PROCESSING BY ELECTROSPINNING OF COMPOSITES MEMBRANES AS POTENTIAL ANTIBACTERIAL DRESSING

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Abstract. A membrane based on PVA-Ag is manufactured using the electrospinning technique. This membrane is characterized by the methods: Scattering Electron Microscopy (SEM), Energy Dispertion Scattering (EDS) and X-Ray Difraction (XRD). In addition, its antibacterial properties are evaluated using two different strains of microorganisms: Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus). The results show that when these microorganisms are put in contact with the membrane to based of PVA with 8 % in silver, they would be resistant to die. However, the electrospinning method allows to easily increase the amount of silver to increase the antibacterial efficiency.

Key words: *Electrospinning, composites membranes, bactericides, triple E.*

INTRODUCTION

The pioneering works of Antonin Formhals in 1934, lead him to patent a process of fiber collection through a metallic device and explain the organization of fibers caused by electrostatic forces. Years later, the studies of Geoffrey Ingram Taylor in 1969, with the handling of electrically operated polymeric jets, formulated the so-called "Taylor cone" which promoted the base of the electrospinning [1]. The year 1974 proposed the use of this technique as a dressing for wounds and when they realized the good functionality of it, in 1978 the first vascular graft made with electros pinned fibers was made in rats. Nowadays, it is investigated how to manufacture active dressings based on biopolymers with the property of being absorbed by the human body by electrospinning [2, 3], or by means of an electro-spray variant [4], which serve as a means of transport of drugs [5]. Inclusive, it looks for how to make this type of dressing's antibacterial [6-9].

In this research, we contribute to this field of science and engineering of biomaterials by proposing an easy-to-make, economical and efficient method (triple E) to manufacture composite membranes based on PVA-Ag. These membranes are characterized by SEM, EDS and DRX. Additionally, its antibacterial properties are evaluated for clinical purposes.

MATERIALS AND METHODS

The reagents used were polyvinyl alcohol of transparent color in filament form with thickness of 1.75 mm (PVA, of the brand Color Plus Premium Quality), silver nitrate (brand Sigma Aldrich, purity at 95 %), ethyl alcohol (brand Sigma Aldrich, purity at 99 %), distilled water.

Fabrication of PVA-Ag membranes: A electrospinning prototype was designed, manufactured and assembled whose scheme is showed in the Fig. 1. This prototype was employed to generate the composite membranes of PVA -Ag by means of the injection of a PVA polymeric solution and a colloid to base of water-Ag through a metallic needle. The preparation method of these solutions is explained later. Both solutions are subjected to high voltage of 22 kV inside the electrospinning prototype. The PVA fused threads formed by the interaction of the polymeric solution with the high voltage (charged jet), are collected by a rotary roller (rotating collector), previously coated with waxed paper (Fig. 1). The rotating collector was kept rotating to 1000 rpm and the distance of the this to the metal needle was fixed to 8 cm, located at a height of 15 cm. It is important to mention that the total operating time of the electrospinning was 30 min.

Preparation method of PVA polymeric solution: a dissolution is obtained with a mixture of 6ml of ethyl alcohol and 4 ml of water, which is heated to a temperature of 120 °C, submitted to constant agitation for 1.5 hours and adding in this mixture the PVA filament.

Preparation method of colloid to base of water-Ag: a dissolution of silver nitrate in distilled water to 1 molar is obtained; later, this is exposed to UV radiation for 1 hour. The colloid resulting is left to stand and the container is changed to remove the remaining silver nitro by decantation.

The physics and chemistry characterization of composites membranes was realized by means of Scattering Electron Microscopy (SEM), Energy Dispersion Scattering (EDS) and X-Ray Diffraction (XRD).

Antibacterial test: In rest of this section is described the antibacterial evaluation method using the follows microorganisms: Gram-negative Escherichia coli (E. coli) and Gram-positive Staphylococcus aureus (S. aureus). 40 grs of tryptic soy medium is dissolved in 1 Lt of distilled water, which was sterilized to 100 ºC in autoclave for 40 min. 20 ml of this tryptic soy agar was deposited in sterilized petri dishes and they were allowed to cool for 1 hour. Then the strains in independent cultures were sown by the differentiated method [10]. The cultures of the strains in Petri dishes were carried out in a sterile environment previously irradiated with ultraviolet light and with constant air flow. These culture media were deposited in an oven at 40 °C for 2 days for optimal growth of the strains. 18 culture medias was realized to put in contact the composites membranes, but two of these was taken as control to monitor any type of

