

Original Article

Comparative Evaluation of Silver, Neem, and Chitosan-Loaded PVA-CMC Electrospun Nanofibrous Mats for Potential Antibacterial Application

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Received: 02 May 2026

Revised: 04 June 2026

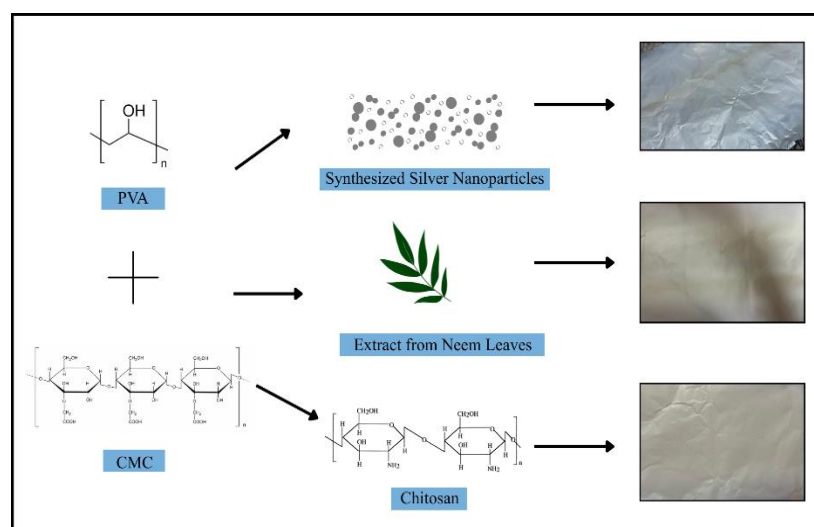
Accepted: 29 June 2026

Published: 13 July 2026

Abstract - Electrospinning has emerged as a versatile and efficient technique for producing antibacterial nanofibrous mats by enabling the incorporation of various bioactive agents into polymeric fiber systems. In this study, nanofibrous mats based on poly(vinyl alcohol) (PVA) and Carboxymethyl Cellulose (CMC) polymers were successfully fabricated with the inclusion of three different Antibacterial Agents, Namely Chitosan, Silver Nanoparticles (AgNPs), and ethanolic extract of neem (*Azadirachta indica*) leaf. The electrospinning solutions were prepared using a fixed volumetric ratio (PVA:CMC:antibacterial additive) in order to establish a comparative evaluation of structural and antibacterial performance under identical conditions. Synthesized AgNPs exhibited a narrower particle size distribution, confirming uniform nanoparticle formation. SEM images revealed the formation of continuous, randomly oriented nanofibrous networks. FTIR spectroscopy confirmed the presence of characteristic functional groups of the antibacterial agents without significant alteration of the base polymeric structures. The agar disk diffusion method was employed to evaluate antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, and the results demonstrated that AgNPs-loaded mats exhibited the highest inhibition zones, followed by neem-loaded mats, while chitosan showed comparatively lower activity. Overall, this study highlights the comparative effectiveness of incorporating different antibacterial agents into PVA-CMC polymer-based nanofibers.

Keywords - Antibacterial nanofibrous mats, Carboxymethyl cellulose, Electrospinning, Silver nanoparticles, Poly(vinyl alcohol).

Graphical Abstract



1. Introduction

Electrospinning is a well-established technique for producing polymeric fibers via electrostatic forces. It can generate fibers with diameters ranging from a few nanometers to several micrometers using both natural and synthetic polymer solutions. In relation to other methods of forming sub-micrometer and nanoscale fibers, electrospinning is considered an economical, scalable, and moderately automated approach that requires minimal labor. In recent years, this method has garnered significant research and commercial attention due to its ability to fabricate nanofibers with unique features. These nanofibers exhibit tunable surface functionality, controllable pore structure, high surface area-to-volume ratio, and enhanced mechanical properties compared to conventional fibers. Such characteristics make them highly suitable for a wide range of applications, including wound dressings, drug delivery, biotechnology, tissue engineering, self-cleaning surfaces, filtration, sensing, and environmental engineering. [1], [2], [3].

Poly(vinyl alcohol) (PVA) is a water-soluble polymer characterized by the presence of abundant hydroxyl groups along its side chains, contributing to its high hydrophilicity. It exhibits excellent film-forming ability, easy spinnability, favorable physical and mechanical properties, biocompatibility, strong chemical resistance, and biodegradability [4], [5]. Recently, attention has focused on its biomedical applications, particularly as electrospun nanofibers, due to its breathability, flexibility, and ability to maintain a moist environment [6], [7], [8], [9].

Despite being one of the most abundantly available natural polymers, cellulose presents limitations in electrospinning owing to the subsequent difficulties in dissolution and spinning [9]. Carboxymethyl Cellulose (CMC), a derivative of cellulose, exhibits excellent water absorption properties; however, its increasing viscosity makes it less suitable for direct electrospinning [4]. Additionally, the presence of specific chain conformations, hydrodynamic behavior, and repulsive interactions among the polyanionic chains of CMC restricts its practical applications and makes the electrospinning process complex. To address these limitations, many studies have employed CMC in electrospinning along with other easily spinnable polymers as support [10]. PVA and CMC together demonstrate strong interaction due to the hydrogen bonding. The ability to blend with water-soluble polymers like PVA has significantly expanded the application potential of CMC-based biomaterials in wound dressing. In this study, PVA and CMC are selected as base polymers due to their water solubility, which facilitates the formation of a homogeneous solution suitable for electrospinning [11], [12].

