

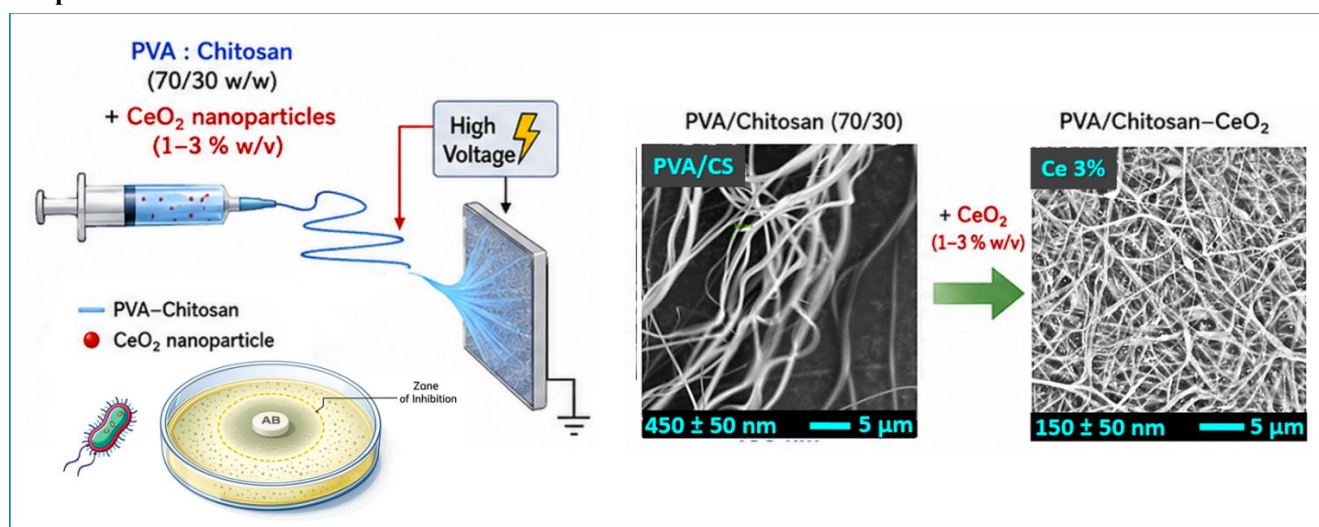
ELECTROSPUN POLYVINYL ALCOHOL - CHITOSAN FIBERS WITH CERIA NANOPARTICLES FOR ANTIBACTERIAL AND ANTIFUNGAL APPLICATIONS

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Graphical Abstract



ABSTRACT

Electrospun Poly(vinyl alcohol)/Chitosan (70/30) fibers embedded with cerium oxide nanoparticles (1 to 3 % w/v) were fabricated and evaluated as antimicrobial fibrous materials. Transmission electron microscopy confirmed CeO₂ nanoparticles with an average size of 10 nm. Incorporation of CeO₂ induced a significant reduction in fiber diameter from 450 nm to 150 nm. Agar diffusion assays against *Escherichia coli* *E. coli* and *Candida albicans* *C. albicans* revealed inhibition zones of 11.21 mm and 22.86 mm, respectively. These results indicate that CeO₂ incorporation was the principal factor associated with the observed antimicrobial activity, while the polymer composition influenced the apparent diffusion-dependent response. The combination of electrospun PVA-chitosan fibers and cerium oxide nanoparticles exhibits preliminary antibacterial and antifungal activity on a single electrospun platform, with potential biomedical applications.

Keywords: Electrospinning, fibers, nanoparticles, antibacterial properties, antifungal properties.

FIBRAS DE ALCOHOL POLIVINÍLICO Y QUITOSANO ELECTROHILADAS CON NANOPARTÍCULAS DE ÓXIDO DE CERIO PARA APLICACIONES ANTIBACTERIANAS Y ANTIFÚNGICAS.

RESUMEN

Se desarrollaron fibras de Alcohol poli-vinílico/Quitosano (70/30) electrohiladas, con nanopartículas de óxido de cerio (1 a 3 % p/v), para evaluar sus propiedades antibacterianas y antifúngicas. La microscopía electrónica de transmisión confirmó la presencia de nanopartículas de CeO₂ con un tamaño promedio de 10 nm. La incorporación de CeO₂ indujo una reducción significativa en el diámetro de las fibras, de 450 nm a 150 nm. Los ensayos de difusión en agar contra *Escherichia coli* (*E. coli*) y *Candida albicans* (*C. albicans*) revelaron zonas de inhibición de 11,21 mm y 22,86 mm, respectivamente, atribuidas a las interacciones aditivas entre el PVA, el quitosano y las nanopartículas de CeO₂. La combinación de fibras de PVA-quitosano electrohiladas y nanopartículas de óxido de cerio presenta actividad antibacteriana y antifúngica preliminar en una única plataforma electrohilada, con potenciales aplicaciones biomédicas.

