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Facile synthesis, characterization, and evaluation of neurotoxicity effect of cerium oxide nanoparticles

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Abstract

Cerium oxide nanoparticles (CeO_2-NPs) were synthesized via the sol–gel method in gelatin media. Long-chain gelatin compounds were utilized to terminate the growth of CeO_2-NPs and stabilize them. The CeO_2-NPs were characterized by a number of techniques, including X-ray diffraction analysis (XRD), UV–vis spectrophotometry, and high-magnification transmission electron microscopy (TEM). The CeO_2-NPs calcined at different temperatures exhibited a cubic fluoride structure with sizes less than approximately 10 nm. The influence of the calcination temperature on the morphology of CeO_2-NPs was also investigated. *In vitro* cytotoxicity studies on neuro2A cells showed a dose-dependent toxicity with non-toxic effect of concentration below 10 μg/mL. The results indicated that gelatin is an interesting material that can be used as a stabilizer in the sol–gel processes for preparing small CeO_2-NPs.

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

1. Introduction

Over the past decade, cerium oxide nanoparticles (CeO_2-NPs) have been widely studied for their structural, chemical, and physical properties, such as non-stoichiometry, reduction behavior, oxygen storage capacity and interactions of metal/CeO_2-NPs. Various preparation methods have been used to obtain dispersed CeO_2-NPs such as hydrothermal [1], pyrolysis [2], precipitation [3], thermodecomposition [4], sol–gel [5], microwave heating [6], sonochemical [7], W/O microemulsions [8], and mechanochemical [9] 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 CeO_2-NPs). Another method for preparing the pure crystalline phase and stable CeO_2-NPs has used cationic surfactant (cetyltrimethylammonium bromide, CTAB) and cerium chloride ($CeCl_3$) at ambient temperatures [10]. However, in this method (surfactant-assisted method), the used reagents are generally unfriendly to the environment. In fact, different types of natural polymers can also be used as bio-templates in the synthesis of CeO_2-NPs. However, there are some studies on the non-surfactant templating route for the preparation of CeO_2-NPs [10,11]. 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 [12].

Gelatin, the protein from collagen, has a three-chain helical structure in which individual helical chains are stranded in a super-helix around the common molecular axis [13–15]. Gelatin contains positively and negatively charged as well as hydrophobic domains folded into a

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stable, non-native state that minimizes hydrophobic interactions with water (as it is dissolved). Gelatin can stabilize surfaces through the formation of a steric barrier [16]. Therefore, the main function of gelatin is as a stabilizer. It is usually used in applications such as food processing [17], the pharmaceutical industry [15], photography [18], and electrochemistry [19]. Gelatin has been used as a bio-template for the synthesis of ZnO nanopowders and Ag-NPs [20–22]. On the other hand, the synthesis of CeO_2-NPs using gelatin as a bio-template is a novel and versatile route that has not yet been reported. In this work, a facile, homogeneous, and modified sol–gel route was applied for preparation of CeO_2-NPs. The CeO_2-NPs were first synthesized with $Ce(NO_3)_3 \cdot 6H_2O$ and gelatin was used as starting material at different calcination temperatures.

2. Materials and methods

2.1. Materials and reagents

Chemical compounds which are used in this work were of analytical grade and used as received without further purification. Cerium (III) nitrate hexahydrate [$Ce(NO_3)_3$ $6H_2O$, Merck], gelatin (type B, Sigma-Aldrich), and ammonium hydroxide solution (NH_4OH, 25 vol%; Merck) were used as starting materials. For the evaluation of neurotoxicity effect, 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 CeO_2-NPs

To prepare the CeO_2-NPs, 0.2 g of gelatin powder was dissolved in 20 ml of distilled water and stirred for 10 min at 40 °C to obtain a clear gelatin solution. Meanwhile, the required amount of 0.5 M cerium nitrate solution was added to the gelatin 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. Initially, 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 divided into 5 parts that were heat treated at 120 °C (S1), 200 °C (S2), 400 °C (S3), and 600 °C (S4) for 2 h each and characterized.

