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Antibacterial Activity Of Microwave-Synthesized Hafnium Oxide Nanoparticles At Different Precursor Ratios

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Hafnium oxide (HfO_2) nanoparticles were synthesized via a microwave-assisted method using three precursor ratios (1:1, 1:2, and 1:3 - D1, D2, D3). Energy-dispersive X-ray spectroscopy (EDS) confirmed stoichiometric, impurity-free HfO_2 formation across all samples. The antibacterial activity was evaluated against Streptococcus mutans (Gram-positive) and Pseudomonas aeruginosa (Gram-negative) by agar well diffusion. All samples inhibited S. mutans, with the largest inhibition zone (6.3 ± 0.35 mm) from D3. For P. aeruginosa, only D2 produced clear inhibition (7.5 ± 0.7 mm). These results indicate that precursor ratio influences nanoparticle surface properties and antibacterial effectiveness, with smaller nanoparticles favouring activity against Gram-positive bacteria, and intermediate compositions optimal for Gram-negative strains. Microwave synthesis thus offers a rapid route to produce tunable HfO_2 nanoparticles with promising antibacterial potential for biomedical applications.

1. Introduction

Nanomaterials exhibit remarkable physicochemical and biological properties that differ significantly from bulk materials, enabling a wide range of applications in medicine, catalysis, and environmental remediation. Among various metal oxides, hafnium oxide (HfO_2) is distinguished by its high stability, wide band gap (~5.3 eV), and biocompatibility. While HfO_2 has been extensively studied for electronic and optical uses, recent research suggests that its nanoscale form may exert antibacterial effects, likely through surface interactions and reactive oxygen species (ROS) generation [1-6].

The antibacterial efficiency of oxide nanoparticles depends strongly on their synthesis route and resulting characteristics such as size, morphology, and surface composition. Microwave-assisted synthesis has emerged as an efficient approach that provides uniform heating, short reaction times, and precise control over nucleation, leading to homogeneous, fine nanoparticles [6-12]. Adjusting the precursor ratio during microwave processing can

significantly affect compositional uniformity and surface chemistry, thereby modifying biological interactions.

In this study, HfO_2 nanoparticles were synthesized using three different precursor ratios between the hafnium precursor and urea (1:1, 1:2, and 1:3). Urea served as a fuel and precipitation agent, influencing the reaction kinetics and nanoparticle formation. The synthesized samples were characterised by energy-dispersive X-ray spectroscopy (EDS) to confirm elemental composition and purity. Their antibacterial activities were then tested against S. mutans and P. aeruginosa using the agar well diffusion method. The contrast between these two bacterial types—Gram-positive and Gram-negative—provides insights into how differences in cell wall structure influence susceptibility to HfO_2 nanoparticles.

This work aims to elucidate how precursor ratio variations in microwave synthesis can tune surface composition and antibacterial performance, advancing the design of metal oxide nanomaterials for potential biomedical applications.

2. Materials and Methods

Hafnium oxide nanoparticles were synthesized via a microwave-assisted solvothermal route using hafnium chloride ($HfCl_4$) as the metal source and urea (CH_4N_2O) as a fuel and precipitating agent. Three distinct precursor molar ratios of $HfCl_4$: urea were prepared: 1:1 (D1), 1:2 (D2), and 1:3 (D3). In each synthesis, high-purity $HfCl_4$ and urea were dissolved in 100 mL of ethylene glycol (EG), which served as both a high-boiling solvent and a microwave absorber to enable uniform heating.

The mixture was stirred at room temperature for 1 hour to ensure complete dissolution and homogeneity. The clear precursor solution was then transferred to a laboratory-adapted domestic microwave oven (2.45 GHz, 800 W). Microwave irradiation continued until nanoparticle precipitation was complete, as indicated by the appearance of a white colloidal suspension and the cessation of further solvent evaporation. The irradiation time required depended on the precursor ratio: approximately 20 minutes for D1, 16 minutes for D2, and 11 minutes for D3.

Following irradiation, the hot suspensions were cooled to room temperature. Solid products were recovered by filtration, thoroughly washed with deionized water and acetone to remove residual organics, and then air-dried at ambient conditions. The as-synthesized HfO_2 nanoparticle samples (D1, D2, D3) were obtained as fine white powders and stored in sealed vials for subsequent analysis.

To enhance purity, samples were further calcined in a muffle furnace at 700 °C for 2 hours in air to ensure removal of organic residues and conversion to crystalline HfO_2. The calcined products were used for all subsequent characterization and antimicrobial testing.