Silver nanoparticles (AgNPs) demonstrate exceptional antimicrobial activity against a variety of pathogenic microorganisms, including multidrug-resistant bacteria, and

have been incorporated into various polymer systems via electrospinning [13], [14], [15], [16], [17]. Similarly, neem (*Azadirachta indica*) is a renowned medicinal plant containing nearly 35 biological active compounds, including azadirachtin, nimbin, alkaloids, flavonoids and phenolic compounds. Its leaves have been reported to have antibacterial properties [18], [19]. Recent studies have investigated the incorporation of neem leaf extracts into electrospun fibers [20], [21], [22].

Chitosan, widely recognized for its antibacterial efficacy, has attracted significant academic and industrial interest since the 1970s [23]. It is known to be difficult to electrospin due to its rigid crystalline structure and strong hydrogen bonding, which result in poor solubility in common organic solvents [24]. To overcome these limitations, it is often blended with PVA, a combination that has been widely explored in electrospinning, as it facilitates the formation of uniform nanofibrous membranes with significant potential for biomedical applications [25], [26], [27].

Although the electrospinning of PVA and CMC has been investigated extensively [4], [10], [28], [29], their combination with different antibacterial agents remains underexplored. Similarly, silver nanoparticles, neem extract, and chitosan have individually been incorporated into electrospun nanofibers in previous studies. However, direct comparative investigations using a common PVA-CMC polymer matrix under identical electrospinning and antibacterial testing conditions remain limited. Most reported studies focus on a single antibacterial agent, making it difficult to assess their relative effectiveness and influence on fiber morphology. Therefore, this study aims to systematically compare AgNPs, neem leaves extract, and chitosan within the same electrospun PVA-CMC system to provide insights into material selection for antibacterial nanofibrous applications. This study adopts a novel approach by incorporating chitosan, silver nanoparticles, and neem extracts into a PVA-CMC base polymer system, allowing a comparative evaluation of their structural and antibacterial properties under equivalent conditions.

2. Materials and Methods

2.1. Materials

Poly(vinyl alcohol) (PVA) powder was purchased from Modern Scientific Co. (Hatkhola, Dhaka, Bangladesh). Carboxymethyl Cellulose (CMC) powder (commercial grade) was obtained from Dolphin Scientific Co. (Hatkhola, Dhaka, Bangladesh). PVA and CMC were used as base polymers for all electrospun samples.

Three antibacterial agents - chitosan, silver nanoparticles (AgNPs), and neem (*Azadirachta indica*) leaf extract - were incorporated into the polymer system. Low-viscosity chitosan (75% degree of deacetylation) was procured from Dolphin Scientific Co. Glacial acetic acid (100%, anhydrous) was used

for chitosan dissolution. Silver nitrate (AgNO_3) was obtained from Dolphin Scientific Co. and used as a precursor for silver nanoparticle synthesis. Sodium hydrosulfite (hydrose) served as the primary reducing agent. Ethanol was used in nanoparticle synthesis and neem extraction. Fresh neem leaves were collected from the *Azadirachta indica* plant in the Bangladesh University of Textiles campus. All reagents were used without further purification.

2.2. Preparation of Base Polymer Solutions

A 10% (w/v) PVA solution was prepared by dissolving 2.5 g of PVA in 25 mL of distilled water under magnetic stirring at 700 rpm and 70 °C until a clear and homogeneous solution was obtained. A 2% (w/v) CMC solution was prepared by dissolving 0.4 g of CMC in 20 mL of distilled water, followed by stirring at 700 rpm at room temperature to ensure complete dissolution.

2.3. Preparation of Functional Agents

To ensure uniform comparison among samples, each antibacterial agent solution was prepared at a concentration of 7% (w/v).

2.3.1. Silver Nanoparticles (AgNPs) Synthesis

Silver nanoparticles were synthesized via chemical reduction. A 7% (w/v) AgNO_3 solution was prepared by dissolving 3.5 g of silver nitrate in 50 mL of distilled water. From this solution, 15 mL was taken and stirred at 250 rpm at 35 °C. Ethanol (5 mL) was added dropwise, followed by the dropwise addition of 2 mL of 5% sodium hydrosulfite solution as a reducing agent. A visible color change from pale yellow to brown indicated nanoparticle formation. The reaction mixture was further stirred at 500 rpm to ensure complete reduction (Figure 1).

