



Research Article

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Antibacterial Potential of Cerium Oxide and Cobalt-Doped Cerium Oxide Against *E. Coli* and *S. Aureus*

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Abstract

This research focuses on combating bacterial infections by synthesizing cerium oxide (CeO₂) and modifying it with 3% and 5% zinc doping, along with 7% cobalt doping, using the co-precipitation method. Structural, morphological, optical, and antibacterial properties were systematically investigated. X-Ray Diffraction (XRD) revealed an increase in crystallite size from 12nm for pure cerium oxide to 13.42nm for 3% cobalt-doped cerium oxide after annealing. Scanning Electron Microscopy (SEM) confirmed agglomerated spheroidal structures for all samples. Diffuse Reflectance Spectroscopy (DRS) showed a widened energy band gap, from 2.76eV for pristine cerium oxide to 3.09eV for the annealed 7% cobalt doped cerium oxide, suggesting potential alterations in electronic properties. Antibacterial activity demonstrated that 7% cobalt doped cerium oxide exhibited the maximum zone of inhibition against *Escherichia coli* and *Staphylococcus aureus*, indicating superior antibacterial activity compared to other synthesized materials. Thus, this study showcases a tailored approach to cerium oxide nanoparticles, highlighting the significance of modifications for enhanced antibacterial applications. The findings in this research contribute to the development of advanced antibacterial agents, leveraging the unique properties of modified cerium oxide nanoparticles.

Keywords: Antibacterial activity, Cerium oxide, Co-precipitation, *E. coli*, *S. aureus*

Introduction

Nanoparticles are emerging in every field of life for various applications i.e. industries, energy storage, medicine and environment [1-4]. Nanotechnology has revolutionized medicine through nanoparticles for diagnostics, DNA sequencing, drug delivery systems and anti-bacterial activities [5]. Recent progress in nanotechnology has notably enhanced the management and spread of bacterial and microbial infections [6]. Recent advancements in nanotechnology have led to the creation of over 1814 nanoparticle-based medications, primarily utilizing metals and metal oxides such as gold, nickel, silver, iron, carbon, zinc, cerium, and more. These nanoparticles are gaining attention for their ability to selectively block metabolic pathways, interact with bactericidal activity, and combat drug-resistant bacteria [7-13].

Nanostructures can be synthesized via many techniques i.e.

laser ablation, hydrothermal, sol-gel, and co-precipitation [14-17]. Co precipitation is a cheap, environmentally friendly and most commonly used chemical synthesizing method [18]. Cerium, a rare earth metal and the first element in the lanthanide series, exhibits a unique ability to exist in both the 3⁺ and 4⁺ oxidation states. Cerium oxide, also known as ceria, possesses a cubic fluorite-type oxide structure with cerium ions occupying the face and vertices, while oxygen ions fill the tetrahedral vacancies within the cubic unit cell [19,20]. Previous studies have demonstrated its antibacterial properties without the need for external stimulation [21-23]. Cerium oxide (CeO₂) has garnered significant attention as an antibacterial agent due to its minimal or absent toxicity to mammalian cells, distinguishing it from other nano materials [24-26]. Renowned for its low toxicity, high stability, ductility, chemical reactivity, and potent oxidation catalytic capabilities, cerium oxide is a focal point in nan-



otechnology research, particularly for its potential in catalytic anti-oxidant applications. Also, cobalt is found to be effective in treating bacterial activities and cancer [27].

In this research, the antibacterial properties of *E. coli* and *S. aureus* are studied thoroughly. *E. coli* is a gram-negative bacterium that causes diarrheal sickness, whereas *S. aureus* is a gram-positive bacterium associated with skin and tissue infections. Recent research has shown that cerium oxide is effective in fighting these two bacteria [28,29]. Research conducted by Y.A. Syed prompt us to investigate 3wt%, 5wt% and 7wt% cobalt doped cerium oxide synthesized via co precipitation instead of hydrothermal synthesis to study the antibacterial activities of *E. coli* and *S. aureus* [30].

