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Role of Pullulan in preparation of ceria nanoparticles and investigation of their biological activities

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ABSTRACT

Throughout this work, a facile, environmental–friendly, and "green" method is delineated for preparing ceria nanoparticles (CNPs), which utilizes nontoxic and renewable degraded polysaccharide polymer including pullulan as a natural matrix. Pullulan behaves as a suitable stabilizing (capping) agent for CNPs that are effectively formed at various high temperatures, while they are structurally analyzed through different techniques such as TGA/DTG, XRD, FESEM, and FTIR instruments. This procedure was found to be comparable to the ones that were acquired from conventional preparation methods that employ hazardous materials, which confirms this approach to be an exquisite alternative in preparing CNPs through the benefit of bioorganic materials. The *in vitro* cytotoxicity studies on Neuro2A cells have mentioned nontoxic particles in a range of concentrations (0.97–125 μg/ml) and thus, we reckon that the prepared particular CNPs will have persistent utilization in various fields of biology and medicine.

1. Introduction

The position of biopolymers and natural materials in fabricating nanoparticles, especially bioceramics, is precisely associated with the relation between green chemistry, nanoscience/nanotechnology, and nature [1–3]. Throughout recent years, synthesizing nano-bioceramics in biological matrices have been contemplated a facile, eco–friendly, safe, easily used, cost-effective, and available as a suitable alternative for the chemical and physical preparation procedures [4–6]. As significant rare–earth oxide materials, Ceria (CeO_2) nanoparticles (CNPs) have caught the interest of many in the past years since they contain unique physicochemical properties that are quite very different in comparison of those in bulk form [7]. For this reason, they have been widely reflected in various fields e.g., catalysis [8,9], fuel cells [10], specific absorbers [11], photo-protective [12], polishing [13], gas sensors [14], biomedical [15,16], and *etc*. A number of fabrication courses have been applied in synthesizing CNPs, involving sol–gel [17,18], hydrothermal [19], polymeric precursor [20], co-precipitation [21,22], sonochemical [23], and microwave–assisted heating [24]. In some of the mentioned processes (e.g., an aqueous phase), surfactants and polymeric materials have been employed for the sake of improving or controlling the shape (structure) and size of CNPs. In actuality, bio-polymers including macromolecules such as polysaccharides of can be utilized as bio–substrates in the bio-synthesis of CNPs as well. When some of these particular bio-based polymers (bio-polymers) are employed as stabilizers, the size of NPs can be rationally controlled [25,26]. Fig. 1 exhibits the molecular structure of pullulan (as a neutral glucan) that contains "maltotriosyl" as a repeating unit that they are connected by α-1,6 linkages. Pullulan as an extracellular, microbial, and water-soluble polysaccharide is known as an important natural material that is can be used in different biomedical/bioengineering utilization due to unique properties e.g., non-toxic, non-mutagenic, non-immunogenic, and non-carcinogenic effects [27].

Nevertheless, there seems to be no report on the polysaccharide polymers such as pullulan as a capping and/or stabilizing agent in

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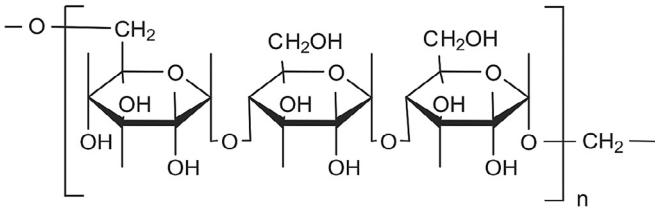

Fig. 1. Molecular structure of Pullulan.

preparing or producing CNPs. In recent years, natural-based polymers including soluble starch and gelatin, and food-based materials such as honey that stand as available, degradable, and eco-friendly templates have been utilized as bio-stabilizers in the synthesizing procedure of various materials in nanoscale [5,28–32]. Thus, pullulan was selected as a new biological template to synthesize CNPs. Thereby in this project, a modified sol-gel route was employed to synthesize of CNPs. These particular samples were first prepared using cerium nitrate hexahydrate, while pullulan was applied as a bio-template material at different temperatures.

2. Experimental

2.1. Synthesis of CNPs

To compose 4.0 g of CNPs, 10.1 g of cerium nitrate hexahydrate was dissolved in 25 ml of double distilled water and stirred for the duration of 30 min. In the meantime, 1.5 g of pullulan was dissolved in 50 ml of double distilled water and stirred for a period of 15 min at 40 °C in order to obtain a clear solution. Subsequently, the cerium precursor was added to the pullulan solution, while the flask was positioned in the bath at about 60 °C with stirring for 8 h. Then, the obtained resin was heated (3°C/min) at different respected temperatures of 400 (S_1), 500 (S_2), and 600 (S_3) for the duration of 2 h to achieve CNPs.