Palabras clave: Electrohilado, fibras, nanopartículas, propiedades antibacterianas, propiedades antifúngicas.

INTRODUCTION

Electrospun polymeric fibers are widely explored as antimicrobial platforms due to their high surface area-to-volume ratio and tunable morphology [1]. Poly(vinyl alcohol) (PVA) offers mechanical stability and processability, while chitosan (CS) contributes intrinsic antimicrobial activity through electrostatic interaction between its protonated amino groups and negatively charged microbial membranes [2]. Additionally, ceria nanoparticles (CeNPs) have demonstrated promising therapeutic, diagnostic, antibacterial, and anti-fungal properties [3]. CeNPs have played an essential role in regenerative medicine and tissue engineering, particularly for theragnostic and bioimaging applications, mainly due to their high oxidant resistance (antioxidant activity) [4]. These properties make CeNPs ideal candidates for embedding in polymeric matrices to enhance their performance as tissue-engineering scaffolds, thereby positioning these complexes as biomaterials with potential for skin grafts and drug delivery systems [5]. CeO₂ nanoparticles-based chitosan/PVA composite films have been reported [6] for gram-positive bacteria, gram-negative bacteria, and filamentous fungi, suggesting that the CeO₂/CS/PVA composite films could be used as effective wrapping materials for food preservation or biomedical applications.

Electrospinning has emerged as a novel fiber fabrication method that enables scalable mass production [7]. Natural and synthetic polymers are used in the electrospinning technique as well as blends of natural/synthetic polymers to fabricate fibers [8]. Motasadizadeh, et al. [9], developed electrospun poly(vinyl alcohol)/chitosan-g-poly(N-vinyl imidazole) containing titanium dioxide/curcumin for biocompatible antibacterial wound-dressing applications. Additionally, Shenata et al. synthesized fluorescent PVA electrospun fibers with CeNPs for applications in bioimaging and environmental monitoring.

Although electrospun PVA/chitosan fibers and ceria-containing wound materials have each been investigated previously, the literature remains limited on electrospun PVA-chitosan fibrous systems loaded with ceria nanoparticles specifically designed for combined antibacterial and antifungal activity. Prior studies have primarily focused on antimicrobial [10], performance, without a dual-microbial evaluation. Therefore, the present work advances the field by integrating the processability of electrospun PVA/chitosan fibers with ceria nanoparticles into a single fibrous platform for broad-spectrum antibacterial and antifungal applications. The incorporation of CeO₂ nanoparticles not only modified the electrospinning behavior, leading to a significant reduction in fiber diameter, but also promoted the formation of an integrated hybrid nanocomposite through interactions with

the hydroxyl and amino groups of the polymeric matrix, as confirmed by FTIR and Raman spectroscopy.

MATERIALS AND METHODS

Chemical Reagents

All chemicals were used as received, without further purification, and were of analytical reagent grade. Polyvinyl alcohol (PVA) (Mw 130,000, 99% hydrolized), chitosan (CS) (high molecular weight, 90% degree of deacetylation), ammonium hydroxide (NH₄OH)(99.8 %), cerium Sulfate (III) (Ce₂(SO₄)₃) (99%) and acetic acid (99 %) were purchased from Aldrich and Sigma Co LLC.

Synthesis of ceria nanoparticles (CeNPs)

For the synthesis of ceria nanoparticles, 0.05 M of cerium sulfate was prepared by dissolving 0.16 g of cerium sulfate in 10 mL of distilled water at 60 °C at 800 rpm. Simultaneously, 10 mL of ammonium hydroxide (25% w/v) was added to 142 mL of distilled water to form a 0.5 M NH₄OH solution. 25 mL of ammonium hydroxide solution was added dropwise until a yellowish precipitate formed. The solution containing CeNPs was sonicated at 60 % power for 30 min, with pulses of 6.0 s followed by 7 s to disperse the nanoparticles.

Preparation of CS/CeNPs

A 4% w/v chitosan solution was prepared by dissolving chitosan (high molecular weight, 90% degree of deacetylation) in an aqueous acetic acid solution under continuous magnetic stirring at 50 °C for 20 h. Ceria nanoparticles were then incorporated into the chitosan solution at concentrations of 1, 2, and 3% w/v. Each suspension was maintained at 50 °C under magnetic stirring at 800 rpm and subsequently sonicated for 45 min to promote nanoparticle dispersion.

