

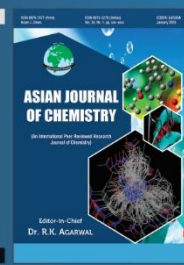


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Biogenic CaO Nanoparticles-Loaded Chitosan/PVA Electrospun Nanofibers for Antibacterial Water Disinfection

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Chitosan-based nanofibers are especially attractive owing to their biodegradability, biocompatibility and intrinsic antibacterial activity. In present study, chitosan/poly(vinyl alcohol) (PVA)/calcium oxide (CaO) composite nanofibers were fabricated by electrospinning using CaO nanoparticles derived from eggshell waste as a sustainable antibacterial additive. Chitosan and PVA were blended to improve solution spinnability, fiber continuity and mechanical integrity, while biogenic CaO nanoparticles were incorporated to enhance antibacterial performance. The optimized formulation produced uniform nanofibers with diameters in the range of approximately 100-250 nm. Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) confirmed the formation of the composite matrix, intermolecular interactions between chitosan and PVA and the incorporation of CaO nanoparticles. Field-emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM) revealed smooth, bead-free fibers with a porous interconnected morphology and thermogravimetric analysis (TGA) indicated the presence of thermally stable inorganic residue. The nanofibers showed pronounced antibacterial activity against *Escherichia coli* in the agar well diffusion assay. Freshly prepared fibers produced an inhibition zone of 2.0 cm, while fibers stored for one month retained substantial activity with an inhibition zone of 1.8 cm. The antibacterial effect is attributed to the combined action of chitosan-mediated membrane disruption and CaO-induced alkaline/oxidative stress. These results indicate that biodegradable chitosan/PVA/CaO electrospun nanofibers have potential as eco-friendly antibacterial materials for decentralized and portable water disinfection applications. Water disinfection performance was further assessed using a most probable number (MPN) assay with contaminated canal water. Following 10 min of direct contact with the nanofiber membrane, coliform-positive responses and gas formation were substantially reduced, supporting the use of the composite for rapid point-of-use water disinfection.

Keywords: Biogenic calcium oxide, Chitosan/PVA, Electrospinning, Eggshell waste, Antibacterial nanofibers, Water disinfection.

INTRODUCTION

Microbial contamination of drinking water remains a major cause of waterborne diseases and contributes substantially to global morbidity and mortality [1]. *Escherichia coli* is widely recognized as an indicator organism for fecal contamination and is routinely used to assess microbial water quality [2]. Due to the rapid population growth, urbanization, industrial development and increasing environmental pollution have intensified the demand for effective and sustainable water purification technologies [3]. Point-of-use (POU) disinfection systems have gained considerable interest as they can provide microbiologically safe water at household and community levels without relying entirely on centralized treatment infrastructure [4].

Conventional methods for microbial water treatment comprise chlorination, ozonation, ultraviolet irradiation, ceramic filtration and membrane filtration [5,6]. Each technology has practical limitations related to operating cost, energy consumption, membrane fouling, material durability or the formation of potentially undesirable disinfection byproducts [7,8]. Many filtration systems rely on non-biodegradable polymers derived from petroleum resources, raising concerns regarding their environmental persistence and disposal [9]. These considerations have increased interest in low-cost, biodegradable and antibacterial materials that can be applied in the decentralized water treatment systems [10].

Electrospun nanofibers have attracted attention in water treatment owing to their small fiber diameters, high porosity, interconnected pore structure and large surface-area-to-volume

ratio [11-13]. Electrospinning provides a relatively simple and adaptable route for fabricating continuous fibers with tunable morphology and surface characteristics [12]. The resulting nanofibrous membranes offer extensive interfacial area for interactions between microbial contaminants and functional groups present on the fiber surface, which can support microbial capture and antibacterial action [14]. Such membranes have been investigated for wastewater treatment, air filtration, biomedical applications and antimicrobial water purification [15].

Natural biopolymers are promising components for nanofiber fabrication due to their renewable origin, biodegradability and biocompatibility [16]. Chitosan, a deacetylated derivative of chitin, has received extensive attention in environmental and biomedical applications [17]. Its amino and hydroxyl groups provide sites for adsorption, film formation and interactions with microbial cells [18]. The antimicrobial activity of chitosan is commonly associated with electrostatic attraction between protonated amino groups and negatively charged bacterial cell membranes. This interaction can alter membrane integrity and promote leakage of intracellular components [19]. Such characteristics make chitosan a suitable candidate for developing biodegradable materials for water purification [20].