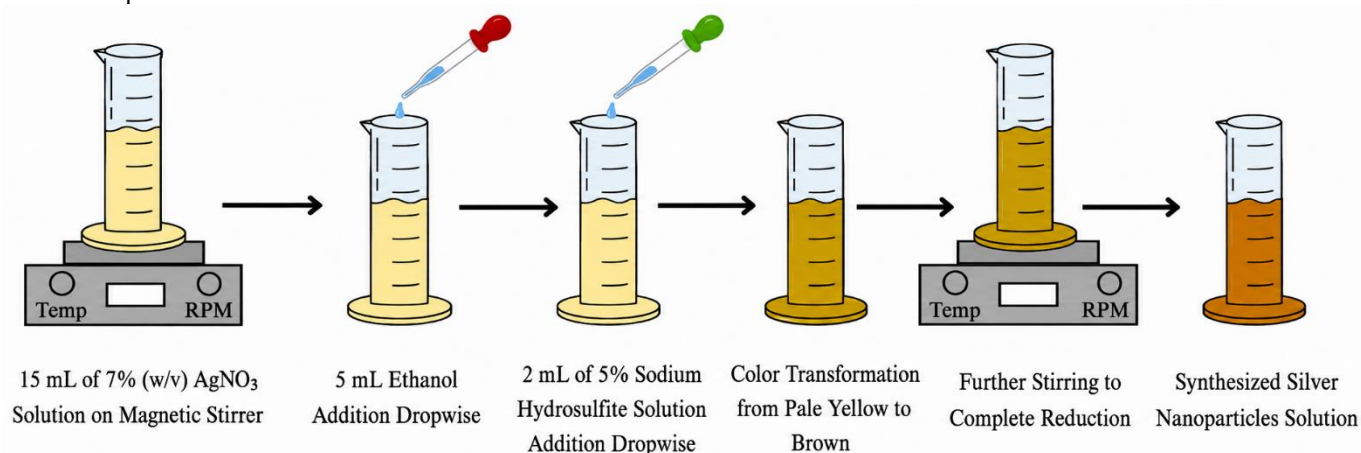


Fig. 1 Flow Diagram of Silver Nanoparticles Synthesis

2.3.2. Neem (*Azadirachta indica*) Leaf Extract

Fresh neem leaves were thoroughly washed, oven-dried at 45 °C for 1.5 h, and ground into fine powder. A 7% (w/v) ethanolic extract was prepared by soaking 1.4 g of powdered neem leaves in 20 mL of ethanol and allowing maceration for 24 h at room temperature. The extract was then filtered and used for further processing (Figure 2).

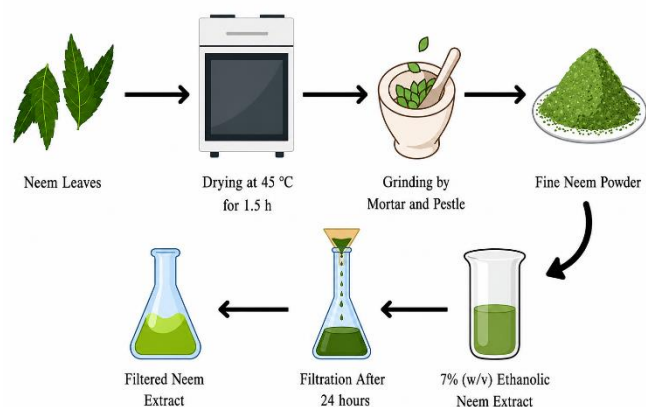


Fig. 2 Process Schematic of Neem Leaf Extract Preparation

2.3.3. Chitosan

A 7% (w/v) chitosan solution was prepared by dissolving 0.7 g of chitosan in a mixture of 9 mL glacial acetic acid and 1 mL distilled water. The solution was stirred at 500 rpm at room temperature to obtain a homogeneous solution.

2.4. Preparation of Electrospinning Solution

A total volume of 20 mL of electrospinning solutions was prepared by mixing PVA, CMC, and the respective antibacterial agent in a volumetric ratio of 12:3:5 (PVA:CMC:Functional Agent). The mixtures were stirred at 600 rpm for 6 h to obtain homogeneous solutions.

The compositions were as follows:

Sample 1 (PVA-CMC-AgNPs): 12 mL of 10% PVA + 3 mL of 2% CMC + 5 mL of AgNPs-containing solution.

Sample 2 (PVA-CMC-Neem Extract): 12 mL of 10% PVA + 3 mL of 2% CMC + 5 mL of 7% Neem extract.

Sample 3 (PVA-CMC-Chitosan): 12 mL of 10% PVA + 3 mL of 2% CMC + 5 mL of 7% Chitosan solution.

2.5. Electrospinning Process

Electrospinning was performed using Nanospinner (Inovenso, Istanbul, Turkey). Each 20 mL electrospinning solution was distributed into two syringes fitted with 18G needles. Electrospinning was conducted under 26 kV voltage, employing a flow rate of 1 mL/h. Nanofibers were collected on aluminium foil (22 cm × 32 cm) attached to a collector drum rotating at a speed of 100 rpm. The horizontal distance between the tip of the needles and the collector drum was 12 cm (Figure 3). The developed electrospun mats were allowed to dry for 24 h before removal from the aluminium foil. The entire electrospinning process was conducted under ambient laboratory conditions.

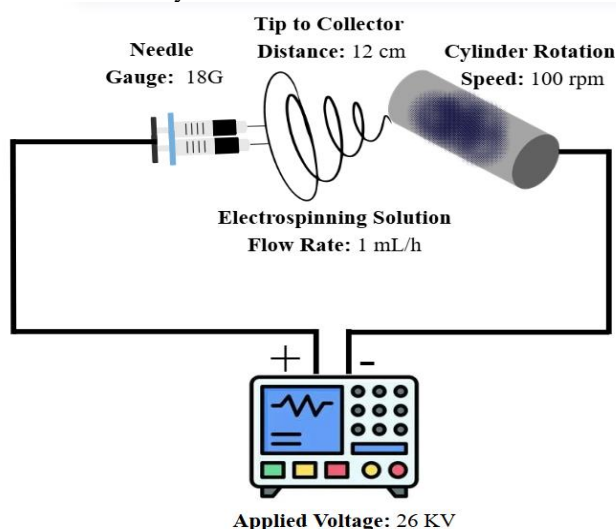


Fig. 3 Schematic Representation of Electrospinning Assembly

2.6. Characterization

The particle size distribution of the synthesized silver nanoparticles was analyzed using laser diffraction (ANALYSETTE 22 Next, FRITSCH, Germany) over a measurement range of 10 nm to 3.8×10^6 nm. The Mie scattering model was applied with a refractive index of 1.54 and an absorption coefficient of 0. All measurements were conducted under identical dispersion conditions (pump speed, ultrasonic intensity, etc.) to ensure consistency. The analysis was conducted thrice to evaluate the reproducibility and distribution uniformity of the synthesized AgNPs. The particle size span was calculated to verify distribution uniformity. RMS (Root Mean Square) error values were also recorded to assess the fitting accuracy of the Mie model.

