

Manufacture of Antibacterial Medical Dressings from Nanofibers Prepared by Electrospinning

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ABSTRACT

Electrospinning has become a preferred method for manufacturing nanofiber membranes quickly and with minimal investment. In this study, polyacrylonitrile (PAN) was dissolved in dimethylformamide (DMF), and a specific weight ratio of polyvinylpyrrolidone (PVP) and gentamicin sulfate was added to the solution to manufacture nanofibers using the electrospinning process. Gentamicin was added to kill germs and bacteria resulting from exposure of the skin to outside air, while PVP was added to make the membrane surface hydrophilic to enhance the filtration rate and efficiency. The prepared samples were spun using a homemade electrospinning device at specific parameters, and the diameters of the spun nanofibers were calculated using a scanning electron microscope (SEM). The samples were analyzed using an infrared (IR) spectrometer, and then the biological effectiveness of the prepared medical dressings was tested on two types of bacteria, showing great effectiveness for use as medical dressings for skin wounds, especially after surgery.

KEYWORDS: Electrospinning, Medical dressings, Nanofibers, Polyacrylonitrile

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INTRODUCTION

Electrospinning is a modern and innovative technique for manufacturing fibers with extremely small diameters due to its ability to produce nanomaterials and structures with unique properties that can be used as drug delivery systems [1]. where nanofiber networks provide high porosity and surface area, biologically active materials/drugs can be encapsulated in polymeric nanofibers by axial electrospinning, non-axial electrospinning, or another method that involves adding medical materials and binding them as nanoparticles to the nanofiber network, such as antimicrobial, antibacterial, and antioxidant materials [2,3]. Polymeric nanofibers have received widespread attention worldwide in nanotechnology, biotechnology, and many other fields [4-6]. Electrospinning is the only technique that uses electrostatic force to produce nanofibers from polymer solutions or melts. Electrospinning is unique and unconventional because it relies on the production of nanofibers from a polymer solution at low temperatures and the ability to easily control the diameters of the resulting nanofibers by changing certain operating conditions, such as controlling the flow rate of the solution over time, controlling the applied electrical voltage, or controlling the chemical composition of the spun solution. This has enabled its wide application in various fields, as any change in one of the working conditions changes the composition of the resulting fibers [7,8]. Figure 1 illustrates the mechanism of action of the electrospinning device and the composition of the nanofibers.

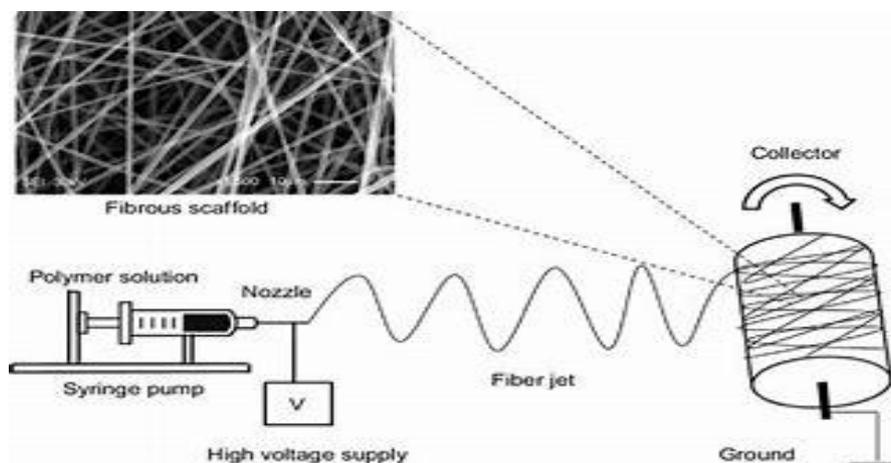


Fig. 1 The electrospinning Machen and the resulted nanofibers.

Nanofibers are a type of nanostructure that, due to their unique properties, can be used in wound dressings and medical patches, and electrospinning is a good method for producing them. The benefits of electrospun nanofiber wound dressings include a

large surface area (area-to-volume ratio), high absorption of wound exudate, high air permeability, mimicry of the extracellular matrix (ECM) of damaged tissue, and the possibility of gradual release of drugs loaded onto the nanofibers [9-11]. The skin is the largest organ in the body and acts as a barrier against bacteria and other organisms. However, the skin can be injured or torn due to various factors or certain surgical procedures. The condition and spread of wounds are expected to worsen, especially in the elderly and diabetic patients. Wound healing is a complex process that aims to restore the normal anatomical structure and function of the skin. Wound healing consists mainly of three stages called the inflammatory stage, the proliferative stage (including protein synthesis and wound contraction), and the remodeling phase. Wounds are treated during the wound healing phase to prevent infection using a strict regimen of antibiotics, which places a significant economic burden on the patient. Therefore, there is an urgent need to prevent the growth of microbes and reduce the possibility of their proliferation by using antibiotics that are safe for human use or herbal extracts to accelerate the wound healing process and reduce the need to use pharmaceutical chemicals such as antibiotics, and thus prevent the side effects of their use. Initially, wound dressings were made from natural materials such as plants, plant fibers, and animal fats.

