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Effect of Cerium Oxide Nanoparticles as Antibacterial on Staphylococcus Aureus Bacteria

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Abstract
In this study the antibacterial impact of cerium oxide nanoparticles(CON) particles on staphylococcus aureus bacteria was investigated. Microwave induced technique were used for synthesis of cerium oxide nanoparticles. Synthesized Cerium oxide nanoparticles were characterized by Scanning electron microscope (SEM) and X-ray diffraction (XRD). It was noted that cerium oxide nanoparticles of 18-29 nanometer size were synthesized. XRD and SEM results found that with increasing synthetization time size of cerium oxide nanoparticles were decreased. The size of CON particles depends upon the time of synthetization. It was also found that CON particles antibacterial activity is size dependent. Cerium oxide nanoparticles improved the effectiveness of antibacterial agents in a disc diffusion investigation. With Terivid, Amikin Grasil, velosef, spraxin and ceftriaxone drugs against the staphylococcus aureus bacteria appreciably.

Keywords: *cerium oxide Nano particles (CON), Staphylococcus aureus, Antimicrobial analysis, Nano size, characterization*

1. Introduction

Cerium oxide is a very common rare earth metal oxide also known as ceria or ceric oxide. Cerium oxide nanoparticles is nowadays used as antibacterial agent, oxidation resistant coatings and in oxygen sensors (Chen et al., 2014; Hilaire et al., 2014; Soykal et al., 2015). Cerium oxide nanoparticles have exceptional physical and chemical properties due to their small size, including a high magnetic moment and highly strong complexation reactivity (Hilaire et al., 2014). Cerium oxide nanoparticles are like pale-yellow- white powder and has Face Centered 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 CO2 and moisture from the atmosphere (Sahu et al., 2013). Microwave energy, sol-gel procedures, precipitation, hydrothermal synthesis technique, and emulsion methods are all employed to synthesize Cerium oxide nanoparticles. Microwave method is a simple, quick, and efficient alternative to other traditional methods (Crespo et al., 2012). For the past ten years, cerium oxide has been used as effective antimicrobials. Cerium oxide nanoparticles have recently attracted more attention than the most extensively studied metallic nanoparticles, such as silver, copper, gold, aluminum, and zinc. Cerium oxide nanoparticles are one of the advanced materials created by nanotechnology to solve medicinal and biological issues. When compared to other metal oxides, it was discovered that cerium oxide material had antibacterial activity at low temperatures against a variety of microorganisms. Cerium oxide nanoparticles has been shown to protect against radiotherapy used during cancer treatments and to have very low or no toxicity when present in exhaust emissions.

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 (Negahdary et al., 2012; Odonkor et al., 2011). Staphylococcus aureus is a gram-positive bacterium found primarily in the epidermis and respiratory systems. These bacteria are the most common cause of skin infections (Lin et al., 2011; Panacek et al., 2006). Cerium oxide nanoparticles are used in combination with several medications to prevent staphylococcus aureus germs from growing (Fu et al., 2005). The primary goal of this study is to look at the Cerium oxide nanoparticles as an antibacterial effect on staphylococcus aureus bacteria.

2. Experimental

2.1. Synthesis of Cerium Oxide Nanoparticles

Cerium oxide nanoparticles is synthesized by cerium nitrate Ce(NO3)3 and urea CH_4N_2O. Combustion of redox mixtures of urea as a reducing agent and cerium nitrate as an oxidising reactant was used to synthesize Cerium oxide nanoparticles. A four decimal electron weight balance was used to precisely measure 0.30 g of cerium nitrate and 0.15 g of urea. These ingredients were put into a 10 ml test tube and diluted with 5 ml distilled water. Using a sonicator set to 50 hertz, the contents of the test tube were correctly mixed for 5 minutes. The subsequent step was to filter the solution using watts-man filter paper with a 0.5-micron opening. After being transferred to a ceramic plate with a 5 cm diameter, the filtered solution was heated in a domestic type microwave oven. The microwave solution was heated for varying time spans 16 to 20 minutes using a 500 W input power setting. It was noteworthy noticed that a 12-minute microwave heating period is sufficient to produce the precipitates. The precipitates obtained hereinafter are referred as cerium oxide nanoparticles (CON). Despite this finding, the CNO duration was increased to 20 minutes in order to determine the ideal time for the reduction followed by the oxidation reaction necessary to change cerium nitrate into cerium oxide

2.2. Characterization

For characterization of Cerium oxide nanoparticles different techniques were used. For the determination of mineralogy of Cerium oxide nanoparticles X-ray diffraction is used.