Electrospinning of pure chitosan remains challenging due to its high solution viscosity, rigid molecular structure and extensive intermolecular hydrogen bonding [21,22]. Blending chitosan with poly(vinyl alcohol) (PVA) provides a practical approach for improving its electrospinning characteristics. PVA is water-soluble, relatively non-toxic and biodegradable and its incorporation can improve polymer-chain entanglement, solution viscosity, flexibility and fiber continuity during electrospinning [23,24]. Chitosan/PVA blends can consequently form more uniform and mechanically stable nanofibrous structures while preserving the functional characteristics of chitosan [25,26].

The incorporation of inorganic antibacterial agents into polymeric nanofibers offers another route for enhancing antimicrobial performance [27]. Metal oxides such as ZnO, TiO₂, MgO and CaO have been investigated extensively due to their antimicrobial properties, chemical stability and relatively low cost [28]. Calcium oxide is of particular interest as an antibacterial additive owing to its low cost, environmental compatibility and bactericidal properties [29]. Its antimicrobial action has been associated with the generation of reactive oxygen species (ROS) and the development of alkaline conditions, which can induce oxidative stress, damage cellular membranes and promote protein denaturation in microbial cells [30]. Calcium oxide can be obtained from calcium-rich biowastes, such as eggshells and mollusk shells, providing an opportunity to convert waste resources into functional materials while supporting circular resource utilization [31,32].

The incorporation of biogenic CaO into a chitosan/PVA nanofibrous matrix may provide complementary antibacterial mechanisms while influencing the structural and thermal characteristics of the resulting membrane. Combining biodegradable polymers with waste-derived inorganic materials is consistent with the development of more sustainable filtration and disinfection technologies [33]. Despite the extensive research on electrospun antimicrobial membranes, studies specifically

addressing biodegradable chitosan/PVA/CaO composite nanofibers derived from eggshell waste for point-of-use water disinfection remain relatively scarce [34]. This research gap warrants investigation of composite nanofibers in which a biodegradable polymeric matrix and a waste-derived antibacterial phase are integrated into a single membrane platform.

The present study reports the fabrication of chitosan/PVA electrospun nanofibers loaded with CaO nanoparticles derived from eggshell waste for antibacterial water disinfection. The fabricated membranes were characterized by Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), field-emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM) and thermogravimetric analysis (TGA) to examine their chemical structure, crystalline characteristics, morphology and thermal behaviour. The antibacterial activity against *E. coli* was assessed using the agar well diffusion method. The persistence of antibacterial activity after one month of storage was examined to obtain preliminary evidence of the storage stability of the developed nanofibrous material.

EXPERIMENTAL

Chitosan (medium molecular weight, degree of deacetylation $\geq 75\%$), poly(vinyl alcohol) (PVA) and other analytical grade chemicals were obtained from Sigma-Aldrich and used without further purification. Waste eggshells were collected from local restaurants and used as the calcium-rich precursor for preparing calcium oxide nanoparticles. Distilled water was used throughout the experimental work.

Preparation of CaO nanoparticles from eggshell waste: Eggshell waste was washed thoroughly with distilled water to remove adhering organic matter and dried in a hot-air oven at 100 °C. The dried shells were crushed, ground into a fine powder and passed through a 100 μm sieve to obtain a uniform precursor. The powder was calcined in a muffle furnace at 800-900 °C for 2 h to convert calcium carbonate into calcium oxide. The colour change from off-white to bright white after calcination was taken as a preliminary indication of CaO formation.

Preparation of chitosan/PVA/CaO blend solution: 1 wt.% chitosan solution was prepared by dissolving chitosan (1 g) in 1% (v/v) acetic acid under continuous magnetic stirring for 6-8 h. 10 wt.% PVA solution was prepared separately in distilled water at 80 °C with constant stirring. The two polymer solutions were then blended at a 70:30 ratio until homogeneous. Calcined eggshell-derived CaO powder (0.5 wt.%) was incorporated into the blend and dispersed by vigorous stirring followed by ultrasonication to obtain a uniform and stable electrospinning solution.