The surface morphology of electrospun mats was examined using a FESEM (JEOL JSM-7610F, Japan) operated at an accelerating voltage of 5 kV. A total of 50 randomly selected fibers per sample were analyzed from SEM (Scanning Electron Microscope) images using ImageJ to determine fiber diameters, and the results were reported as mean $\pm$ standard deviation. Statistical analysis for the diameter distribution of the electrospun mats was performed using Origin software.

Fourier Transform Infrared (FTIR) Spectroscopy was employed to identify the functional groups present in the electrospun samples and to confirm and compare the incorporation of different antibacterial functional materials into the base polymer system. FTIR spectra were recorded in the range of 400 cm^{-1} to 4000 cm^{-1} with Origin software.

The agar disk diffusion method was followed to evaluate antibacterial activity at the Bangladesh Council of Scientific and Industrial Research. Samples were tested against Gram-positive *Staphylococcus aureus* ATCC 6358 and Gram-negative *Escherichia coli* ATCC 25922. These strains were grown in nutrient broth at 37°C for 24 h. Bacterial growth suspension was compared with a 0.5 McFarland turbidity standard, which denotes 1.5×10^8 CFU/ml. The agar disk diffusion method, with slight modification, was performed to assess the antimicrobial activity [30]. Electrospun samples were placed on inoculated Mueller-Hinton (Condalab, Spain) agar plates and incubated at 37°C for 24 h. Antibacterial activity was assessed by measuring the Diameter of the inhibition zones surrounding the samples.

3. Results and Discussion

3.1. Analysis of Synthesized Silver Nanoparticles

Particle size analysis was conducted to verify the presence of nanoparticles in the synthesized solution. The diameter distribution of the synthesized particles is stated in Table 1.

Table 1. Characteristic Particle Diameters Derived from the Cumulative Undersize Distribution

Cumulative Undersize (%)	Mean Diameter (nm)	CV%
5 (D5)	80	0.11
10 (D10)	90	0.06
25 (D25)	100	0.01
50 (D50)	110	0.05
75 (D75)	120	0.01
90 (D90)	150	2.73

The results confirmed that the particles predominantly lie within the nanoscale range, specifically, 90% of particles were below 150 nm, indicating minimal formation of larger aggregates. The coefficient of variation (CV%) for D5 to D75 ranged between 0.01% and 0.11%, demonstrating excellent repeatability across measurements. Although the CV% for D90 cumulative undersize was comparatively higher (2.73%), this deviation could be attributed to a minor fraction of larger particles.

$$\text{Particle Size Span} = \frac{D_{90} - D_{10}}{D_{50}} \quad (1)$$

The particle size span was calculated from Equation 1 [31]. The value of the size span was obtained as 0.55, signifying that the synthesized AgNPs possessed a narrow

distribution. Low RMS error values (0.056 to 0.072) confirmed the reliability of the particle size measurements. These findings demonstrate that the adopted synthesis method produced AgNPs with a narrow size distribution and high reproducibility.

3.2. Surface Morphology of Electrospun Mats

The SEM micrographs of the electrospun nanofiber mats composed of AgNPs-PVA-CMC, Neem-PVA-CMC, and

Chitosan-PVA-CMC are presented in Figure. 4. The observed surface morphology reveals the formation of randomly oriented, continuous fibrous networks for all samples, indicating successful electrospinning [32].

The variation in fiber diameters across the developed samples can be correlated with changes in solution properties, including viscosity, surface tension, conductivity, and molecular interactions [2], [3].