With the advancement of science, they began to contain medicinal substances that accelerate the healing process [8,12]. Due to the adverse side effects of some orally administered drugs, researchers have developed a technology for delivering drugs through drug-loaded nanofibers with long-term release, through the electrospinning of various polymer solutions with different drugs according to the desired application [13-16]. This enhances the ability to heal wounds and treat burns on the skin through a number of substances such as proteins, lipids, amino acids, vitamins, and enzymes [17]. Figure 2 illustrates how nanomedicinal dressings work on wounds.

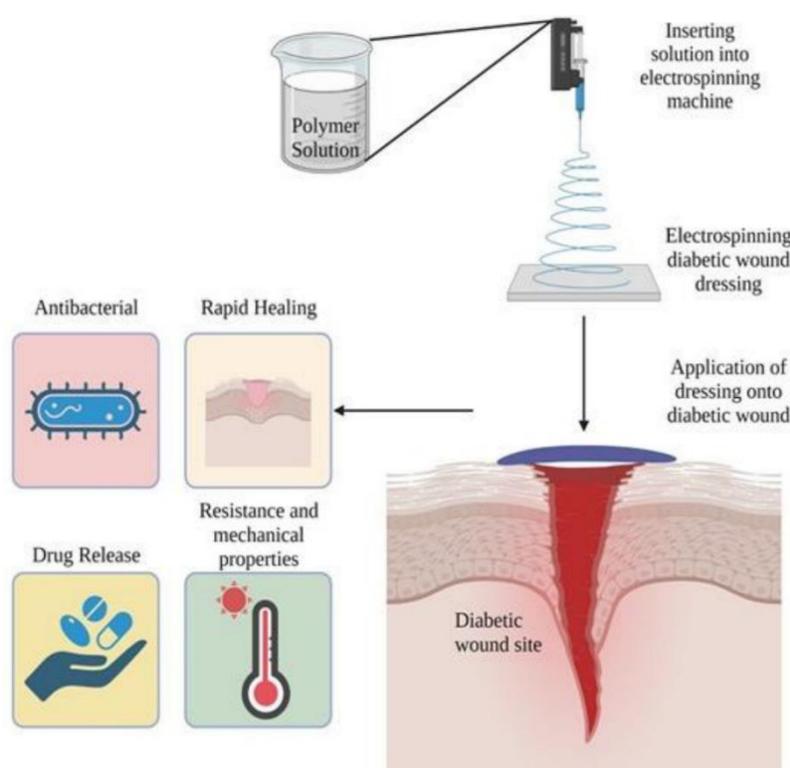


Fig. 2 Illustrates how the nanomedical dressings work on wounds.

Research in this area remains scarce and scattered [18,22]. Gentamicin is considered an effective and powerful antibiotic that kills germs and fights bacteria [18-21]. It is used to treat severe or dangerous bacterial infections and helps eliminate acne-causing bacteria and treat bacterial skin cracks and eczema. [23,24]. Figure 3 shows the chemical structure of gentamicin, which is symbolized by the code GM.

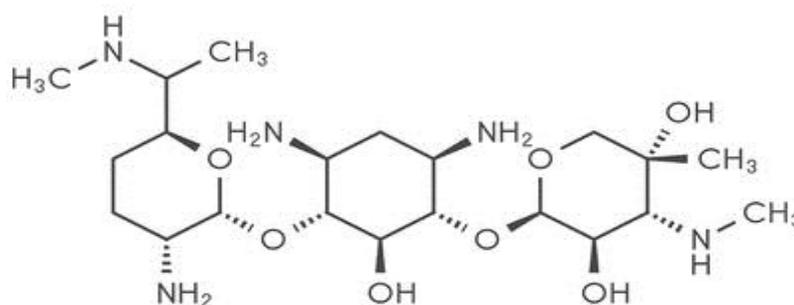
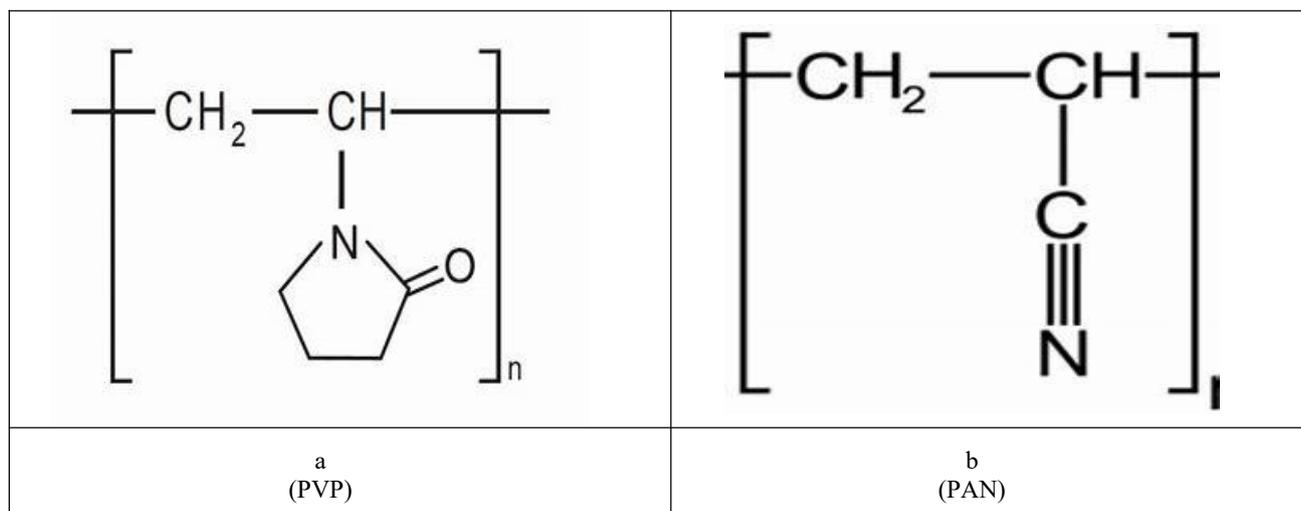