Preparation of PolyVinyl Alcohol/Chitosan (PVA/CS)

To prepare the 8 % w/v PVA polymeric solution, 8 g of Polyvinyl Alcohol (PVA) was dissolved in 100 mL of distilled water under magnetic stirring and maintained at 50 °C for 4 h. Separately, 2 g of Chitosan (CS) was dissolved in a 50 % w/v acetic acid at 50 °C under constant stirring

for 20 h to obtain a 4 % w/v CS solution. PVA and CS solutions were mixed at a 70/30 volume ratio, stirred at 40 °C and 600 rpm for 4 h to obtain a homogeneous PVA/CS polymer mixture.

Preparation of PVA/CeNPs and PVA/CS/CeNPs

PVA and PVA/CS polymeric solutions were embedded with (1, 2, and 3% w/v) of CeNPs. The solutions were held at 50 °C, stirred at 800 rpm to remove excess solvent by evaporation, and finally sonicated for 45 min.

Preparation of PVA/CS/CeNPs fibers

Poly(vinyl alcohol)/Chitosan (70/30) fibers with CeNPs were fabricated using the SprayBaseT M electrospinning technique [11]. The fibers were deposited onto an aluminum foil, which was the collector. A DC voltage supply was used to release the PVA/CS/CeNPs solutions at a controlled flow rate between 0.010 mL/h and 0.500 mL/h. An electrical potential from 3.80 kV to 13.84 kV was applied to the solutions to evaporate the solvents. The distance between the emitter and collector ranged from 9 to 14 cm. The electrospinning process was realized at 16 °C.

Agar Diffusion Test

Luria broth (LB) agar was prepared according to the method described by Liu [12] and stored at 4 °C until further use. 20 mL of LB liquid medium was prepared and added to a test tube. *E. coli* ATCC 25922 culture was grown overnight in 1 mL of LB broth. Square-shaped samples, 4 mm per side, containing PVA fibers, or CeNPs 3% w/v embedded in PVA/chitosan (70/30) fibers, were placed on the plate as treatments. Amikacin (100 µg/mL) was used as a positive control, and acetic acid as a negative control. The plate with the cells, controls, and treatments was incubated at 37 °C for 24 hours.

Yeast peptone dextrose (YPD) agar solid culture medium was prepared according to the method proposed by Liu [13] and stored in the refrigerator at 4 °C until further use. 20 mL of YPD liquid medium was prepared and put in a test tube. Immediately, 1 mL of *C. albicans* preculture was

inoculated into YPD liquid medium and grown at 37 °C with constant agitation at 210 rpm for 1.5 hours. A 1/20 dilution of *C. albicans* cultured cells was prepared to accurately measure the concentration. The YPD solid medium was left under UV light for 15 min to ensure sterilization. Square-shaped samples, 4 mm per side of PVA/chitosan (70/30) fibers were placed on the plate as treatments. Nourseothricin (25 µg/mL) was used as a positive control, and acetic acid as a negative control. The plate with the cells, controls and treatments were left in an incubator at 37°C for 24 h.

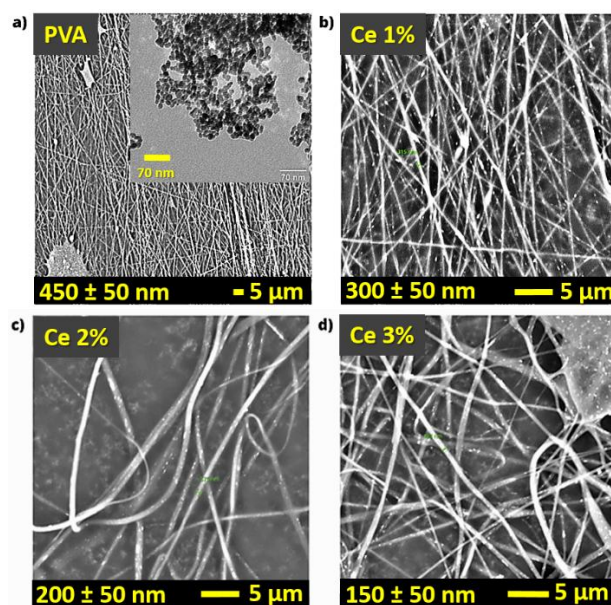
Characterization

Infrared measures of the samples were acquired using an Agilent Cary 630 FTIR spectrometer over the range of 4000 to 400 cm^{-1} , at a spectral resolution of 4 cm^{-1} . Raman spectra were recorded using a HORIBA LabRAM HR Evolution spectrometer with a 532 nm excitation laser over the range of 3500 to 400 cm^{-1} . Scanning electron micrographs were acquired using a Phenom ProX microscope operated at 10 kV. Fiber diameters were measured using ImageJ software. Transmission Electron micrograph of CeNPs was obtained at 120 kV using an FEI-Tecna G20 Spirit Twin microscope with an Eagle 4k HR camera. Thermogravimetric analysis was performed using a Discovery TGA 55 thermogravimetric analyzer (TA Instruments, USA), equipped with an automatic sample loader and an infrared furnace. The instrument has an operating temperature range from ambient temperature to 1000 °C, a temperature accuracy of ± 1 °C, and a programmable linear heating-rate range of 0.1–100 °C min^{-1} . The SprayBaseT M electrospinning equipment was used to fabricate the fibers.

