GREEN SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL PROPERTIES OF CEO_2 NANOPARTICLES

Abstract

Green synthesis of various metal and metal oxide nanoparticles for various applications drew the attention of researcher around the world. Ceria nanoparticles (CeO_2 NPs) or ceria can be synthesized using various plant and biological sources including bacteria and fungi. Ceria nanoparticles are one of the important bioactive nanoparticles due to their efficiency against various pathogenic bacteria, fungi, and cancer cells. In this chapter, it is aimed to discuss the potential of ceria and their antibacterial, antifungal, anticancer, antidabetic, and anti-inflammatory activities. Definitely, this study may useful to the scientific society to understand the importance of Ceria nanoparticles.

Keywords: Ceria nanoparticles, Plant extract, Green synthesis, Biosynthesis, Biological Activities.

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I. GREEN SYNTHESIS

Nanoparticles (NPs) are nanostructures used in nanotechnology that range in size from 1 to 100 nm. These nanoparticles are used in many areas of science and technology and exhibit peculiar physiochemical properties [1]. Nanoparticles have their potential applications in agricultural, food, energy harvesting, environmental remediation, cosmetics and medicine. Nanoscience provided solution for the packing materials in food products. Among the metal oxide, CeO_2 NPs have been attracted by the researcher due to their properties and applications [2]. Applications of CeO_2 NPs have been explored in fuel cells, gas sensor, and catalysts [3]. Ceria nanoparticles were produced using physical, chemical and biological methods [4]. Numerous medical uses, including anti-inflammatory, anti-apoptotic, cancer therapy, neurological illnesses, tissue regeneration, and eye problems, have been suggested for the potential use of nanoceria made with diverse plant extracts [5].

However, ceria nanoparticles produced by chemical methods are harmful to the human health and environments. Furthermore, when nanoparticles are produced via physical or chemical methods, it is necessary to evaluate their safety and interaction of nanoparticles with biological systems. Synthesis of metal, bimetallic, metal oxide, bimetal oxide and metal-metal oxide nanocomposites using extracts of various parts of plants is known as green synthesis. This synthesis produces no toxic byproducts, making it an environmentally beneficial process [6]. Matussin et al. graphically represented (Figure 1) the various applications in the fields of environment and biological activities of CeO_2 NPs [7].

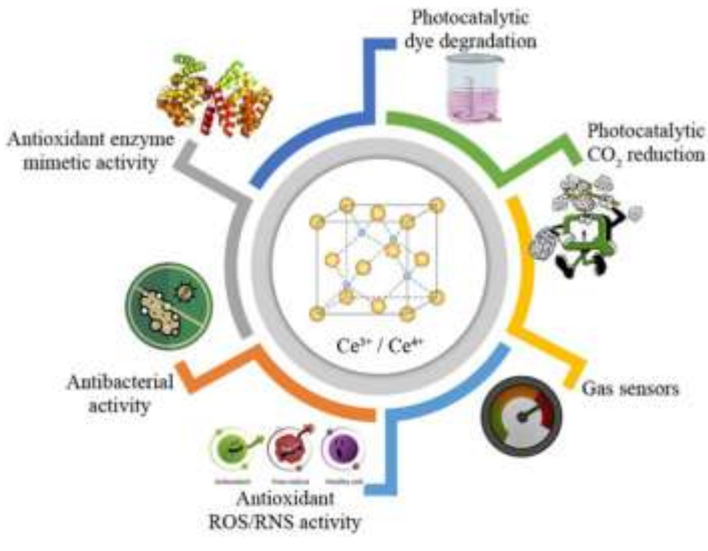

Figure 1: Various Applications of Ceria Nanoparticles [7]

It is known that trees, herbs, shrubs, bacteria, Algae, and Fungi are rich in phytochemicals such as phenolics, flavonoids, phenolic acids, carbohydrates, alkaloids, and lignin. Some of phenolic group containing phytochemicals are freely soluble in hot water and hence such phytochemicals act as reducing agent in the synthesis of ceria nanoparticles. Different parts of plants can be used as reducing agent when the extract is prepared in hot water. Natural materials used as reducing agent is represented in Figure 2.