Experimental Section

Fabrication

The experimental technique utilized in this research was the co-precipitation technique. To synthesize cerium oxide nanoparticles and cobalt-doped cerium oxide nanoparticles, molar solutions of the starting materials were prepared. For cerium oxide nanoparticles, a 0.1M solution of cerium chloride hepta-hydrate was combined with a 0.3M solution of sodium hydroxide, resulting in a light-yellow colloidal suspension after 4hours of reaction time. The precipitates formed were washed thrice with distilled water and ethanol and then dried at 100°C for 3hours to obtain ceria (CeO₂) nano powder. For cobalt-doped cerium oxide nanoparticles, three different molar concentrations of cobalt chloride salts (0.03M, 0.05M, and 0.07M) were prepared and added gradually to a stirred 0.1M cerium chloride hepta-hydrate solution. Sodium hydroxide was used as a precipitating agent to maintain pH during the process. The resulting pale-yellow colloidal solutions were aged at room temperature for 2days, followed by separation of the precipitates via centrifugation. These precipitates were washed several times with ethyl alcohol and distilled water to remove impurities, dehydrated, ground, and subjected to further characterization.

Antibacterial Activity

The effectiveness of newly synthesized Nano formulations against pathogenic bacteria causing various infections was assessed using the disc diffusion method. Pure strains of *E. coli* and *S. aureus* were isolated from clinical samples of infected patients. Cultures of these pure strains were grown overnight and inoculated into tubes of nutrient broth to achieve a final inoculum of 1.6×10^4 CFU ml⁻¹ after 24hours of incubation at 37°C. To ensure even distribution, 10µl of the cultures were spread onto Muller-Hinton (MH) plates using a glass spreader. Sterile forceps were used to place discs containing equal concentrations (5µg/ml) of Nano formulations onto the MH plates. Additionally, different concentrations of Ce-Co Nano composite were tested against the pathogenic bacteria. After incubating the plates for 24hours at 37°C, the zone of inhibition was observed. All experiments were conducted in triplicate, with three

independent experiments performed to confirm the findings.

Characterization

Structural analysis of thin films deposited on a glass substrate was conducted using an X-ray diffractometer (XPRT-3 with Cu K_α radiation), with the apparatus operating at a voltage of 40kV and a current of 40mA. The diffractometer had a wavelength of 1.540589Å. Morphological examination of nanostructures was carried out via scanning electron microscopy (JSM-6490) at an accelerating voltage of 20kV, with photos analyzed at a resolution of 1µm. Optical characteristics were assessed using a spectrophotometer (Schimadzu 2700-UV).

Results and Discussions

X-Ray Diffraction

The XRD technique was employed to analyze the crystal structure and morphology of the samples under investigation. This method enabled the examination of the crystal structure, crystallite size, and the impact of cobalt on cerium oxide nanoparticles. (Figure 1) shows the XRD patterns of four prepared samples, encompassing pure cerium oxide and cobalt-doped cerium oxide nanoparticles with dopant concentrations of 3 mole %, 5 mole %, and 7 mole %, were studied. The results revealed well-indexed and consistent peaks of cerium oxide nanoparticles, with no additional peaks corresponding to cobalt detected, indicating complete dissolution of cobalt into the ceria lattice. The single-phase cubic fluorite structure of ceria remained unchanged with doping. The observed peaks were identified at planes indexed by (111), (200), (220), (311), (222), (400), (331), and (420), in accordance with the JCPDS card no. 34-03940.