RESULTS AND DISCUSSION

Scanning Electron Microscopy (SEM)

SEM micrographs of PVA fibers with different percentages of CeNPs are presented in Figure 1 (a to d). PVA fibers reveal a network of continuous, randomly oriented fibers with smooth surfaces and uniform morphology with an average diameter of 450 ± 50 nm. The inset of Figure 1a shows the transmission electron micrograph of CeNPs, with an average particle size of 10 nm. In this Figure, CeNPs appear as brighter points on the fiber surface, with some aggregation. In Figure 1 (e to h), CeNPs were dispersed on the PVA/CS fibers with a noticeable increase in fiber uniformity and an average diameter of 450 ± 50 nm. In general, the average diameter of the fibers decreases with the CeNPs concentration from 450 ± 50 nm to 150 nm. This is mainly due to the increased solution conductivity due to CeNPs, the exertion of a high electric field during the electrospinning process, and the susceptibility of CeNPs to interact with the electric field [14].



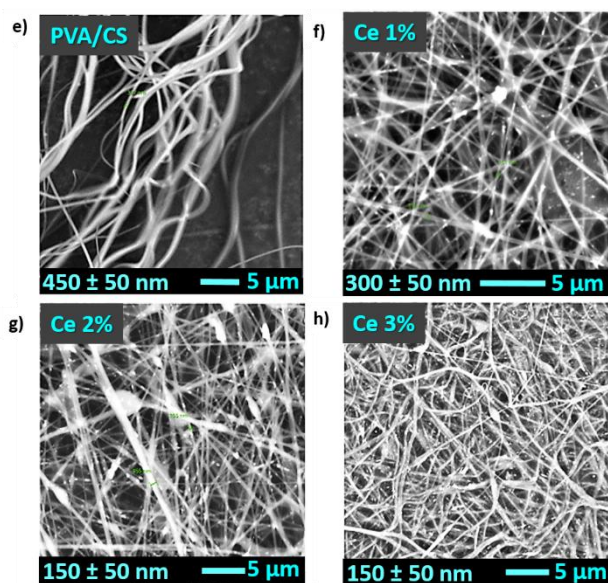


Figure 1: Scanning Electron micrographs of a) PVA fibers and the inset shows the Transmission electron micrograph of CeNPs, b) PVA fibers with 1 % w/v of CeNPs, c) PVA fibers with 2% w/v CeNPs, and d) PVA fibers with 3% w/v CeNPs, e) PVA/CS (70/30) fibers, f) PVA/CS fibers with 1% w/v CeNPs, g) PVA/CS fibers with 2% w/v CeNPs h) PVA/CS fibers with 3% w/v CeNPs.

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of CeNPs with PVA and PVA/CS (70/30) fibers are shown in Figure 2 and confirm the incorporation of cerium oxide nanoparticles into the PVA and PVA/CS matrices through the PVA hydroxyl groups at 3300 cm^{-1} and the overlapping of the chitosan amine groups in Figure 2b. The appearance of characteristic Ce–O vibrations ($500\text{--}700\text{ cm}^{-1}$), along with the shift and broadening of O–H and N–H bands, indicates intermolecular interactions between ceria nanoparticles and the functional groups of PVA and chitosan. These results suggest the formation of a chemically integrated hybrid nanocomposite rather than a simple physical mixture [15].