Electrospinning of chitosan/PVA/CaO blend solution: The chitosan/PVA/CaO blend solution was electrospun using a high-voltage power supply, a syringe pump and a grounded collector covered with aluminium foil. Electrospinning was carried out under optimized conditions: applied voltage approximately 19 kV, tip-to-collector distance approximately 10 cm and feed rate approximately 1 $\mu\text{L min}^{-1}$. The fibers were collected as thin mats on the aluminium foil, dried at room temperature to remove residual solvent, carefully peeled off and

stored in a desiccator until characterization and antibacterial testing.

Characterization: XRD and FTIR were used to characterize the synthesized CaO and identify its crystalline phase and functional groups. FTIR analysis of the composite nanofibers assessed functional-group interactions among chitosan, PVA and CaO, while XRD was used to determine crystallinity and crystallite size using the Scherrer equation. TGA was performed to evaluate the thermal stability and degradation behaviour of the nanofiber membranes.

Antibacterial activity: The antibacterial activity of the electrospun nanofibers was evaluated against *E. coli* using the agar well diffusion method. Nutrient agar was prepared, sterilized and poured into sterile Petri dishes. A freshly cultured *E. coli* suspension was adjusted to 0.5 McFarland turbidity and uniformly spread over the agar surface using a sterile cotton swab. Wells of 6 mm diameter were punched into the agar, and 100 μ L of each test sample was aseptically added to the respective wells. Sterile distilled water and nalidixic acid served as the negative and positive controls, respectively. The plates were incubated at 37 °C for 24 h, after which the diameter of the inhibition zones was measured. All experiments were performed in triplicate and the mean inhibition zone diameter was calculated.

Most probable number (MPN) assay for liquid phase disinfection: The dynamic water disinfection efficiency of the electrospun composite membrane was evaluated using a most probable number (MPN) test with contaminated canal water. A section of the chitosan/PVA/CaO nanofiber mat was immersed directly in the contaminated water for 10 min under gentle agitation. Untreated canal water served as the control. Both control and membrane-treated samples were inoculated into lactose broth tubes containing inverted Durham tubes and incubated at 37 °C for 24-48 h. Coliform presence was assessed from gas production and acid formation during incubation.

RESULTS AND DISCUSSION

FTIR analysis of CaO nanoparticles: The FTIR spectrum of the synthesized CaO nanoparticles was recorded in the range of 4000–400 cm^{-1} (Fig. 1). A broad absorption band at 3640–3400 cm^{-1} is attributed to O-H stretching vibrations from adsorbed moisture and surface hydroxyl groups, reflecting the hygroscopic nature of CaO. A weak band around 1640 cm^{-1} corresponds to the bending vibration of water molecules. Intense bands in the region of 1470–1420 cm^{-1} , along with a band near 870 cm^{-1} , are assigned to carbonate CO_3^{2-} groups formed due to partial carbonation of CaO after exposure to atmospheric CO_2 . The presence of Ca-O lattice vibrations in the low-frequency region around 710 cm^{-1} confirms the successful formation of calcium oxide.

XRD analysis of CaO nanoparticles: The crystalline nature of the calcined eggshell powder was investigated by XRD (Fig. 2). The XRD pattern recorded over the 2θ range of 30–90° exhibited sharp, well-defined diffraction peaks, which confirmed the crystalline nature of the calcined CaO. The observed reflections are consistent with the thermal decomposition of CaCO_3 and its conversion to CaO. Crystallite sizes