However, a minor reduction in size was observed with the incremental increase in cobalt doping concentration, denoted at 3, 5, and 7 moles %. A gradual peak shift towards lower angle side in the (111) plane was observed. This diminution in size was attributed to the substitution of smaller Co²⁺ ions having radii of 0.74Å in place of larger Ce⁴⁺ ions having atomic radii of 1.30Å within the CeO₂ crystal lattice [31]. This decrease in particle size lead to peak broadening [32,33]. The crystalline sizes or average particles sizes were calculated using Debye-Scherrer's formula and their sizes were found to be lie in the range 7–13 nm that is in accordance with prior literature [34]. The calculated crystalline sizes are given in (Table 1) (Figure 1).

Table 1: Crystallite size of all samples.

Samples	Crystallite Size
CeO ₂	13nm
3% cobalt doped cerium oxide	12.42nm
5% cobalt doped cerium oxide	9.54nm
7% cobalt doped cerium oxide	7.88nm

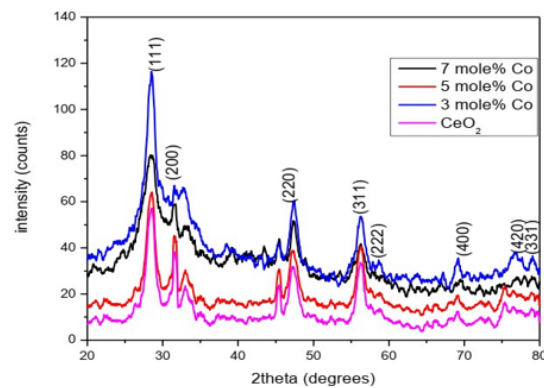


Figure 1: XRD spectrum of all samples.

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) was employed to analyze the structure of ceria nanoparticles and to identify any functional groups present in the prepared samples. The graphical representation in (Figure 2) illustrates the formation of ceria nanoparticles and the presence of chemical functional groups, as

determined by FTIR spectroscopy. The FTIR analysis involved plotting a graph of transmittance against wave number. The ceria nanoparticles synthesized via the precipitation technique exhibited a prominent broad peak at 3385cm^{-1} , corresponding to the O-H stretching mode of water molecules adsorbed on the surface of the nanoparticles [35].

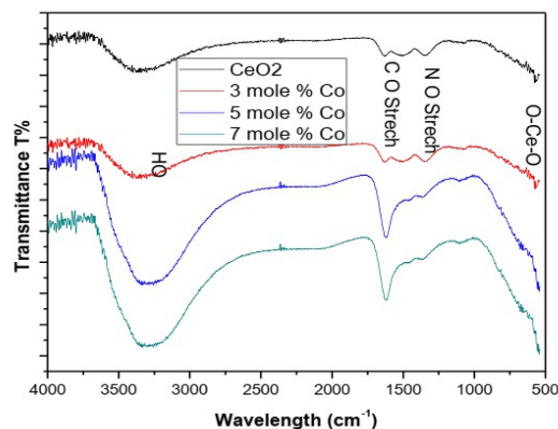


Figure 2: FTIR spectrum of samples.

Additionally, a peak at approximately 1356cm^{-1} was observed, attributed to the nitro (N-O) stretching vibrations, while peaks at around 1526cm^{-1} and 1079cm^{-1} were identified as stretching and bending vibrations of the interpolated C-O and N-O, respectively [36]. Furthermore, the vibrational band observed at 558cm^{-1} was associated with the O-Ce-O stretching mode of vibration [37]. These findings collectively confirm the successful synthesis of cerium oxide nanoparticles via the co-precipitation technique. The obtained spectra clearly indicate that the NO and OH bending nature increased with increasing the Co-doping concentration in steps of 3, 5 and 7 mole %. Hence, we get a clear picture about the chemical structure and other functional groups present in prepared samples (Figure 2).

