Electrospun Polystyrene in Limonene Fibrous Structures for Medical Applications

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This study focuses on possible applications of polystyrene in the medical field. Nonwoven fiber mats were produced through electrospinning, using limonene or, as control, the conventionally used ethyl acetate. Subsequently, different types of dilutions were tested. Electrospinning (ES) was performed with different parameters to study the effect upon the fibrous materials resulted, in view of obtaining structures with high porosity and a specific desired morphology. Thin fibers were obtained using both solvents, with fibers average diameter of 1.98-2.58 μm for limonene and 8.87-13.81 μm for ethyl acetate. The obtained various materials are a result of the different solvent densities, surface tensions and polymer concentrations. Best quality fibers, when using limonene, were obtained for 35 % polystyrene, while when using ethyl acetate the high quality fibers were obtained for 60 % polystyrene in solution. The mats morphology and structure were compared and analyzed through optical microscopy, scanning electron microscopy and Fourier Transform Infrared Spectroscopy. This study proposes the fabrication of nonwoven fibrous mats from polystyrene using a natural, economical, biocompatible and environmentally friendly solvent that can successfully replace the common and toxic solvents in medical applications involving wound dressing or tissue reconstruction.

Keywords: electrospinning, limonene, polystyrene, ethyl acetate, wound dressings

There are several methods for providing fiber mats to serve as support structures for Regenerative Medicine and wound dressing, such as electrospinning or fiber bonding. The difference between these two is that, while the first leads to manufacturing of nonwoven mats, the second one is used to bind the individual fibers together. Both methods have advantages such as the high porosity or the small fiber thickness, but they have several drawbacks as well, their main disadvantage being the poor mechanical proprieties for particular applications. Electrospinnig (ES) was found to be more suitable for wound dressings, due to its capacity to produce porous nanometer to micron – scale fibrous structures, which provide nonwoven textiles with desirable properties, best accepted by the extracellular matrix [2-6].

This method, derived from the electrostatic spraying of polymer coatings, can produce fibers that are successfully used in biomedical applications, such as drug delivery, wound dressing, implants and membranes for the recovery of the harmed tissue. The obtained structures possess sufficient mechanical strength for these particular applications [2-6].

Polystyrene (PS) is a thermoplastic, inexpensive, stiff material, transparent, with high electrical resistance and low dielectric loss. PS can be used in biomedical application, for example for cell cultures and can be easily processed into fibers through Electrospinning [7]. Limonene is a monoterpenic compound extracted from citrus peels, being available in both enantiomeric forms: R(+) and S(-) [8]. As a non-polar organic compound, it may be used as a solvent for non-polar organics, including polystyrene. Due to its external and internal double bonds exposed to polymerization, limonene cannot be radically co-polymerized with vinyl monomers [10].

D-limonene was used in preclinical studies due to its chemotherapeutic activity and minimal toxicity in treating patients with advanced cancer. D-limonene was found to be well tolerated in cancer patients [13].

Other application involves the use of limonene as solvent for PS, for improving the quality of recycling polystyrene and reducing the transportation costs in the process. Shin and Chase [11] used *d*-limonene to dissolve expanded polystyrene in the recycling process. They have chosen this natural solvent because it is environmentally friendly, biodegradable, biocompatible, safe, and can replace some other organic solvents like acetone, ethyl acetate, toluene.

This study aims to analyze the fabrication of nonwoven fibrous structures from polystyrene and limonene, as natural, economical, biocompatible and environmentally friendly solvent in order to replace the common and toxic solvents for medical applications such as wound dressings. This fibrous structure is designed to be used as a skin wound dressing support, which can be easily replaced without causing any trauma to the patient, or as a support structure, for Regenerative Medicine.

Experimental part

Materials and Methods

Polystyrene powder (Castform, 3D Systems, Germany), having particle size under 50μm, with 0.86 g/cm^3 density, was used to elaborate nonwoven fibers mats through ES. Limonene was used to dissolve polystyrene and obtain homogenous and clear solutions to be further processed into nonwoven fiber mats. Limonene ($C_{10}H_{16}$), Mw 136.24 g/mol, 0.841 g/cm^3 density, was extracted from orange peels through a steam distillation process. The white pith of the orange peels was removed and the orange peels were chopped into pieces of about 1 cm^2, placed inside a glass flask and covered with distilled water. A heating mantle (Raypa) was used for the extraction, using a glass distillation unit. The condensed liquid contained a light phase (limonene) at the surface of water. The separation of limonene was made using a glass separation funnel the

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resulted limonene was employed as prepared for the dissolution of PS. The output ratio (efficiency of extraction) η was found to be 1.882 %, close to the maximum value of 2% [9], due to several white residues remained on the orange peels. Different concentrations of PS in limonene were prepared: 10 %, 25 % and 35 % PS. For more than 35 % PS in solution, the resulted viscosity was too high, further processing being impossible. The solutions were prepared at room temperature, then stirred for 30 min and left for 24 h, allowing the polymer to dissolve completely. Small quantities of ethyl acetate (10 μL in 1 mL PS-limonene dilution) were mixed into the 25 % and 35 % solutions, as they were found to increase the solvent evaporation rate and to improve the surface tension and homogenization.

