, making it suitable candidate for various biological applications.

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