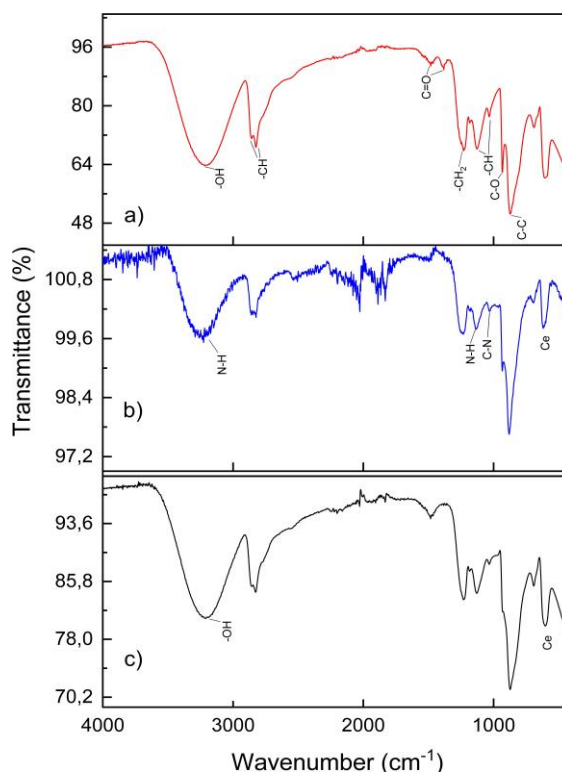


Figure 2: Fourier Transform Infrared spectra of a) PVA fibers, b) CeNPs with PVA/CS (70/30), and c) CeNPs with PVA.

Raman spectroscopy

Raman spectroscopy further corroborates the structural modifications observed by FTIR. Figure 4 shows the Raman spectra of PVA and PVA/CS with CeNPs. In Figure 4a, two well-defined, strong peaks are observed at wavenumbers of 2914 and 1442 cm^{-1} ; these peaks correspond to the stretching vibrations of the $-\text{CH}_2$ and $-\text{CH}$ groups of PVA, respectively, while peaks observed in the region of $850\text{--}1150\text{ cm}^{-1}$ are associated with C–C and C–O stretching modes of the PVA chain [16]. In Figure 4b, PVA/CS shows broadening and an increase in intensity at 2900 cm^{-1} , indicating intermolecular interactions, by hydrogen bonding between the hydroxyl groups of PVA and the amino groups of chitosan. The appearance and enhancement of bands at 1147 cm^{-1} and 926 cm^{-1} are consistent with glycosidic linkages and saccharide ring vibrations from chitosan. The Raman spectra confirm the incorporation of ceria nanoparticles into the PVA and

PVA/CS matrices, as evidenced by the characteristic vibrational mode of Ce-O at 494 cm^{-1} .

The observed shifts and intensity changes in polymer-related bands indicate intermolecular interactions between ceria nanoparticles and the hydroxyl and amino functional groups of PVA and chitosan, respectively, suggesting the formation of a chemically integrated nanocomposite describe in Figure 4. There have been reported [27] that the incorporation of CeNPs modifies the local dielectric

environment and the conformational mobility of PVA and chitosan chains. Interactions between CeO₂ nanoparticle surface sites and polymer –OH/–NH₂ groups alter the electron density and polarizability derivatives associated with C–O, C–C, C–N, CH, and CH₂ vibrations. These effects produce small peak shifts and changes in relative Raman intensity. Band broadening may arise from a distribution of interfacial environments, overlapping vibrational contributions, or increased local disorder.