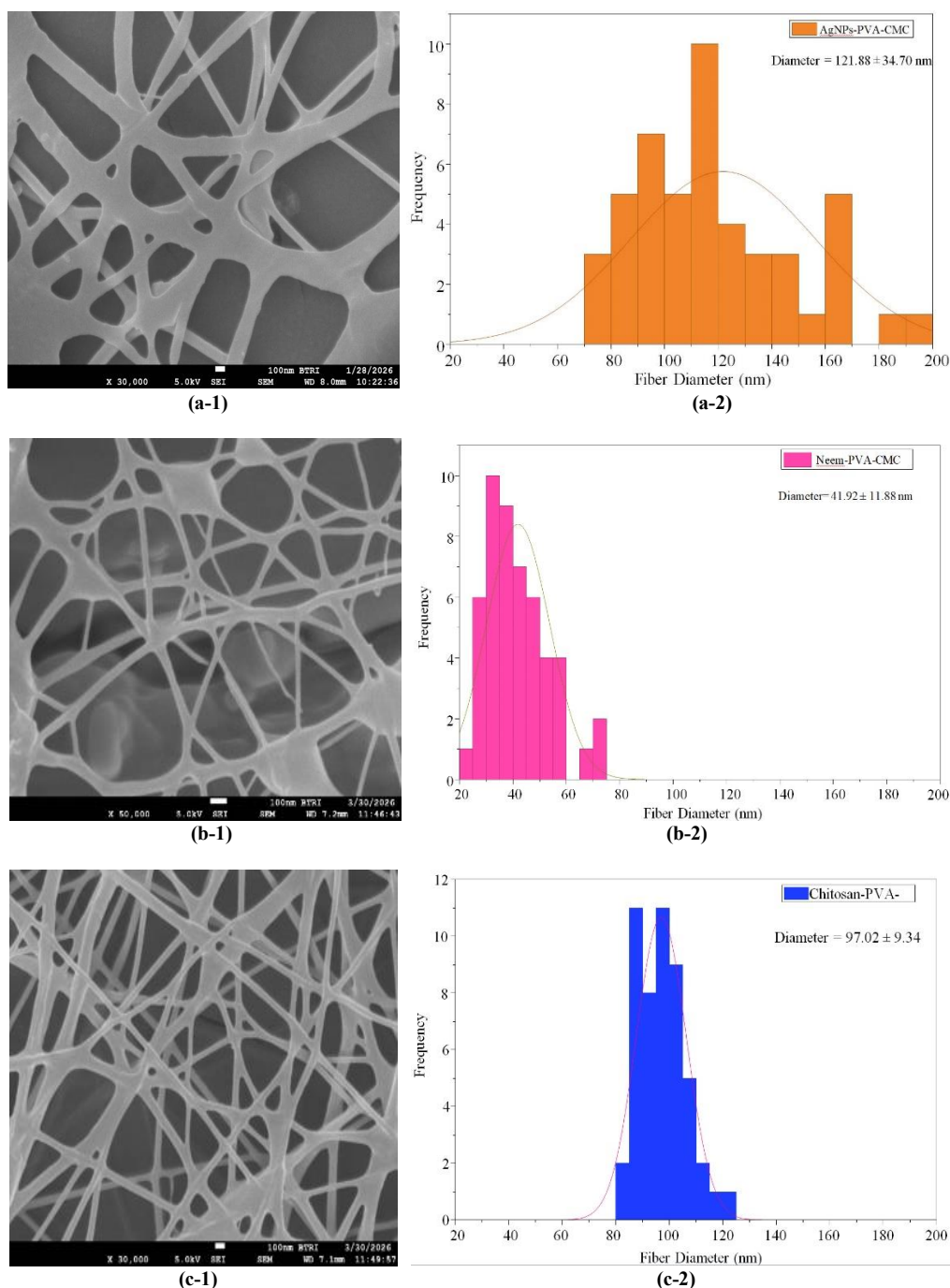


Fig. 4 SEM Micrographs of the Developed Electrospun Nanofibrous Mats (a) AgNPs-PVA-CMC, (b) Neem-PVA-CMC, (c) Chitosan-PVA-CMC

Table 2 summarizes the average fiber diameter and diameter distribution of the developed electrospun nanofibrous mats as determined from SEM micrographs. The results reveal that the type of antibacterial agent incorporated into the PVA-CMC matrix affected the morphology and uniformity of the resulting nanofibers.

Table 2. Fiber Diameter Distribution of the Developed Electrospun PVA-CMC Nanofibrous Mats Derived from SEM Analysis

Sample	Mean Fiber Diameter (nm)	SD (nm)
AgNPs-PVA-CMC	121.88	34.70
Neem-PVA-CMC	41.92	11.88
Chitosan-PVA-CMC	97.02	9.34

The AgNPs-PVA-CMC nanofibers exhibit relatively uniform and smooth fibers with slight variations in diameters. The incorporation of AgNPs does not significantly interrupt the fiber formation.

However, minor surface irregularities as well as beads were observed, which could be attributed to the addition of nanoparticles within the base polymer mixture [13].

Fiber diameters averaged 121.88 ± 34.70 nm, spanning from 75.52 nm to 200 nm. These findings are comparable to those reported in earlier studies involving AgNPs-integrated electrospun PVA nanofibers [13], [33].

In contrast, the neem-PVA-CMC nanofibers demonstrate significantly finer and more uniform fibers. The fibers exhibited an average diameter of 41.92 ± 11.88 nm, with a distribution between 22.53 nm and 74.99 nm, which is considerably lower than that of the other samples and closely corresponds to the findings reported in a previous study on neem-loaded electrospun nanofibers [34].

The chitosan-PVA-CMC nanofibers show relatively smooth and continuous fibers with a more homogeneous structure compared to other samples. A mean diameter of 97.02 ± 9.34 nm was observed for the fibers, with sizes varying from 81.37 nm to 123.81 nm, indicating a narrow distribution and improved uniformity.

This is in good agreement with existing literature on chitosan-containing electrospun PVA-based nanofibers [35], [36], [37]. The incorporation of chitosan enhances intermolecular interactions within the PVA solution, potentially contributing to stable fiber formation and low diameter variation [38], [39].

3.3. FTIR Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy was employed to identify the functional groups present in the developed nanofibrous mats and to explore the possible interactions among the constituent compounds. The FTIR spectra of the composite nanofibers are presented in Figure. 5.

