

ANTIBACTERIAL ELECTROSPUN MEMBRANE PREPARED FROM POLY (VINYLIDENE FLUORIDE)-CO-HEXAFLUOROPROPYLENE WITH LAURIC ACID MONOACYLGLYCEROL

Jana ZELENKOVA¹, Petra PEER¹, Pavel PLEVA², Magda JANALIKOVA², Jana SEDLARIKOVA³, Petr FILIP¹

¹*Institute of Hydrodynamics of the Czech Academy of Sciences, v. v. i., Prague, Czech Republic, EU, zelenkova@ih.cas.cz, peer@ih.cas.cz, filip@ih.cas.cz*

²*Faculty of Engineering, Tomas Bata University in Zlin, Department of Environmental Protection Engineering, Zlin, Czech Republic, EU, ppleva@utb.cz, mjanalikova@utb.cz*

³*Faculty of Engineering, Tomas Bata University in Zlin, Department of Fat, Surfactant and Cosmetics Technology, Zlin, Czech Republic, EU, sedlarikova@utb.cz*

<https://doi.org/10.37904/nanocon.2020.3741>

Abstract

The aim of this study was to prepare an antibacterial nanofibrous membrane using electrospinning technique. The nanofibrous membranes were spun from polymer solution of poly(vinylidene fluoride)-co-hexafluoropropylene (PVDF-co-HFP) dissolved in N,N'-dimethylformamide. Monoacylglycerol of lauric acid (MAG C12) was used as an antimicrobial agent at the concentrations ranging from 1 to 3 wt%. The impact of MAG C12 incorporation on the rheological, structural and antibacterial properties was investigated. The rheological tests of polymer solutions, as steady shear and oscillatory shear, proved that addition of MAG C12 changed marginally rheological quantities such as viscosity, elastic (storage) and viscous (loss) moduli. Measurement of mean nanofibres diameter indicated a slight decrease with increasing MAG C12 concentration. Antimicrobial activity of PVDF-co-HFP nanofibre membranes with incorporated MAG C12 against Gram-positive bacteria *Staphylococcus aureus* and Gram-negative *Escherichia coli* was studied. An antibacterial activity was revealed for the samples containing MAG C12 at all concentrations against Gram-positive bacteria *Staphylococcus aureus* by the disk diffusion method.

Keywords: Nanofibres, electrospinning, antibacterial membrane, rheology, polymer solution

1. INTRODUCTION

Usage of membrane technology is very common in many industrial fields as electro-technology [1,2], water treatment [3-5] or fluids separation [6]. At present, the fabrication of nanofibrous membranes by an electrospinning method is the common approach. Nanofibres are created in an electric field between polymer drop and a collector under optimized conditions. Fluorinated polymers as poly(vinylidene fluoride) (PVDF) or polytetrafluorethylene (PTFE) [7] are suitable for membrane fabrication, among other things also due to their high resistance to chemical and mechanical impact of environment. In recent years, the copolymer of PVDF with hexafluoropropylene (HFP) proved its potential due to better mechanical properties, lower crystallinity, better solubility and higher hydrophobicity compared with pure PVDF [3,4]. The modification of characteristics of electrospun nanofibers, e.g. hydrophobicity, is achieved by blending the polymer with such ingredients like monoacylglycerols to control wettability and obtain antimicrobial activity. Monoacylglycerols (MAGs) are organic compounds consisted of glycerol linked to fatty acid via an ester bond. The antibacterial (bacteriostatic and bactericidal) properties of saturated fatty acids and their derivatives, such as lauric acid monoacylglycerol with 12 carbon atoms, have been investigated by many researches [8-11]. Although the mechanism of inhibition is not completely known, Yoon et al. [12] present that the antimicrobial activity is given by three cells

processes based on cell lysis, disruption of electron transport in oxidative phosphorylation process and inhibition of membrane enzymes.

The aim of this contribution was to prepare the electrospun antibacterial membranes based on PVDF-co-HFP dissolved in N,N'-dimethylformamide. Different concentrations of MAG C12 and solvent were used and rheological characteristics were investigated with an emphasis paid to correlation with nanofibres quality.

