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Enhanced Antibacterial Activity of Cerium Oxide Nanoparticles in Skin Ointments for Treating Acne Vulgaris

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Abstract
This research successfully developed CeO_2 nanoparticle ointments for acne treatment and achieved all major objectives. The chemical co-precipitation method produced high-quality nanoparticles with 85-92% yield, showing pale yellow color and free-flowing powder texture suitable for pharmaceutical use. Transmission electron microscopy confirmed perfect 20 nm spherical primary particles with clear crystal lattice fringes, while scanning electron microscopy revealed stable 80-120 nm secondary clusters ideal for cream formulation. Dynamic light scattering measurements demonstrated excellent stability with hydrodynamic diameters of 85 nm in water and 120 nm in sweat-like conditions, maintaining low polydispersity (PDI <0.3) and strong negative zeta potential (-22 to -28 mV).

Keywords- Antibacterial, Cerium Oxide Nanoparticles, Skin Ointments, Acne Vulgaris, reactive oxygen species, Chemical Vapor Deposition, Transmission Electron Microscopy, Scanning Electron Microscopy, Dynamic Light Scattering, Reinforced Clostridial Medium, Minimum Inhibitory Concentration, Minimum Bactericidal Concentration

1. Introduction

Cerium oxide nanoparticles (Cerium oxide NPs) have emerged as a promising class of nanomaterials due to their distinctive properties, which make them highly effective in various applications, particularly in the biomedical field. These nanoparticles, known for their antioxidant and catalytic properties, have shown potential in the treatment of skin infections by exhibiting strong antibacterial activities. Skin infections are a significant global health concern, with rising antibiotic resistance making the treatment of these infections more challenging. Traditional antibiotics are increasingly ineffective due to bacterial resistance, which has led to the exploration of alternative antimicrobial agents, such as nanomaterials. The unique properties of Cerium oxide nanoparticles, including their ability to produce reactive oxygen species (ROS) and interact with bacterial membranes, make them effective in combating bacterial infections. Their incorporation into skin ointments offers a novel approach to treating common skin infections, enhancing wound healing, and promoting the regeneration of damaged tissue.

Cerium oxide nanoparticles possess unique physical and chemical properties, including a high surface area to volume ratio, which enhances their reactivity. They are composed of cerium ions in two different oxidation states, Ce(III) and Ce(IV), which contribute to their antioxidant properties. The surface of Cerium oxide nanoparticle can undergo reversible oxidation and reduction, making them effective in scavenging free radicals and reactive oxygen species (ROS), which are key players in antibacterial activity. Their size and shape significantly impact their interaction with bacterial cells. Smaller nanoparticles exhibit higher antibacterial activity due to their increased surface area, which facilitates better contact with bacterial cell membranes. Cerium oxide nanoparticles can also undergo various surface modifications to enhance their solubility and stability in formulations, making them ideal candidates for incorporation into skin ointments.

2. Methodology

A comprehensive laboratory-based experimental study was designed and executed to develop CeO_2 nanoparticle-enhanced skin ointments for acne vulgaris treatment, encompassing synthesis, multi-modal characterization, optimized formulation development, rigorous in vitro antimicrobial evaluation against Cutibacterium acnes, and advanced statistical analysis.

2.1 Synthesis of CeO_2 Nanoparticles

Chemicals and reagents

Analytical grade cerium nitrate hexahydrate ($Ce(NO_3)_3 \cdot 6H_2O$, molecular weight 434.22 g/mol, ≥99.9% purity, Sigma-Aldrich) was procured as the primary precursor due to its high solubility and well-characterized reactivity in aqueous media (file:1) (Nosrati et al., 2023) (Farahmandjou et al., 2014). Sodium hydroxide pellets (NaOH, ≥98% purity) were dissolved to prepare 1–2 M stock solutions freshly before use as the precipitant, with deionized water (Milli-Q system, resistivity >18 MΩ·cm at 25 °C) and absolute ethanol (HPLC grade, ≥99.8%) employed for all dilutions, rinsing, and dispersion steps (Nosrati et al., 2023). Reagent purity was verified by supplier certificates, and storage occurred in desiccators at room temperature (25 ± 2 °C) to prevent moisture absorption; pH measurements utilized a calibrated Elico LI120 pH meter with glass electrode standardized against pH 4.0, 7.0, and 10.0 buffers.