Figure 2: Green Synthesis of CeO_2 Nanoparticles Using Various Parts of Plants

Various metal oxide nanopartiles such as ZnO, CuO, ZrO_2, TiO_2, Fe_2O_3, Co_3O_4, and CeO_2 have been synthesized for various applications. Due to surface properties, ceria nanoparticles have been most extensively for further applications. However, both methods use chemicals such as sodium hydroxide or ammonium hydroxide as reducing agent to produce nanoparticles [8, 9]. These chemicals are toxic to environment and human health [10]. Whereas the natural sources such as plant extract, biological extracts and microbes are safe and less toxic method, which methods are called as green synthesis [11]. These natural extracts are rich in secondary metabolites, especially water soluble biomolecules thought to be responsible for the reduction metal salts into respective nanoparticles [12, 13].

Various parts of plants have been used for the synthesis of ceria nanoparticles [14]. Leaves are especially rich in phenolic compounds when compared with other parts of the plant extract. Therefore, leaf extract predominantly used in the green synthesis of various metal oxides [14-16]. The phytochemicals present in the plant react with metal salt solution to produce metal nanoparticles in case of silver, gold, palladium and platinum. Whereas, in case of metal oxide, phytochemicals react with metal salt solution and produce hydroxide. After annealing at higher temperature hydroxide lose water molecule to form metal oxide such as ZnO, CuO, ZrO_2, CeO_2 and [17].

1. **Biosynthesis:** Biosynthesis classified into Microbes and other biological sources. Microbes are rich source of phytochemicals. Hence, metal and metal oxide nanoparticles can be synthesized using microbes extract. For examples, *Curvularia lunata, Humicola sp, Fusarium solani* and *Aspergillus niger* have been used as stabilizing agent to produce CeO_2 NPs [18-21].

   Spherical CeO_2 NPs with size ranges from 5–20 nm were synthesized using *Curvularia lunata* extract. *Fusarium solani* extract stabilized spherical CeO_2 NPs of size ranges from 20–30 nm were prepared for the purpose of antibiofilm against many pathogenic bacteria. Spherical shape CeO_2 NPs with the size range of 12–20 nm using a fungus *Humicola* extract as capping agent [19]. Bacteria, *Bacillus subtitles* extract was also used to synthesis the spherical CeO_2 NPs with an average size of 8 nm, which exhibited good antioxidant activity. Egg protein, Honey, Agarose, Starch, Polyethylene glycol, Chitosan, and Pectin are some of the biological sources used for the fabrication of CeO_2 NPs [22-27], *Acalypha indica* [28], *Petroselinum crispum* [29] *Gloriosa superba* [30], *Aloe barbadensis* [31], *Olea europaea* [12], *Hibiscus sabdariffa* [32], and *Azadirachta indica* [33], *Manilkara zapota* fruit peel extract [34], aqueous extract of *Salvadora persica* [35], and *Olocasia esculenta* [36] are some of natural and biological sources used in the synthesis CeO_2 NPs.

II. SYNTHESIS OF CeO_2 NPs

Cerium nitrate hexahydrate ($Ce(NO_3)_3{\cdot}6H_2O$) [31], Cerium (III) acetate hydrate [37] and Cerium chloride heptahydrate [28] are commonly used precursor for the synthesis of CeO_2 NPs. Few researchers have used sodium hydroxide as reducing agent. Most of the researchers have not given any information regarding the reducing agent. Some researchers used ammonia solution as reducing agent [22]. They did not express the clear details about the usage of sodium hydroxide. Various concentrations (0.01, 0.1, 0.2, and 0.5M) of cerium nitrate are used as a precursor. General schematic diagram of green synthesis of CeO_2 NPs is shown in Figure 3.