Scanning Electron Microscopy (SEM)

Scanning electron microscopy was done for examining morphology of as prepared samples of Ceria nano particles. A suitable scale of 500nm was chosen in order to get a clear picture of effect of doping on ceria nano powders. The obtained SEM micrographs exposed the spherical and agglomerated nature of nanoparticles. It was observed from (Figures 3) that the morphology of the nanoparticles exhibited variation depending on the doping percentage of the dopant. With the increase in doping concentration the sizes of the Nano particles decreased and agglomeration increases. It is also clear from the SEM images of synthesized cobalt doped CeO_2 samples were porous in nature (Figure 3).

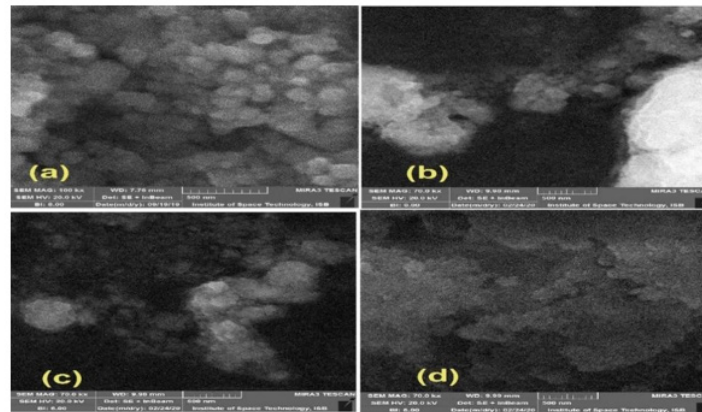


Figure 3: Surface morphology of all samples.

Antimicrobial Activity

The antimicrobial efficacy of various synthesized combinations was assessed using the disc diffusion method against resistant strains of *E. coli* and *S. aureus*. In this study, three compounds were investigated, and the nano composite with a 7 mole % doping concentration exhibited the highest efficiency, characterized by a zone of inhibition measuring 23mm ±0.67, as detailed in (Tables 2,3).

The observed zones of inhibition and their corresponding antibacterial activity patterns suggest the potential of these compounds as alternative therapeutic agents against challenging infections that pose difficulties for current treatment strategies. (Figure 4) illustrates the antibacterial activity of pure cerium oxide nanoparticles against both selected pathogens, while (Figure 5) depicts the impact of the three doped materials on determining effective antibacterial activity (Figure 4) (Figure 5) (Table 2).

Table 2: Showing zone of inhibition of cobalt doped ceria nanoparticles.

Antimicrobial agents	Zone of inhibition (mm)	
	<i>E. Coli</i>	<i>S. aureus</i>
Pure CeO ₂	7	6
CeO ₂ -Co 5 mole %	10	12
CeO ₂ -Co 7 mole %	13	17

Table 3: Increasing concentrations of pure cerium oxide Nano particles (mg).

Tested strains	Increasing concentrations of Pure cerium oxide Nano particles (mg)		
	7 mg	10mg	12mg
<i>E. coli</i>	15	17	20
<i>S. aureus</i>	19	21	23

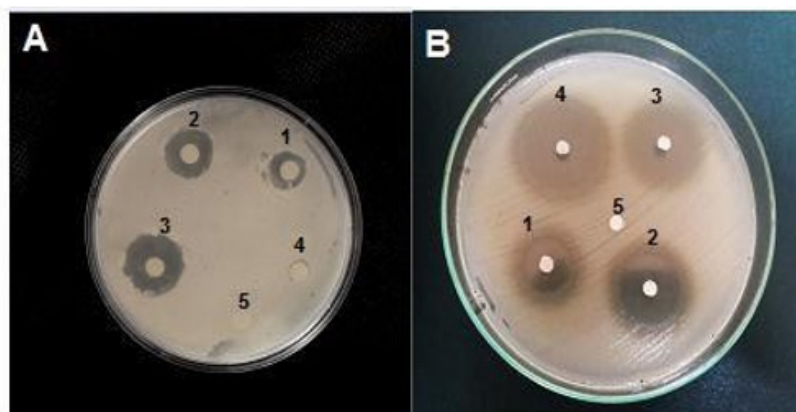


Figure 4: Increasing concentrations of pure cerium oxide nano particles.