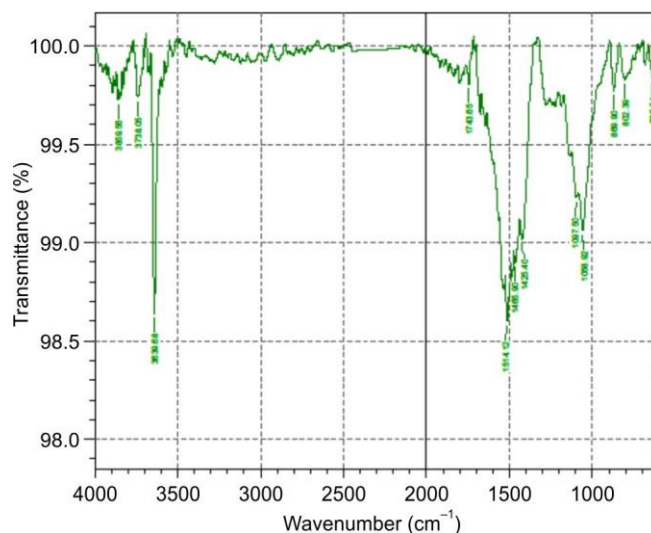


Fig. 1. FTIR spectrum of CaO nanoparticles

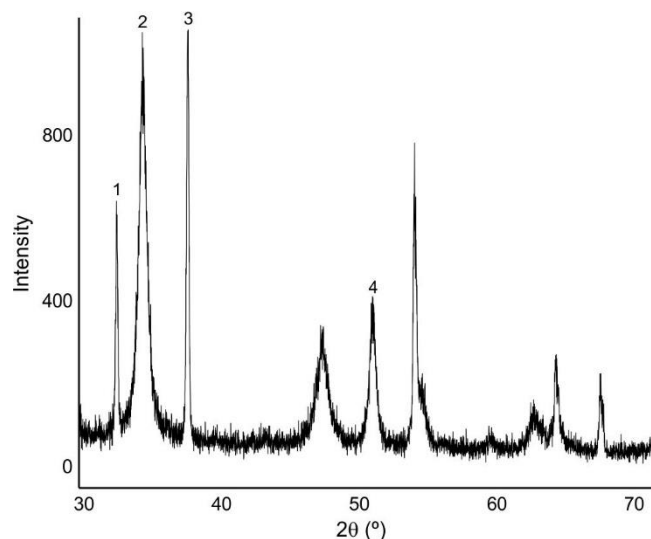


Fig. 2. XRD spectrum of CaO nanoparticles

calculated from the prominent diffraction peaks using the Scherrer equation were 11.36, 11.17, 2.48 and 28.29 nm, with an average crystallite size of approximately 13.33 nm. The results confirm the formation of nanoscale CaO crystallites. The diffraction peaks observed within the 2θ range of 30–90° are consistent with the face-centered cubic (FCC) structure of CaO and agree with the reference JCPDS pattern No. 37-1497.

FTIR analysis: The FTIR spectrum of the chitosan/PVA/CaO nanofibers (Fig. 3) shows the characteristic absorption bands of the composite system. The broad peak at 3282 cm^{-1} corresponds to the overlapping O-H and N-H stretching vibrations and indicates hydrogen bonding between chitosan and PVA. The absorption peak appearing at 1705 cm^{-1} is attributed to carbonyl (C=O) stretching vibrations associated with residual acetate groups from the PVA polymer backbone and intermolecular hydrogen bonding interactions between chitosan and PVA matrices, which is well-documented in blended biopolymer systems [35]. The band at 1433 cm^{-1} is due to the carbonate-related species associated with CaO, while the peak around 1102 cm^{-1} corresponds to the C-O-C stretching vibrations of the chitosan/PVA backbone. These spectral fea-

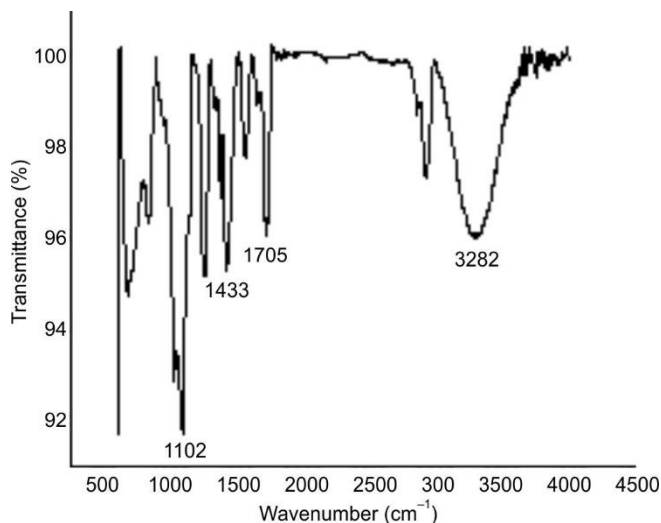


Fig. 3. FTIR spectrum of chitosan/PVA/CaO nanofibers

tures confirm the successful incorporation of CaO and the formation of an integrated composite nanofiber matrix.

