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Bioorganic polymer-based synthesis of cerium oxide nanoparticles and their cell viability assays

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Abstract

A simple one-step, eco-friendly, and "green" approach for the preparation of monodispersed cerium oxide nanoparticles (CeO_2-NPs) are described. This process uses nontoxic and renewable degraded agarose as a natural matrix and mild reaction conditions. The agarose acted as a stabilizing and/or capping agent for the CeO_2-NPs. The CeO_2-NPs were successfully grown at different calcination temperatures within the agarose matrices, and their crystallite structures were characterized using various methods, including FESEM, PXRD, FTIR, TGA/DTA and UV–vis spectroscopy techniques. This process was found to be comparable to those obtained from conventional preparation methods that use hazardous materials proving to be an excellent alternative for the preparation of CeO_2-NPs, using bio-organic materials. *In vitro* cytotoxicity studies on L929 cells, a non-toxic effect in all concentration (up to 800 μg/ml) was illustrated and we believe that these samples will have viable applications in different fields in medicine.
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1. Introduction

In recent years, the synthesis of nanopowders in bio-based matrices has been considered a green route, reliable, eco-friendly, safer, easy to use, availability, and more cost-effective alternative to chemical and/or physical routes of preparation [1–3]. Cerium oxide nanoparticles (CeO_2-NPs), as important rare-earth oxide materials, have attracted interest in recent years due to its unique physical and chemical properties that are significantly different from those of bulk materials [4]. Therefore, it has been widely considered in different fields e.g., catalysis [5], fuel cells [6], gas sensors [7], ultraviolet absorbers [8], polishing materials [9], medicine [10], and other fields. A number of preparation methods have been used to synthesis of CeO_2-NPs, including hydrothermal [11], sol–gel [12], co-precipitation [13], polymeric precursor [14], microwave-assisted heating [15], sonochemical [16], and other methods. Some of these routes have been used in solutions containing polymers and surfactants in order to improve or control the size and shape of CeO_2-NPs. In fact, natural polymers as a category of macromolecules can also be used as bio-templates in the green synthesis of CeO_2-NPs. When these polymers are used as a capping/stabilizing agent, the diameter of nanoparticles can be logically controlled [17]. We have also reported the green synthesis of cerium oxide nanoparticles, stabilized with gum molecules via a sol–gel method [18]. The ease with which nearly monodisperse gum mediated CeO_2-NPs were synthesized, provided an encouragement for the controlled synthesis of plant extract protected CeO_2-NPs.

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Agarose is an oxygen rich naturally occurring straight-chain polysaccharide extracted from red purple seaweeds or other vegetables, which is widely used in biomedical and bioengineering applications. The basic repeating units (disaccharide) of agarose consists of (1,3) linked β-D-galactose and (1,4) linked α-L-3,6-anhydrogalactose [19] (Fig. 1). On heating to above 90 °C, the agarose powder is normally dissolved in water and when the temperature dropped to 35–40 °C, a good semi-solid gel will be formed. This is the main advantage and feature for a variety of application.

Gel formation occurs when cooling the hot aqueous agarose solution to ambient temperature which is stable over a wide range of pH from 3 to 9. The gelation phenomena in agarose created by the presence of H-bonds can be destroyed by any factor lead to the destruction of H-bonds. It is because of this particular gelation properties combined with the considerable stability, shrink, and swell features, agarose gel has been widely used in different subjects such as pharmaceutical, medicine, textile, chemical industries, etc. [20–22]. In our recent studies, bioorganic polymers such as gelatin, starch, and honey as "green", ecofriendly degradable materials, and abundant in nature have been used as the capping and/or stabilizing agents for the synthesis of different nanomaterials [23–25]. Therefore, agarose was chosen as a new bio–based medium for the synthesis of water-soluble CeO_2-NPs. Caruso et al. [26] used the agarose biopolymer matrix as a sacrificial media to prepare macroporous metal oxides. Agarose is a green matrix, easily available, eco-friendly and cost-effective as compared to other stabilizers available on the market. Indeed, a general and simple method to use of biopolymer as a template for preparing of metal oxide nanoparticles would be advantageous given their potential application in many areas. In this work, an improved sol–gel route was used for synthesis of CeO_2-NPs. The CeO_2-NPs were first synthesized with cerium nitrate hexahydrate and agarose was used as starting material at different heating treatments.