Ethyl acetate (Sigma Aldrich), with a density of 0.902 g/mL at 25°C, was used to obtain solutions with 60% and 70% PS concentrations, which were previously found appropriate. The homogenous and clear solutions were prepared by dissolving PS into ethyl acetate, at room temperature, followed by 30 min stirring. The solutions were left for 24 h, allowing the polymer to dissolve completely.

Electrospinning process

The electrospinning setting (fig. 1) consists of a high-voltage generator (GN 1090 Sames) connected to a syringe metallic needle and to a stainless steel collector plate placed within the spin coater, at 8 cm from the tip of the needle, according to the solutions being used. The spin-coater spun the metallic collector during different time intervals. Each polymeric solution was drown into a 10 cm^3 glass syringe, at a constant feed rate of 0.1 mL/min using a syringe pump (WRI SP 230 IWZ) to prevent the air bubbles formation. The flow rates were controlled by the syringe pump, producing a continuous stream towards the grounded metallic collector. The process took place at room temperature. Different morphologies of fibers / mats were obtained by changing the voltage, the flow rate and the spinning periods.

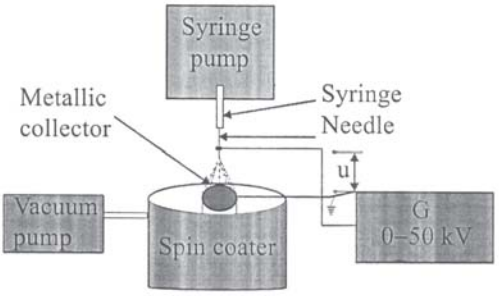

Fig. 1. Schematic illustration of the ES setting used in this study

In this study, nonwoven fiber mats were obtained from both solvents at voltages between 25 and 30 kV, while the optimal found flow rates were set at 0.01 mL/min for limonene and 0.05 – 0.1 mL/min for ethyl acetate.

Fibers characterization

The fibers morphology and diameters were investigated through optical and electron microscopy (EUROMAX Winfast PV2, Optika Lab 1, Scanning Electron Microscopy SEM, JEOL - JSM 5600 LV).

A single-beam Fourier Transform Infrared (FT-IR), Spectrum BX FTIR spectrometer from Perkin Elmer was also used. The measurements were made using an ATR (Attenuated Total Reflectance) accessory, both for limonene and ethyl acetate. Square 5 mm fiber mats samples and unprocessed polystyrene raw material was investigated.

The composition of limonene in the resulted extract was analyzed by GC-FID (GC:Agilent 7890A) on a HP5 column (30 m, 0.320 mm, 0.25 mm), using a 100:1 split ratio, 0.5 mL/min He flow and the following temperature program: 40°C (5 min); 15 °C /min to 70°C for 10 min; 10°C /min to 120°C for 3 min; 15°C /min to 200°C for 10 min.

The sample for GC-FID analysis was prepared by dissolving 0.25 mL limonene extract in 0.75 mL ethanol (S.C. Reagents).

Results and discussions

Morphology characterization

Table 1 shows the optimal parameters chosen after preliminary tests together with the fibers average diameters. The images were imported into Olympus Microscope Software to manually measure and calculate the fiber average diameters of 30 fibers per image. Important differences were observed by varying the parameters, especially concerning the fiber average diameters.

The 10 and 25% polystyrene in limonene solutions were impossible to process into fibers through electrospinning. Oily beads did not allow solvent evaporation in time. This is due to the low evaporation rate, low surface tension of the solvent, and low polymer concentration.

The 35 % PS in limonene solution was found to produce fine and thin fibers. Two sets of parameters produced the best results: 25 kV, 0.01 mL/min, 600 s (2.58 μm average diameter) and at 30 kV, 0.01 mL/min 900 s (1.98 μm average diameter). The distance between the needle and the metallic collector was 8 cm. Best fibers in what concerns diameter and morphology were obtained at 30 kV, 0.01 mL/min, 900 s (fig. 2 a and b). Limonene was found to be very restrictive as solvent of PS in what concerns flow rate as well: the best flow rate for the 35 % PS solution was of 0.01 mL/min. The higher the voltage for the same flow rate, the thinner the fibers and the denser the nonwoven mesh.