For the XRD pattern, all produced nanoparticles samples were scanned from 20 to 80 at 2o/min speed and 40 KV with EVA programme. The surface morphology of cerium oxide nanoparticles was studied using a scanning electron microscope.

2.3. Disc Diffusion Method

The disc diffusion method was used to assess the behaviour of staphylococcus aureus bacteria against antibiotic medicines in the absence and presence of Cerium oxide nanoparticles particles. The media for microbe cultivation was made by mixing 15 g of nutrient agar with 70 ml distilled water. In an autoclave, the solution was heated to 121°C for 20 minutes. After that, a specified amount of Cerium oxide nanoparticles was added to the nutritious agar solution and thoroughly mixed. Following that, equal amounts of nutritional agar solution with or without Cerium oxide nanoparticles were placed onto five petri dishes and allowed to dry for 10 minutes. With the use of a cotton swab, Staphylococcus aureus was placed to dried nutritional agar. In the petri dishes centre, a small disc of 8mm diameter was impregnated with antibiotic drug containing 25 mg. The petri dishes were then incubated for 48 hours at 37°C.

2.4. Zone Inhibition Measurement

Kirby-Bauer chart is used to analyze susceptibility of bacteria to Cerium oxide nanoparticles to measure diameter of zone inhibition by using Vernier caliper. Susceptibility of bacteria may be poor, intermediate or high. The magnitude of zone inhibition listed in Table 1 was used to assess Staphylococcus aureus susceptibility with and without Cerium oxide nanoparticles.

Table 1: Diameter of Zone Inhibition

S. No	Diameter of zone inhibition (mm)	Susceptibility of organism	Explanation
1	0 to 2	Resistant	Bacteria are resistant to specific antibiotics; therefore, the medicine could not effectively restrict their growth.
2	+1 to 3	Intermediate	Bacteria are resistant to specific antibiotics; therefore, the medicine could not effectively restrict their growth.
3	+4	Susceptible	Bacteria are resistant to specific antibiotics, therefore the medicine could not effectively restrict their growth.

3. Results and Discussion

3.1 Cerium Oxide Nanoparticles Phase Analysis

XRD technique was used to analyse the phase analysis of Cerium oxide nanoparticles. The pattern of synthesize nanoparticles at various time intervals is shown in Fig.1. The distinctive peaks of CON particles are shown in Fig 1 at 2 ϴ =28.60, 34.41, and 48.49. It was noted that no any peak corresponding to any other phase was developed in the XRD patterns of samples synthesized at 16 min, 18 min and 20 min which meant that pure cerium oxide nanoparticles was developed Peaks grow more pronounced as the synthesis time is increased, as shown in Fig 1.

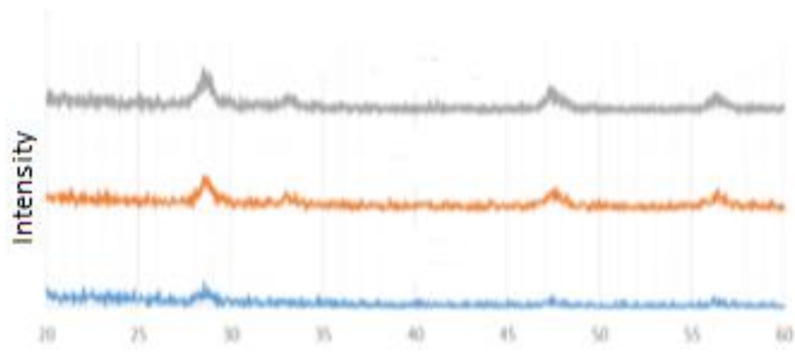

Fig.1: Patterns of Cerium Oxide Nanoparticles on XRD

3.2 Particle Size Analysis

The scherrer equation (dXRD= 0.9/ cos) was used to calculate the particle size of CON particles given in Table 2. As shown in the table, the average crystalline size of nanoparticles spans from 28.91 nm to 17.99 nm. As the synthesis time was increased, the size of the nanoparticles shrank significantly.

Table 2: Particle size analysis of Cerium Oxide Nanoparticles

Time of Synthesis (min)	**FWHM (Deg)**	**2Θ (Deg)**	**Wavelength (A)**	**Particle size (nm)**
16	0.71	28.60	1.5405	13.65
	0.286	34.41	1.5405	31.58
	0.212	48.49	1.5405	41.50
		Average		**28.91**
18	0.521	28.60	1.5405	5.01
	0.983	34.41	1.5405	8.78
	0.24	48.49	1.5405	32.11
		Average		**24.49**
20	0.776	28.60	1.5405	4.01
	0.546	34.41	1.5405	6.67
	0.497	48.49	1.5405	20.47
		Average		**17.50**

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. 3 (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 20.47 nm were synthesized.