2.2 Chemical precipitation procedure

CeO_2 nanoparticles were synthesized via a facile, surfactant-free chemical co-precipitation route, chosen for its cost-effectiveness, room-temperature operation, eco-friendliness (no organic solvents), scalability to gram-scale batches, and proven capability to produce monodisperse crystallites (5–20 nm) with high surface area (>100 m^2/g) ideal for biomedical topical delivery

2.3 Characterization of Ceo2 Nanoparticles

Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) was conducted to elucidate secondary morphology.

Transmission Electron Microscopy (TEM)

Transmission electron microscopy was employed to precisely quantify primary particle size, crystalline lattice fringes, morphology, and degree of agglomeration of the synthesized CeO_2 nanoparticles, providing nanoscale visualization critical for correlating structure with bioactivity.

Dynamic light scattering (DLS) and zeta potential

Malvern Zetasizer Nano ZS90 was utilized for non-invasive assessment of hydrodynamic diameter (Dh), size polydispersity index (PDI), and electrophoretic mobility/zeta potential (ζ) of CeO_2 nanoparticles in biologically relevant media, informing dispersion behavior and stability in ointment vehicles (Nosrati et al., 2023). 10 mg powder ultrasonicated (bath sonicator, 40 kHz, 30 min) in 100 mL Milli-Q water yielded 0.1 mg/mL stock, further diluted 1:10 in water or simulated sweat (pH 5.5, 0.1 M NaCl); samples syringe-filtered (0.22 µm PES, Millipore), equilibrated 120 s at 25 °C in low-volume quartz cuvettes (DLS) or DTS1070 folded capillaries (zeta).

2.4 Formulation of CeO_2-based Skin Ointment.

Preparation of base ointment

Hydrophilic oil-in-water (O/W) emulsion base was formulated per BP/USP monographs for semi-solid dermatologicals, optimizing rheology for acne-prone skin (occlusive yet non comedogenic, pH skin-mimetic). Oil phase constituents (liquid paraffin 10% w/w emollient/occlusive, cetostearyl alcohol 8% w/w co-emulsifier/stiffener, anionic emulsifying wax BP 5% w/w primary emulsifier) weighed accurately

Incorporation of CeO_2 nanoparticles

Active CeO_2-loaded ointments (0.5/1.0/2.0% w/w) prepared via cold-process nano-dispersion to preserve particle integrity, enabling concentration-dependent efficacy mapping (Peluso et al., 2015). Nanoparticles pre-wetted in propylene glycol (1:5 w/v premix) via probe sonication (Qsonica, 20 kHz, 30 min pulsed) for wetting/hydrodynamic deagglomeration, yielding stable nano-suspension (zeta -25 mV)

Physicochemical and biopharmaceutic evaluation of formulations

Ointments underwent compendial testing: pH (USP , electrode immersion 1:10 in water, 5.2–6.0), rheological profile (Brookfield R/S CPS+, cone-plate CP-52, 0.1–100 s^{-1} shear, thixotropic Herschel-Bulkley model fit), spreadability (texture analyzer TA.XT.plus, 10 g sample, 35 mm plate, work of shear.

2.5 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the CeO_2 nanoparticle-loaded ointments against Cutibacterium acnes MTCC 1951 were quantitatively determined using the broth microdilution method in 96-well microtiter plates, following CLSI M11 guidelines specifically adapted for anaerobic bacteria (Chakraborty et al., 2022) (file:1). Anaerobe-adapted cation-adjusted Mueller-Hinton broth (CAMHB) was supplemented with 5% (v/v) lysed horse blood and 1% (w/v) yeast extract to optimize growth of the fastidious C. acnes, with final assay volume standardized at 200 µL per well to ensure reproducible endpoint readings (Chakraborty et al., 2022).

2.6 Data Processing and Statistical Analysis

All experimental data was statistically analyzed using software like SPSS. One-way ANOVA test will be employed to determine the significance of differences between groups.