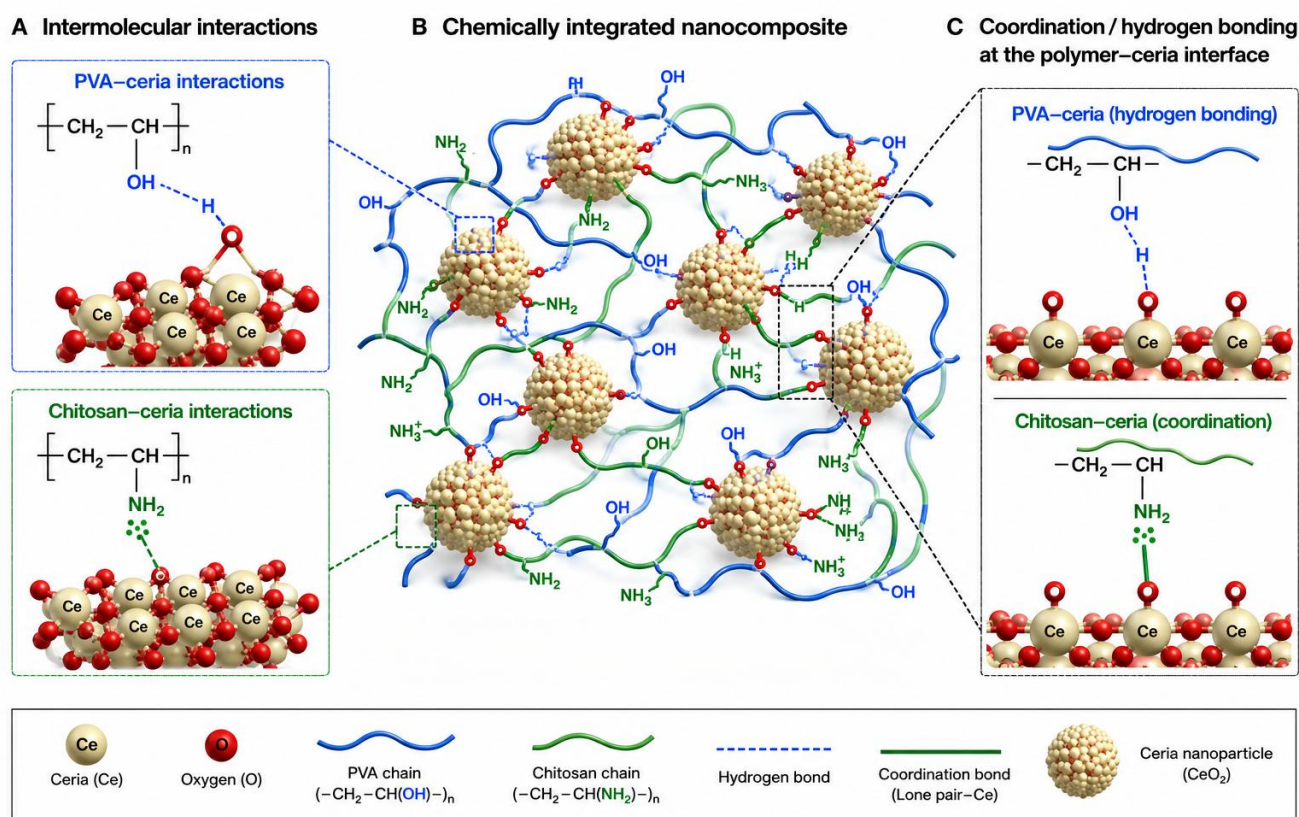


Figure 3. Schematic representation of the intermolecular interactions responsible for the formation of PVA/chitosan–ceria nanocomposite. (A) Proposed molecular interactions between CeO₂ nanoparticles and the polymeric matrix, (B) Three-dimensional representation of the nanocomposite network showing the distribution of ceria nanoparticles within the PVA/chitosan matrix. (C) Enlarged views illustrating the two principal interfacial interaction mechanisms: hydrogen bonding between PVA hydroxyl groups and the ceria surface, and chemical bonding between chitosan amino groups and exposed cerium atoms. Created with the assistance of ChatGPT (OpenAI) and scientifically reviewed by the authors.

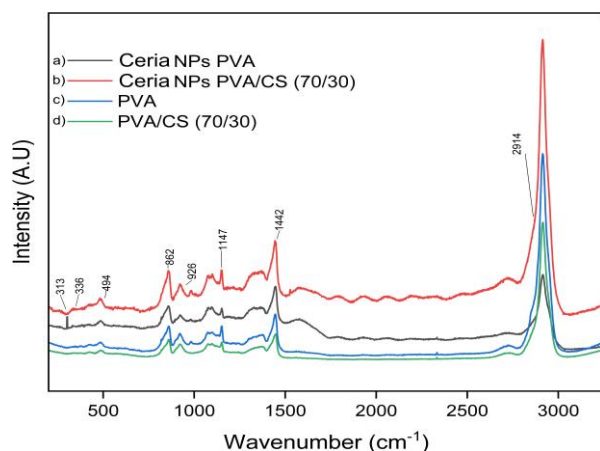


Figure 4: Raman spectra of a) CeNPs with PVA, b) CeNPs with PVA/CS (70/30), c) PVA fibers, and d) PVA/CS (70/30).

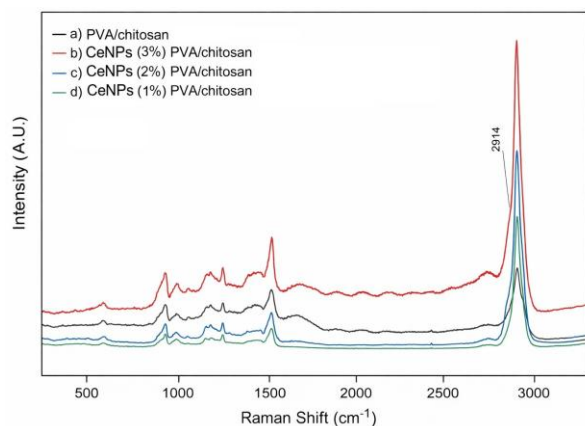


Figure 5: Raman spectra of (a) PVA/chitosan (70/30) and (b) PVA/CS fibers with 3% w/v CeNPs, (c) PVA/CS fibers with 2% w/v CeNPs and (d) PVA/CS fibers with 1% w/v CeNPs.