2.3. Evaluation of neurotoxicity effect

The cytotoxicity of nanoparticles was evaluated by the method using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [23]. 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 nanoparticles in the presence of 10% FBS. The sample S3 was suspended in a stock solution at 5 μg/ml in a solution of dimethyl sulfoxide (DMSO)/DDW 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 was 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 mean ± SD of triplicates.

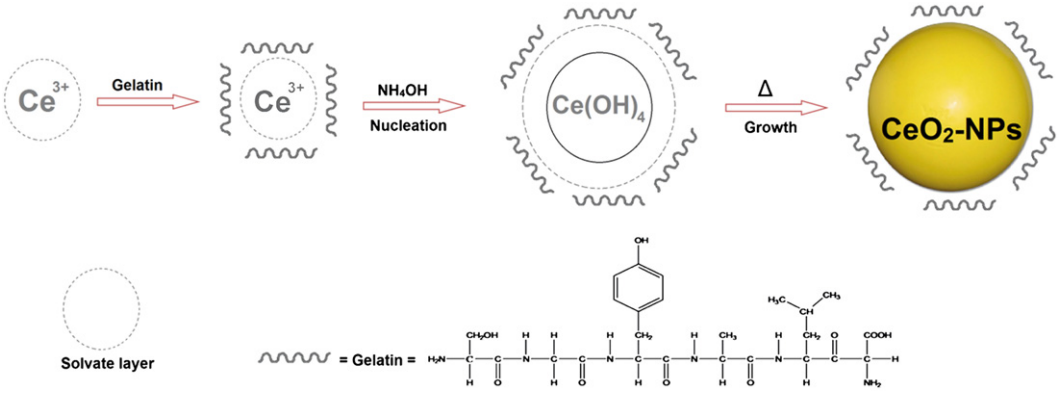
2.4. Characterization of CeO_2-NPs

The synthesized CeO_2-NPs 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 nanoparticles were determined using the UTHSCSA Image Tool® Version 3.00 program.

3. Results and discussion

Based on this experiment, a tentative responsible mechanism for the formation of CeO_2-NPs has been proposed and illustrated in Scheme 1. Initially, the gelatin molecules were adsorbed onto the solvated Ce^{3+} ions in water with the coordination between cerium cations and the oxygen atoms in gelatin. Hydroxyl ions were released through the hydrolysis of the dropped ammonium hydroxide. Under such a basic condition, the simultaneous oxidation transfer of Ce^{3+} to Ce^{4+} took place due to the oxygen in air [24], and the color of the initial solution changed from colorless to light yellow. The subsequent sol–gel procedure and heat treatment afforded enough energy for the complete conversion of the $Ce(OH)_4$ nuclei into CeO_2 nuclei via dehydration and the subsequent growth of highly crystallized CeO_2-NPs, which were stabilized by gelatin.

This condition-dependent experiment and our previous research [25,26] demonstrated that the introduction of gelatin played double roles—stabilizing agent for initial ions and final products—in many preparations. Above all, gelatin greatly stabilized the as-formed $Ce(OH)_4$ nuclei and the as-obtained CeO_2-NPs through the bonding between the carbonyl groups and surface hydroxyls while simultaneously preventing them from agglomeration.



Scheme 1. Schematic plan of sol–gel mechanism for synthesis of CeO_2-NPs.