An important parameter in agreement with the solvent evaporation rate was the surface tension. The surface tension of limonene needs to be modified in order to

Solvent	Polystyrene (%)	Flow rate (mL/min)	Voltage (kV)	Revolution (rpm)	Time (s)	Fibers average diam. (μm)
Limonene + Ethyl Acetate	35	0.01	25	50	600	2.58
	35	0.01	30	80	900	1.98
Ethyl Acetate	60	0.1	25	200	120	9.30
	60	0.05	30	200	120	8.87
	70	0.05	30	200	90	13.81

Table 1
THE ELECTROSPINNING PARAMETERS AND OBTAINED RESULTS

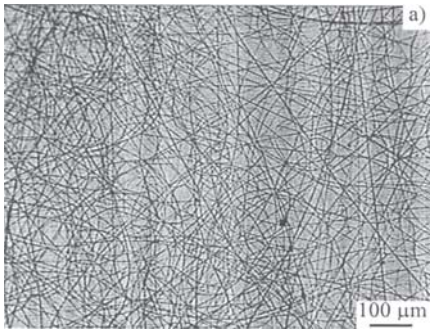

Fig. 2a. Optical of limonene and 35% polystyrene nonwoven fiber mats obtained through electrospinning at 30 kV, 0.01 mL/min, 900 s

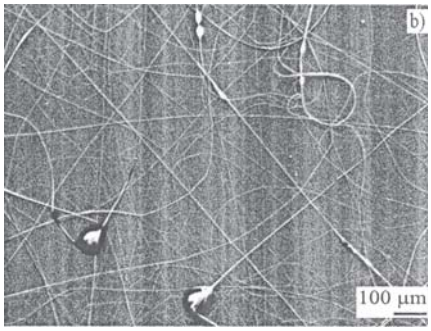

Fig. 2 b) SEM aspects of limonene and 35% polystyrene nonwoven fiber mats obtained through electrospinning at 30 kV, 0.01 mL/min, 900 s. SEM pictures were made at 20 kV, magnitude of 100 and 300 microns

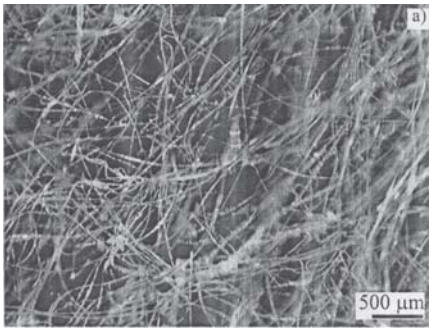

Fig. 3a. Optical stereoscopic images of electrospun PS fibers obtained from 60% PS, 25 kV, 0.1 mL/min, 120 s

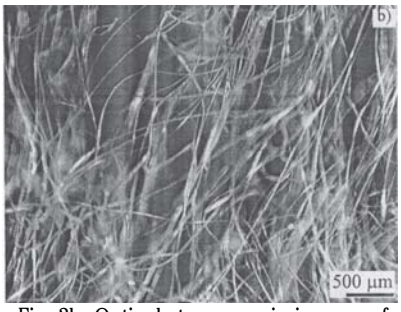

Fig. 3b. Optical stereoscopic images of electrospun PS fibers obtained from 70% PS solution at 30 kV, 0.05 mL/min, 120 s

increase the solution evaporation rate. By increasing the polymer concentration, the solution became more viscous and the surface tension too high. Therefore, by adding a small amount of organic solvent with low surface tension, (1 % ethyl acetate) the solution became more volatile, while still keeping its non toxic feature.

The 35% PS solution can be processed into micro and nanofibers, with a few number of beads, as it can be seen from the optical and SEM images (fig. 2 a and b). This is due to the factors combination during the process of solvent evaporation – fibers formation. By increasing the voltage from 25 kV to 30 kV, the fibers became more continuous, with smaller diameter and lower number of beads. By increasing the revolution from 50 rpm to 80 rpm, the fibers became more continuous.

By increasing the voltage, the spinning period, the distance between the collector and the needle and the revolution, more homogenous and continuous fiber mats were obtained.