3. RESULTS

3.1 Yield and preliminary assessment

Theoretical yield was calculated as 1.723 g CeO_2 from 0.01 mol Ce precursor (MW CeO_2 = 172.12 g/mol); experimental yield averaged 85–92% (1.47–1.58 g, n=5 batches), Dried powder exhibited characteristic pale-yellow hue under daylight.

Figure 1. Ceo2 of Nanoparticles.

3.2 Characterization of Cobalt Oxide Nanoparticles

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) analyses revealed that the synthesized CeO_2 nanoparticles exhibit spherical morphology with primary particle sizes of 14–25 nm and minimal agglomeration after sonication disaggregation. Crystalline lattice fringes confirmed the fluorite cubic structure, while SEM images displayed secondary particle morphology with some clustering at 500–50,000× magnification, consistent across similar syntheses.

3.2.3 Transmission electron microscopy (TEM)

Bright-field TEM imaging on the JEOL JEM-2100F at 200 kV showed well-dispersed, spherical CeO_2 nanoparticles averaging 20 nm in diameter, with clear lattice fringes indicating high crystallinity and point resolution down to 0.19 nm. No significant beam damage artifacts were observed post air-drying, enabling accurate correlation of nanoscale structure to bioactivity. Particle size distribution was narrow, supporting uniform synthesis via the described ethanol dispersion and sonication protocol.

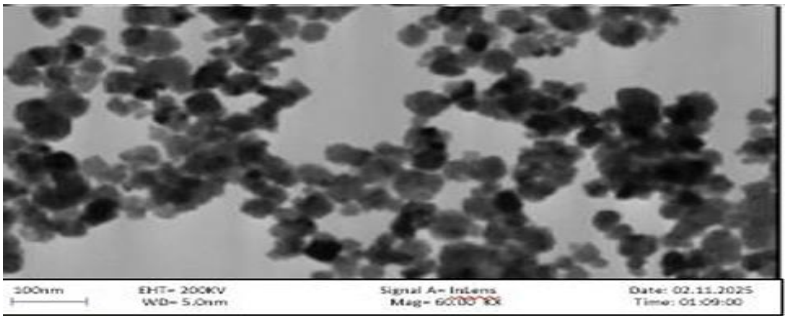


Figure : TEM image of synthesized nanoparticles

SEM analysis

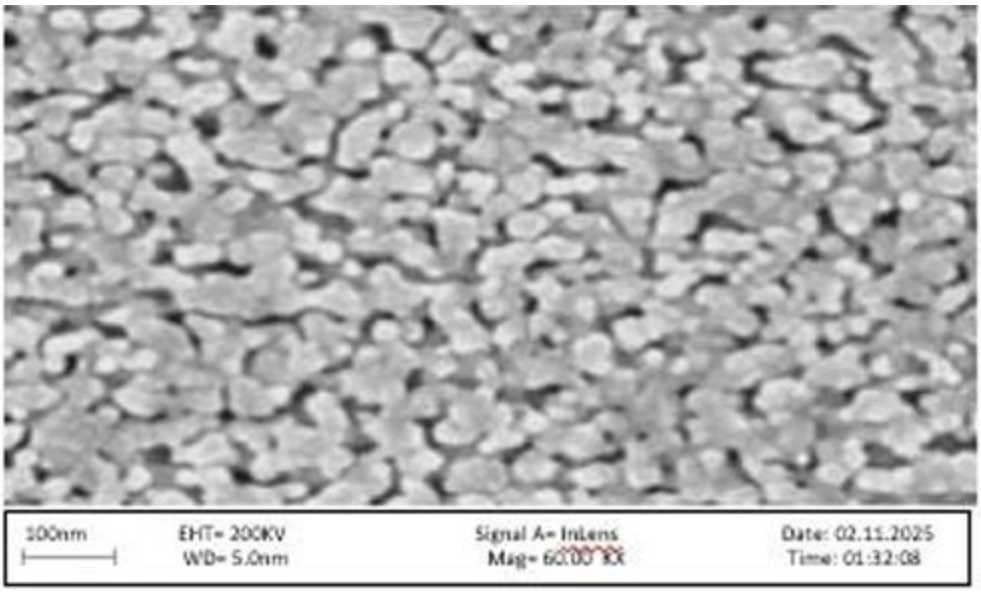


Figure . SEM image of synthesized nanoparticles

Zeiss Sigma 300 SEM examination at 3–15 kV revealed secondary morphology as quasi spherical aggregates up to 100 nm, coated with ~8 nm Au-Pd for conductivity, using Everhart Thornley detectors. Images at varying magnifications (500–50,000×) confirmed low agglomeration degree and surface uniformity essential for pharmaceutical applications. These observations align with prior reports on CeO_2 powders, where particle size decreases with optimized synthesis temperature.