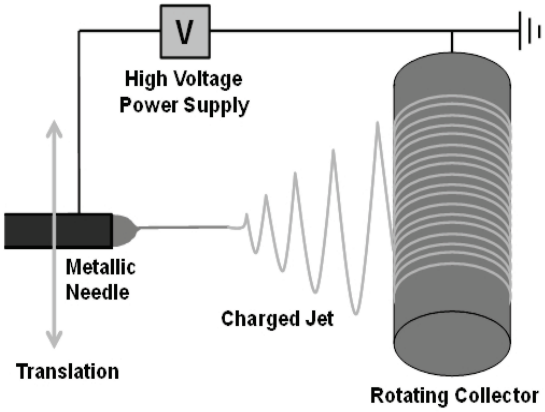


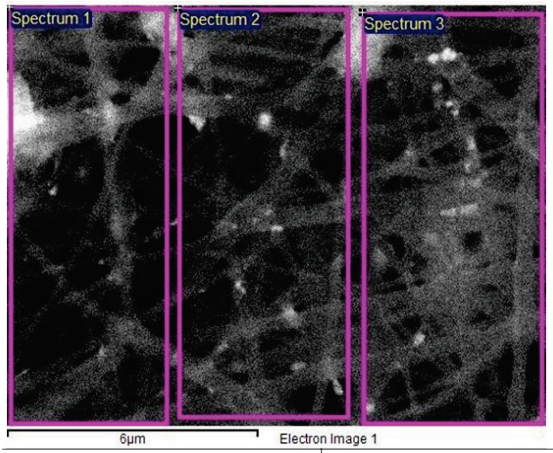
Figure 1. Electrospinning prototype scheme where are showed their parts

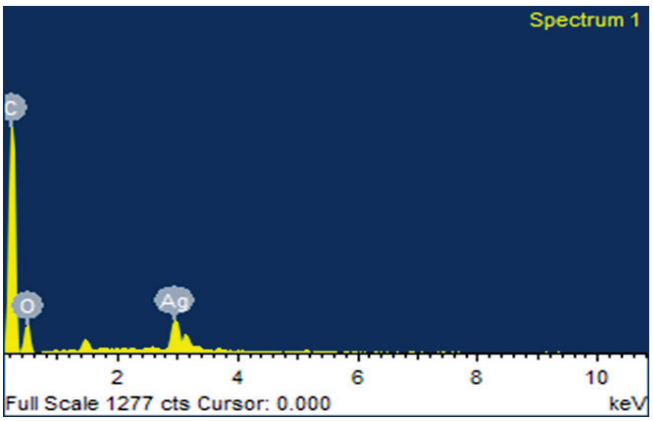
pollution. With the help of the controls it was corroborated that the incubation and the contact of the membranes with the strains was done without generating any growth caused by external agents. The bacterial activity was determined by the classification of the American Type Culture Collection (ATCC) whose principle is based on demonstrating its activity by the presence of inhibition halo in the samples [10, 11].

RESULTS

The characterization of composite membranes tissue to base of PVA-Ag is showed in the left image of Figure 2. The morphogy of these membranes is composed of PVA threads intertwined with wide of 140 nm to 416 mn (see left image of Fig. 3). Therefore the morphological structure of membranes is of micrometric scale. And is very similar to the extracellular matrix of human cutaneous tissue (see left image of Fig. 2).

The presence of the silver inside the membrane structure is confirmed by characterization by EDS and XRD. In right image of Figures. 2 and 3, are showed the results of count by EDS and the diffraction pattern of membranes, respectively. The EDS results reveal that there is 8.21 % of the total silver; the count was made on three independent measurements (see right image of Fig. 1). The diffraction pattern obtained by XRD of membranes, presents the characteristic diffraction peaks of metallic Ag in angles of 38.31, 44.4, 64.6, 77.5, 81.6 degrees that can be attributed to diffraction planes (111), (200), (220), (311) and (222) integrated in the JCPDS tab 04–0783.





Spectrum	In stats.	C	O	Ag	Total
Spectrum 1	Yes	66.71	23.75	9.54	100.00
Spectrum 2	Yes	69.83	22.55	7.62	100.00
Spectrum 3	Yes	69.87	22.64	7.48	100.00

Figure 2. left image presents the PVA-Ag membranes morphology and the three measure of EDS, right image presents the results of count obtained by EDS

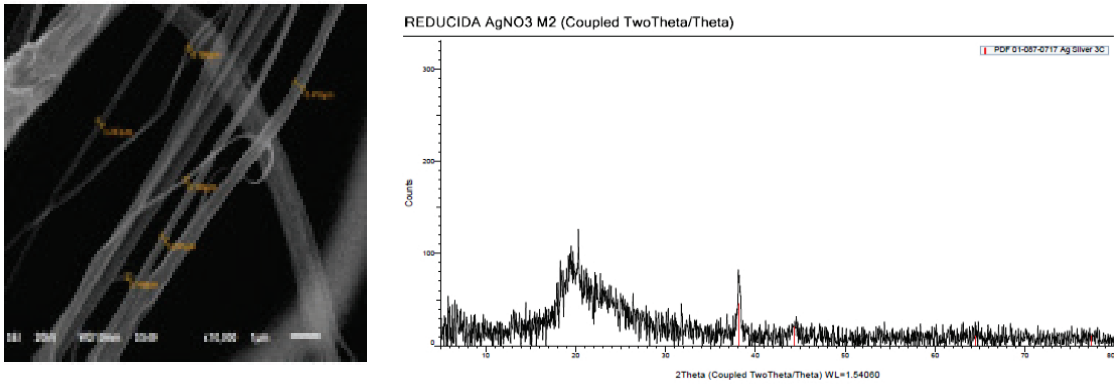


Figure 3. Left image shows a zoom of membrane morphological structure, right image exhibit the diffraction pattern of crystalline phase of metallic silver