In Figure 5, Raman spectroscopy was employed to evaluate the effect of ceria nanoparticles at different concentrations (1%, 2%, and 3%) on the PVA/CS matrix. The spectrum of undoped PVA/CS fibers exhibits the characteristic vibrational features of both polymers. The prominent band at 2914 cm^{-1} is attributed to symmetric and asymmetric stretching of $-\text{CH}_2$ groups. An increase in the intensity of this band is observed with the highest CeNPs concentration, as seen in the 3% CeNPs sample, indicating a stronger contribution from CeO_2 containing regions and modification of the polarizability of the surrounding

polymer chains in agreement with the SEM observations that revealed localized aggregation of CeNPs.

Antibacterial and Antifungal Activity Assessment

The bacterial and fungal inhibition tests of the CeNPs 3% w/v with PVA and CeNPs 3% w/v with PVA/chitosan (70/30) fibers against *E. coli* ATCC 25922 and *C. albicans*, were performed by the disc diffusion susceptibility test after 24 h incubation. To confirm whether CeNPs 3% w/v with PVA/Chitosan (70/30), and CeNPs 3% w/v with PVA fibers have an effective antibacterial and antifungal activity, pure PVA fibers and acetic acid (solvent of chitosan) were used as negative controls. As shown in Figure 6 (a and b), both *E. coli* ATCC 25922 and *C. albicans*, clearly showed a zone of inhibited growth of the bacteria and fungi around the CeNPs 3% w/v PVA/Chitosan (70/30) and CeNPs 3% w/v PVA fibers. In contrast, in the absence of CeNPs and chitosan, the PVA/CS fibers showed no antibacterial or antifungal activity, and neither the controls nor the controls exhibited any activity, with no zone of inhibition.

The PVA/CS/CeNPs formulation showed antimicrobial activity against both microorganisms; however, its antifungal inhibition zone was smaller than that of PVA/CeNPs, potentially because of differences in nanoparticle accessibility, matrix retention, or diffusion through agar. Our results demonstrated that bacterial and fungal growth were inhibited by the presence of CeNPs and chitosan in PVA/CS fibers. These results suggest that *E. coli* and *C. albicans* are affected by CeNPs and chitosan, as they are lethal to these particular bacteria and fungi. The results are consistent with those of Muzzarelli et al. (2019) [17,18], who tested the bactericidal effect of chitosan on 289 strains of Gram-positive and Gram-negative bacteria, observing morphological damage, weakening and thinning of cell walls, and modifications and disappearance of internal cell structures.

Also, Hardiansyah et al. (2015) [19] demonstrated that chitosan-blended poly(lactic acid) fibers with a content of 3.2 % w/v killed *E. coli* on the first day. This could be explained by the presence of highly positively charged chitosan, which is known to inhibit biosynthesis and energy transport across the cell wall and to kill bacteria [20]. The positively charged -NH_3^+ would bind to the negatively charged cell walls of *E. coli* and cause their enzymes to break down [19]. This is consistent with previous research, which confirmed that chitosan inhibits the growth of several Gram-positive and Gram-negative bacteria and exhibits high antimicrobial activity against *E. coli* [20]. Furthermore, a report on chitosan-PVA fibers showed higher antimicrobial activity against *E. coli* for larger diameters, due to a greater contact surface area and a greater abundance of amino groups, which facilitate microbial growth inhibition. Additionally, incorporating CeNPs into electrospun chitosan fibers improves antibacterial performance. Ceria nanoparticles, owing to their outstanding physical and chemical properties, are promising for biomedical applications, as demonstrated by

their antibacterial and antifungal activities [21]. For studies of antifungal activity [21], CeNPs were used against clinical strains of *C. albicans*, where the minimum inhibitory concentration of CeNPs (0.12 – 0.48 $\mu\text{g/mL}$) was lower than that of Nourseothricin (25 $\mu\text{g/mL}$) drug (1.75 – 25 $\mu\text{g/mL}$) against clinical strains of *C. albicans*. Our results show that electrospun PVA/CS/CeO₂ nanofibers exhibit antifungal activity against *C. albicans* that exceeds that typically reported for CeNPs alone, while maintaining antibacterial performance comparable to that of silver-based systems, suggesting an additive interaction among PVA, chitosan, and CeNPs.

Table 1 highlights that the inhibition zones obtained are consistent with literature-reported values for CeNPs and chitosan-based antimicrobial systems, confirming that PVA/CS with CeNPs fibers exhibit competitive antimicrobial performance, particularly against *C. albicans*, where their activity approaches that of conventional antifungal agents.

Table 1: Comparison of inhibition zones (mm) for CeO₂-based and chitosan-based antimicrobial systems against *E. coli* and *C. albicans*.