Fig. 3 Chemical structure of Gentamicin GM

Ultra-thin and uniform nanofibers can be produced from polyacrylonitrile (PAN), and these nanofibers are hydrophobic in nature [25-31]. The wettability of the membrane surface depends on its microstructure and chemical nature; however, surface modification can be applied to the membrane to increase its hydrophilicity [32-36]. The addition of polyvinylpyrrolidone (PVP) has excellent wetting properties due to its high surface tension [37]. The important goal of adding PVP to the PAN polymer solution is to make the membrane more hydrophilic in order to facilitate effective filtration. A homogeneous polymer solution for electrospinning can be made by dissolving PVP in a suitable solvent, such as deionized (DI) water, dichloromethane (DCM), tetramethylammonium chloride, and dimethylformamide (DMF) [38-42]. Figure 4 shows the chemical structures of polyvinylpyrrolidone (PVP) and polyacrylonitrile (PAN), respectively.



EXPERIMENT

1. Materials used:

Polyacrylonitrile synthetic fibers of known molecular mass (100,000 g/mol), containing the following monomers: Acrylonitrile $\text{CH}_2=\text{CH}-\text{C}\equiv\text{N}$ at 85% by weight, acrylamide $\text{CH}_2=\text{CH}-\text{CO}-\text{NH}_2$, and vinyl acetate $\text{CH}_2=\text{CH}-\text{O}-\text{CO}-\text{CH}_3$. Dimethylformamide (DMF) in 99.5% purity (GC) \leq MERCK. Gentamicin sulfate with a purity of 99.5% (GC) \leq MERCK. Polyvinyl pyrrolidone (PVP) (Mw 360,000) was obtained from Sigma–Aldrich (St. Louis, USA), Gentamicin (GM) 80, Exir Pharmaceutical Co.

2. Synthesis of PAN/PVP nanofibers:

Electrospinning has been used in recent research to produce polymeric nanofibers incorporated with additives such as antibacterial agents. Nanofibers have attracted considerable attention due to their unique properties. They are called ultra-fine fibers and possess some unique features, such as nanometer-scale fiber diameter, cross-sectional area, and microscopic length along the fiber axis. The fibers have a high surface area, flexibility, and tremendous porosity, which allows for many more sites for separation processes [43-46].

In this study, PAN was dissolved in DMF at a weight percentage of 10 w% and 5 w% by weight of gentamicin was added to the PAN/DMF mixture. The purpose of using PVP and gentamicin was to increase the filtration rate and reduce the biofouling of the membrane, respectively. Several different polymer solutions were prepared based on different ratios of PVP and gentamicin. We obtained homogeneous solutions for all the samples studied. By placing them in a hot water bath for one hour at 70°C at 500 rpm. Each sample of the completely mixed polymer solution was then transferred to a 10 ml syringe with an inner diameter of 0.5 mm and placed in a KD Scientific syringe pump at a flow rate of 0.5 ml/hour. One end of a copper electrode with a diameter of 0.25 mm was connected to a high DC power source with a voltage of 30 kV, and the other end was connected to the syringe. A distance of 15 cm was maintained between the capillary tube, which was covered with aluminum foil. This experimental setup for electrospinning was carried out using a homemade electrospinning device. As shown in Figure 5, this process was carried out at a temperature of 250°C. The samples spun with the electrospinning device were then left to dry for 24 hours before being removed from the aluminum foil.



Fig. 5 The electrospinning device

Figure 6 also illustrates the process of droplet extrusion and Taylor cone formation, without which electrospinning would not occur. This leads to the accumulation of charges on the upper part of the droplet formed from the droplet formed and extends at an angle of 49.3°C , forming what is known as a Taylor cone, where tangential electrical stress causes the polymer solution to move, generating hydrodynamic pressure on the surface of the droplet, and the interaction between electrical and hydrodynamic pressures causes the droplet to deform [47,48].

