Studying the Anti-Microbial and Alpha Amylase Inhibition Activity of Cerium Oxide Nanoparticles For Biomedical Applications

Sneha Kumari[1], Shivam Pandey[2], Sushant Singh[3*]

[1,2]Amity Institute of Biotechnology, Amity University Chhattisgarh, Raipur-493225, India.
[3]Department of Life Sciences, School of Basic Science and Research, Sharda University, Greater Noida-201310, Uttar Pradesh, India
*Corresponding Author

Abstract:
Cerium oxide nanoparticles (CNP) have been produced using a wet chemical technique. The therapeutic capacities of cerium oxide nanoparticles are determined based on the analysis of their anti-diabetic and antibacterial activities. The study below demonstrated the antibacterial characteristics of cerium oxide nanoparticles (CNP) against several bacteria. Specifically, the inhibition zones observed for *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Enterococcus gergovae* were 14.5mm, 11mm, 17mm, and 15mm, respectively. The results obtained indicate that CNP has the potential to address the urgent problem of hospital-acquired microbial infections, particularly those caused by antibiotic-resistant microorganisms. Additionally, the anti-diabetic property of CNP was evaluated and found to have a 66% alpha amylase inhibition, which suggests a significant reduction in glucose levels. Therefore, it has the potential to be utilized as an antidiabetic medication as well. The study has produced a compound with potent biological capabilities that can be enhanced through a synthesis technique and utilized in the pharmaceutical industry.

Keywords: Cerium oxide nanoparticles, anti-diabetic, anti-microbial, alpha-amylase inhibition, anti-oxidative.

Introduction:

Cerium is a rare metallic element and the initial element of the lanthanide series. The rare earth metal's 4f orbitals are effectively protected by the presence of 5p and 4d electrons, resulting in intriguing features (Xu & Qu, 2014). Cerium is unique among rare-earth metals because it has the ability to exist in both the 3+ and 4+ oxidation states. Cerium is present in a bulk state as CeO2 and Ce2O3 (Rico et al., 2011). At the nanoscale, cerium oxide nanoparticles exhibit the presence of both 3+ and 4+ oxidation states on their surface. As the diameter of nanoparticles decreases, the quantity of oxygen atoms is significantly reduced because the increase in surface area leads to the formation of more 3+ sites (Singh et al., 2020). Cerium oxide nanoparticles exhibit several biologically important activities, including SOD, peroxidase, Catalase (H_2O_2 inhibition), oxidase, phosphatase, and others. Free radicals are generated as a result of metabolic distortion commonly associated with oxidative stress. ROS, or reactive oxygen species, are the main signaling molecules involved in physiological processes (Singh, 2017).

Cerium oxide nanoparticles possess biorelevant properties that make them suitable for applications in pharmacology, drug transport, and bio-scaffolding. The physiological activities of CeNPs are primarily driven by the thermodynamic eminence of the cycling from 3+ to 4+ states. (Krishnamoorthy et al, 2014). Additionally, CeNPs possess a distinctive ability to release and absorb oxygen. Studies have concluded that all antioxidant activities of CeNPs can be attributed solely to redox cycling. Therefore, the ratio of $Ce3+/Ce4+$ on the surface is crucial for all bio-relevant processes (Wang & Wang, 2014). The physiochemical properties of any nanoparticle are determined by the synthesis method used. For a nanoparticle that is important to biology. In order to attain optimal physiochemical characteristics during in vivo synthesis, it is crucial to meticulously adjust the synthesis parameters. Various synthesis techniques have been documented thus far, which result in variations in parameters like as size, morphology, and agglomeration rate (Seil JT et al, 2014).Several techniques for producing cerium nanoparticles have been documented, including solution precipitation, hydrothermal, and sol-gel methods. However, there is a growing significance in the use of bio-directed synthesis methods for cerium nanoparticles, which involve the utilization of natural matrices as stabilizing agents (Tarnuzzer et al, 2005). These methods are particularly valuable as they enhance the biocompatibility of the nanoparticles. The following research study demonstrates the biological capabilities of CNP as a potent antibacterial and ant diabetic agent (Tsai et al, 2007). These nanoparticles possess remarkable qualities that make them suitable for usage as pharmaceutical agents in the development of future treatments.