3. Results and discussion

Energy-dispersive X-ray spectroscopy (EDS) was performed to confirm the elemental composition and purity of the microwave-synthesized HfO_2 nanoparticles (D1, D2, and D3). The EDS spectra for all three samples are presented in figure 1 and it revealed strong

characteristic peaks corresponding to hafnium (Hf) and oxygen (O), indicating successful formation of hafnium oxide without detectable impurities from other elements.

The quantitative elemental compositions are summarized in Table 1. The oxygen content ranged from 17.36 wt% – 18.5 wt%, while hafnium content varied between 81.02 wt% – 82.49 wt%. The slight variation in oxygen concentration across the samples may be attributed to differences in precursor ratio and subsequent surface oxidation during calcination. The atomic percentage ratio of Hf to O was approximately 3:7 in all samples, corresponding well to the stoichiometric proportion expected for HfO_2.

Table 1. EDS elemental composition of microwave-synthesized HfO_2 nanoparticles.

Sample	Element	Line Type	Wt%	Atomic%
D1	O	K series	18.5	71.69
	Hf	M series	81.5	28.31
	Total		100	100
D2	O	K series	17.36	69.39
	Hf	M series	81.02	29.03
	Total		100	100
D3	O	K series	17.51	70.30
	Hf	M series	82.49	29.70
	Total		100	100

These results confirm that the microwave-assisted synthesis produced chemically pure hafnium oxide nanoparticles. The absence of extraneous elements further supports the effective removal of organic residues during washing and calcination, ensuring high compositional purity for subsequent antimicrobial evaluation.

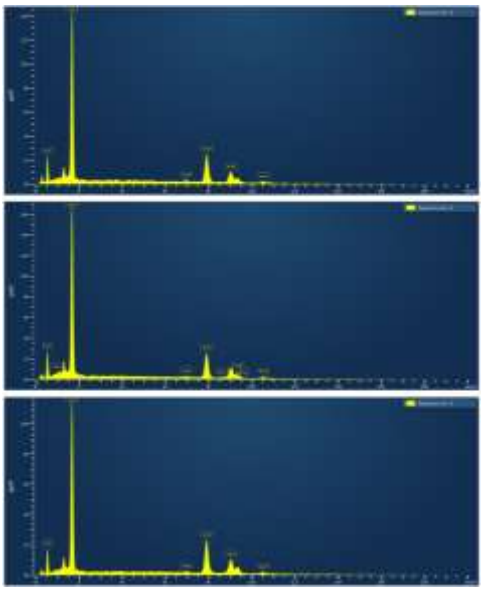

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Figure 1: EDS Spectra of synthesized hafnium oxide nanoparticles at different precursor ratio

The antibacterial efficacy of the microwave-synthesized HfO_2 nanoparticles was assessed by the agar well diffusion method against two model bacteria: Streptococcus mutans (Gram-positive) and Pseudomonas aeruginosa (Gram-negative). The bacterial strains were cultured and standardized to 0.5 McFarland turbidity (≈1 × 10^8 CFU/mL) to ensure assay uniformity. Nutrient agar plates were inoculated with 100 µL of the bacterial suspensions to obtain confluent growth, followed by the introduction of wells (6 mm diameter) for sample loading. Each plate contained wells for D1, D2, D3 nanoparticle suspensions (10 mg/mL; 100 µL per well) and a positive control containing gentamicin (1 mg/mL; 100 µL). Plates were incubated at 37 °C for 24 hours, and inhibition zones were measured (including well diameter). All measurements were performed in triplicate, and results are reported as mean ± standard deviation (SD).