2. EXPERIMENTAL

PVDF-co-HFP Kynar® 2801 was purchased from Arkema (Arkema Inc. France). N, N'-dimethylformamide p.a. (DMF) used as a solvent was purchased from P-LAB (Czech Republic). Both chemicals were used without further purification. 1-monocaprin (MAG C12) was prepared by direct addition of decanoic acid into glycidol by the epoxide ring opening in a double skin reactor at the temperature of 90 °C [13]. The product was then recrystallized from ethanol to the purity of 99 %.

2.1. Sample preparation

Four samples differing in concentration of MAG C12 were prepared. Each sample contained 23 wt.% of PVDF-co-HFP, the remaining 77 wt.% were shared by DMF and MAG C12 with the ratios 77-0, 76-1, 75-2, and 74-3 wt.%. All samples were mixed at 25 °C using a magnetic stirrer Heidolph MR Hei-Tec (Heidolph, Germany) for 24 hours.

2.2. Rheological characterization of samples

The steady shear and oscillatory tests were applied for the sample characterization. The measurements were carried out using a rotational rheometer Physica MCR 501 (Anton Paar, Austria) equipped with concentric cylinders geometry (the inner and outer diameters were 26.6 and 28.9 mm, respectively) at temperature 25 °C. Strain sweep measurement was carried out in the range 0.1 – 100 % and frequency sweep measurement was carried out in the range 0.1 – 100 Hz at 1 % strain. The phase angle δ was determined by means of the definitoric relation, $\tan \delta = G''/G'$, where G'' is the loss (viscous) modulus and G' is the storage (elastic) modulus. Consequently, the viscosity was measured in the range 0.1 – 300 s⁻¹ and shear rate value 0.12 s⁻¹ (in linear viscoelastic (LVE) region) was chosen as the reference point.

2.3. Electrospinning process

Nanofibrous mats were electrospun using a laboratory device (details are introduced in [14]) with the tip-to-collector distance 10 cm under voltage 18 kV (a high voltage power supply Spellman SL70PN150, USA). The volume of the processed polymer sample attained approximately 0.2 mL. The experiments were carried out under ambient conditions (temperature 23 ± 1 °C, relative humidity 34 ± 1 %).

2.4. Scanning electron microscopy

The scanning electron microscopy measurement was used for characterization of electrospun fibres. The analysed samples were sputtered by a conductive coating (gold) layer using a sputter Quorum Q150R (Quorum Technologies Ltd., UK) and fibres quality was evaluated by a scanning electron microscope Vega 3 Tescan (Tescan, Czech Republic). The mean fibres diameter was determined using Adobe Creative Suite software by means of which 300 fibres were analysed from 3 different samples.

2.5. Wettability

The wettability of the nanofibrous membranes was analysed by gauging contact angles according to the sessile drop method using the Surface Energy Evaluation System by the Advex Instruments (Czech Republic) at laboratory temperature. The mean values were calculated as an average from five independent

measurements. Demineralized water was employed as the reference liquid; the volume of each deposited droplet attained 3 μL .

2.6. Antibacterial tests – agar disk diffusion method

In order to observe the antibacterial activity, the agar disk diffusion method was carried out: circular samples (9 mm in diameter) of nanofibres from neat PVDF-*co*-HFP or PVDF-*co*-HFP enriched with MAG C12 (1, 2, 3 wt.%) were placed on agar plates previously inoculated with 1 mL of 0.5 McF turbid bacterial suspension (*Escherichia coli* CCM 3954, *Staphylococcus aureus* CCM 3953) in sterile saline solution. Moreover, sterile paper disks (9 mm in diameter) were loaded by 10 μl MAG C12 (5 wt.% in ethanol) and tested in the same way as nanofibres. The plates were incubated at 37 °C for 24 hours and the whole experiment was repeated three times. The inhibition zones as well as growth under the samples were evaluated.