The results of bacteriological evaluation of composite membranes are showed in the Figure 4. By way of example in this figure, the culture media with the strains of E. coli and S. aureus inside the Petri dishes are presented. The images of Figure 4 exhibit a halo of similar inhibition for each strains in culture media, which is measured with a Vernier. The diameter of inhibition halo in both culture media with the two strains is approximately of 8 mm. It means that the membrane presents a behavior similar to antibiotics: trimethoprim or sulfamethoxazole, in the presence of E. coli and S. aureus. The strains used have resistance to these drugs, which means that they are not effective in preventing an infection for these microorganisms. However, to increase the effectiveness of the antibacterial properties of the PVA-Ag membrane, it is necessary to increase the concentration of silver micro particles in the structure of the membrane. But the concentration should be appropriate since it could eliminate healthy tissue when applied, for example in skin wounds at the level of muscular facial, which is motive of futures studies.

In recent studies, new biocompatible composite materials have been developed to transport drugs within the active dressing (nanofibers of the membrane) based on silica and PVA Nano capsules [6], silica nanoparticles decorated with silver nanoparticles incorporated in poly-caprolactone (PLC) [9], silver sulfadiazine with cellulose [7] and this same drug mixed with PCL-PVA. Nevertheless, in all these cases the method employed for fabrication of active dressing, is difficult to perform despite using the electrospinning technique. Conversely, our fabrication method of composites membranes is easier of carry out, and theses present a inhibition halo larger than active dressing that has been published in [7]. The biocompatibility property of our PVA-Ag membranes is due to the PVA biopolymer. Therefore, the behavior of active dressings of these type of membranes is justified, which is similar at biomaterial membrane presented in [9].

CONCLUSION

An improved methodology of easy-to-make, economical and efficient for fabrication of PVA-Ag membranes is proposed. The behavior of our membranes is as a antibacterial active dressing with biocompatibility properties. The efficient of antibacterial membranes of PVA-Ag is modified increasing the concentration of silver in the morphological structure. These composite membranes may be a promising application to skin wound healing.

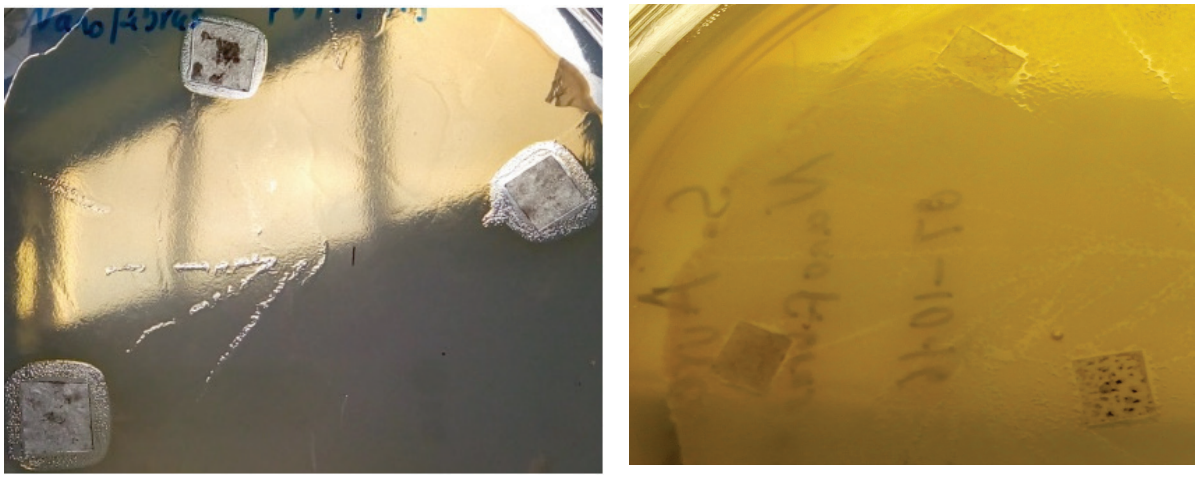


Figure 4. left image present a culture media with inhibition halo in presence of E. coli inside of a Petri dishes, right image shows the same that in left image but in presence of S. aureus

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