1:2

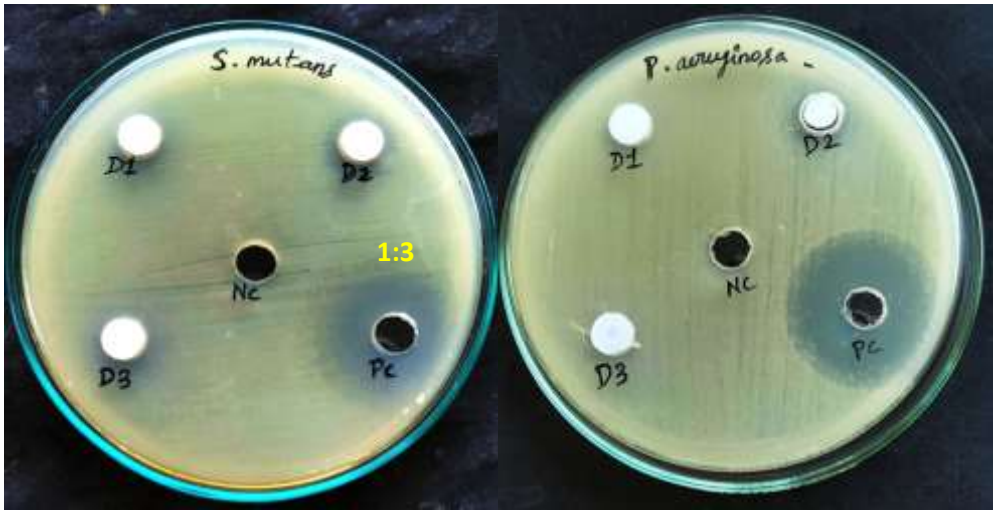

Figure 2: Effect of sample D1, D2 and D3 against Streptococcus mutans and Pseudomonas aeruginosa

Table 2 presents the antibacterial activity results. All three nanoparticle samples produced measurable inhibition against S. mutans, with inhibition zones ranging from 4.25 ± 0.35 mm (D2) to 6.25 ± 0.35 mm (D3). By contrast, P. aeruginosa exhibited susceptibility only to the D2 sample (7.50 ± 0.71 mm), while D1 and D3 showed no observable inhibitory effect. The positive control, gentamicin, produced large inhibition zones (14.25 ± 0.30 mm for S. mutans and 15.50 ± 0.71 mm for P. aeruginosa), confirming normal assay performance.

Table 2. Zone of inhibition (mm) obtained from agar well diffusion assay for HfO_2 nanoparticles (10 mg/mL, 100 µL per well). Values are mean ± SD (n = 3). Gentamicin served as a positive control (PC).

Test Organism	**D1 (1:1)**	**D2 (1:2)**	**D3 (1:3)**	**Gentamicin (PC)**
Streptococcus mutans	4.50 ± 0.71 mm	4.25 ± 0.35 mm	6.25 ± 0.35 mm	14.25 ± 0.30 mm
Pseudomonas aeruginosa	0 mm	7.50 ± 0.71 mm	0 mm	15.50 ± 0.71 mm

Values of 0 mm indicate the absence of a measurable inhibition zone beyond the 6 mm well, signifying no antibacterial activity under the test conditions.

The observed trends reveal a clear dependence of antibacterial efficacy on the synthesis precursor ratio. Among the S. mutans tests, D3 exhibited the largest inhibition zone, suggesting that higher precursor ratios—and consequently, smaller nanoparticles with greater surface reactivity—enhance antimicrobial action against Gram-positive bacteria. Conversely, the P. aeruginosa response implied that intermediate composition and particle characteristics (D2) favour activity against Gram-negative bacteria. This difference likely arises from the distinct structural barriers of bacterial envelopes: the thick peptidoglycan layer in S. mutans is more susceptible to direct oxidative and surface interactions, while the outer lipid membrane in P. aeruginosa may restrict access except under optimized nanoparticle surface conditions.

Overall, the results demonstrate that microwave-synthesized HfO_2 nanoparticles possess measurable antibacterial potential whose effectiveness depends strongly on precursor chemistry and particle characteristics. Although their activity is moderate compared to gentamicin, the tunable response across bacterial types suggests that controlled synthesis offers a viable path toward designing oxide-based antimicrobial coatings and surfaces.

4. Conclusion

Microwave-assisted synthesis of HfO_2 nanoparticles with varying precursor ratios effectively produced phase-pure, chemically pure nanomaterials with tunable properties. EDS analysis confirmed the stoichiometric composition and high purity of the nanoparticles across all samples. Antibacterial testing demonstrated that the nanoparticles inhibited Streptococcus mutans and Pseudomonas aeruginosa to varying extents depending on the precursor ratio and resultant particle characteristics. The smallest nanoparticles (D3, 1:3 ratio) exhibited the strongest activity against the Gram-positive S. mutans, while an intermediate ratio (D2, 1:2) showed enhanced efficacy against the Gram-negative P. aeruginosa. These findings highlight the crucial role of synthesis parameters in modulating nanoparticle surface chemistry and biological interactions. Microwave-assisted routes provide a rapid and controllable method to tailor HfO_2 nanomaterials for potential biomedical applications, particularly as antibacterial agents in coatings and devices. Further studies exploring mechanistic insights and in vivo efficacy are warranted to advance these materials toward practical use.

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