XRD analysis: The crystalline characteristics of the electrospun chitosan/PVA/CaO nanofiber membrane were examined by XRD (Fig. 4). The pattern recorded in the 2θ range of $30\text{--}90^\circ$ exhibits distinct reflections together with broadened peaks, indicating the semi-crystalline nature of the composite nanofibers. The broad features are associated with the polymeric chitosan/PVA matrix, while sharper reflections arise from CaO-containing crystalline domains. Crystallite sizes calculated using the Scherrer equation for three prominent peaks were 7.70, 10.35 and 5.14 nm, with corresponding FWHM values of 1.04, 0.77 and 1.55, respectively. The average crystallite size of approximately 7.73 nm confirms the presence of nanocrystalline domains within the electrospun membrane. CaO nanoparticles may act as nucleating centres and promote interfacial interactions with the polymer matrix, thereby contributing to structural reinforcement.

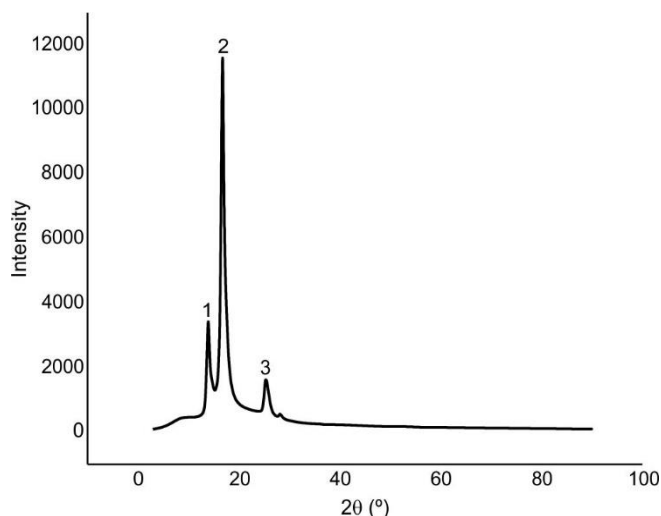


Fig. 4. XRD spectrum of chitosan/PVA/CaO nanofibers

FESEM analysis: The surface morphology of the chitosan/PVA/CaO nanofiber membrane was examined by FESEM. The image (Fig. 5a) reveals a continuous, randomly oriented

and highly interconnected fibrous network with a uniform, bead-free morphology. The electrospun membrane exhibited smooth and continuous fibers with relatively uniform morphology. No pronounced beads, fiber breakage or major structural defects were evident in the examined region. The observed morphology is consistent with favourable spinning conditions and adequate polymer-chain entanglement in the chitosan/PVA system. The porous web-like architecture is favourable for water purification as it provides a high surface-area-to-volume ratio and facilitates contact between waterborne microorganisms and antibacterial active sites. Although individual CaO nanoparticles are not clearly visible at the selected magnification, the absence of large agglomerates suggests satisfactory dispersion within the polymer matrix.

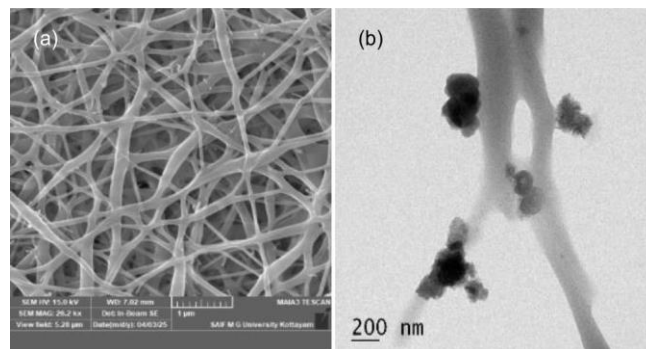


Fig. 5. (a) FESEM and (b) TEM images of chitosan/PVA/CaO nanofibers

TEM analysis: TEM analysis further confirmed the formation of smooth and continuous fibers with diameters in the range of approximately 100–250 nm (Fig. 5b). The lighter regions correspond to the chitosan/PVA polymer matrix, whereas darker electron-dense spots are attributed to CaO nanoparticles embedded within or attached to the fiber surface. The observed morphology supports the successful incorporation of the inorganic phase into the electrospun nanofibrous matrix.

Thermal stability: The thermal stability and decomposition behaviour of the chitosan/PVA/CaO nanofibers were evaluated by TGA. The TGA curve (Fig. 6) shows three main stages of weight loss. The initial weight loss of approximately 12.52% between 31.74 and 162.33°C is attributed to the evaporation of physically adsorbed moisture and low molecular weight volatile components. The major weight loss of 54.87% in the range of $214.20\text{--}388.14^\circ\text{C}$ corresponds to the thermal degradation of the polymeric components, mainly chitosan and PVA. A minor weight loss of 3.66% between 674.74 and 746.93°C may be associated with residual organic matter and reactions involving the inorganic phase. The residual mass of approximately 9.31% at higher temperature confirms the presence of thermally stable inorganic content, primarily CaO, in the nanofibrous membrane.