3. RESULTS AND DISCUSSION

Proper electrospinnability of polymeric materials is closely related to their viscoelasticity. The following rheological measurements were carried out for four polymeric materials differing in MAG C12 content (0, 1, 2, and 3 wt.%). First, oscillatory strain and frequency sweep measurements describing a degree of elasticity of the studied materials are depicted in **Figure 1**. Based on these measurements, the phase angle δ relating a mutual ratio of viscous and elastic moduli was determined, see **Figure 2** (left). The values of shear viscosity obtained for the value of shear rate 0.12 s^{-1} in the linear LVE region are presented in **Figure 2** (right) jointly with the mean fibres diameters. **Figure 3** provides visual evaluation of all four nanofibrous mats. Unlike e.g. dodecyltrimethyl ammonium bromide used as antibacterial agent in [15], monoacylglycerol MAG C12 does not change the structure of the fibres.

Apparently, monoacylglycerol MAG C12 had a negligible impact to the storage and loss moduli and mean nanofibre diameter contrasting to its influence on hydrophobicity (hydrophilicity) of nanofibrous membranes. Their wettability was evaluated by the sessile drop method as presented in **Table 1**. The mean contact angles document hydrophilic behaviour for the MAG C12-modified nanofibrous membranes while the pure PVDF-*co*-HFP membrane exhibits hydrophobic behaviour.

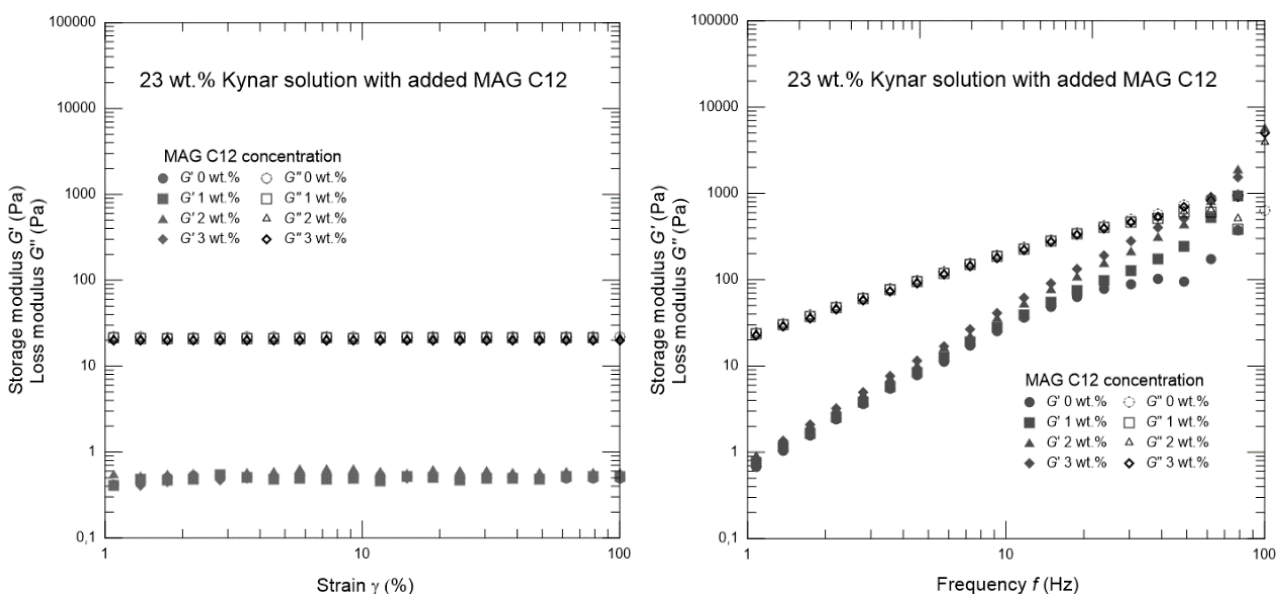


Figure 1 Oscillatory strain (left) and frequency (right) sweep measurements characterizing viscoelastic nature of polymer solutions

