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Antibacterial Potential of Cerium Oxide Nanoparticles against Mycobacterium tuberculosis: A Novel Approach for Tuberculosis Treatment

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ABSTRACT

This study investigated the antibacterial effects of cerium oxide nanoparticles (CON) on Mycobacterium tuberculosis. Cerium oxide nanoparticles were synthesised using a microwave-induced technique and characterized by Scanning Electron Microscopy. Results indicated that an increase in synthesis time led to a reduction in nanoparticle size, demonstrating that the duration of the synthesis process influenced particle size. Additionally, the antibacterial activity of CON particles was found to be size-dependent. In a disc diffusion assay, cerium oxide nanoparticles enhanced the efficacy of antibacterial agents, significantly improving the effectiveness against Terivid, Amikin, Grasil, Velosef, Spraxin, and Ceftriaxone against Mycobacterium tuberculosis.

Keywords: *Cerium Oxide Nano Particles (CON), Mycobacterium Ttuberculosis, Antimicrobial Analysis, Nanosize*

1. Introduction

Cerium oxide is a prevalent rare earth metal oxide known as ceria or ceric oxide. Oxygen sensors use Cerium oxide nanoparticles as antibacterial agents and oxidation-resistant coatings [1-2]. Cerium oxide nanoparticles have exceptional physical and chemical properties due to their small size, high magnetic moment, and highly reactive complexion [3]. Cerium oxide nanoparticles appear pale-yellow to white powder and have a Face Centred Cubic Crystal structure. Cerium oxide nanoparticles are more stable than rare earth oxides like bismuth dioxide, Thorium dioxide, and zirconia. Because of their hygroscopic nature, cerium oxide nanoparticles absorb some CO_2 and moisture from the atmosphere [4]. Microwave energy, sol-gel procedures, precipitation, hydrothermal synthesis techniques, and emulsion methods are all employed to synthesize Cerium oxide nanoparticles. The microwave method is a simple, quick, and efficient alternative to traditional methods [6].

Mycobacterium tuberculosis, Staphylococcus aureus, Listeria monocytogenes, pseudomonas, and Escherichia coli are only a few of the bacteria that are toxic to humans and cause diseases in various ways [7-8]. Mycobacterium tuberculosis (M. tuberculosis) is a species of bacteria that causes tuberculosis (TB) in humans. A slow-growing, rod-shaped, aerobic bacterium primarily affects the lungs but can also spread to other body parts. It has a unique waxy cell wall rich in mycolic acids, which makes it resistant to many antibiotics and disinfectants [9-10]. Cerium oxide nanoparticles are used in combination with several medications to prevent Mycobacterium tuberculosis germs from growing [11]. The primary goal of this study is to look at the Cerium oxide nanoparticles as an antibacterial effect on Mycobacterium tuberculosis bacteria.

2. Experimental

2.1 Synthesis of Cerium Oxide Nanoparticles

Cerium oxide nanoparticles were synthesized via combustion of redox mixtures, using urea as the reducing agent and cerium nitrate as the oxidizing agent to prepare Cerium oxide nanoparticles. The redox combination of 0.17 g urea and 0.35 g cerium nitrate was diluted with 7 ml of distilled water, agitated for 8 minutes with a sonicator set to 50 Hz, filtered, and heated for various periods in a microwave oven at 700 watts.

2.2 Characterization

Various techniques were employed to characterize cerium oxide nanoparticles. X-ray diffraction (XRD) was used to determine their mineralogical composition. The XRD analysis was conducted by scanning all nanoparticle samples from 20° to 80° at a speed of 2°/min and 40 kV using the EVA program. Additionally, the surface morphology of the cerium oxide nanoparticles was examined using a scanning electron microscope (SEM).

2.3 Disc Diffusion Method

The disc diffusion method was employed to evaluate the response of Mycobacterium tuberculosis bacteria to antibiotic drugs, both in the absence and presence of Cerium oxide nanoparticles. To prepare the microbial growth medium, 20 g of nutrient agar was dissolved in 100 ml of distilled water. The solution was then autoclaved at 121°C for 15 minutes. After sterilization, a specified quantity of Cerium oxide nanoparticles was added to the nutrient agar and thoroughly mixed.

Equal portions of the prepared nutrient agar, with and without Cerium oxide nanoparticles, were poured into five petri dishes and left to dry for 10 minutes. Mycobacterium

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tuberculosis was then applied to the dried agar using a cotton swab. In the center of each petri dish, a small disc with a diameter of 7 mm, impregnated with 30 mg of an antibiotic drug, was placed. The petri dishes were subsequently incubated at 37°C for 48 hours.