Antibacterial activity and shelf stability of electrospun nanofibers: The electrospun chitosan/PVA/CaO nanofibers demonstrated pronounced antibacterial activity against *E. coli*, as evidenced by the well diffusion assay. Nalidixic acid was used as the positive control and the antibacterial efficiency of the nanofibers was evaluated by measuring the diameter of the inhibition zones formed around the wells. The clear inhibition zones confirmed that the active components

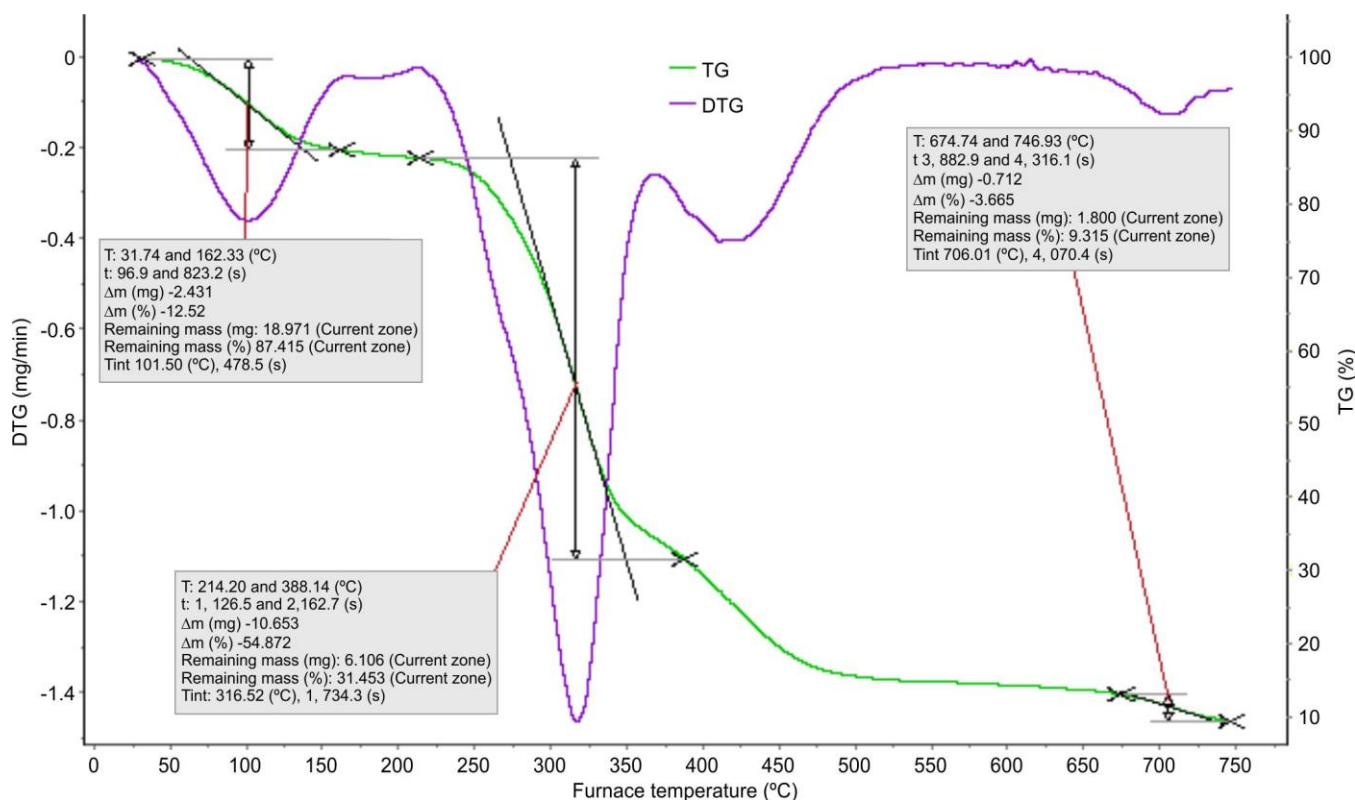


Fig. 6. TGA curve of chitosan/PVA/CaO nanofibers

within the nanofibrous matrix were able to diffuse effectively through the agar medium and suppress the growth of *E. coli*.

