ANTIMICROBIAL PROPERTIES OF POLYMERIC NANOFIBROUS MEMBRANES CONTAINING FERROUS SULPHATE

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Abstract

This paper reposts on nanofibers of polymers doped with ferrous sulphate ($FeSO_4$) possibly in combination with quaternary ammonium salt (QAS) prepared by electrospinning. Three types of polymers (polyvinylidene fluoride/PVDF, polylactic acid/PLA and polyurethane/PU) with good electrospinning processability and good mechanical properties of nanofibers were chosen. The prepared nanofibrous membranes were characterised in terms of morphology of the fibres assessed by SEM, and the pore sizes were determined by porometry. The leaching test showed a firm anchoring of the additive in the nanofiber structure. Antimicrobial activity was monitored after 0 h, 4 h and 24 h using *Staphylococcus aureus (CCM 4516)* and *Klebsiella pneumoniae (CCM 4415)* strains. Furthermore, aerosol filtration efficycy was dermined and also quality factor of filtration.

The membrane prepared from PU doped with $FeSO_4$ showed the best antibacterial efficiency. The porosity and morphology of the nanofibrous membrane effectively contributed to the trapping of microorganisms. This system was also evaluated as the most suitable for the electrospinning process from the effectivity point of view. Spinning ang dopping process of preparation is easily applicatbole to the the industrial conditions of production which is very importatnt aspect of this submitted scientifique work.

Keywords: Antimicrobial properties, doped nanofibers, $FeSO_4$, filtration

1. INTRODUCTION

In recent years, polymeric nanofibers have been demonstrated as effective tool for aerosol filtration applications. They have a great ability to aerosol filtration and trapping microorganisms that have became extremely relevant due to the COVID-19 occurrence [1]. The size of SARS-COV-2 virus is 60–140 nm with nanospikes coated on its spherical viral capsid/envelop with heights of 9–12 nm [2]. The most beneficial properties in this field of study are: high surface area to weight ratio, low density, high pore volume and besides other also small pore size [3]. As increase requirement for antimicrobial effect across nanofiber applications it needs to be mention also another valuable advantage; possibility of their relatively easy surface coating or doping with various species, which can provide nanofibers with specific properties, including antimicrobial effects [4].

As demands for functionality of nanofibers membrane are more and more complex, many research groups have prepared already wide range of surface modified nanofibers, including antimicrobial [5]. However, it needs to be said that some of methods are very complicated, expensive or not stable during usage time of membrane. Therefore, in our study we developed easy procedure for antimicrobial nanofibers doping with $FeSO_4$ possibly in combination with QAS as antimicrobial agents. Our method of spinning and doping of nanofibers is easily applicable in conditons of industrial production.

In out work, after detailed characterization of prepared marials the antimicrobial effect was evaluated on two species of bacteria, *Staphylococcus aureus* and *Klebsiella pneumoniae*. Apart from the pathogenic effects of these two bacterial strains, another reason for testing them is that they are fundamentally different in the arrangement of outer membranes. So obtained results provide general information on the interaction between a biocide compound and other Gram-positive and Gram-negative bacteria [6]. Furthermore, aerosol filtration efficacy at different particle sizes was proved via automated filter tester Model 3160. An ideal air filter should have high particle-removal efficiency, low-pressure drop, and a long lifetime as well as high quality factor number. [7]

Nanofibrous membranes doped with an active antibacterial component directly in the mass of nanofibers are the next step towards products that actively eliminate microorganisms and thus protect human health.This work focused on developing nanofibrous membrane doped with antimicrobial compounds for aerosol filtration, which is the next step towards products that actively eliminate microorganisms and thus protect human health.

2. MATERIALS AND METHODS

We prepared three polymeric nanofibrous membranes based on PVDF, PU, and PLA deposited on polypropylene (PP) non-woven as collecting support. All types of successfully electrospun nanofibers were doped via 1 % of $FeSO_4$. Then PU nanofibers were doped with both 1 % of $FeSO_4$ and also 7.5 % of QAS incorporated into PU chains. Basic weights of all prepared nanofibers were at a maximum of 1 g/cm^2, as shown in **Table 1**.