Figure 3: Green Synthesis Method of CeO_2 Nanoparticles Using Plant Extract

1. **Characterization Techniques:** Structure of the CeO_2 can be confirmed by UV-Visible spectroscopy (UV-Vis), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), Energy dispersive X-ray diffraction study (EDAX), Transmission Electron Microscope coupled with Selected Area Diffraction (SAED), and X-ray photoelectron spectroscopy (XPS). Absorption and functional group identification can be done by UV-Vis and FTIR. Formation of particles and elemental composition of can be analyzed by SEM-EDAX. Shape, size and morphology can be analyzed by TEM images. Average size of the particles can be

calculated from the XRD data. Oxidation state of Ce^{3+} and Ce^{4+} can be identified by XPS spectroscopy. And many more advance techniques nowadays available for the analysis of structural studies.

2. **Structural Confirmation of CeO_2 NPs:** Structure of green synthesized CeO_2 NPs is confirmed by various spectral techniques. UV-Visible is a primary method to confirm its structure. The absorption maximum and bandgap energy of CeO_2 NPs can be determined by UV-Vis DRS spectroscopy. The energy difference between valence band and conduction band is called as bandgap energy. Electron density is high in valence band and less is conduction band. Sharma et al. reported bandgap energy *Azadirachta indica* stabilized CeO_2 NPs (2.57eV), which is smaller than the bulk (3.19 eV) [33]. The bandgap energy is crucial for deciding the semiconductor's performance and photocatalyst. The electrons jump from the valance band to conduction band is caused by small bandgap energy. However, higher bandgap energy will inhibit electron jump so it will stop the flow of electrons [38, 39].

III. BIOLOGICAL ACTIVITIES

CeO_2 NPs having various biological activities due to their size, shape, morphology and surface area. They act as Antioxidant, Antibacterial and Anticancer agent against various pathogenic bacteria, various radicals produced in human body, and against various cancer cells.

1. **Antibacterial and Antifungal Activities:** Many researchers have studied the antibacterial and antifungal activities of CeO_2 NPs against many pathogenic bacteria such *Escherichia coli (E. coli), Staphylococcus aureus (S.aureus), Pseudomonas aeruginosa (P.aeruginosa),* and *Bacillus subtilis (B.subtilis)*. It was proved that CeO_2 NPs at the concentration of 10 µg/mL to 50 µg/mL able to inhibit the growth of bacteria. Arumugam et al. synthesiszed CeO_2 NPs using *Gloriosa superba L* and tested the antibacterial efficiency against *S. aureus, Streptococcus pneumonia (S.pneumonia)* and *B. subtilis, P. aeruginosa, Proteus vulgaris (P.vulgaris)* and *Klebsiella pneumonia (K.pneumonia)* [30]. Similarly, Munusamy et al (2014) have derived CeO_2 NPs using *Curvularia lunata* and tested the antibacterial efficiency against *S. aureus. S. pneumonia, E.coli, P. vulgaris, K. pneumonia, Shigella dysenteriae (S.dysenteriae)* and *P. aeruginosa* [18, 20].

   Moringa oleifera leaf extract stabilized CeO_2 NPs exhibited antibacterial and antifungal activities against *E.coli, P. aeruginosa, C. albicans* and *A. fumigatus* [40]. They also included many bacteria and fungi in their article for the purpose of comparison. Antibacterial activity mechanism of CeO_2 NPs is shown in Figure 4.