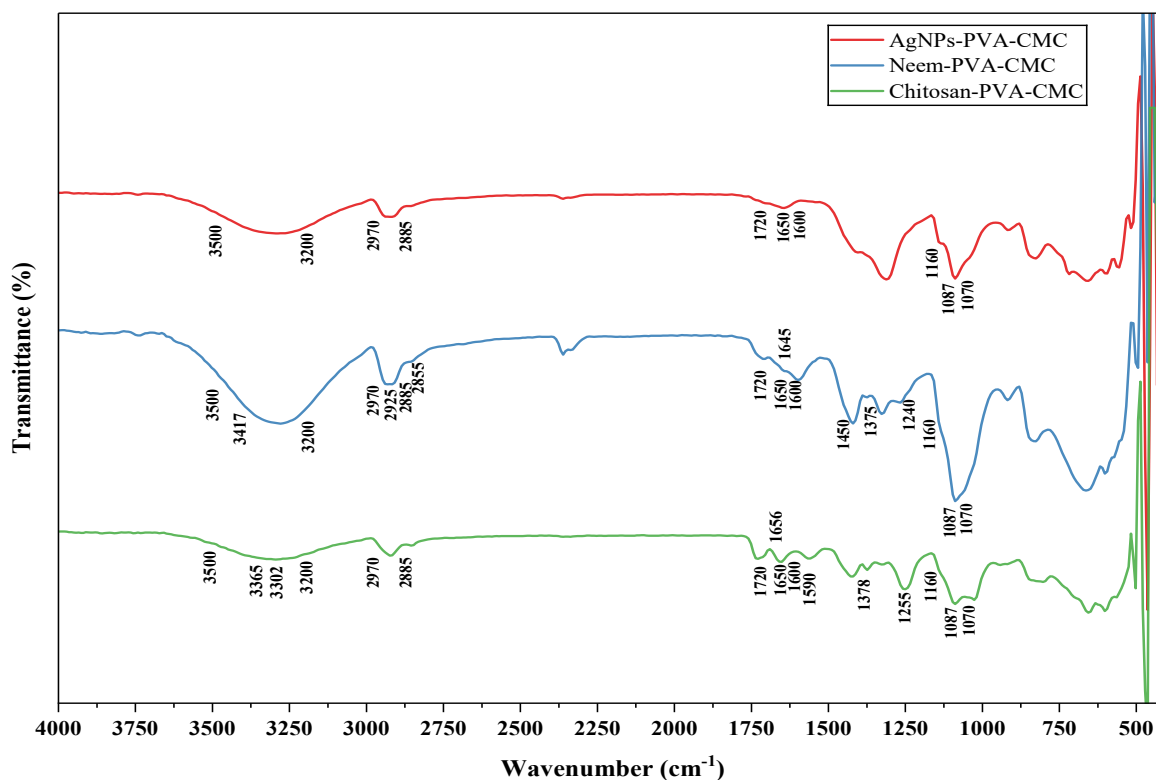


Fig. 5 FTIR Spectrum of the Developed Electrospun Nanofibrous Mats

A broad absorption band observed in the range of 3200 cm^{-1} to 3500 cm^{-1} is attributed to the stretching vibrations of OH groups, primarily originating from PVA and CMC and contributing to hydrophilicity. The broadening of this peak indicates extensive hydrogen bonding across the samples [10], [13], [38]. Other characteristic peaks of PVA were identified at approximately 2885 cm^{-1} to 2970 cm^{-1} and $\sim 1087 \text{ cm}^{-1}$, related to C-H stretching vibrations and C-O stretching vibrations, respectively. Additionally, a peak near $\sim 1720 \text{ cm}^{-1}$, indicating C=O stretching vibration, may be associated with residual acetate groups present in partially hydrolyzed PVA [10], [13], [21], [38]. The CMC component exhibited a distinct absorption band near 1600 cm^{-1} to 1650 cm^{-1} , corresponding to the stretching vibration of carbonyl groups. Peaks within the 1070 cm^{-1} to 1160 cm^{-1} region further confirm C-O stretching vibrations associated with alcohol as well as other functionalities in the cellulose backbone [10], [13], [40].

For the chitosan-containing sample, the peaks due to O-H and N-H stretch remain evident at $\sim 3365 \text{ cm}^{-1}$ and $\sim 3302 \text{ cm}^{-1}$ [22]. Other significant peaks of chitosan are observed at $\sim 1656 \text{ cm}^{-1}$ (amide I) and $\sim 1590 \text{ cm}^{-1}$ (amide II). Moreover, the peaks at $\sim 1378 \text{ cm}^{-1}$ and $\sim 1255 \text{ cm}^{-1}$ are related to C-H and N-H bonds, respectively [38]. In the case of neem-integrated sample, additional peaks were observed near $\sim 3417 \text{ cm}^{-1}$ (O H stretching), $\sim 2925 \text{ cm}^{-1}$ (C-H stretching), $\sim 2855 \text{ cm}^{-1}$ (C H stretching), $\sim 1720 \text{ cm}^{-1}$ (C=O stretching), $\sim 1450 \text{ cm}^{-1}$ (C H bending), $\sim 1375 \text{ cm}^{-1}$ (C-H stretching), and $\sim 1240 \text{ cm}^{-1}$ (C OH stretching), which are characteristic features of bioactive compounds such as natural polyphenols and flavonoids. Furthermore, bands around $\sim 1645 \text{ cm}^{-1}$ indicate C=C stretching vibrations of aromatic structures [21], [22]. The FTIR spectrum of the AgNPs-loaded sample does not show any characteristic peak, particularly for silver [41], [42]. It may indicate that the incorporation of nanoparticles does not significantly alter the chemical structure of the polymers.