Material and methodology:

The study utilized various chemicals from different suppliers: Cerium Nitrate Hexahydrate and 30% Hydrogen Peroxide were sourced from Hi-Media, as were Nitroblue Tetrazolium, Methionine, LB Broth ,α-Amylase and Ascorbic Acid was obtained from Loba Chemie. Di-nitrosalicylic Acid and Acarbose were procured from Sigma-Aldrich.Agar powder, $H2O2$ 30%, Ammonium hydroxide, LB agar pre mix powder, LB broth premix powder, ethanol, methanol.

Fabrication:

Cerium Nitrate Hexahydrate was used as the starting material to create cerium oxide nanoparticles. The specified quantity of Cerium Nitrate Hexahydrate was mixed in 35 ml of distilled water, and obtained mixture was agitated until it achieved complete transparency. Once a definitive solution was reached, a 30% aqueous solution of Ammonium Hydroxide, which served as both an oxidant and mineralizer, was introduced and agitated until a transparent nanoparticle solution was achieved. The resultant mixture was further purified using centrifugation at a speed of 6,000 revolutions per minute. The synthesis was completed by calcination at 400°C for 60 minutes.

Characterization:

UV spectroscopy:

UV visible spectroscopy is used to determine the oxidation state of synthesized cerium oxide nanoparticles as the absorption peak shows the oxidation state hence the biochemical properties of cerium oxide nanoparticles can be studied based on the results obtained through absorption peaks. the synthesized material is characterized through a wavelength range of 220- 500 nm and then according to the OD of sample the primary confirmation of synthesis is done (Charbgoo et al, 2017)..

Alpha amylase degradation property:

Amylase is an enzyme responsible for degradation of complex sugar into simple sugar in body and hence may lead to increase in free sugar in blood. To study the degradation capacity of CNP the following assay was performed. The material and starches were dissolved in a 25mM buffer solution of sodium phosphate with 6mM NaCl at a pH of 6.9 in this experiment. A starch solution is developed as well using the same buffer. The solution is incubated at 37°C for 15 minutes, after which 250µl of a 0.5% starch solution is introduced. After adding the starch, the alpha-amylase stock solution is allowed to incubate for 60 minutes. Following the first period of incubation the mixture is vigorously mixed and then incubated once again at 37°C for 15 minutes. In order to stop the reaction from occurring, 1µl of DNS (color) solution is introduced, and the test tubes are thereafter immersed in a warm water bath equipment at 65°C for a duration of 20 minutes. Once the substance has cooled down to the temperature of the surrounding environment, the optical density, or OD, is determined by measuring its absorbance at a wavelength of 540nm.

Antimicrobial efficacy:

The antibacterial effectiveness of nanoparticles of cerium oxide was assessed using a well defined procedure that included introducing samples into a solution of saline, and then applying them to appropriate growth conditions. At first, each test microorganism was separately introduced into a container holding 5 mililitre of a saline sol with a concentration of 85%. The bacterial suspensions were further modified to attain a turbidity that corresponds to the 0.5 McFarland scale, which is roughly equal to 1.5×10^8 colony-forming units per milliliter (CFU/ml) (Farias et al, 2018). After maintaining the standard concentration of microorganism in the broth culture. Agar well plates were prepared and kept in a sterile condition. Later the wells were poured with different concentration sof microbial inoculums and left for incubation.

Result and discussion of the study:

Synthesis and characterization of the material:

After following the above mentioned protocol for synthesis of material , a fine yellow colored powder of CNP was obtained and primary screening of the material was done by using the UV visible spectroscopy technique in which the peaks obtained at 310nm it can be concluded that the oxidation ratio of CNP obtained is Ce^{4+}/Ce^{3+} which may lead to higher anti-oxidative property to scavenge super radicals.