Table 1 Basic weights of all prepared nanofibrous materials

Sample	Basic weight (g/cm^2)
PVDF/$FeSO_4$	0.77
PLA/$FeSO_4$	0.56
PU/$FeSO_4$	0.45
PU/$FeSO_4$/QAS	1.00

Polymeric nanofibers were prepared by electrospinning device SpinLine 40 (SPUR, Czech Republic) equipped with two sets of moving nanofibers forming jets. The applied voltage during the spinning process was 75 kV, and the distance between electrodes was 19 cm for all samples. Other parameters for polymeric solution were different for each sample. Summarisation of these parameters is completed in **Table 2**.

Table 2 Summarization of parameters of polymeric solutions.

Sample	Solvent	Basic weight (g/cm^2)	Viscosity (Pa.s)	Conductivity (µS/cm)
PVDF/$FeSO_4$	DMF	0.77	1.60	25.10
PLA/$FeSO_4$	DMF/aceton	0.56	0.75	110.3
PU/$FeSO_4$	DMF/H_2O	0.45	3.25	120.20
PU/$FeSO_4$/QAS	DMF/H_2O	1.00	0.80	310.00

The morphology of the electrospun nanofibers was observed using scanning electron microscopy (SEM; Vega 3, Tescan, Czech Republic). The average diameter of the electrospun nanofibers was determined by analysing the SEM images using a custom code image analysis program. As well as the pore size of nanofibrous materials was performed by porometry device (SPUR, Czech Republic) according to ASTM F316-03.The antimicrobial activity of the prepared nanofiber's mats was tested according to the testing method for the antibacterial activity of textiles using *Staphylococcus aureus (CCM 4516) and Klebsiella pneumoniae (CCM 4415)* strains. Then antimicrobial activity was calculated via ISO 22196:2011.

Antimirobial activity (A) calculation:

A = log N0 - log Nx

Where:

- x - incubation time with bacterial suspension (0, ½, 4 a 24h)
- A - antibacterial activity of sample
- N - number of viable bacteria (CFU/cm^2)

The samples were cut into 20 x 20 mm squares and the polypropylene cover foil was also cut into 20 x 20 mm. Prior to testing, the samples were disinfected with UV radiation for 30 minutes on both sides (sterilization in an autoclave or disinfection with ethanol wasn't possible due to potential damage of sample). The cover foils were disinfected with 70 % ethanol. The steriled samples were prepared in triplicated and put in to the sterile glass petri dishes and covered byf 0.1 ml of bacterial suspension, and this was covered with PP foil. Prepared samples were incubated for 4h and 24h at 35 °C with a relative humidity of 95 %. Samples in time 0h was immediately processed. Triplicates of one sample including foil were transferred in to the sterile PP container. Neutralization medium (SCDLP) was added in a total volume 15 ml (5 ml per sample). Incubation time was 30 s. Then all saples were decimally diluted nad mixed with agar media (PCA) and incubated for 24h at 35 °C.

3. RESULTS AND DISCUSSION

The samples were tested after optimising the spinning process of all the polymer solutions leading to repetable structures. The SEM images in **Figure 1** show the porous structure of the prepared nanofibrous materials with randomly oriented nanofibers having a smooth surface without beads-like or any other morphological defects. Although the electrospinning process was adequately optimised, in the case of sample PU/$FeSO_4$ the produced nanofibers were not straight and were tangled. The fibres also had a larger diameter compared to the other materials. These deficiencies were corrected by adding 7.5 % QAS to the polymeric solution containing 1 % $FeSO_4$. On the contrary, PVDF and PLA polymeric nanofibers were straight even without adding QAS into the solution.