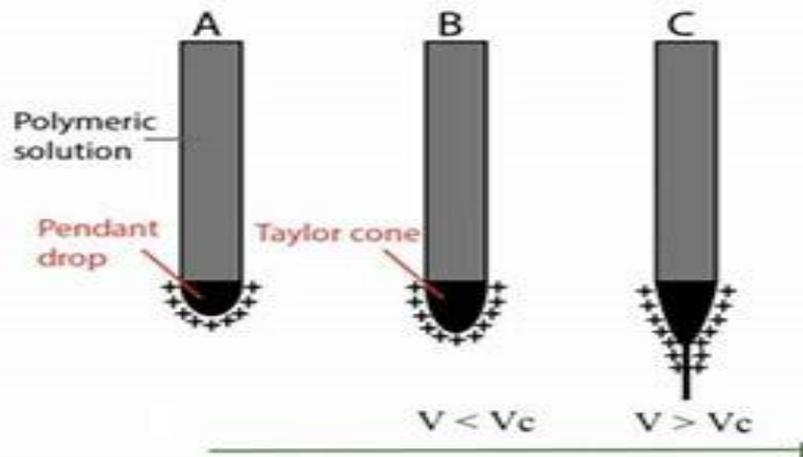


Fig. 6 Taylor cone for droplet emergence.

Figure 7 also shows the extrusion and collection of fibers on the rotating cylindrical collector, where the injector head is connected to the positive pole and the collector is connected to the negative pole. Figure 5a also shows the process of extruding and collecting the fibers on the rotating cylindrical collector, where the injector head is connected to the positive pole and the collector is connected to the negative pole. Figure 7 shows the shape of the fibers produced after the electrospinning process on an aluminum plate [49].

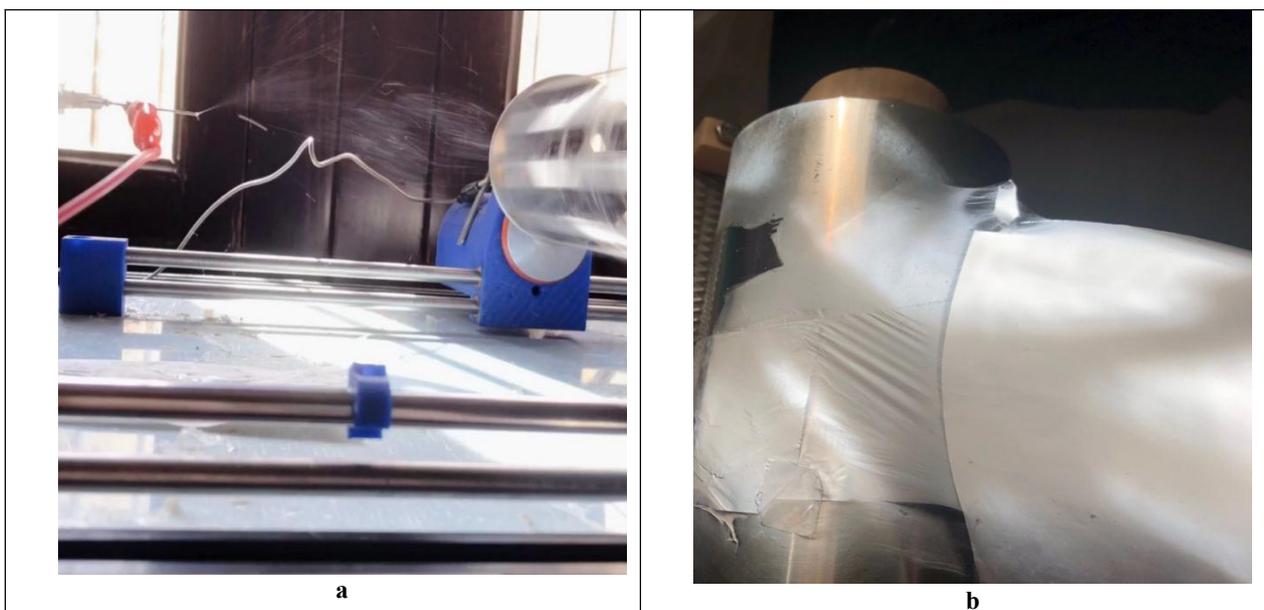


Fig. 7 a; the extrusion of the fibers, b; the shape of the resulting fiber

RESULTS AND DISCUSSION

1. Measurement of contact angle with water

The contact angle values of PAN fibers were determined. The samples were determined using an optical contact angle meter, a compact video-based instrument for measuring contact angles between 1° and 180° with an accuracy of $\pm 1^\circ$. It accurately measures contact angles and captures images of contact angles. The samples were dried in a vacuum chamber overnight after being removed from the assembly screen. The samples were then placed on the angle gauge sample holder. A drop of water was gently dropped onto the sample from a syringe connected to the angle gauge. Figure 8 shows schematic views of superhydrophobic, hydrophobic, and highly hydrophilic surfaces. The contact angle measurements of the samples used in this study are shown in Figure 9. As can be seen, all samples are hydrophilic and have a constant water contact angle of less than 90° degrees. The sample without PVP shows a higher water contact angle. However, the addition of PVP makes the fibers hydrophilic due to the hydrophilic nature of PVP. Hydrophobic and hydrophilic are terms related to the behavior of solid surfaces when they come into contact with water droplets. A hydrophobic surface is one on which a water droplet forms a contact angle greater than 90° degrees, while a hydrophilic surface is one on which a water droplet forms a contact angle less

than 90 degrees. Polymer surfaces with contact angles between 150 and 180 degrees are called superhydrophobic. This phenomenon is also known as the “lotus effect,” which exhibits self-cleaning, anti-fouling, and anti-contamination properties. The water repellency or wettability of a membrane plays an important role in its performance because a highly wettable membrane can wet the membrane surface and thus increase filtration efficiency [50-52]. Figure 9 shows that water droplets on PAN nanofibers with different percentages of PVP are spherical in shape, indicating the semi-hydrophilic properties of the nanofiber surface. As shown in Figure 9A, the average water contact angle on both sides of the droplet was 350°C. In Figure 9b, the average water contact angle of both sides of the droplet was 350°C. In Figure 9C, the average water contact angle of both sides of the drop was 350°C. The presence of gentamicin has no effect on the wettability of the PAN membrane. PVP has wettability properties because the surface tension of PVP is 75 mN/m.