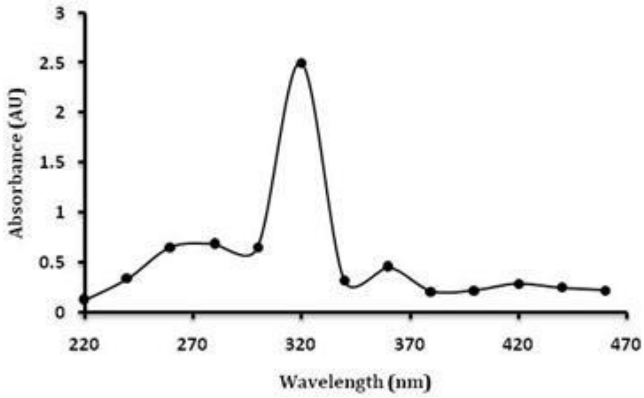

Figure 1 UV spectroscopy.

Alpha- amylase degradation capacity:

The degradation capacity of alpha amylase can be co- related to the capacity to reduce the amount of free glucose in blood as the major function of amylase enzyme is to degrade complex sugar into simple sugar. The rise of free glucose in blood may lead to elevated diabetic problems hence regulating the function of alpha amylase is a challenge in diabetic patients world wide. Therefore the above mentioned assay was performed to see the activity of CNP and it was observed that in reference to the control taken the activity of CNP was 66% which is a good result and further optimization in synthesis process can lead to better activity.

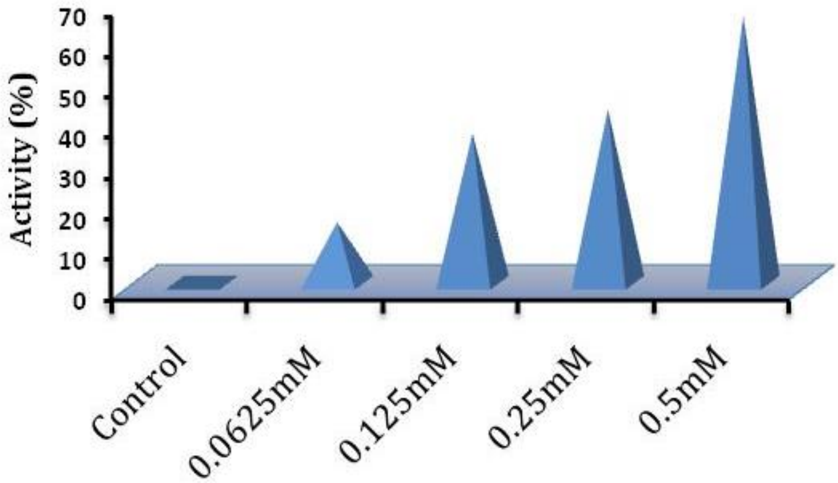

Figure 2 Alpha amylase inhibition.

Antimicrobial property:

The antimicrobial efficacy of obtained CNP was done by studying its effect on four microorganism – *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Enterococcus gergovae*and a notable result was obtained through the observation of Zone of Inhibition obtained in each plate as: *Staphylococcus aureus*- 14.5mm, *Pseudomonas aeruginosa*-11mm, *Escherichia coli*- 17mm, and *Enterococcus gergovae*- 15mm. the above results show that the microorganisms are well inhibited by the obtained CNP and the nanoparticle can be considered as effective anti microbial agent. As antimicrobial resistance is the persistent issue world wide and hence the study of nanoparticles to combat these microorganisms can prove to be very eminent to next gen pharmaceutical industries.

