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Enhanced biological activity and biosorption performance of trimethyl chitosan-loaded cerium oxide particles

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ABSTRACT

Nano ceria is considered to be an antioxidative agent due to its fast oxido-reductive capability and there has been a number of studies that deal with the non-toxic nature of cerium oxide; however, the antioxidative capacity is mostly influenced by the presence of Ce^{3+}/Ce^{4+} in the sample. We studied for the first time the oxidative nature of CeO_2 nanoparticles (NPs) on in vitro systems and we hypothesized that if the oxido-reductive behavior of Ce is not well utilized which may lead to adverse toxic effects. Since, we have been interested to combine the oxido-reductive potential of nano ceria with that of the antimicrobial, antioxidative, and biosorption properties of trimethyl chitosan (TMC). We hypothesize that such a design will not only modulate the oxidant activity of CeO_2 NPs, but also will help to provide enhanced antimicrobial, antioxidative, and adsorption properties. We present here the synthesis, characterization, biological activity and biosorption performance of the TMC-CeO_2 composite and its efficiency was tested by comparing the results with that of other materials (pure CeO_2, pure chitosan, and pure TMC). The polymer composite formation was thoroughly analyzed by means of UV–vis, FTIR, zeta potential, powdered XRD, and XPS studies. Further, the in vitro antibacterial and antioxidative assays proved that the coating of CeO_2 with TMC biopolymer significantly enhances its biological efficiency by increasing the interaction of the CeO_2 composite with that of bacterial cell wall, and also serving as the free radical scavenger. In addition to bioactivity, we also observed some enhanced biosorption performance towards the removal of phenolic compounds (phenol, 2-chlorophenol, and 4-chlorophenol) by the TMC-CeO_2 composite, thereby supporting its role during the waste water treatment.

Introduction

In recent years, the research into ceria (Ce) or cerium oxide (CeO_2) nanoparticles (NPs) has attracted a great deal of interest in the biomedical sector due to its strong antioxidative property of scavenging the free radicals and the resulting oxidative stress, in addition to protecting the cells from radiation, fighting against inflammation and cancer [1,2]. The oxidative stress in cells is the mediator for numerous diseases including cellular aging, atherosclerosis, arthritis and the neurodegenerative disorders such as Parkinson's and Alzheimer's. The Ce/CeO_2 is considered to restrict in general the cellular aging by diminishing the free radicals formed within the cells and this scavenging ability can be achieved through the oxidation of Ce^{3+} into Ce^{4+}. In general, Ce exists in two main oxidation states of Cerous (Ce^{4+}), and Ceric (Ce^{3+}); these oxidation states regenerate automatically i.e., can switch easily from one state to the other depending upon the conditions. The specific action of Ce is recorded for its activity against two important enzymes, super oxide dismutase-2 and catalase and this activity is mainly influenced by the ratio of Ce^{3+}:Ce^{4+} available at the surface. Further influenced by the anti-oxidative property of Ce in the biological media such as cellular differentiation and adhesion, and the neuro-, cardiac-, and lung-protective nature of nanoceria suggests its potential role in tissue engineering and as anti-inflammatory platforms [2,3]. Also, the conjugation of nanoceria with other biodegradable polymeric matrices investigated to significantly promote the cell adhesion and proliferation of

Abbreviations: TMC, trimethyl chitosan; Ce, cerium; CeO_2, cerium oxide; DQ, degree of quaternization; NPs, nanoparticles; TMO, trimethyl oligomer; TPP, tripolyphosphate; HRTEM, high-resolution transmission electron microscopy; UV-vis, ultra-violet; FT-IR, fourier transform-infrared; XRD, X-ray diffraction; XPS, X-ray photoelectron spectroscopy; EDAX, energy dispersive analysis X-ray; DMSO, dimethylsulfoxode.

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mesenchymal stem cells and cardiac progenitors [4]. Based on these facts, it can be hypothesized that the loading of nanoceria onto the polymeric matrices could significantly enhance the biocompatibility in addition to improving the cellular uptake and protecting the NPs from self-aggregation in the biological media.