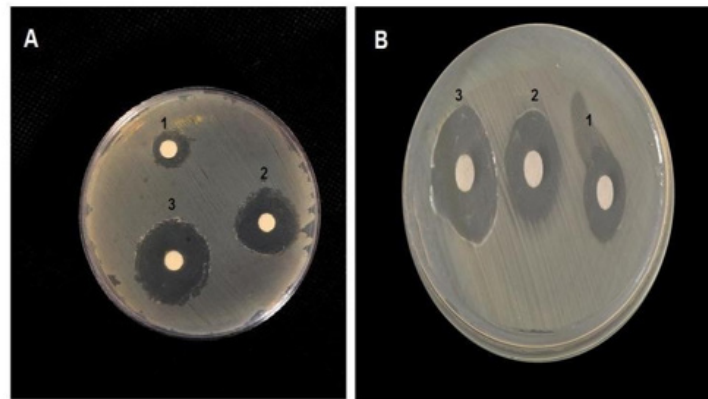


Figure 5: Zone of inhibition against *S. aureus*, B: zone of inhibition against *E. coli*.

Diffuse Reflectance Spectroscopy

Diffuse reflectance spectroscopy was performed to measure optical properties including reflectance, transmittance and band gap. Reflectance spectra of all samples of cerium oxide with pure cerium oxide and different doping concentration of cobalt were investigated in the wavelength from 220nm to 800nm at room temperature. UV-Vis transmittance spectra of pure ceria nanoparticles

and different concentrations of cobalt-doped cerium nanoparticles is shown in (Figure 6) spectrum analysis showed a decrease in transmittance in visible region by increasing dopant concentration. These results recommended that the transmittance depends on the particle dimensions and the combinations of the particles [38]. This property of ceria nanoparticles can be utilized as UV shield due to increase of UV light absorption and visible light transmittance property [39] (Table 3) (Figure 6).

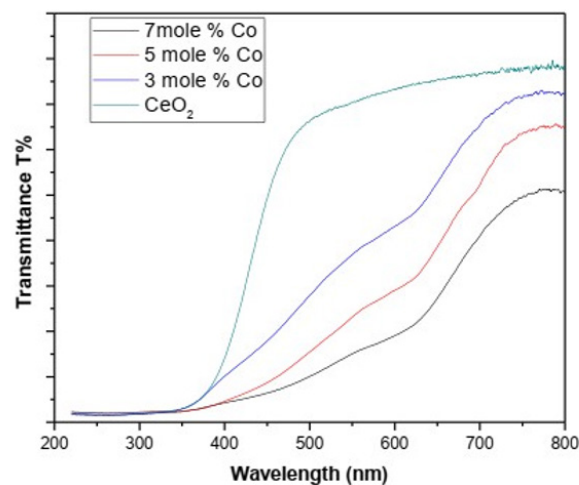


Figure 6

The optical band gap of the powdered samples was measured by DRS (Diffuse Reflectance Spectroscopy) in UV-Visible range. The optical band gap of samples was determined using Tauc method [40]. In order to investigate band gap of as prepared samples Kubelka-Munk function was found from the DRS Measurements. Kubelka-Munk transform gives a relationship between incident photon energy ($h\nu$) and bandgap energy (E_g) which is given in equation 4.2:[41].

$$F(R) = (1-R)/2R$$

$$F(R) h\nu = (h\nu - E_g)^n$$

where h is Planck constant, $F(R)$ is the absorption coefficient, E_g represents band gap and A represent proportionality constant. The exponent n represents the nature of electronic transition. Direct or indirect transitions can be obtained by substituting different values of n . For direct transition n is substituted as 2 and for indirect transitions n is equal to $1/2$. In order to do this the value of the optical band gap was obtained by linearly extrapolating the linear portion to $y=0$. The function of $(F(R)h\nu)^2$ vs photon energy for cerium oxide with different concentration of cobalt is given in (Figure 7) The estimated optical band gap of ceria nanoparticles was found to be 2.76eV with different concentrations of cobalt (i.e. 0.03 mole

%, 0.05 mole %, 0.07 mole %) was found to be 2.92eV, 3eV, 3.05eV respectively (Figure 7). The band gap values clearly indicate the rise of band gap which can be explained as the captivity of electrons

and holes in between valence and conduction band with decrease in particle size [42,43] (Figure 7).