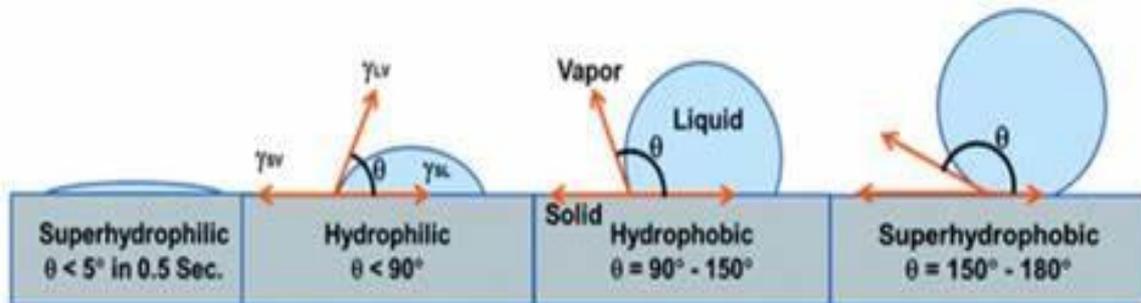


Fig. 8 Schematic views of super hydrophilic, hydrophilic, hydrophobic, and superhydrophobic surfaces (left to right)

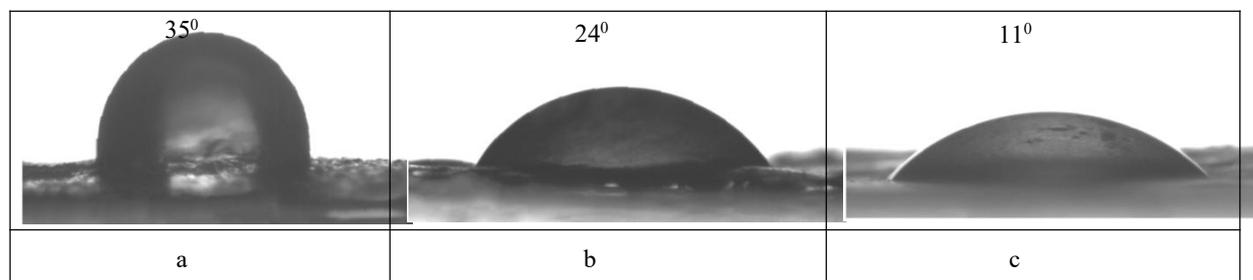
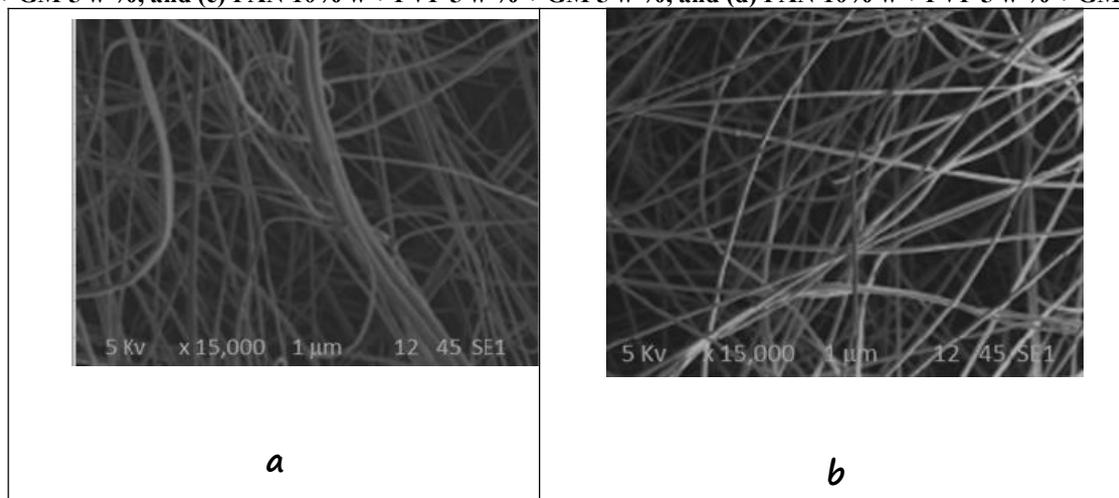