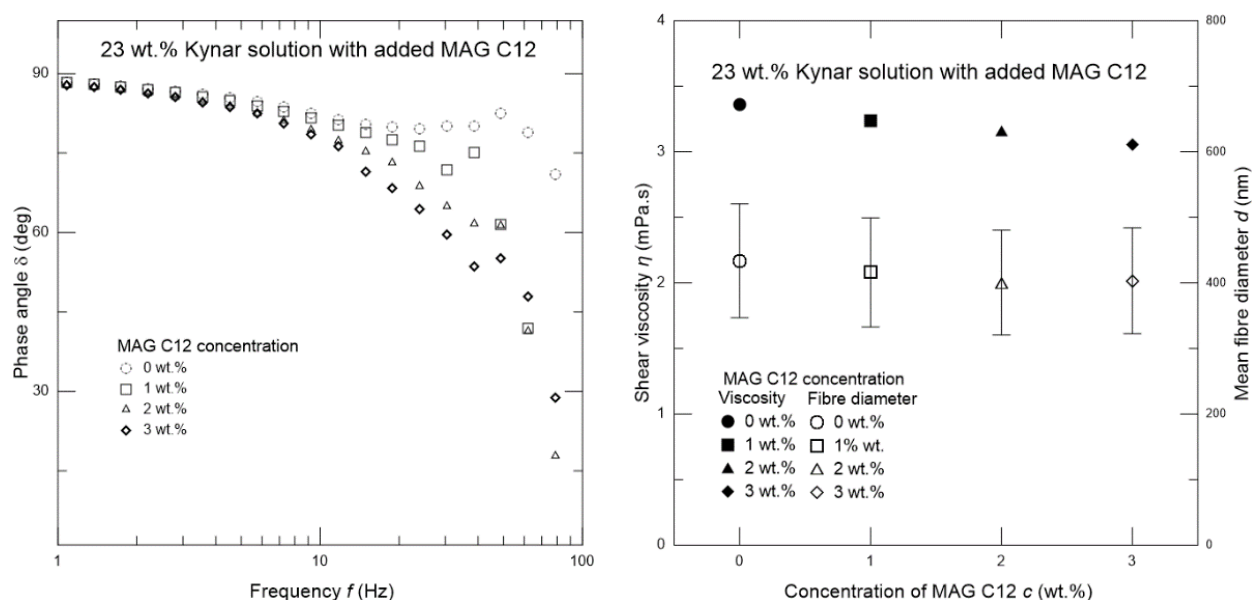


Figure 2 Phase angle (left), shear viscosity of polymer solutions (right) and mean fibre diameter of electrospun nanofibres (right)

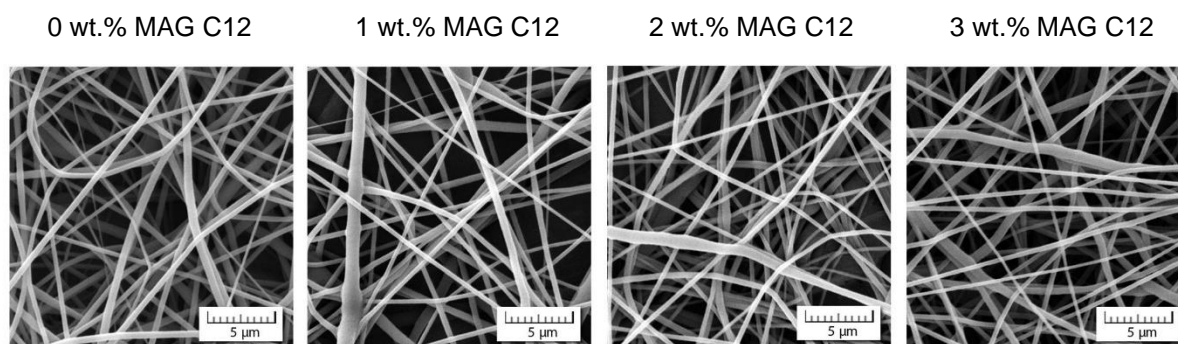


Figure 3 The quality of nanofibrous mats without/with added MAG C12

Table 1 Contact angle for PVDF-*co*-HFP nanofibrous membranes in dependence on MAG C12 content