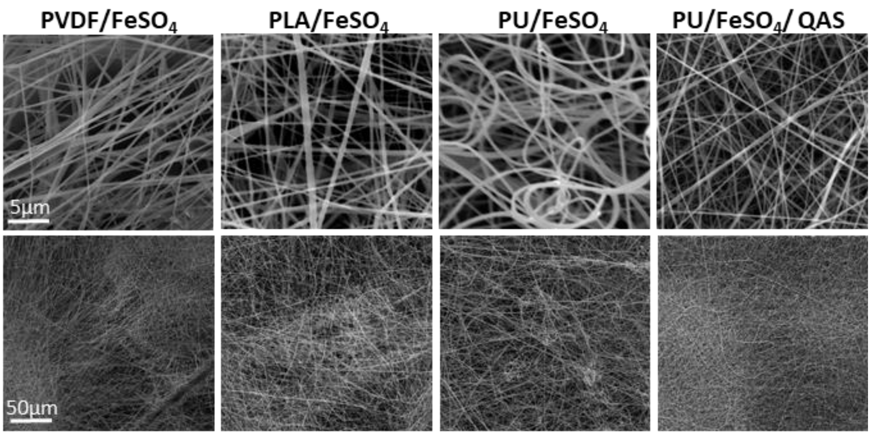

Figure 1 SEM images of four different types of electrospun nanofibers: PVDF doped with 1 % of $FeSO_4$, PLA doped with 1 % of $FeSO_4$, PU doped with 1 % of $FeSO_4$ and PU doped with 1 % $FeSO_4$ in combination with 7.5 % of QAS

Among other characterisation methods, the measurement of fibre diameter is crucial for predicting the properties of nanofiber structures. The diameter of fibres depends on the surface tension, flow rate, viscosity and electric conductivity of polymer solution [8]. **Figure 2** represents the fibre diameter distribution of the electrospun fibres onto the non-woven PP substrate, indicating a range of diameter between 0.16 µm and 0.33 µm. The PVDF/$FeSO_4$ sample shows the largest average nanofiber thickness, which is due to the low conductivity of the polymer solution. However, this sample also contains a significant proportion of fibres with a diameter of around 0.1 µm, which could improve the filtration properties of the material. For polyurethane samples, the addition of QAS shifts the distribution of fibre diameters towards lower values and the average fibre diameter value drops from 0.33 to 0.16 µm. Usage of nanometric fibres has proven advantageous in air filtration since their small diameter, and high surface to volume ratios can enhance the capture of particles through interception.