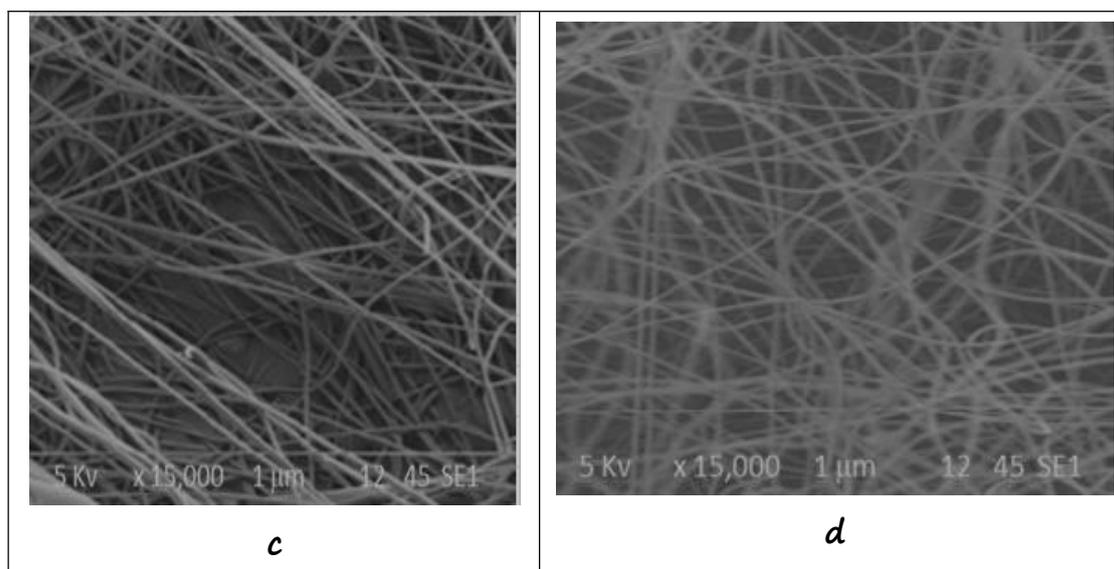
Fig. 9 Water contact angle values for electro spun nanofibers: (a) PAN + 0 w% PVP + 5 w% GM, (b) PAN + 5 w% PVP + 5 w% GM, and (c) PAN + 10 w% PVP + 5 w% GM.

2. SEM images of the studied samples:

Figure 10 shows SEM images of the prepared samples of PAN nanomembranes used in this study. The average PAN fiber diameter is approximately 210 nm. No significant change was observed when 5% by weight of gentamicin sulfate was added, with the average PAN fiber diameter being approximately 215 nm, as shown in Figure 10b. This means that gentamicin has no significant effect on the diameter of the spun nanofibers. A slight increase in fiber diameter was also observed, with an average fiber diameter of approximately 240 nm, after adding 5% by weight of PVP, as shown in Figure 10c. The average fiber diameter also increased significantly to 300 nm after adding 10% by weight of PVP, as shown in Figure 10d.

Fig. 10 SEM images of electro spun nanofibers(a) PAN 10% w + PVP 0 w % + GM 0 w %, (b) PAN 10% w + PVP 0 w % + GM 5 w %, and (c) PAN 10% w + PVP 5 w % + GM 5 w %, and (d) PAN 10% w + PVP 5 w % + GM 5 w %




Table 1: The Average Diameter of the Nanofibers for Each of the Samples Studied.

samples	I. Composition	II. Diameter(nm)
a	PAN 10% w + PVP 0% w + GM 0 % w	210
b	PAN 10% w + PVP 0 % w + GM 5 % w	215
c	PAN 10% w + PVP 5 % w + GM 5 % w	240
d	PAN 10% w + PVP 10 % w + GM 5 %w	300

3. Fourier Transform Infrared (FTIR) Analysis:

This is a useful tool for determining the chemical reaction of PAN. With the help of spectra, it is possible to study the relationship between chemical changes and strength [53]. FTIR spectroscopy was performed to verify the interaction between the PAN/PVP composite polymers by comparing the individual polymers and the previously prepared virgin polymers. Figure 11 shows the FTIR spectra of (1) 10% pure PAN nanofibers, (2) 10%w PAN+5%w PVP nanofibers, and (3) 10%w PAN+10%w PVP nanofibers.

The FTIR spectra of PAN fibers contain several peaks, which are related to the presence of CH₂, CN, C=O, CO, and CH bonds. A peak was observed at 2,916 cm⁻¹, which is related to CH bonds in CH, CH₂, and CH₃. Another peak was observed at 2,243 cm⁻¹, which indicates the presence of nitrile (CN) bonds, showing the presence of a nitrile group in the PAN chain. The intensity corresponding to 1,668 cm⁻¹ is due to the cyclic C=O peak of the homogenous methyl acrylate compound. The peak corresponding to 1,456 cm⁻¹ is due to CH. Figure 11(2) shows the FTIR spectra of the PAN 10%w+PVP 5%w network. As can be seen, a peak is observed at 2,916 cm⁻¹, similar to that shown in Figure 11(1), which is related to the stretched CH/CH₂ vibration. There is another peak at 1,456 cm⁻¹, which is caused by CH distortion in cyclic CH₂ groups. A peak corresponding to 1,662 cm⁻¹, which can also be seen in Figure 15, is caused by the contribution of C=O from the PVP film. The vibrational range related to the pyrrolidone C=O group is at 1,662 cm⁻¹ and corresponds to the C=O of the PVP polymer film. The peak corresponding to 534 cm⁻¹ could be attributed to the fourth amide range. Figure 11(3) shows the FTIR spectra of the PAN 10%w+PVP 10%w network. As can be seen, the band related to the pyrrolidone C=O group is located at 1664 cm⁻¹. The band corresponding to the vibrational band at 1,698 cm⁻¹ is consistent with the C=O of the PVP polymer film, and the absorption band at 2,920 cm⁻¹ indicates the asymmetric stretching of CH₂. The band at 1,664 cm⁻¹, which is due to the C = O vibration band, indicates the presence of some H-bonded carbonyl groups in PVP. The bands at 1,290 cm⁻¹ and 1,450 cm⁻¹ are attributed to the stretched CN vibration and the bent C-H vibration of PVP, respectively [56-54].