2.4 Zone Inhibition Measurement

The Kirby-Bauer chart is used to evaluate bacterial Susceptibility to cerium oxide nanoparticles by measuring the diameter of the zone of inhibition with a Vernier caliper. Bacterial susceptibility can be classified as poor, intermediate, or high. The inhibition zone sizes listed in Table 1 were used to determine the susceptibility of Mycobacterium tuberculosis with and without cerium oxide nanoparticles.

Table 1: Diameter of zone inhibition

S. No	Diameter of zone inhibition (mm)	Susceptibility of organism	Explanation
01	0 to 3	Resistant	Certain bacteria resist specific antibiotics, preventing the medication from effectively inhibiting their growth.
02	+1 to 4	Intermediate	Certain bacteria resist specific antibiotics, preventing the medication from inhibiting their growth.
03	+5	Susceptible	Bacteria resist certain antibiotics, preventing the medicine from effectively inhibiting their growth.

3. Results and Discussion

3.1 Cerium Oxide Nanoparticles Phase Analysis

The XRD technique was used to analyse the phase composition of Cerium oxide nanoparticles. The pattern of produced nanoparticles at various time intervals is shown in Fig.1. The distinctive peaks of CON particles are shown in Fig. 1 at 2 = 29.50, 35.45, and 49.50. Peaks grow more pronounced as the synthesis time increases, as shown in Fig. 1. Only a peak of pure Cerium oxide nanoparticles was developed, and no additional peaks were observed.

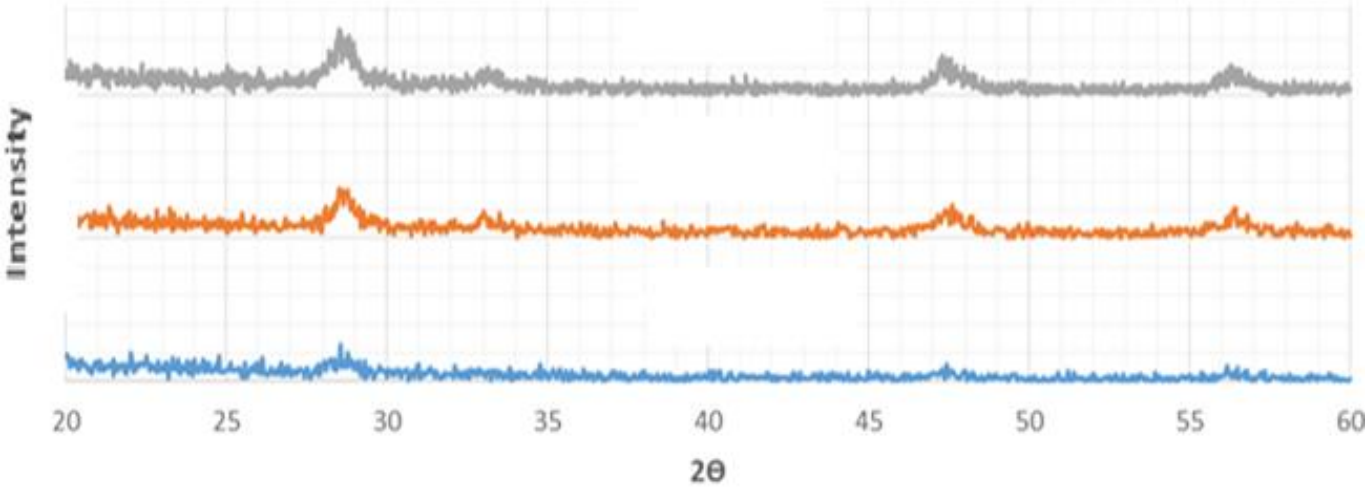


Fig.1: Patterns of Cerium Oxide Nanoparticles of XRD

3.2 Analysis of Particle Size

The Scherrer's equation ($d_{XRD} = 0.9/\beta\cos$) was applied to determine the particle size of CON nanoparticles, as presented in Table 2. According to the table, the average crystalline size of the nanoparticles ranges from 27.14 nm to 16.3 nm. Notably, as the synthesis duration increased, the nanoparticle size decreased significantly.

Table 2: Particle size analysis of Cerium Oxide Nanoparticles

Time of Synthesis (min)	FWHM (Deg)	2Θ (Deg)	Wavelength (A)	Particle size (nm)
17	0.72	29.50	1.540	11.75
	0.288	35.45	1.540	29.18
	0.215	49.50	1.540	40.50
	Average			**27.14**
19	0.524	29.50	1.540	8.01
	0.985	35.45	1.540	17.78
	0.22	49.50	1.540	30.11
	Average			**18.63**
21	0.778	29.50	1.540	6.01
	0.556	35.45	1.540	14.78
	0.487	49.50	1.540	28.11
	Average			**16.3**

3.3 Morphology of Cerium Oxide Nanoparticles

Morphology of CON particles was inspected by using scanning electron microscope. SEM images of CON particles are shown in Fig. 2 (i, ii and iii) denotes porous structure. Figures i to ii show that increasing the synthetization period increases porosity and decreases irregularity in cerium oxide nanoparticles, and Figure iv shows that well-regular spherical particles with a size of 16.3 nm were synthesized.