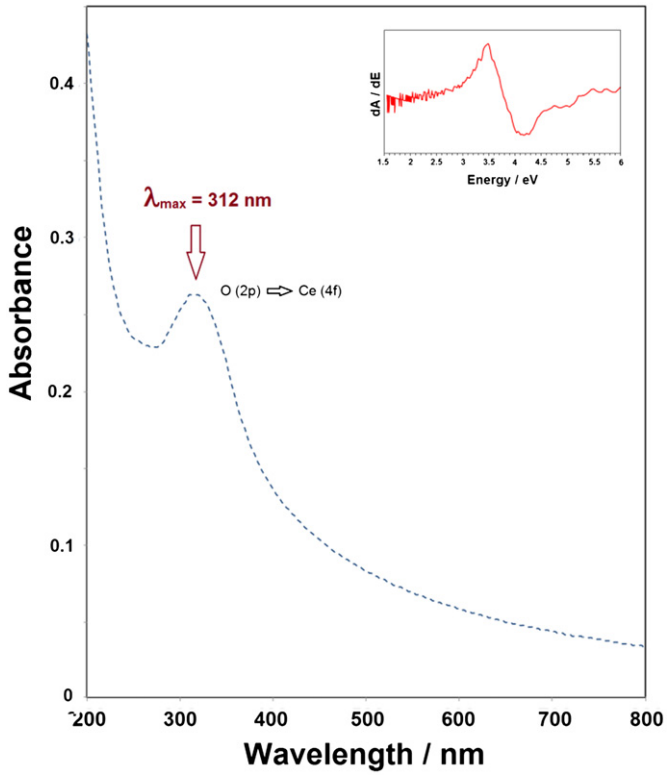


Fig. 1. UV–vis spectrum and band gap estimation (inset) of S3.

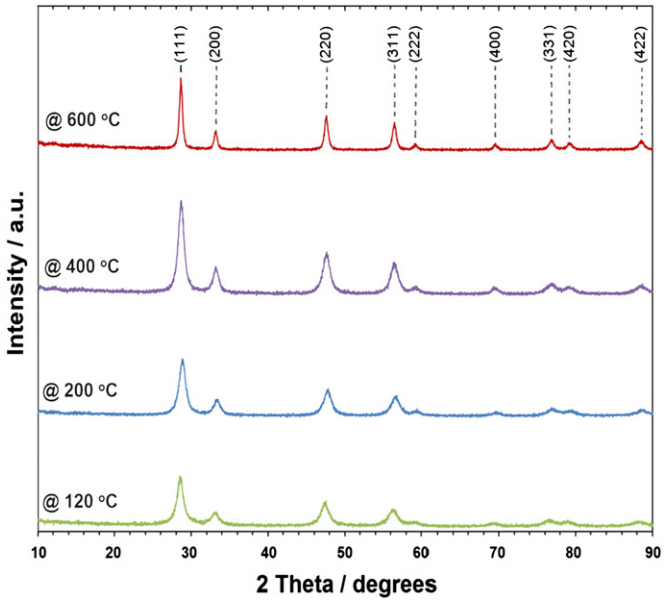


Fig. 2. XRD patterns of synthesized CeO_2-NPs at different temperatures.

Typical UV–vis absorption spectrum of S3 is shown in Fig. 1. The CeO_2-NPs were dispersed in water, and the suspension was then used to perform the UV–vis measurement in the wavelength range of 200–800 nm. The spectrum revealed a characteristic absorption peak at wavelength of 312 nm for S3, which can be assigned to the intrinsic band-gap absorption of CeO_2-NPs due to the electron transitions from the valence band to the conduction band. In other words, the band at approximately 300 nm is caused by the absorption of the charge-transfer transition from O 2p to Ce 4f in CeO_2 [27,28]. As shown in Fig. 1, the maximum peak in the absorbance spectrum does not correspond to the true optical band gap of the CeO_2-NPs. A common way to obtain the band 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 [29,30]. The derivative of the absorbance of the CeO_2-NPs shown in the inset of Fig. 1, indicates a band gap of 3.54 eV for the sample.

CeO_2 has a kind of typical calcium fluoride (CaF_2) structure with space group Fm^3m. Fig. 2 shows the XRD patterns of the dried and calcined CeO_2-NPs prepared in the gelatin media. The same crystalline structure for all conditions was observed. All of the detectable Bragg peaks with Miller indices (111), (200), (220), (311), (222), (400), (331), (420), and (422) can be indexed as fluorite cubic structures (JCPDS # 00-043-1002). The broadening of the peaks indicates that the crystallite sizes are below 10 nm, according to the literature [31]. This result indicates that the size of the obtained sample is small, as confirmed by the TEM image and its corresponding size distributions of S3 (Fig. 3). After the as-prepared CeO_2-NPs was calcined