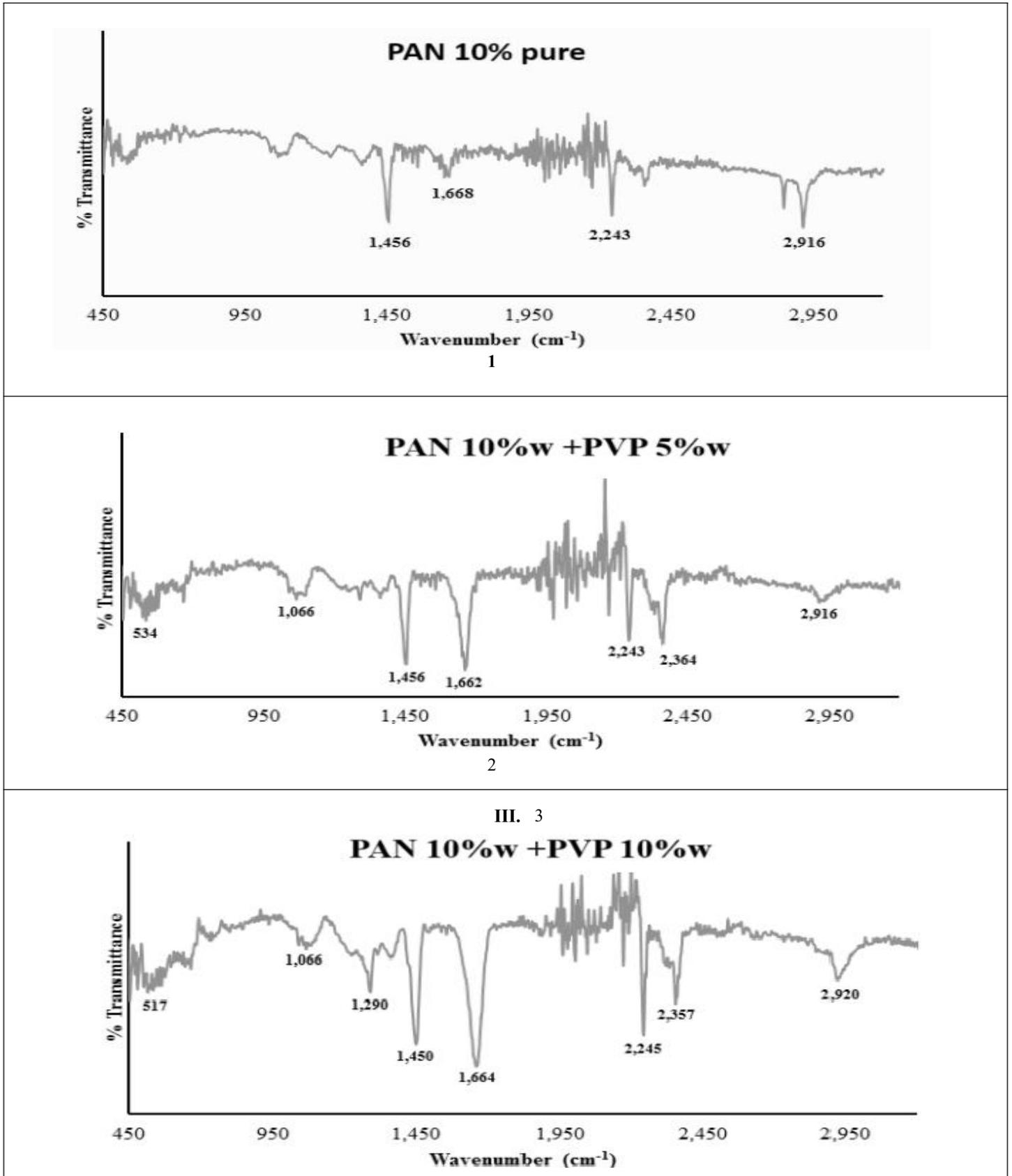


Fig. 11 FTIR spectra (1) PAN nanofibers 10% pure, (2) PAN nanofibers 10%w+PVP 5%w, (3) PAN nanofibers 10%w+PVP 10%w.