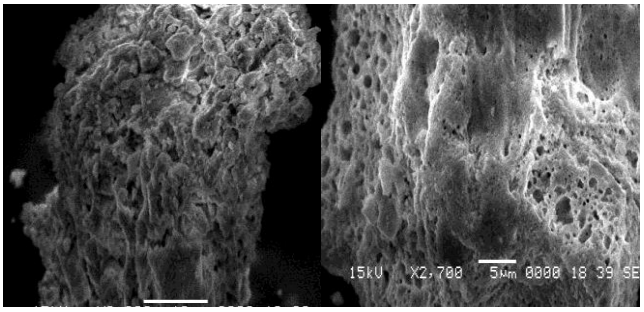
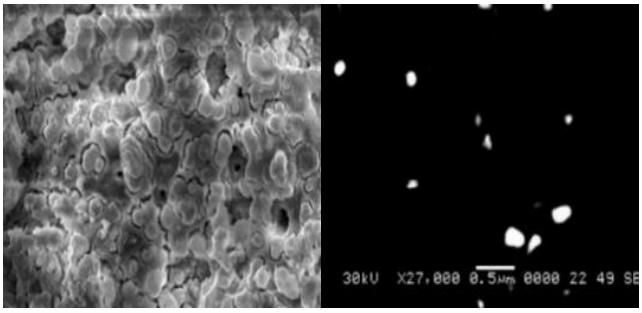


Fig 2. (i) Synthesis 17 min (ii) Synthesis 19 min



(iii) Synthesis 21 min (iv) spherical particles

3.4 Cerium Oxide Nanoparticles Antimicrobial Analysis

The antimicrobial effect of cerium oxide nanoparticles (CNO) against Mycobacterium tuberculosis was assessed. Nanoparticles synthesized at 17, 19, and 21-minute intervals were tested for their antibacterial activity. As shown in Table 3, a concentration of 10 mg/ml CNO17min, when combined with 30 mg of antibiotics such as Amikin Grasil, Terivid, Spraxin, Velosef, and ceftriaxone, was ineffective in inhibiting the growth of Mycobacterium tuberculosis.

The antimicrobial activity of CNO17min, as shown in Table 3, suggests that when amikin grasil and terivid medicines were added with CNO17min, the width of the zone of inhibition was increased to some extent. Furthermore, adding CNO17min to velosef and spraxin antibiotics did not improve their efficacy.

Antimicrobial analysis using CNO21min is extremely promising compared to Cno17min and Cno19min. Table 3 shows that the addition of CNO21min significantly increased the magnitude of zone inhibition in the cases of grasil, terivid, and spraxin. It's worth noting that the inhibition zone did not rise with the addition of CNO21min but rather reduced, as it did with CNO17min and CNO19min.

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Table 3: Cerium Oxide Nanoparticles (CNO) Antimicrobial activity

CNO dose (10mg/ml)	CNO_{17min}		CNO_{19min}		CNO_{21min}	
Antibiotic (30mg/l)	Zone Inhibition (mm)	Resistance/ Susceptibility	Zone Inhibition (mm)	Resistance/ Susceptibility	Zone Inhibition (mm)	Resistance/ Susceptibility
Amikin Grasil	0	Resistance	3.5	Resistance	5	Susceptibility
Terivid	0	Resistance	03	Resistance	5	Susceptibility
Spraxin	0	Resistance	03	Resistance	5	Susceptibility
Velosef	0	Resistance	01	Resistance	3.5	Resistance
Ceftriaxone	0	Resistance	0	Resistance	0	Resistance

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4. Conclusion

Cerium oxide nanoparticles were studied for their antibacterial properties. Synthetization time is important since it reduces the amount of nitrogen-based chemicals in Cerium oxide nanoparticles and particle size. The porosity of nanoparticle samples was enhanced by increasing the synthetization time, and uneven and rough particles were transformed into spherical-shaped particles, according to the SEM analysis. When cerium oxide nanoparticles were tested alongside antibiotic medications. Results indicate that nanoparticles synthesized over CNO21min was more effective than CNO17min and CNO19min.

5. Limitations of Current Study

The study focused on a limited range of synthetization times and did not explore the long-term stability or cytotoxicity of the cerium oxide nanoparticles. Additionally, antibacterial testing was limited to a few combinations with antibiotics.

6. Future Work

Future research should investigate a broader range of synthesis conditions, assess biocompatibility and long-term

effects, and explore the mechanisms behind the enhanced antibacterial activity of optimized nanoparticles

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