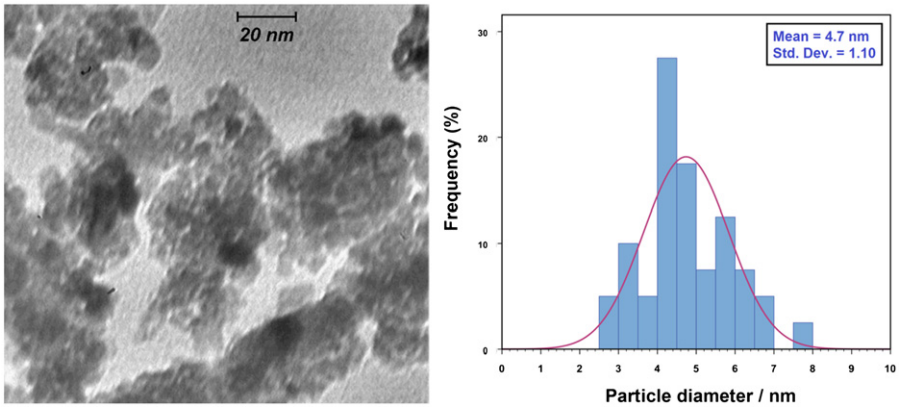


Fig. 3. TEM image and corresponding size distribution of S3.

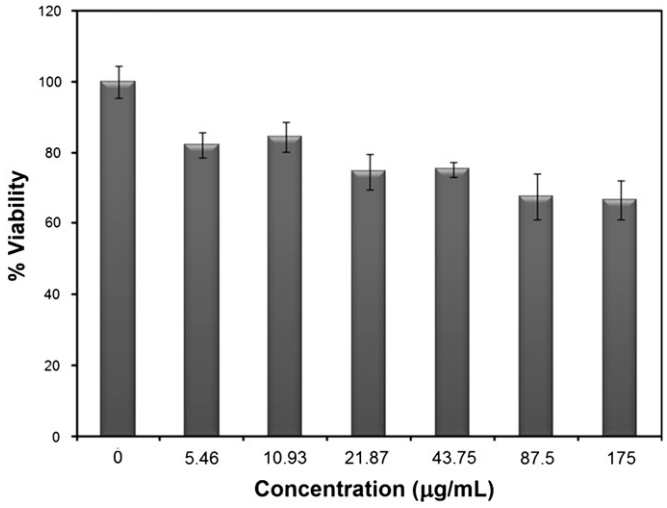


Fig. 4. Cell viability of neuro2A cells measured by the MTT assay. Cells were incubated for 24 h with the indicated concentrations of the nanoparticles.

at 200 °C, 400 °C, and 600 °C for 2 h, XRD peaks became sharper with increasing calcination temperatures and FWHM decreased, indicating that the crystallinity of CeO_2-NPs is accelerated by the calcination process. Moreover, no other peaks related to an impurity for prepared CeO_2-NPs at different calcination temperatures, indicating that the final nanopowders were relatively pure. Thus, the pure crystallized CeO_2-NPs were prepared by gelatin at a low temperature (120 °C).

The results of *in vitro* cytotoxicity studies after 24 h of incubation with different concentrations of nanoparticles, ranging from 0 to 175 µg/mL, are shown in Fig. 4. Concentrations below 10 µg/mL were not cytotoxic in the MTT assay. The level of cytotoxicity as a function of the concentration decreased as the concentration decreased.

4. Conclusion

A simple method to synthesize highly pure CeO_2-NPs by sol–gel method at low temperatures was reported. This method is interesting not only for the low temperature used, but also for applying and extending the green chemistry rules in preparation of nanoparticles. The other advantages of the method are simple synthesis, in a normal atmosphere, and low cost, giving a potential avenue for further practical scale-up of the production process and applications. It is expected that these nanoparticles can find potential applications in different fields such as catalysts, cosmetics, and optical or electrical devices as well as medicinal applications.

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