Overall, the FTIR results confirm the successful fabrication of the nanofibrous mats and demonstrate the presence of the major functional groups associated with PVA, CMC, chitosan, and neem leaf extract.

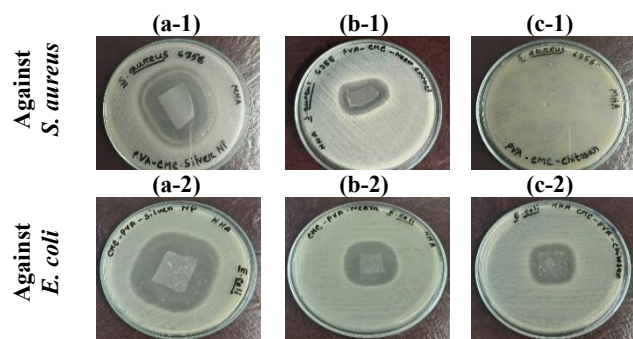


Fig. 6 Antibacterial Performance of the Developed Electrospun Nanofibrous Mats (a) AgNPs-PVA-CMC, (b) Neem-PVA-CMC, (c) Chitosan-PVA-CMC

3.4. Antibacterial Performance

The antibacterial activity of the developed electrospun samples was evaluated by measuring the zone of inhibition obtained from the agar disk diffusion method against Gram-negative *Escherichia coli* ATCC 25922 and Gram-positive *Staphylococcus aureus* ATCC 6358. The results presented in Figure. 6 revealed varied antibacterial performance depending on the incorporated bioactive agent.

The silver nanoparticle-loaded nanofibrous mats exhibited the largest inhibition zone, 51 mm against *E. coli* and 51.5 mm against *S. aureus*, indicating the highest antibacterial activity. The superior antibacterial performance of silver nanoparticle-loaded nanofibers is generally attributed to multiple synergistic mechanisms. AgNPs or released silver ions (Ag^+) show the potential to enter bacterial cells and interact with phosphorus- and sulfur-containing biomolecules, inhibiting DNA replication, and therefore, causing cell death [43], [44]. AgNPs can also induce the formation of reactive oxygen species (ROS), which produce free radicals with strong bactericidal effects [43], [45].

Compared with the silver nanoparticle-incorporated sample, neem-loaded PVA-CMC mats exhibited moderate antibacterial activity, with inhibition zone diameters of 33 mm and 32 mm against *E. coli* and against *S. aureus*, respectively. The observed activity is likely associated with bioactive constituents of neem, such as azadirachtin, nimbin, and flavonoids, which can potentially disrupt the bacterial cell wall [18], [19].

The chitosan-loaded sample formed an inhibition zone of 27 mm against *E. coli*, indicating a comparatively smaller inhibition zone. Unfortunately, the sample was unable to produce a measurable inhibition zone against *S. aureus*, suggesting limited antibacterial activity under the tested conditions. Similar findings against *S. aureus* have also been reported in previous studies involving chitosan [46], [47], [48]. This difference may be attributed to the variations in the cell wall compositions of Gram-negative and Gram-positive bacteria. The antibacterial activity of chitosan is mainly associated with its protonated amino groups, which conduct electrostatic interaction with negatively charged components on the bacterial cell surface.

Gram-negative bacteria contain a more negatively charged and hydrophilic surface due to the presence of lipopolysaccharides attached to phosphorylated groups in the outer membrane, facilitating the adsorption of cationic chitosan. This interaction can sufficiently enhance membrane permeability, causing leakage of intracellular compounds and eventually bacterial cell death. In contrast, Gram-positive bacterial strains possess a comparatively thicker peptidoglycan layer that can hinder the interaction of chitosan with the cell membrane, thereby reducing its antibacterial effectiveness [49], [50].

A comparative assessment of the three antibacterial agents indicates that the effectiveness followed the order under the selected experimental conditions:

AgNPs-PVA-CMC > Neem Extract-PVA-CMC > Chitosan-PVA-CMC

The results demonstrate that the choice of antibacterial additive plays a crucial role in determining the antibacterial performance of electrospun PVA-CMC nanofibrous mats. While silver nanoparticles provided the highest antibacterial efficacy, neem extract also exhibited promising antibacterial activity and may represent a more sustainable and plant-based alternative. Chitosan contributed moderate antibacterial functionality and may offer additional advantages such as biocompatibility and biodegradability for biomedical applications.

4. Limitations

Despite the promising findings, the current investigation primarily focused on morphological characterization, functional group analysis, and antibacterial performance of the developed electrospun nanofibrous mats only. Mechanical properties, release behavior, and durability of the developed materials were not evaluated.

The antibacterial performance was assessed using the agar disk diffusion method against only two bacterial strains, namely *Escherichia coli* and *Staphylococcus aureus*. Although these microorganisms are widely used as representative Gram-negative and Gram-positive bacteria, a broader evaluation involving additional pathogenic strains would provide a more comprehensive understanding of antibacterial effectiveness.

Furthermore, the present study did not investigate cytocompatibility, toxicity, or wound-healing performance, which are important considerations for prospective biomedical applications. Therefore, further studies are necessary to comprehensively evaluate the practical applicability of the developed nanofibrous materials.