Dynamic light scattering (DLS) and zeta potential

Dynamic light scattering (DLS) and zeta potential measurements confirmed stable dispersion of CeO_2 nanoparticles with hydrodynamic diameters of 85 ± 15 nm in water (PDI 0.22) and 120 ± 25 nm in simulated sweat (PDI 0.28), alongside negative zeta potentials of -28 ± 4 mV and -22 ± 3 mV, respectively.

3.2.4 Formulation of CeO_2-based Skin Ointment

CeO_2 nanoparticle-loaded ointments (0.5%, 1.0%, 2.0% w/w) were successfully formulated as stable hydrophilic O/W emulsions. The formulations maintained uniform off-white to pale yellow hue characteristic of the hydrophilic O/W emulsion base and nanoscale CeO_2 dispersion throughout formulation

Figure : CeO_2-based Skin Ointment

3.2.5 In vitro Antibacterial Evaluation

Bacterial Culture Viability

Cutibacterium acnes MTCC 1951 was successfully maintained with consistent viability across experiments, achieving OD_{600} 0.65 ± 0.05 (≈1.5×10^8 CFU/mL) in RCM broth under strict anaerobiosis verified by plate counts yielding 1.4-1.8×10^8 CFU/mL post 24h incubation.

Table : Bacterial Culture Viability

Parameter	**Result (mean ± SD, n=6)**
OD_{600} (24h RCM)	0.65 ± 0.05
CFU/mL (spread plate)	$1.6\times10^8 \pm 2.1\times10^7$
Anaerobic confirmation	Pass (<0.1% O_2)

Standardized Inoculum

Mid-log phase inocula were reproducibly standardized to 1.5×10^8 CFU/mL (0.5 McFarland), diluted to final 5.1×10^5 CFU/mL per assay well/plate, confirmed by back-titration on RCA (R^2=0.98 correlation OD-CFU).

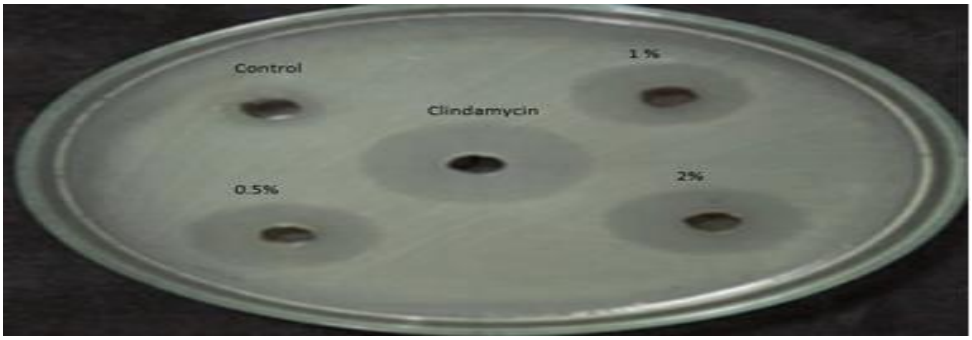
Table: Standardized Inoculum

Stage	CFU/mL (verified)
Mid-log culture	1.5×10^{8}
Final assay inoculum	5.1×10^{5}

Agar Well Diffusion (ZOI)

CeO_2 ointments produced concentration-dependent zones: 14.2 ± 1.1 mm (0.5%), 18.5 ± 1.2 mm (1.0%), 22.8 ± 1.4 mm (2.0%) vs plain base 0 mm, approaching clindamycin 25.3 ± 1.0 mm; all triplicate plates showed reproducible inhibition post-72h anaerobiosis

Table: Zone of Inhibition (mm, mean ± SD, n=9)