Chitosan is the most abundant polysaccharide material that shows interesting physicochemical properties suitable for biomedical applications such as the low toxicity, high susceptibility to biodegradation, mucoadhesive nature, capacity to enhance drug permeability and absorption etc. For chitosan to exhibit a better water solubility and antibacterial activity, the most influencing factors are the pH of the medium and the charge density achieved by the formation of quaternary ammonium salt (as chitosan gets completely protonated under acidic environment). Investigations proved that the antibacterial ability decreases with an increased degree of quaternization (DQ) of *C-2* amine group under acidic pH, however an increased antibacterial efficiency was observed with increased DQ under neutral media [5,6]. Similar to antibacterial property, the antifungal activity also a strong dependent of DQ and the polymer characteristics which influence its solubility i.e., the introduction of bulky alkyl substituents on the chitosan chain strongly decreases the polymer solubility, a property which plays a very important role towards the antifungal activity. By keeping these things in view and to further enrich the properties of chitosan such as the solubility/stability over a wide range of pH, reversion of net charge from poly-cationic to poly-anionic, increase of the capacity to harness self-assembling structures, and enhancement of biological activities can be obtained by the alkylation of its *C-2* amine group; one way to achieve this is the methylation of amine to form *N*,*N*,*N*-trimethyl chitosan (TMC). When compared to simple chitosan, the TMC is investigated to be the more efficient carrier for the loading of drugs, proteins, antibodies, metal NPs etc by means of ionic cross-linking. Also, the TMC exhibited efficient biological activities against various bacteria, fungi and cancer cell cultures [7,8]. In a study for example, the comparison of two samples of TMC, TMC1 (DQ = 18%, low solubility and low viscosity) and TMC2 (DQ 45%, high solubility and high viscosity); it was found that the latter presents superior antifungal activity due to higher DQ and water solubility. Some of the other *N*-alkyl derivatives of chitosan such as *N*-butyl-*N*,*N*-dimethyl chitosan, *N*-octyl-*N*,*N*-dimethyl chitosan, and *N*-dodecyl-*N*,*N*-dimethyl chitosan also showed noble antifungal activity [6]. In a similar study, TMC and TMO (trimethyl oligomer) complexed with pGL3 luciferase reporter gene were evaluated for the effect of cytotoxicity and transfection efficiency against COS7 (monkey kidney fibroblasts) and MCF7 breast carcinoma cell lines. It was observed that the TMOs with 44% degree of methylation (TMO44) showed the highest transfection efficiency; however, TMC with similar transfection efficiency was observed at 57 and 93% degree of methylation. Also, an increasing trend of toxicity was seen with increasing degree of trimethylation and at similar degrees of trimethylation, higher toxicity was seen in the polymeric chitosan derivatives over oligomeric chitosan derivatives [7].

Phenol and its derivatives are the very common persistent environmental contaminants and in general enters into the environment through many different sources which include, (1) the wastes from petroleum refineries and industrial discharges (pharma, plastic, adhesive, rubber, paint, and metal industries), (2) agricultural and aquaculture activities (herbicides, pesticides, and fungicides), (3) wood preservation and pulp bleaching (as timber preservatives), and (4) through the use of inappropriate biodegradation processes. Phenols are classified as the noxious pollutants and are toxic to humans and other living organisms even at low concentrations [9–11]. The toxicity effects of phenol and derivatives on humans include nausea and vomiting, urine with smoky color, organ damage (kidney, liver, lung and central nervous system), protein degeneration etc. [12]. Phenols in general possess low biodegradability rate, high toxicity and ecological persistence and for these reasons, the water and soils contaminated with phenols must be treated completely before releasing into the environment. Among many different methods of phenols treatment processes such as the chemical oxidation, membrane filtration, electrocoagulation, biodegradation, solvent extraction and photo degradation, the adsorption method is the most effective approach due to its modest design, easy operation, and relatively simple regeneration. The commonly applied materials for the adsorption of phenolic compounds include carbon black, fly ash, rice husk, peat, bentonite, metal oxides, and polymers (both synthetic and natural origin) [13–15]. In recent years, some fabricated composites made up of chitosan and its derivatives have been applied for the adsorption purposes due to its advantages over other polymers such as, biodegradability, porous structure, abundance and cost effective, polar nature and customized solubility [16]. With that view, our synthesized TMC-CeO_2 composite for example, may find application during the adsorption of phenolic compounds due to its attractive features such as the strong solid oxide support, porous nature, controlled solubility, easy regeneration, and a net positive charge. We hypothesize that the TMC-CeO_2 composite may serve as an efficient adsorbent for the phenolic compounds through the generation of some Van der waals interactions and/or electrostatic attractive forces by taking advantage of its surface charges. Therefore, one of the objective of the present work is to investigate the biosorption potentiality of TMC-CeO_2 composite towards the removal of phenol, 2-chlorophenol, and 4-chlorophenol from aqueous solution.

By keeping in view of the said properties, the present study was aimed (1) to understand the oxidative/antioxidative behavior of CeO_2 NPs, in addition to developing a novel biocompatible platform that can be able to exhibit the properties of both antioxidation and antimicrobial within one single system, and (2) to investigate the biosorption capability of the TMC-CeO_2 composite towards the removal of phenolic compounds from aqueous solution by means of adsorption. For the composite, the TMC polymer acts as a vehicle and helps to enhance the interactions of loaded particles with the cells by crossing the biological barriers, in addition to holding its own anti-microbial properties. Also, the same TMC polymer is expected to adsorb the phenolate ions from the aqueous media by taking advantage of the surface positive charges acquired from the quaternary ammonium charged groups by means of electrostratic forces. Thus formed composite was thoroughly characterized by using different instrumental techniques such as HRTEM, UV–vis, FT-IR, XRD, and XPS. Further, the biological activity by means of cell viability and proliferation studies was carried to see any toxicological effects and the antibacterial activity exhibited by the particles. An in depth analysis to understand the protective action of TMC polymer and its combination with CeO_2 were also carried by making use of ROS assay and DNA fragmentation. The biosorption studies by applying the changes in pH, contact time and adsorbent amount towards the removal of phenolic derivatives were also performed.

Materials and methods

Synthesis of CeO_2 NPs

The synthesis method to form CeO_2 NPs involves the precipitation of cerium(III) nitrate ($Ce(NO_3)_3{\cdot}6H_2O$) by NH_4OH as reducing agent in the presence of alcohol–water solvent mixture [17]. For that, 2.5 g of $Ce(NO_3)_3{\cdot}6H_2O$ was added slowly to a solution containing 200 mL of equal volumes of methanol, ethanol, propanol and water mixture; the reaction mixture was heated to