4. Preparation of *Staphylococcus epidermidis* vaccine in liquid medium:

Prepare the nutrient broth, sterilize it in an autoclave, and leave it to cool in a laminar flow cabinet to a temperature of 45°C. Then inoculate the prepared medium with *Staphylococcus epidermidis* bacteria (*Staphylococcus epidermidis*), and then incubated in an incubator at a temperature of 32-35°C for 24 hours. Then, solid nutrient agar was prepared and inoculated with

bacteria using nutrient agar medium in sterile distilled water. The pH of the medium was adjusted to 6.6, sterilized in an autoclave for 15 minutes at a temperature of 121°C, left to cool in the sterile chamber to a temperature of 45°C. The mixture was poured into Petri dishes and left to solidify. The dishes were then inoculated with the previously prepared bacterial vaccine using a cotton swab and incubated for 24 hours in an incubator at a temperature of 32-35 °C. We find from Figure 12.1 that the membrane loaded with gentamicin sulfate proved effective in killing and inhibiting the growth of *Staphylococcus epidermidis*, where the diameter of the inhibition zone was (20±2) mm, which indicates the high effectiveness of the membrane loaded with the drug gentamicin sulfate in killing bacteria [57-62]. A bacillus vaccine was also prepared in the liquid medium, and gentamicin sulfate loaded onto a PAN fiber membrane was tested for killing another type of bacillus (*Bacillus* SP), following the same steps as before. Figure 12.2 shows a Petri dish containing a membrane composed of PAN nanofibers loaded with gentamicin sulfate and inoculated with rod-shaped bacteria. We find from the figure that the membrane loaded with gentamicin sulfate has proven effective in killing and inhibiting the growth of bacilli, with an inhibition zone diameter of (27±2) mm, which indicates the high effectiveness of the gentamicin sulfate-loaded membrane in killing and inhibiting the growth of bacilli. As in the gentamicin test loaded on PAN fibers, a membrane of electrospun PAN fibers loaded with gentamicin in the form of 6 mm diameter discs is placed on an agar plate inoculated with *Staphylococcus epidermidis* bacteria, then the plate is placed in the refrigerator for one hour to allow the gentamicin to spread in the medium. The inhibition zone surrounding the discs is then measured, as shown in Figure 12 [63-68].

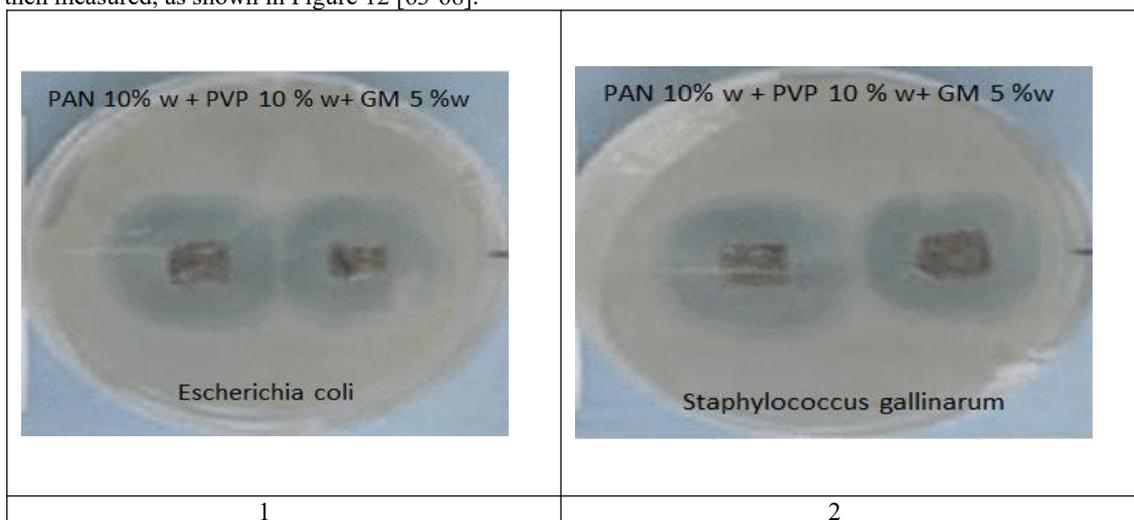


Fig. 12 1- Petri dish containing gentamicin sulfate-loaded membrane inoculated with bacillus bacteria, and 2- Petri dish containing gentamicin sulfate-loaded membrane inoculated with staphylococcus bacteria.

CONCLUSIONS

The growing interest in advanced nanomaterials, such as electrospun medical nanofibers, as a solution for the topical application of therapeutic medical materials, led us to research and study the integration of antibacterial and antimicrobial medical materials with electrospun nanofibers and their use as medical dressings. In this study, PAN nanofiber membranes incorporated with PVP and gentamicin were fabricated for use as medical dressings for skin wounds and ulcers. PVP was added to make the membrane hydrophilic, and the wettability before and after adding PVP was studied, which showed that its addition resulted in high wettability for the spun nanofibers, while gentamicin was added to kill bacteria and germs that could attack wounds. The produced nanofibers were characterized using FTIR spectroscopy and SEM to confirm the chemical nature of the fibrous membrane and its nanopores. Indeed, the ability to kill germs and bacteria was observed by applying Petri dishes loaded with bacillary and staphylococcal germs to electrospun PAN nanofibers combined with PVP and loaded with the germicidal medical substance gentamicin sulfate.

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