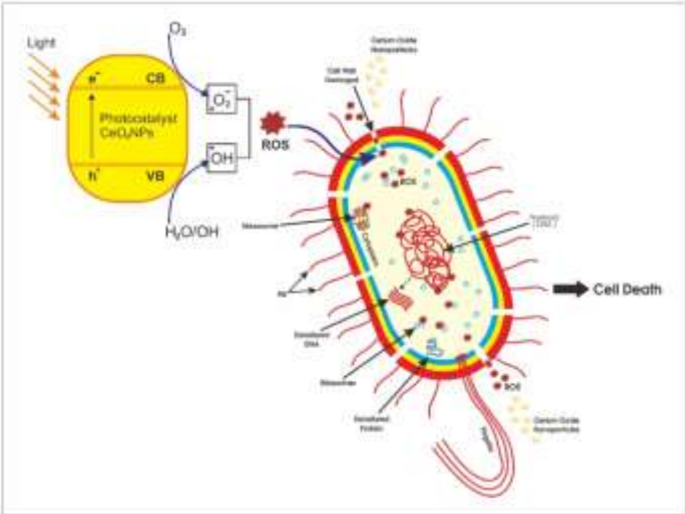

Figure 4: Mechanism of Antibacterial Activity of CeO_2 NPs [41]

Negatively charged bacterial cell surfaces tend to attract positively charged CeO_2 NPs and causes the cell death due to toxicity. Due to this interaction between bacteria and nanoparticles, reactive oxygen species (ROS) are produced, which ultimately kill the bacteria [41]. Highly reactive hydroxyl radical, singlet oxygen, superoxide anions, and photogenerated hydroxyl radicals are responsible for the production ROS [42, 43].

The fungicidal properties of the nanoceria have been tested against *Aspergillus niger*, *Humicola sp*., *Curvularia lunata A. flavus, F. solani*. CeO_2 NPs showed good antifungal activity against tested fungus at the concentrations of 100 µg/mL. The mechanism of antifungal activity of CeO_2 NPs is due to the generation of ROS and surface charge attraction. When the smallest nanoparticles penetrate into the cell wall of the bacteria, causes the cell death [11].

2. **Antioxidant Activity:** Free radical species formed through physical processes and cellular respiration due to oxidation. Antioxidant activities of CeO_2 NPs have been studied using Ferric reducing antioxidant power (FRAP) assay, DPPH free radical scavenging assay, Hydroxyl radical (OH^-) Scavenging activities. The natural antioxidants present in the human body decrease in efficiency due to the oxidative process that happens in the body. However, the natural antioxidants present within the system fail to compensate for ROS production. Proteins, mRNA, enzymes, and endomembrane systems attach to free radicals carrying species and speed up their lipid peroxidation, which alters their chemical composition and properties. Natural antioxidant processes keep the balance between the creation and eradication of diverse free radical species. Additionally, antioxidants are crucial in the management of oxidative stress, degenerative diseases, and autoimmune diseases like arthritis. Natural antioxidants produced by the body, which protect body from the free radical attack. Exactly, we can say that natural antioxidant act as a natural defence system to human body [43, 44]. The process of interaction between the antioxidants and free radical are shown in Figure 5.

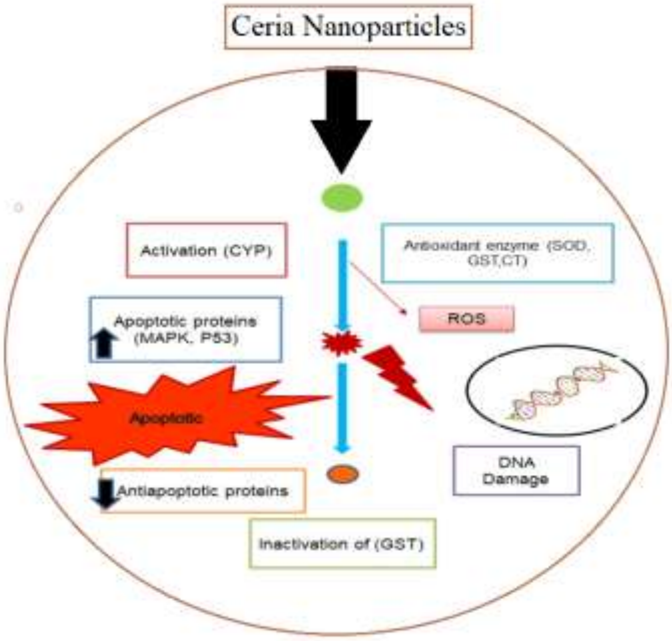

Figure 5: Antioxidant Potential of Cerium Oxide Nanoparticles [40]