5. Conclusion

In this study, electrospun PVA-CMC-based nanofibrous mats incorporated with silver nanoparticles, neem extract, and chitosan were successfully fabricated and comparatively evaluated. The developed nanofibers exhibited continuous and interconnected fibrous structures, indicating the suitability of

the selected polymer system and electrospinning conditions for producing nanofibrous mats.

Particle size analysis confirmed the successful synthesis of silver nanoparticles with a narrow size distribution. SEM analysis revealed that the incorporation of different antibacterial agents significantly affected fiber morphology and diameter distribution. Neem-loaded nanofibers exhibited the smallest average fiber diameter, whereas AgNPs-loaded nanofibers showed comparatively larger diameters with minor bead formation. Chitosan-loaded fibers demonstrated relatively uniform morphology and a narrow diameter distribution. FTIR analysis verified the presence of characteristic functional groups associated with PVA, CMC, neem extract, and chitosan, confirming the successful incorporation of antibacterial agents without substantial alteration of the base polymer structure.

The antibacterial evaluation demonstrated that silver nanoparticle-loaded nanofibrous mats exhibited the strongest antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus*, producing inhibition zones of 51 mm and 51.5 mm, respectively. Neem-loaded mats displayed moderate antibacterial activity, whereas chitosan-loaded mats showed comparatively lower antibacterial performance under the tested conditions. The findings indicate that the nature of the incorporated antibacterial agent plays a decisive role in determining the functional performance of electrospun PVA-CMC nanofibers. The developed nanofibrous mats show potential for applications in antibacterial wound dressings, biomedical textiles, protective healthcare materials, and antimicrobial packaging systems.

Future studies might focus on mechanical characterization, antibacterial durability, controlled release behavior, cytocompatibility assessment, and in vivo performance evaluation. Further optimization of antibacterial agent concentration and electrospinning parameters may also contribute to the development of high-performance multifunctional nanofibrous materials for biomedical and healthcare applications.

Conflicts of Interest

The authors declare no conflicts of interest.

Funding Statement

Not applicable.

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Appendix 1

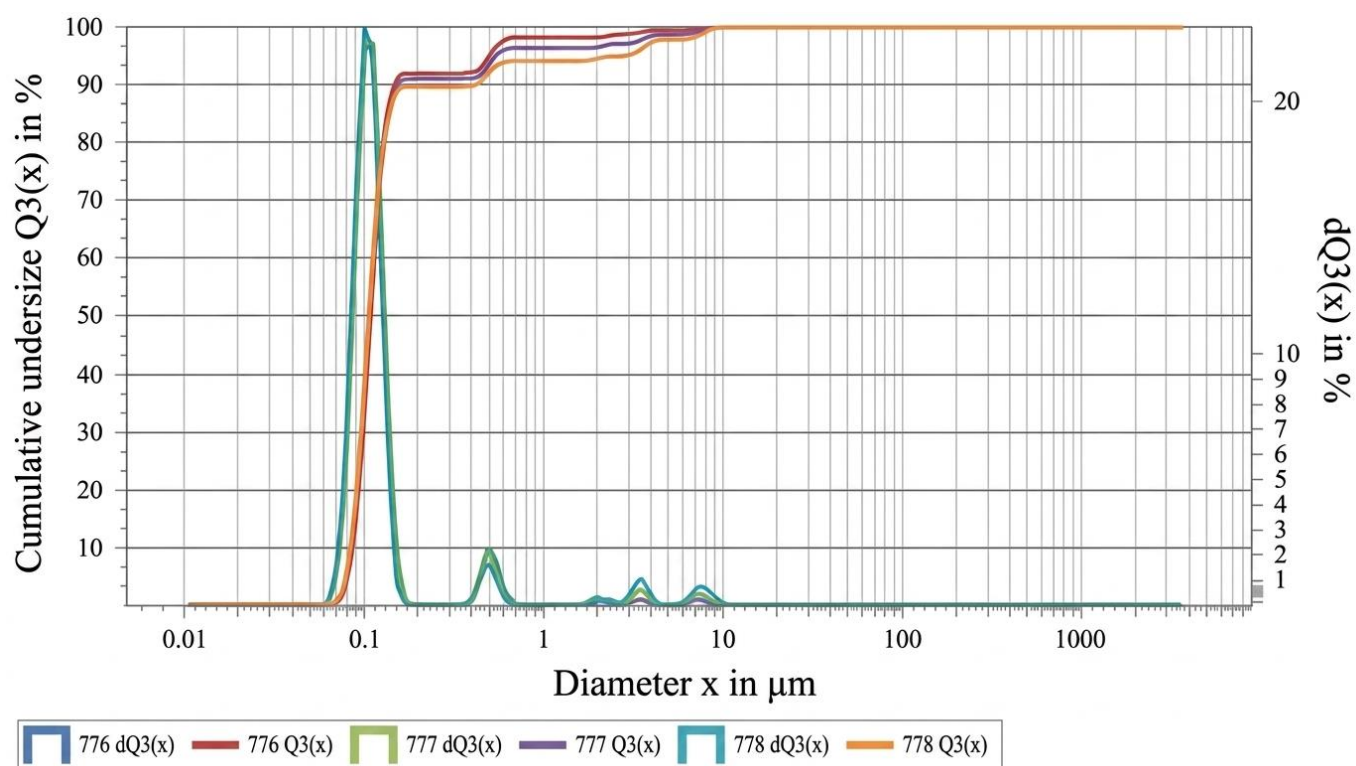


Fig. A1 Particle Size Analysis of Synthesized Silver Nanoparticles