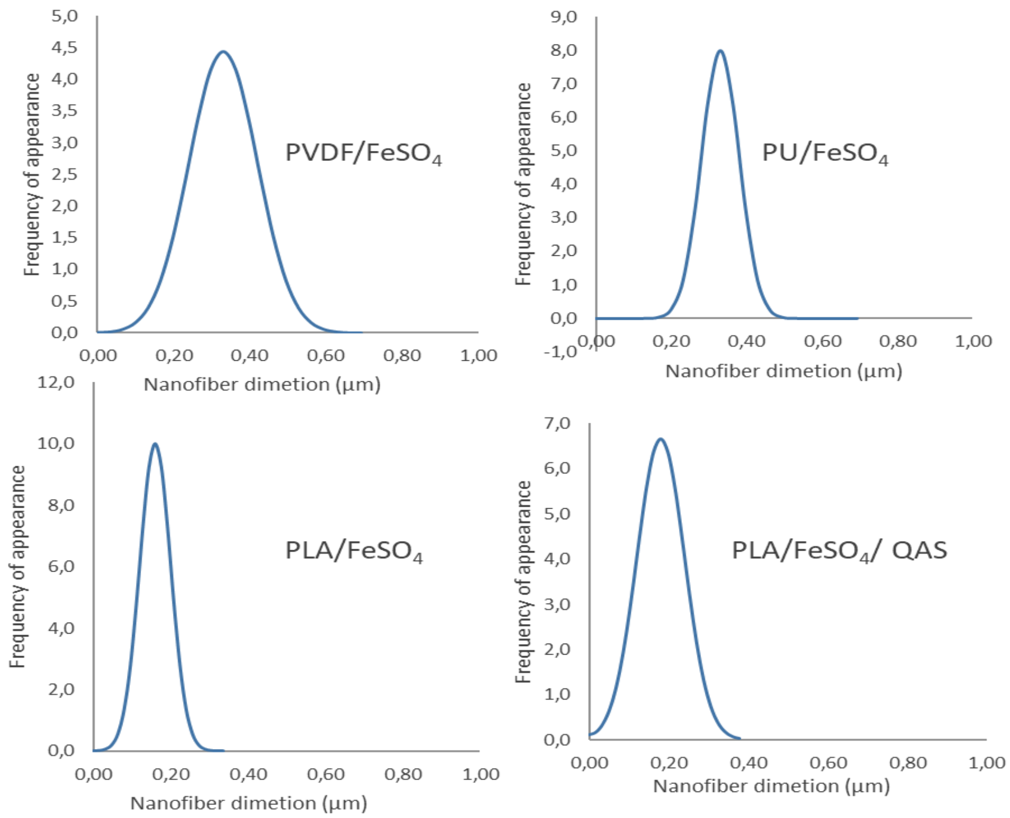

Figure 2 Nanofibers diameter distributions of PVDF/ FeSO4 (average dismeter 0.33 µm), PLA/ FeSO4 (0.16 µm), PU/ FeSO4 (0.33 µm) and PU/ FeSO4/QAS (0.18 µm)

Another important measurement for revealing the properties of prepared samples was porometry. The flow porometry technique is a useful tool for testing nanofiber structures. **Figure 3** presents the pore size distributions of all tested samples. While the distributions of PVDF and PLA have a width of 0.75 µm and 0.85 µm, respectively, and a mean pore size of 0.92 µm and 0.84 µm, the width of the distribution curve for PU/$FeSO_4$ is up to 1.6 µm and the mean pore size reaches 2 µm. However, using a combination of $FeSO_4$ and QAS results in a significant narrowing of the distribution (width of 0.64 µm) and a decrease in the mean pore size to 0.91 µm. The narrower pore size distribution suggests that the material will be more homogeneous. The more homogenous nanofiber layer means lower pressure drop, which is a characteristic playing a significant role in the application of electrospun nanofibers in air filtration [9].