3. **Antidiabetic Activity:** In India, diabetic is type chronic disease facing lots of problem in terms of various health aspects. It gives trouble many old aged people as well as youngster also. Therefore, we are in a position to find out the suitable medicine to overcome the diabetic. Many nanomaterials have been applied and evaluated for the antdiabetic activity. In this connection, CeO_2 NPs found to be a potential metal oxide nanoparticle can be used to combat the diabetic mellitus.

   The α-amylase, the enzyme that causes diabetes mellitus, can be inhibited by the green CeO_2 NPs that were generated. Because of their ability to block the target enzymes, natural sources of inhibitors, such as the leaves of various plants, are of interest [45]. This potential may be related to the presence of particular phenols. Thus, anti-diabetic treatment including CeO_2 NPs suggests inhibitory effects on glycogenolysis and gluconeogenesis, mechanisms that are active when a person is fasting [46].

4. **Anticancer Activity:** Nowadays, cancer is serious diseases among the people due various food habits and other chronic disease. The cytotoxic effect of biosynthesized CeO_2 NPs was determined using MTT ((3-[4, 5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay against colon (HT- 29) cancer cell line. The result of the cytotoxic effect of CeO_2 NPs in the concentration range of 0–800 μg/mL. Anticancer activity of CeO_2 NPs against HT- 29 cell line also reported [47]. Similarly, Akhtar et al. reported the anticancer activity of CeO_2 NPs against MCF- 7 and HT- 1080 cell lines (200 μg/mL) [48].

   Abdolhossein Miri et al. (2019) have evaluated anticancer activity of CeO_2 NPs against colon cancer cell lines through MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay [36]. They proved that CeO_2 NPs at the rate 800 μg/mL able to kill the colon cancer cell line in vitro method.

Rahdar et al. (2019) have reported the cytotoxic effect against MCF-7 breast cancer lines of Fe-doped ceria NPs exhibited and proved as a potential of an anticancer agent [49]. Sridharan et al. (2019) reported anticancer activity of nanoceria on breast cancer cell (MCF 7) in the IC_{50} value was 298.17 µg/ml [50].

5. **Anti-Infammatory Activity:** CeO_2 NPs have the unique potential to be thought of as an anti-inflammatory drug due to their radical scavenging and auto regeneration capabilities [51]. The body quickly produces free radicals as a result of anti-inflammatory enzymes when ROS activate the protein in iNOS, macrophages release NO. Since ROS are crucial for normal cellular processes and their depletion might have detrimental repercussions. Since ROS are crucial for normal cellular processes and their depletion might have detrimental repercussions. When CeO_2 nanoparticles were tested on J774A.1 cells, they reduced the generation of iNOS and ROS, acting as an anti-inflammatory agent [52].

IV. CONCLUSIONS

In conclusion, it is possible to synthesize CeO_2 NPs by a simple and eco-friendly method. The phytochemicals present in the plant extract, bacteria, fungi extract and other biological sources is responsible for the reduction of cerium salt into ceria nanparticles. Various phytochemicals such as phenols, flavonoids, carbohydrates, alkaloids and other phenolic compounds mainly responsible for the bioreduction process. The green or biosynthesized CeO_2 NPs can be by UV–Vis DRS, PL, FTIR, XRD, SEM-EDAX and TEM-SAED pattern analysis. The synthesized CeO_2 NPs showed bactericidal effect against pathogenic bacteria. Similarly, green synthesized CeO_2 exhibited antifungal, antioxidant, antidiabetic and anticancer activities. Therefore, CeO_2 NPs can be used as an suitable candidate for the development drug for various diseases.

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