The antibacterial activity of the electrospun nanofibers can be attributed to the combined contribution of chitosan and CaO nanoparticles incorporated within the fibrous matrix. Chitosan can interact with negatively charged bacterial cell membranes through its protonated amino groups, altering membrane integrity and promoting the leakage of intracellular components. CaO nanoparticles may contribute through reactive oxygen species generation and the development of alkaline conditions, which can increase cellular stress and damage. The porous nanofibrous structure provides a large interfacial area that can facilitate contact between the antibacterial components and bacterial cells, contributing to the observed inhibition of *E. coli*.

The storage stability of the chitosan/PVA/CaO nanofibers was assessed by comparing the antibacterial activity of freshly prepared membranes with that of samples stored under ambient conditions for one month. The fresh nanofibers exhibited an inhibition zone of 2.0 ± 0.06 cm against *E. coli*, while the stored samples produced an inhibition zone of 1.8 ± 0.05 cm. This corresponds to a relatively small reduction of approximately 10% in inhibition zone diameter after one month of storage. The retained antibacterial activity indicates that the active components remained functionally effective within the nanofibrous matrix during the storage period.

The inhibition observed after one month, together with the activity recorded for the freshly prepared membrane, supports the short-term storage stability of the chitosan/PVA/CaO system. The results provide preliminary evidence for its use as an antibacterial membrane for water-treatment applications. However, longer storage periods, repeated-use studies, leaching analysis

and quantitative microbial reduction tests would be required to establish its long-term performance and practical applicability.

Comparative evaluation of individual components and synergistic antibacterial mechanism: To assess the contribution of the individual components to the antibacterial performance, comparative tests were conducted using pure chitosan, chitosan/PVA blends, biogenic CaO nanoparticles and the chitosan/PVA/CaO nanocomposite. As presented in Table-1, pure chitosan and the chitosan/PVA blend exhibited inhibition zones of 1.2 and 1.6 cm against *E. coli*, respectively. The antibacterial activity of chitosan is primarily associated with electrostatic interactions between its protonated amino groups and the negatively charged bacterial cell surface [36]. Biogenic CaO nanoparticles produced a smaller inhibition zone of 0.7 cm, which may be related to localized ROSs generation and alkaline stress following contact with moisture. After incorporation of CaO into the chitosan/PVA matrix and subsequent electrospinning, the resulting nanofibers exhibited a substantially larger inhibition zone of 2.0 cm. The enhanced inhibition relative to the individual components suggests that the combined presence of chitosan, PVA and CaO provides a

TABLE-1
ZONE OF INHIBITION OF DIFFERENT
COMPONENTS AGAINST *E. coli*

Component	Zone of inhibition (cm)
Nalidixic acid	2.0 ± 0.05
Chitosan	1.2 ± 0.04
Chitosan + PVA	1.6 ± 0.04
CaO (ESP)	0.7 ± 0.03
Chitosan + PVA+ CaO (ESP)	1.0 ± 0.05

more effective antibacterial response against *E. coli*. This improvement may arise from the simultaneous contribution of chitosan mediated membrane interactions, CaO-associated oxidative and alkaline effects and the high interfacial area of the electro-spun nanofibrous structure.

Evaluation of water disinfection efficiency by MPN analysis: Microbiological quality of the contaminated water from canal before and after direct contact disinfection using the chitosan/PVA/CaO nanofiber membrane was established through the most probable number (MPN) technique. Prior to the direct contact experiment, canal water showed positive results in the MPN method, as indicated by the considerable amount of gas formation inside the Durham tube in the lactose broth test. This result signifies the existence of live coliform bacteria, in the untreated canal water. Direct immersion of the nanofiber membrane in the canal water for 10 min resulted in a dramatic decrease in the number of positive test tubes and gas formation (Fig. 7).