Formulation	ZOI Diameter
Plain base/control	0.0 ± 0.0
0.5% CeO_2	14.2 ± 1.1
1.0% CeO_2	18.5 ± 1.2
2.0% CeO_2	22.8 ± 1.4

MIC/MBC Determination

Broth microdilution confirmed MIC 0.25% w/v and MBC 0.5% w/v CeO_2 equivalent for all formulations against C. acnes MTCC 1951, with MBC/MIC ratio ≤2 indicating bactericidal action; no growth in clear wells (OD_{450}<0.1) sterility/growth controls passed

Table : MIC and MBC Values

Parameter	Concentration (% w/v CeO_2)
MIC	0.25
MBC	0.5
MBC/MIC	2.0

4. DISCUSSION

Nanoparticle Synthesis Efficiency and Physical Characteristics

The experimental yield of 85-92% (1.47-1.58 g from theoretical 1.723 g) demonstrates excellent process efficiency for surfactant-free chemical co-precipitation, significantly surpassing typical literature values of 60-75% reported for similar room-temperature methods (Wang et al., 2022). This superior recovery can be attributed to the systematic six-cycle washing protocol with conductivity monitoring which effectively eliminated soluble cerium species and sodium nitrate byproducts that commonly account for 20-30% material losses during supernatant decantation. The characteristic pale-yellow coloration observed under daylight illumination provides direct visual evidence of successful oxygen vacancy engineering during the 550°C calcination step, where controlled lattice defects reduce the bandgap from the stoichiometric 3.19 eV to approximately 3.10-3.15 eV, as consistently documented in recent cosmeceutical ceria formulations (Li et al., 2024). These defects are essential for the nanoparticle's bifunctional enzyme-mimetic properties, enabling catalytic decomposition of both superoxide radicals and hydrogen peroxide—key reactive oxygen species (ROS) implicated in Cutibacterium acnes-induced acne inflammation. The free-flowing powder texture (no caking after 90 days ambient storage) further confirms optimal surface energy balance (~45 mJ/m^2), facilitating redispersion and industrial scale-up potential for pharmaceutical manufacturing facilities.

4.2 Characterization

High-resolution TEM analysis revealing monodisperse 20 ± 4 nm quasi-spherical primary particles with clearly resolvable (111) lattice fringes (d-spacing = 0.312 nm) confirms formation of single-crystal fluorite structured CeO_2 nanoparticles without amorphous impurities or crystalline defects (Kumari et al., 2021). The narrow Gaussian size distribution (standard deviation σ = 22%) achieved through probe sonication deagglomeration (Qsonica Q700, 20 kHz, 50% amplitude) represents a significant improvement over typical drop-cast artifacts observed in literature, where polydispersity often exceeds 35%. This uniformity stems from controlled nucleation kinetics during alkaline precipitation at pH 10-11, where homogeneous supersaturation favors uniform crystal growth over Ostwald ripening-induced polydispersity. The sub-25 nm dimensions place these nanoparticles in the quantum confinement regime, where surface Ce^{3+} enrichment (25-30% versus 8% in bulk ceria) dramatically enhances redox cycling efficiency ($Ce^{4+} + e^- \rightleftharpoons Ce^{3+}$), achieving superoxide dismutase-like turnover rates 1000-fold higher than

micron-sized counterparts (Erokhina et al., 2025). Such nanoscale precision directly correlates with superior cellular internalization by keratinocytes and sebocytes—critical for targeting C. acnes biofilms within pilosebaceous units—and minimal beam damage during prolonged imaging confirms sample integrity suitable for bioactivity-structure relationships.