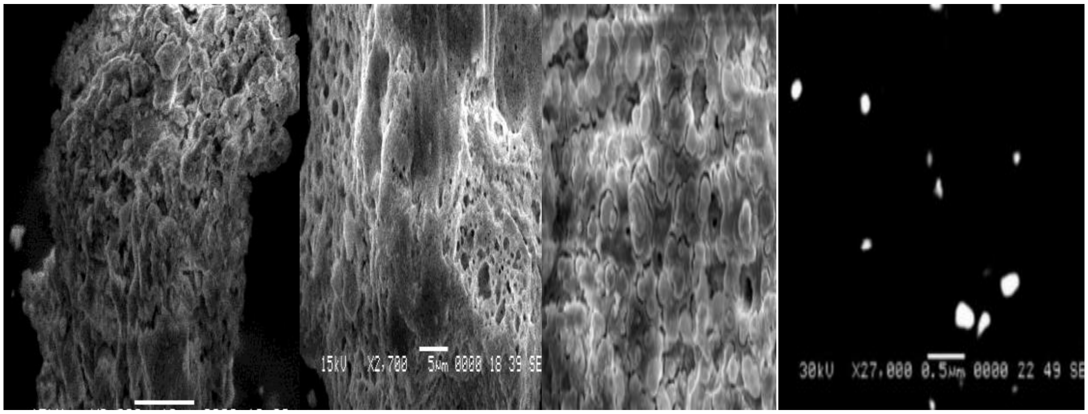

Fig 3. (i) Synthesis 16 min; (ii) Synthesis 18 min; (iii) Synthesis 20 min; (iv) spherical particles

3.4 Cerium Oxide Nanoparticles Antimicrobial Analysis

Cerium oxide nanoparticles antimicrobial effect was evaluated against Staphylococcus aureus bacteria. Nanoparticles produced at 16, 18, and 20 minute intervals were tested for antibacterial activity. Table 3 shows that 10 mg/ml CNO16min in combination with 25 mg antibiotic medicines such as Amikin Grasil, Terivid, Spraxin, Velosef, and ceftriaxone failed to prevent the development of staphylococcus aureus.

Table 3: Cerium Oxide Nanoparticles (CNO) Antimicrobial activity

CNO dose (10mg/ml)	**CNO_{16min}**		**CNO_{18min}**		**CNO_{20min}**	
Antibiotic (25mg/l)	Zone Inhibition (mm)	Resistance/ Susceptibility	Zone Inhibition (mm)	Resistance/ Susceptibility	Zone Inhibition (mm)	Resistance/ Susceptibility
Amikin Grasil	0	Resistance	+2.5	Resistance	+4	Susceptibility
Terivid	0	Resistance	+2.5	Resistance	+5	Susceptibility
Spraxin	0	Resistance	+02	Resistance	+4	Susceptibility
Velosef	0	Resistance	0.5	Resistance	2.5	Resistance
Ceftriaxone	0	Resistance	0	Resistance	0	Resistance

The antimicrobial activity of CNO16min as shown in table 3 suggests that when Amikin Grasil and Terivid medicines were added with CNO18min, the width of zone inhibition was raised to some amount. Furthermore, adding CNO18min to velosef and spraxin antibiotics did not improve their efficacy.

When compared to CNO16min and CNO18min, antimicrobial analysis using CNO20min is extremely promising. Table 3 shows that the addition of CNO20min 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 CNO20min, but rather reduced, as it did with CNO16min and CNO18min.

4. Conclusion

In this study it is investigated that the antibacterial effect of cerium oxide nanoparticles synthesizing it through the reaction of cerium nitrate and urea. During the synthetization process of cerium oxide, it was observed that the synthetization time plays a vital role in the efficient synthesis of the cerium oxide nanoparticles. 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 while also reducing particle size. During the synthesis process, optimal time noted for the synthetization of CON is 20 min. For the characterization of cerium oxide particles, SEM analysis showed that the porosity of CON samples increased as the synthetization duration was increased. Additionally, when the synthetization period increased, rough and irregular particles were transformed into well-regular, spherical-shaped particles. The characterization of cerium oxide nanoparticles was studied using SEM and XRD to identify the presence of various compounds. Antibacterial action of CON evaluated along with antibiotic drugs revealed that NCO20min is more effective as compared to NCO16min and NCO18min. The poor response of NCO16min and NCO18min was due to presence of nitrogen based compounds namely4 N-H and NH3 which worked as nutrient of Staphylococcus aureus microorganism.

5. References

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