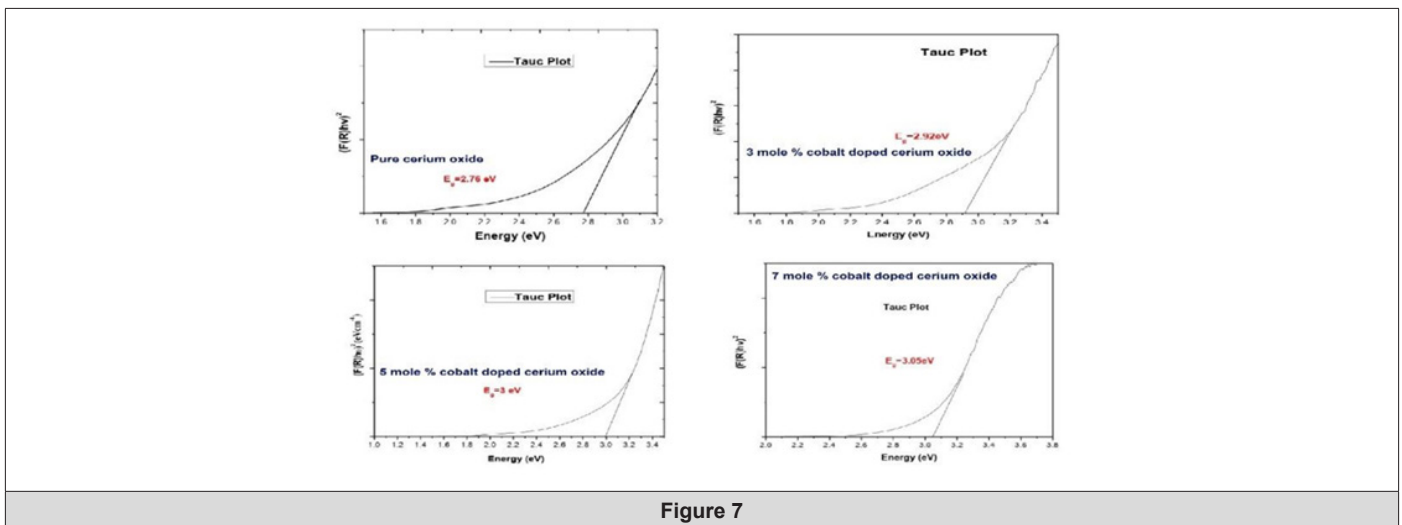


Figure 7

Conclusion

In conclusion, the co-precipitation synthesis and modification of cerium oxide nanoparticles, incorporating cobalt doping (3% and 5%) and 7%, exhibit promise in the treatment of bacterial infections. The increased crystallite size, particularly in 7% cobalt doped cerium oxide post-annealing, signifies structural improvements for enhanced stability. Scanning electron microscopy confirms non uniform agglomerated spheroidal structures. Diffuse reflectance spectroscopy indicates shifts in the energy band gap, influencing optical properties. Antibacterial assessments highlight the superior efficacy of 7% cobalt doped cerium oxide, emphasizing its potential in augmenting antibacterial properties. These findings underscore the potential of tailored cerium oxide nanoparticles for advanced antibacterial applications, warranting further exploration in this dynamic field.

Acknowledgement

None.

Conflict of Interest

None.

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