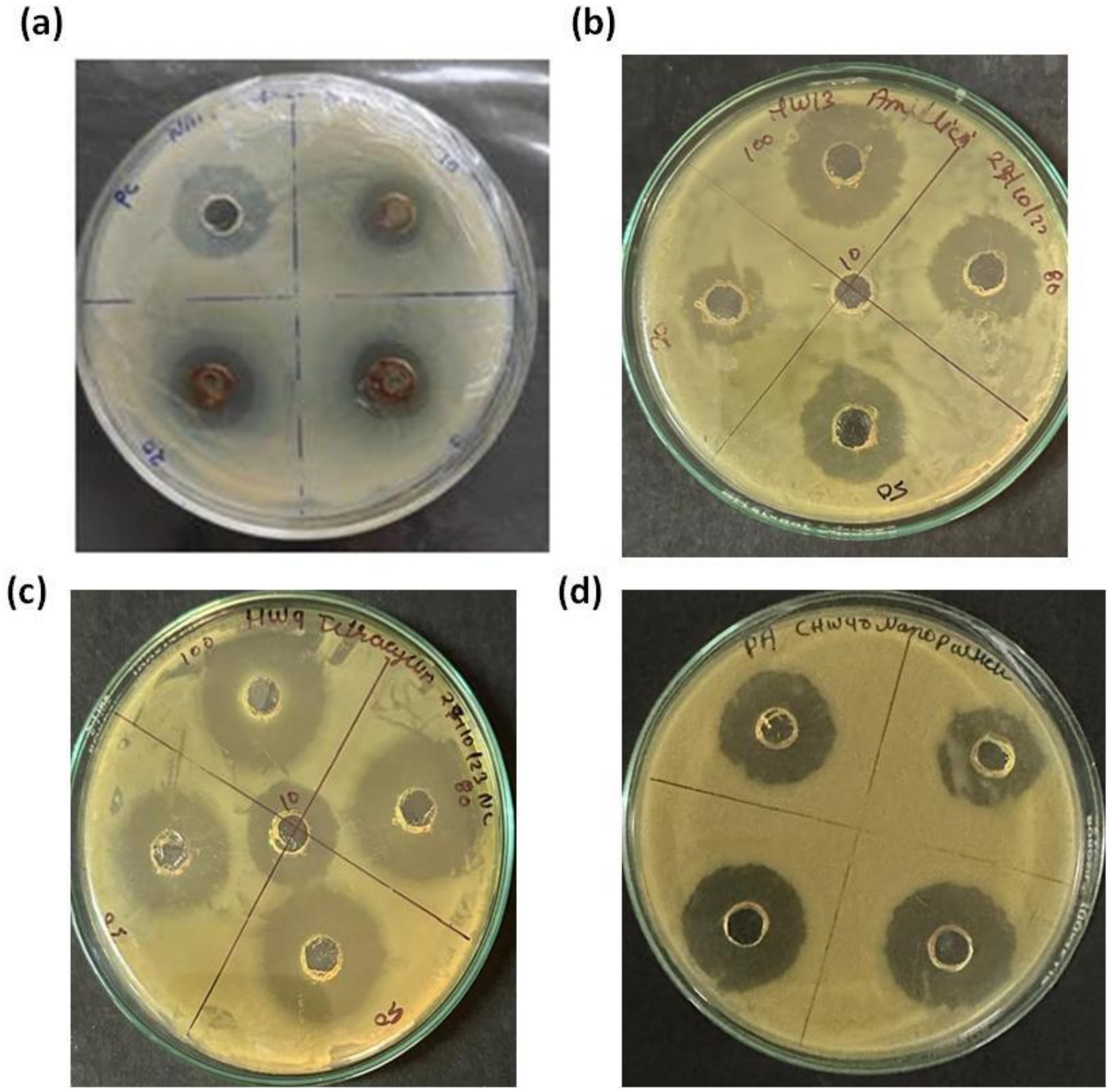

Figure 3 Anti microbial activity in *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Enterococcus gergovae.*

Table1. Anti microbial efficacy of bare Cerium oxide nanoparticles at 0.5mili molar (mM)

Microorganism	**Obtained zone of inhibition (mm) at 0.5mM**
Staphylococcus aureus	14.5
Pseudomonas aeruginosa	11
Escherichia coli	17
Enterococcus gergovae	15

Conclusion:

The proposed study gives a glance of Cerium oxide nanoparticles as effective antimicrobial and anti-diabetic compound as it can be seen above that it has remarkable capacity to inhibit the growth of microorganisms and a good inhibition capacity to regulate the activity of amylase enzyme which is responsible for breaking complex sugar into simple sugar. Antibiotics available in market today are losing its capacity as many species of microorganism are evolving and developing resistance towards the commercially available antibiotics, hence in this persistent scenario study and development of new antimicrobial agents will help the pharmaceutical studies in same direction whereas on the other hand diabetes is reported to be one of the major issues in health care department. In condition of diabetes the cells are under oxidative stress and the action of alpha amylase enzyme can lead to elevated levels of sugar hence development of a effective compound with anti-oxidative property and capacity to lower glucose level can serve as important factor. Hence it can be concluded from the above obtained results that obtained CNP can be a potential candidate as antimicrobial and anti-diabetic compound.

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