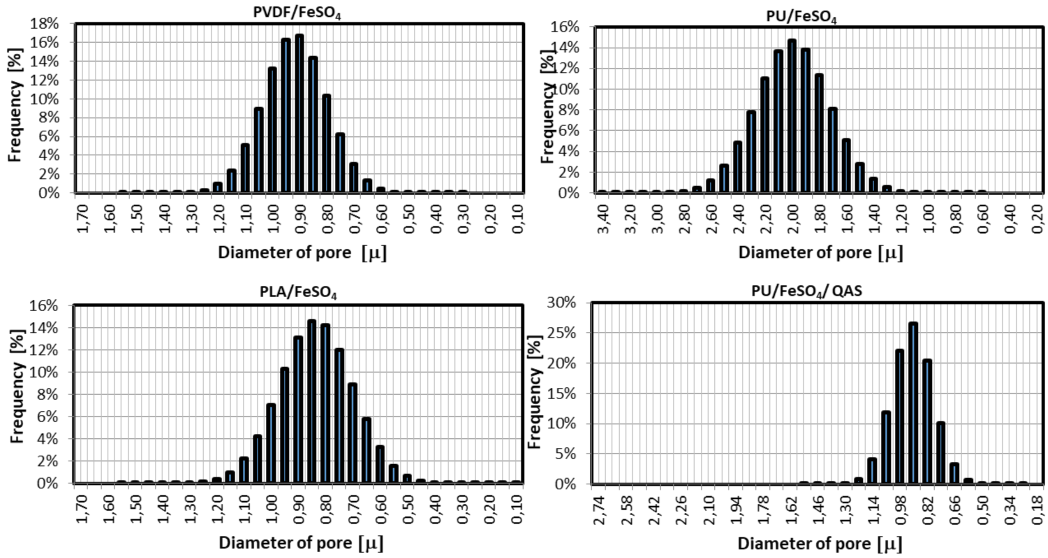

Figure 3 Flow porometry of spun nanofibers doped with $FeSO_4$

After detailed characterisation of prepared nanofibrous materials, we proceeded to antimicrobial activity tests according to -SO 22196:2011. Antimicrobial properties were investigated using *S. aureus* and *K. pneumoniae* as model microorganisms. After treatment time with bacterial solution (4h, 24h) final CFU of bacteria was counted and converted to antimicrobial activity (A) described above. Then it was possible to evaluate precisely the efficacy of antimicrobial activity of the tested materials. **Table 3** describes the exact rate of effectivity of antimicrobial activity according to the A values given in **Figure 4**. It seems that hydrophilicity/hydrophobicity has a significant influence on the antimicrobial activity of the polymer because electrospun nanofibers from PVDF and PLA polymers have low antimicrobial activity compared with more hydrophilic PU nanofibers. Furthermore, adding QAS to polymer solution shows an essential effect on bacteria elimination. The antimicrobial effect is strongest in the case of both bacterial strains (A ≥ 3).

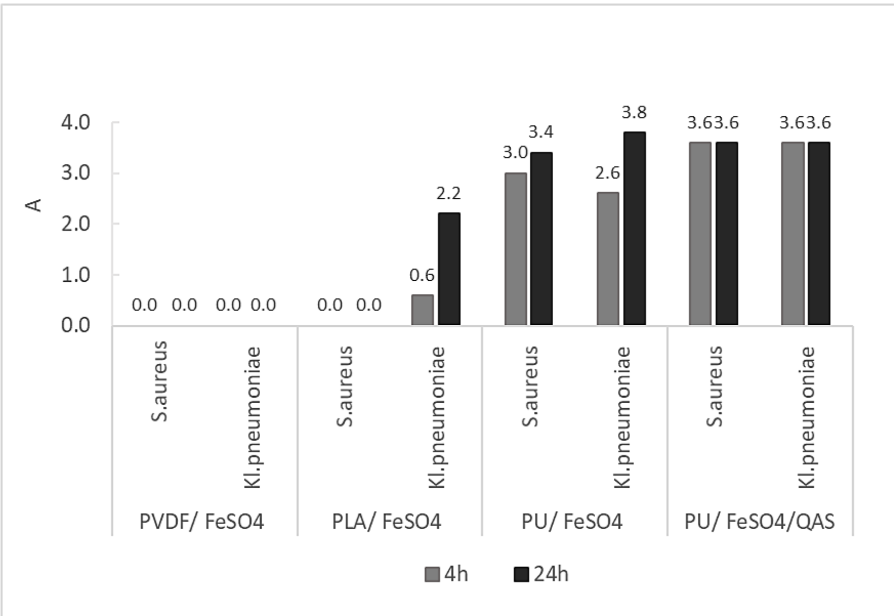

Figure 4 Antimicrobial activity of prepared nanofibrous materials using *S. aureus* and *K. pneumoniae* as model microorganisms, and evaluation of antimicrobial activity (A) after 4h and 24h

Table 3 Explanation of effectivity of antimicrobial activity (A) values

Efficacy of antibacterial properties	Value of antimicrobial activity A
Weak	1 < A < 2
Significant	2 ≤ A< 3
Strong	A ≥ 3

4. CONCLUSION

Electrospun nanofibers offer a number of advantages when used in air filters. There are extensive studies that demonstrate improvement in filteration efficiency without increasing pressure drop. In this research, we successfully doped various nanofibrous mats (PVDF, PLA, PU) designed for air filtration purposes with potential antimicrobial species $FeSO_4$ and QAS.

Characterisation analyses such as SEM, porometry or measurement of the diameter of nanofibers were performed. These characteristics may help to elucidate correlations between antimicrobial activity and the nature and structure of the nanofibrous material. Changes in conductivity of electrospinning polymer solutions significantly impact the diameter and the distribution of nanofiber's diameters as well as porosity of nanofibrous material and its distribution. Furthermore, we proved that adding QAS to PU/$FeSO_4$ polymer solution increased the antimicrobial effect, which was observed for a total period of 24h. Prepared nanofibrous materials with the best effect against bacteria, i.e. PU/$FeSO_4$ and PU/$FeSO_4$/QAS, was selected for a further study dealing with optimising of nanostructure properties for air filtration.

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