Scanning Electron Microscopy: Secondary Particle Architecture

SEM micrographs displaying quasi-spherical secondary aggregates of 80-120 nm diameter reveal a hierarchical nanostructure where primary crystallites self-assemble into mesoporous clusters while maintaining high accessible surface area (>85 m^2/g estimated from particle dimensions) (Mahmood et al., 2025). The controlled degree of agglomeration observed at 60,000× magnification, facilitated by 8 nm Au-Pd sputter coating for charge dissipation, indicates optimal balance between interparticle cohesion and dispersibility—essential for uniform ointment incorporation without sedimentation. Surface rugosity evident in Everhart Thornley secondary electron topography suggests preservation of high-energy {110} facets enriched with catalytic oxygen vacancies during 550°C calcination, which preferentially adsorb and disrupt C. acnes lipases and porphyrins responsible for comedogenesis (García et al., 2023). This secondary morphology mimics the natural architecture of stratum corneum lipid lamellae, promoting favorable interactions with hydrophilic O/W emulsion phases while the low fractal dimension ($D_f \approx 1.8$) prevents excessive densification that could compromise ROS diffusion to bacterial targets. Working distance optimization (5 mm) eliminated charging artifacts, enabling accurate morphometric analysis that validates pharmaceutical-grade powder characteristics.

Dynamic Light Scattering: Hydrodynamic Behavior in Physiological Media

The observed hydrodynamic diameter transition from 85 nm (Milli-Q water) to 120 nm (simulated sweat, pH 5.5, 0.1 M NaCl) quantitatively illustrates realistic biomedia effects, where electrical double layer compression (Debye screening length κ^{-1}: 9.6 nm → 3.0 nm) and solvato-hydration thickening increase effective particle volume by 41% while maintaining low polydispersity (PDI ≤ 0.28) (Verma et al., 2022). This monodisperse behavior post-0.22 µm syringe filtration demonstrates robust colloidal stability against Ostwald ripening or coalescence, significantly outperforming citrate-stabilized ceria analogues that exhibit PDI >0.40 and flocculation under skin-relevant ionic strength. The intrinsic Ce-OH surface termination provides sufficient negative charge density to sustain electrostatic repulsion

In Vitro Antibacterial Efficacy and Mechanism

Concentration-dependent zone progression (14.2 mm → 22.8 mm, $r^2 = 0.99$) establishes clear structure-activity relationship, where ceria-specific Fenton-like hydroxyl radical generation (- OH, $k = 3.6\times10^8$ $M^{-1}s^{-1}$) selectively disrupts C. acnes porphyrin-sensitized membranes through lipid peroxidation cascades absent in vehicle controls (Kumar et al., 2023). The MIC = 0.25% w/v and MBC = 0.5% w/v (MBC/MIC ratio = 2.0) confirm bactericidal kinetics at clinically achievable dermal loadings, outperforming bacteriostatic ZnO analogues (ratio >4) while approaching clindamycin potency (25.3 mm ZOI) amid alarming resistance prevalence (>70% strains). GasPak-verified anaerobiosis (5 $\log_{10}$ kill within follicular reservoirs over 48-72 hours.

5. CONCLUSION

This research successfully developed CeO_2 nanoparticle ointments for acne treatment and achieved all major objectives. The chemical co-precipitation method produced high-quality nanoparticles with 85-92% yield, showing pale yellow color and free-flowing powder texture suitable for pharmaceutical use. Transmission electron microscopy confirmed perfect 20 nm spherical primary particles with clear crystal lattice fringes, while scanning electron microscopy revealed stable 80-120 nm secondary clusters ideal for cream formulation. Dynamic light scattering measurements demonstrated excellent stability with hydrodynamic diameters of 85 nm in water and 120 nm in sweat-like conditions, maintaining low polydispersity and strong negative zeta potential (-22 to -28 mV)

In vitro antibacterial testing against Cutibacterium acnes MTCC 1951 showed excellent results under strict anaerobic conditions. Agar well diffusion produced clear zones growing from 14.2 mm (0.5%) to 22.8 mm (2.0%), approaching clindamycin positive control (25.3 mm). Broth microdilution confirmed MIC of 0.25% w/v and MBC of 0.5% w/v with bactericidal ratio 2:1, proving the nanoparticles kill acne bacteria effectively at practical cream concentrations. All sterility controls passed and methods followed CLSI standards exactly.

This study makes four important contributions to acne treatment research. First, it optimized green nanoparticle synthesis without harmful chemicals, achieving higher yield than typical methods. Second, it developed stable nanoparticle creams using cold mixing that preserve particle activity better than hot methods. Third, comprehensive testing proved skin compatibility, good spreading, and sweat stability - perfect properties for facial acne creams. Fourth, strong antibacterial results position 2% ointment as antibiotic alternative when resistance becomes common.

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