2. Materials and methods

2.1. Materials and reagents

All the materials used were of analytical grade and were used without any purification. Cerium (III) nitrate hexahydrate was purchased from Fluka (Germany) and agarose was purchased from Sigma-Aldrich (USA). All glasswares used in the laboratory experiments were cleaned with fresh solutions of HNO_3/HCl (3:1, v/v), washed thoroughly with doubly distilled water, and dried before use. Double distilled water was used in all experiments.

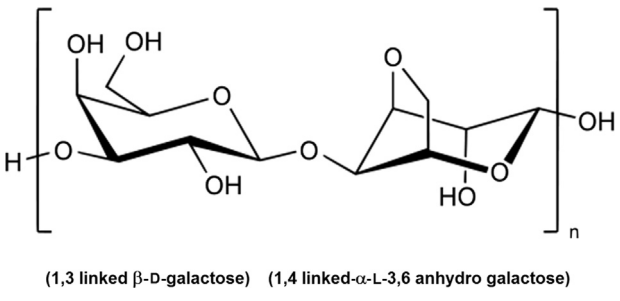

Fig. 1. Chemical structure of repeating unit of agarose.

2.2. Synthesis of CeO_2-NPs

To prepare 5.0 g of CeO_2-NPs, 12.6 g of $Ce(NO_3)_3 \cdot 6H_2O$ was dissolved in 30 ml of distilled water and then stirred for 30 min. Meanwhile, 2.0 g of agarose was dissolved in 50 ml of distilled water and stirred for 15 min at 70 °C to achieve a clear agarose solution. Afterwards, the cerium nitrate solution was added to the agarose solution, and the container was placed in an oil bath with a temperature at 60 °C. Through stirring for 8 h, a white color resin was obtained and divided into 4 parts to be individually heated at a rate of 4 °C/min up to the respective temperatures of 200 (C_1), 400 (C_2), 600 (C_3), and 800 °C (C_4), then the products was maintained for 2 h at the specified temperatures in air to obtain CeO_2-NPs.

2.3. Characterization of CeO_2-NPs

The prepared CeO_2-NPs were characterized by PXRD (Philips, X'pert, Cu Kα), FTIR (ST-IR\ST-SIR spectrometer), TGA/DTA (Q600), UV–vis (Evolution 300® Thermo Fisher Scientific), and FESEM (Carl Zeiss Supra 55VP).

2.4. Evaluation of neurotoxicity effect

L929 cells were obtained from Pasteur Institute (Tehran, Iran). Cells were maintained at 37 °C in a humidified atmosphere (90%) containing 5% CO_2. Cells were cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM) (4.5 g/l) with 10% (v/v) fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin. The cell viability was determined using a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) assay [27]. For MTT assay, 5000 cells were seeded in each well of a 96-microwell plate and treated with various concentrations of Seo agarose (0–800 μg/ml) for 24 h. MTT solution in phosphate-buffered saline (PBS, 5 mg/ml) was added to a final concentration of 0.05%. After 3 h, the formazan precipitate was dissolved in DMSO. The absorbance at 570 and 620 nm (background) was measured using a StatFAX303 plate reader. All experiments were carried out in triplicate; the percentage of viable cells was calculated as the mean ± SD and as a percentage of non-treated control groups, which was assumed to be 100% and morphological deformations of the cells were also examined.

3. Results and discussion

Research on polymer–metal oxide nanoparticles received much attention in recent years owing to their application in wide and different fields. Among the wide variety of polymer matrices, biopolymers become the preferred choice as they are readily available, inexpensive, environmentally green and more amenable to scale up. Moreover, the oxygen rich

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