2.2. Characterization of CNPs

The arranged CNPs were distinguished by XRD (Philips, X'pert, Cu K_α), FTIR (ST–IR\ST–SIR spectrometer), TGA (Q600), and FESEM (Mira 3, TESCAN).

2.3. Cytotoxicity effect

Neuro2A cells were sustained in a humidified atmosphere (90%) that contained 5% CO_2 at 37 °C. Then they were cultured in Dulbecco's modified Eagle's medium (DMEM) (4.5 g/l) with fetal bovine serum (10% v/v), streptomycin (100 μg/ml), and penicillin (100 Units/ml). The cell viability was ascertained through the usage of the modified 3–(4,5– dimethylthiazol–2–yl)–2,5–diphenyl tetrazolium (MTT) assay [33]. Regarding the MTT test, about 5000 cells were seeded and processed in a range of Seo agarose concentrations (0–800 μg/ml) for a period of 24 h. The MTT in phosphate–buffered saline (PBS, 5 mg/ml) was supplemented 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 calculated through the utilization of a plate reader (StatFAX303). All the experiments were performed in triplicate.

3. Results and discussion

The inquiry on polymers and metal oxide NPs have caught the concern of many in last decay due to their unique properties in a large number of different applications. Furthermore, containing oxygen-rich functionalities and the affinity towards metals and metal oxides have turned biopolymers into the best possible candidates to stabilize nanoparticles [34]. The thermogravimetric analysis (TGA/DTG) of the as-formed gel that contains pullulan and cerium derivatives are demonstrated in Fig. 2. Thermal analysis was started from about 25 °C (ambient temperature) and then was heightened up to 850 °C (10°C/min). The first weight loss observed at ~186 °C (mass loss of about 6% w/w), which would be ascribable to the volatilization of adsorbed water molecules and dehydration/condensation of formed cerium hydroxide in the gel [32]. Later on, the thermal graph of pullulan revealed a second weight loss (about 85% w/w) in some higher temperatures (240–410 °C) due to degradation of saccharide rings [35], forming and decomposing of pyrochlore phases along with the formation of crystalline CNPs and further oxidation of cerium components [36]. There is almost no weight loss that could be observed between 500 and 850 °C, indicating on the formation of thermally stabled CNPs.

Fig. 3 display the XRD patterns of obtained CNPs in diverse temperatures (400, 500, and 600 °C) that were arranged in the pullulan. The CNPs is a type of ordinary calcium fluoride crystallite structure with the space group of $Fm\bar{3}m$. Similar crystallinity is perceived for all samples at different calcination temperatures. Bragg's peaks such as (111), (200), (220), (311), (222), (400), (331), and (420) can be confirmed the fluorite cubic crystallite structure (JCPDS #00–043–1002). The dried gel was converted into crystallite phase resulting from the heat treatment that took an increase at 400 °C (S1). In higher calcination temperatures (500 and 600 °C), the NPs demonstrate well–defined crystallinity and the XRD patterns become sharper as the calcination temperatures heighten and peaks width decreases; this fact suggests that the crystallinity of CNPs are accelerated through the higher temperatures. The peaks width were broader for all the samples because of the combined effects of small crystal dimension and the relatively affiliated higher crystal lattice strain and defects [37,38]. Also, the broadening of XRD peaks indicate that the prepared CNPs are nanocrystalline form in nature (<20 nm) in accordance with the literature [39], while this result impresses that particles diameter of the achieved CNPs are small which is affirmed through the FESEM image of S2 (Fig. 4).

Moreover, no diffraction features were perceived that would stand as a characteristic of byproducts e.g., $Ce(OH)_4$, $Ce(OH)_3$, and *etc*, denoting on the purity of CNPs. The typical mean crystallite size of the composed CNPs (S2) was calculated from the FWHM of (111) reflection (Fig. 3b) using Scherrer formulism as rendered in Eq. (1)

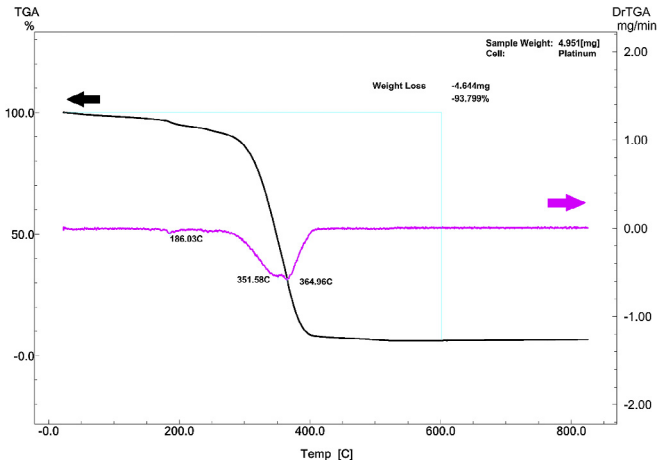

Fig. 2. TGA–DTG analysis of the Pullulan gel containing cerium cations from 25 to 850 °C.