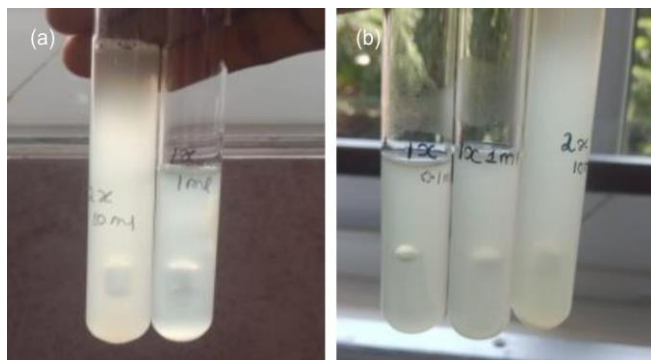


Fig. 7. MPN test results for canal water samples: (a) untreated canal water and (b) canal water after exposure to nanofiber

The significant disinfection can be contributed to the combined effect of the bioactive compounds encapsulated in the nanofibrous matrix. The electrostatic attraction between the protonated chitosan and the microbial membrane causes structural damage and the loss of cell contents and, at the same time, the presence of biogenic CaO nanoparticles provides oxidative stress conditions and a basic pH environment. In addition, the large surface area and interconnected pores of the electrospun matrix ensure effective contact of the antibacterial sites with the waterborne pathogens.

Monitoring of water pH changes during treatment:

The pH of the canal water was measured before and after exposure to the electrospun chitosan/PVA/CaO nanofiber membrane to assess changes in water chemistry. As shown in Table-2, the initial pH of the untreated canal water was 6.82 ± 0.05 . After direct contact with the nanofiber membrane for 10 min, the pH increased to 7.54 ± 0.04 , representing a shift toward mildly alkaline conditions. This change can be associated with the presence of biogenic CaO within the chitosan/PVA matrix. Upon contact with water, CaO undergoes hydration to form $\text{Ca}(\text{OH})_2$, which can release hydroxyl ions and increase the pH of the surrounding medium. The observed shift from mildly acidic to slightly alkaline conditions is consistent with the alkaline nature of CaO and its interaction with water during the treatment period. The pH remained within a near-neutral to mildly alkaline range after treatment, which is

TABLE-2
EFFECT OF NANOFIBER SACHET
FILTER ON pH OF WATER SAMPLE

Water sample	pH
Untreated canal water	6.82 ± 0.05
Treated canal water	7.54 ± 0.04

relevant to the suitability of the membrane for the water disinfection applications.

Aqueous stability and Ca^{2+} leaching considerations:

For water purification applications, the aqueous stability of the nanofiber membrane and potential leaching of Ca^{2+} ions are important considerations. Upon contact with water, CaO incorporated within the chitosan/PVA matrix may undergo hydration and contribute to the observed increase in water pH. In this study, the pH increased from 6.82 ± 0.05 to 7.54 ± 0.04 after 10 min of treatment. However, quantitative determination of Ca^{2+} release and membrane mass loss was not performed in the present investigation.

Conclusion

Biodegradable chitosan/PVA/CaO composite nanofibers were successfully fabricated by electrospinning for potential application in point-of-use water disinfection. The optimized chitosan/PVA formulation enabled the formation of relatively uniform, bead-free nanofibers, while eggshell-derived CaO nanoparticles provided a biogenic inorganic antibacterial component. FTIR, XRD, FESEM, TEM and TGA analyses established the chemical, structural, morphological and thermal characteristics of the composite nanofibrous matrix and confirmed the incorporation of nanoscale CaO. The composite nanofibers exhibited pronounced antibacterial activity against *E. coli*. This activity can be associated with the combined contribution of chitosan-mediated interactions with bacterial cell membranes and CaO-associated alkaline and oxidative effects. The interconnected porous structure of the electrospun membrane provides a large interfacial area that can facilitate contact between the antibacterial components and bacterial cells. Comparative experiments showed that the chitosan/PVA/CaO nanofibers produced a larger inhibition zone than the individual chitosan, chitosan/PVA and CaO samples, which confirmed enhanced antibacterial response from the composite system. The water-disinfection performance was further assessed using contaminated canal water. MPN analysis showed a reduction in coliform-positive responses after 10 min of contact with the nanofiber membrane. The treatment was accompanied by an increase in water pH from 6.82 ± 0.05 to 7.54 ± 0.04 , which is consistent with the hydration of CaO and the resulting alkaline contribution. The membrane retained most of its antibacterial activity after one month of ambient storage, with the inhibition-zone diameter decreasing from 2.0 to 1.8 cm. These findings provide preliminary evidence for the use of eggshell-derived nano CaO-loaded chitosan/PVA nanofibers as biodegradable antibacterial materials for point-of-use water-treatment applications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language and no data, results or scientific conclusions were generated by AI. All analyses, interpretations and conclusions are the authors' own. The authors reviewed and edited the content and take full responsibility for the published work.

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