MAG C12 concentration (wt.%)	0	1	2	3
Contact angle (deg)	128	19	25	26

The antibacterial activity of MAG C12, neat PVDF-*co*-HFP and PVDF-*co*-HFP/MAG C12 nanofibrous membranes against *Escherichia coli* and *Staphylococcus aureus* was performed by the agar disk diffusion method. MAGs were previously reported to be effective against various Gram-positive and Gram-negative bacteria [12]. In this work, it was found that MAG C12 (5 wt.%) against *E. coli* is effective and against *S. aureus* has antibacterial activity, see **Table 2**. Neat PVDF-*co*-HFP nanofibrous membranes exhibited no growth inhibition using the disk diffusion method, on the other hand MAG-modified membranes proved antibacterial activity against *Staphylococcus aureus*, see **Figure 4**. Antibacterial activity of MAG C12 incorporated into nanofibrous membranes was higher with increasing concentration, however the effect of 2 wt.% and 3 wt.% PVDF-*co*-HFP/MAG C12 nanofibrous membranes seemed to be comparable. PVDF-*co*-HFP/MAG C12 nanofibrous membranes did not prove to be inhibitory against *Escherichia coli*. Nevertheless, it is possible to expect antifouling activity against all bacteria as it was proved for the similar system of PVB/MAG C10 nanofibrous membranes [16].

Smaller inhibition zones observed in the case of PVDF-*co*-HFP/MAG C12 nanofibrous membranes in comparison with MAG C12 can be explained either by lower concentration of applied MAG C12 or/and by lower amount of MAG C12 diffused from membranes. Monocaprin (MAG C10) have been reported to exhibit very similar antibacterial activity as monolaurin (MAG C12). It was recently published that MAG C10 from PVB/MAG C10 nanofibrous membranes was not diffused almost at all [16]. On the other hand, this work proves a release of MAG C12 from PVDF-*co*-HFP/MAG C12 nanofibrous membranes.



Figure 4 The inhibition zones of *S. aureus* (SA) nanofibrous membranes without/with added MAG C12 (from left to right: 0 wt.%, 1 wt.%, 2 wt.%, 3 wt.%)

Table 2 The inhibition zones of nanofibrous membranes PVDF-*co*-HFP/MAG C12 (disk diameter 9 mm)

	SA (mm)	EC (mm)
MAG C12 (5 wt.%)	27	11
PVDF- <i>co</i> -HFP / 0 wt.% MAG C12	9	9
PVDF- <i>co</i> -HFP / 1 wt.% MAG C12	13	9
PVDF- <i>co</i> -HFP / 2 wt.% MAG C12	19	9
PVDF- <i>co</i> -HFP / 3 wt.% MAG C12	18	9

4. CONCLUSION

The application of MAG C12 as an antimicrobial agent exhibited non-negligible advantages. As documented above, no changes in rheological characterization of primary solutions were evoked by adding this agent in units of percentage. The courses of loss and storage moduli, and shear viscosity were practically unchanged as well as the mean nanofibre diameters. The addition of MAG C12 had other positive aspects. First, a purely hydrophobic behaviour of neat PVDF-*co*-HFP membranes was abruptly converted to hydrophilic character with adding as low as 1% of MAG C12, which is suitable for filtration applications. Moreover, the antimicrobial efficiency of PVDF-*co*-HFP/MAG C12 membranes against *Staphylococcus aureus* was proved.

ACKNOWLEDGEMENTS

This research was supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project LTC 19034), and within Program NPU I (LO1504). Institutional support was provided by the Czech Academy of Sciences, Czech Republic (RVO: 67985874).

REFERENCES

- [1] CAO, J-H., ZHU, B-K., XU, Y-Y. Structure and ionic conductivity of porous polymer electrolytes based on PVDF – HFP copolymer membranes. *Journal of Membrane Science*. 2006, vol. 281, no. 1- 2, pp. 446 – 453.