Material/System	<i>E. coli</i> (mm)	<i>C. albicans</i> (mm)	Reference
CeO ₂ nanoparticles	9–15	14–18	[22], [23]
Chitosan films	2–8	11–13	[24], [25]
Chitosan-PVA/CeO ₂	10–14	–	[26]
CS/PVA electrospun nanofibers	10–12	10–12	[27], [28]
This work: CeNPs–PVA	10.74	22.86	This work
This work: CeNPs–PVA/CS	11.21	13.14	This work
Positive control	15.06 (Amikacin)	15.85 (Nourseothricin)	This work

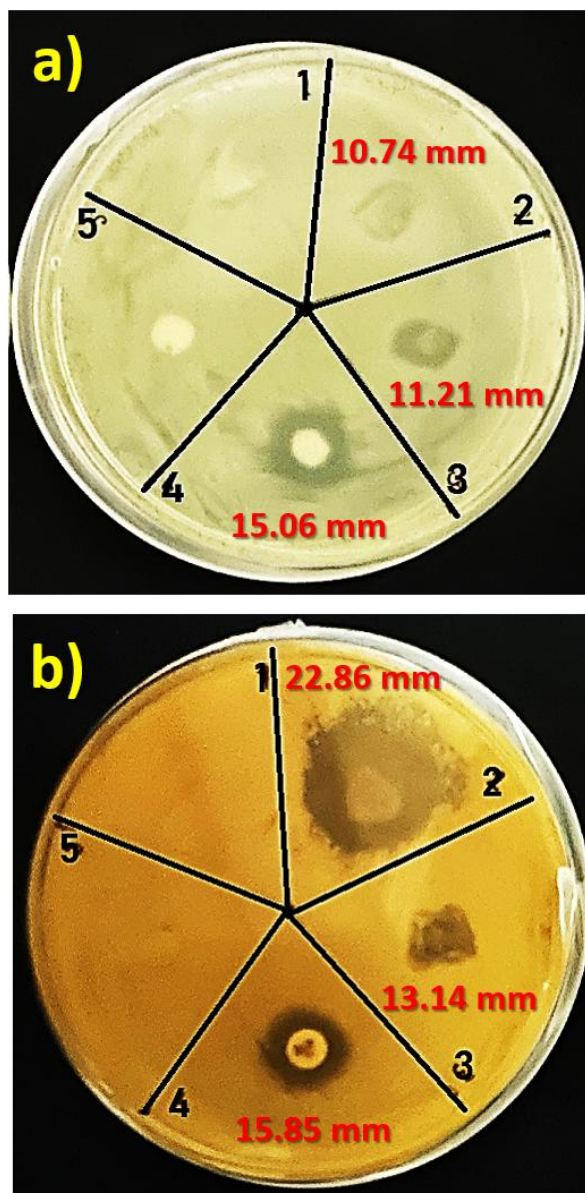


Figure 6: a) Antibacterial susceptibility test of CeNPs 3% w/v with PVA and PVA/CS (70/30) fibers against *E. coli* ATCC 25922. Labels 1 = PVA fibers negative control; 2 = CeNPs 3% w/v with PVA fibers; 3 = CeNPs 3% w/v with PVA/CS (70/30) fibers; 4 = (1:1000) dilution of Amikacin (100 µg/mL) as positive control; and 5 = 3% w/v acetic acid. b) Antifungal susceptibility test of CeNPs 3% w/v with PVA and PVA/CS (70/30) fibers against *C. albicans*. Labels 1 = PVA fibers negative control; 2 = CeNPs 3% w/v with PVA fibers; 3 = CeNPs PVA/CS (70/30) fibers; 4 = (1:1000) dilution of Nourseothricin (25 µg/mL) positive control; and 5 = 3% w/v of acetic acid.

CONCLUSIONS

Electrospun PVA and PVA/chitosan fibers with CeO₂ nanoparticles were fabricated and characterized to evaluate the antibacterial and antifungal properties. TEM showed CeO₂ nanoparticles with an average size of approximately 10 nm, while SEM revealed that incorporation of CeNPs was associated with a progressive reduction in fiber diameter from 450 ± 50 nm to 150 ± 50 nm. FTIR and Raman spectroscopy confirmed the presence of CeO₂ and indicated interfacial interactions between the nanoparticles and the hydroxyl and amino-containing groups of the polymer matrices. CeNPs containing PVA and PVA/chitosan fibers produced measurable inhibition zones against *E. coli* ATCC 25922 and *C. albicans*. The results indicate that CeNPs incorporation was the main factor associated with the observed agar-diffusion activity. These results support the potential of CeO₂-containing electrospun fibers as antimicrobial materials, although additional release, cytocompatibility, contact-killing, and quantitative microbiological studies are required before biomedical application can be established.

Authors' Contributions

The authors contributed equally to writing the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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