- [2] STEPHAN, A. M., NAHM, K. S., KULANDAINATHAN, M. A., RAVI, G., WILSON, J. Poly(vinylidene fluoride – hexafluoropropylene) (PVdF-HFP) based composite electrolytes for lithium batteries. *European Polymer Journal*. 2006, vol. 42, no. 8, pp. 1728–1734.
- [3] TIAN, X., JIANG, X. Poly(vinylidene fluoride-co-hexafluoropropylene) (PVDF-co-HFP) membranes for ethyl acetate removal from water. *Journal of Hazardous Materials*. 2008, vol. 153, no. 1 – 2, pp. 128 – 135.
- [4] GARCÍA-PAYO, M. C., ESSALHI, M., KHAYET, M. Effects of PVDF-HFP concentration on membrane distillation performance and structural morphology of hollow fiber membranes. *Journal of Membrane Science*. 2010, vol. 347, no. 1–2, pp. 209–219.
- [5] LALIA, B. S., GUILLEN-BURRIEZA, E., ARAFAT, A. H., HASHAIKEH, R. Fabrication and characterization of polyvinylidene fluoride-co-hexafluoropropylene (PVDF-HFP) electrospun membranes for direct contact membrane distillation. *Journal of Membrane Science*. 2013, vol. 428, no. 1, pp. 104–115.
- [6] WANG, X., XIAO, C., LIU, H., HUANG, Q., HAO, J., FU, H. Poly(vinylidene fluoride-hexafluoropropylene) porous membrane with controllable structure and applications in efficient oil/water separation. *Materials*. 2018, vol. 11, no. 3, p. 443.
- [7] KANG, G-D., CAO, Y-M. Application and modification of poly(vinylidene fluoride) (PVDF) membranes – A review. *Journal of Membrane Science*. 2014, vol. 463, no. 1, pp. 145–165.
- [8] KODICEK, E., WORDEN, A. N. The effect of unsaturated fatty acids on *Lactobacillus helveticus* and other Gram-positive Micro-organisms. *Biochemical Journal*. 1945, vol. 39, pp. 78–85.
- [9] KABARA, J. J., SWIECZOWSKI, D. M., CONLEY, J. A., TRUANT, J. P. Fatty acids and derivatives as antimicrobial agents. *Antimicrobial Agents and Chemotherapy*. 1972, vol. 2, no. 1, pp. 23–28.
- [10] SCHLIEVERT, P. M., DERINGER, R. J., KIM, M. H., PROJAN, S. J., NOVICK, R. P. Effect of glycerol on bacterial growth and toxin production. *Antimicrobial Agents and Chemotherapy*. 1992, vol. 36, no. 3, pp. 626–631.
- [11] OH, D. H., MARSHALL, D. L. Antimicrobial activity of ethanol, glycerol monolaurate or lactic acid against *Listeria Monocytogenes*. *International Journal of Food Microbiology*. 1993, vol. 20, no. 4, pp. 239–246.
- [12] YOON, B. K., JACKMANN, J. A., VALLE-GONZÁLES, E. R., CHO, N-J. Antibacterial free fatty acids and monoglycerides: Biological activities, Experimental Testing and Therapeutic Applications. *International Journal of Molecular Sciences*. 2018, vol. 19, no. 4, pp. 1–40.
- [13] JANIS, R., KLASEK, A., KREJCI, J., BOBALOVA, J. Influence of some chromium complexes on the conversion rate of glycidol – fatty acid reaction. *Tenside Surfactants Detergents*. 2005, vol. 42, no. 1, pp. 44–48.
- [14] PEER, P., STENICKA, M., PAVLINEK, V., FILIP, P. The storage stability of polyvinylbutyral solutions from an electrospinnability standpoint. *Polymer Degradation and Stability*. 2014, vol. 105, no., pp. 134-139.
- [15] CAPKOVA, P., KORMUNDA, M., KOLSKA, Z., TROGL, J., MUNZAROVA, M., RYSANEK, P. Electrospun antimicrobial PVDF-DTAB nanofibrous membrane for air filtration: Effect of DTAB on structure, morphology, adhesion, and antibacterial properties. *Macromolecular Materials and Engineering*. 2018, vol. 303, no. 3, 1700415.
- [16] PEER, P., SEDLARIKOVA, J., JANALIKOVA, M., KUCEROVA, L., PLEVA, P. Novel polyvinyl butyral/monoacylglycerol nanofibrous membrane with antifouling activity. *Materials*. 2020, vol. 13, p. 3662.