All the solutions with polystyrene dissolved in ethyl acetate were processed through ES. Thin fibrous mats were obtained in the best conditions from the 60 % PS solution at 25 kV, 0.1 ml/min and 30 kV, 0.05 mL/min. The distance between the collector and the needle tip was maintained at 8 cm. Although the fibers were thin, many beads could be observed (fig. 3 a). This is due to the combination of factors during the process of solvent evaporation – fibers formation. The applied voltage was found to be more important for the resulted mats compactness and fibers thickness than the flow rate. For a twice smaller flow rate (0.05 mL/min vs. 0.1 mL/min) and a 16% higher voltage (30 kV vs. 25 kV), the mats were much denser, the fibers were thinner (9.30 µm vs. 8.87µm diameter at the average), while the beads content increased.

The solution with 70% PS was found to produce fine and thin fibers (13.81µm diameter at the average) in a nonwoven mesh, at approximately 30 kV and 0.05 mL/min (fig. 3 b).

The polymer concentration is important in this experiment as well: by increasing the polystyrene concentration, the number of beads is decreased and the fibers are more continuous. This observation was made as well by [14] and [11].

The solvent characteristics and the solution concentration determine the parameters of the electrospinning process. Compared to the mixtures of polystyrene and ethyl acetate, the flow rate for the polystyrene and limonene solution was reduced with up to 70 %, while the polymer concentration within the solution was 50 % lower. Fine and thin fiber mats were obtained using both solvents, with diameters ranging between 600 nm – 4.5µm (fiber average diameters of 1.98 for higher voltage and 2.58 µm for lower voltage, as seen in table 1 for limonene and between 3µm – 40µm (fiber average diameters of 8.87 for lower PS concentration, lower flow rate and higher voltage and 13.81µm for higher PS concentration, as seen in table 1 for ethyl acetate. The fibers obtained using polystyrene and limonene were smaller in diameter than the ones obtained using polystyrene and ethyl acetate. This was due to the difference in solvents densities, surface tensions and polymer concentrations. Best quality fibers, when using limonene, were obtained for 35 % polystyrene, while when using ethyl acetate, best quality fibers were obtained for 60 % polystyrene.

Chemical characterization

The polystyrene used in the process of electrospinning of fibers, limonene, ethyl acetate and the fibers obtained were analyzed by FT-IR. The spectrum of polystyrene raw material is indicated in figure 4.

Compared with the polystyrene data base spectrum given from Perkin Elmer, the spectrum of polystyrene raw material is very similar. The absorption peaks and bands correspond to the bonds in pure polystyrene: 2921.67 cm^{-1} – CH_2 – methylene groups, 1451.83 cm^{-1} – methyl group asymmetrical/symmetrical, 753.63 cm^{-1} – *cis*-C-H; 1601.15 cm^{-1} – aromatic ring stretch.

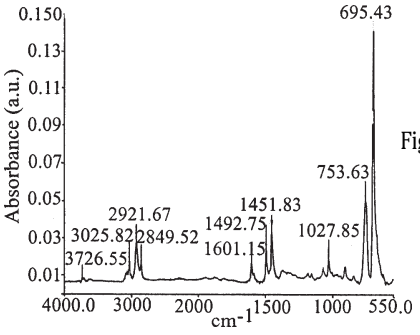

Fig. 4. FT-IR spectrum for the polystyrene raw material

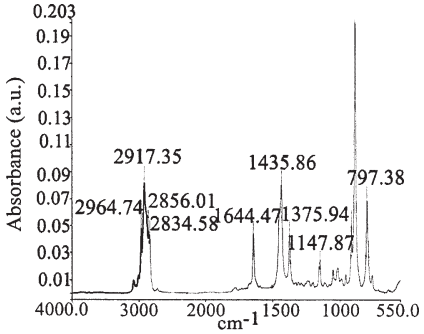

Fig. 5. FT-IR spectrum of the obtained limonene

The FT-IR spectrum of the obtained limonene is presented in figure 5. One can see that all the absorption peaks belong to the limonene. There were not found absorption peaks for other bonds of functional groups in the limonene spectrum, leading to the conclusion that high purity limonene has been obtained.

GC-FID chromatogram of limonene is shown in figure 6. The peak at retention time (R_t) of 23.040 min corresponds to limonene, while the one of 3.483 min corresponds to ethanol used as solvent. The small peaks around limonene peak (retention times between 18 and 33) are related to some impurities. The concentration of limonene was found to be 91.06 %. Amanzadeh et al. [12] analyzed limonene obtained from orange peels on GC-MS, limonene also contains small quantities of β- Myrcene (3.89 %), α-Pinene (0.94 %), Sabinene (0.48 %); Decanal (0.38 %).

The FT-IR analysis of the fibers obtained by electrospinning of PS solutions, using as solvents both limonene and ethyl acetate, revealed that the obtained fibers consist mainly of polystyrene. There were not found peaks of aliphatic double bonds from limonene or ester groups (from ethyl acetate) in the spectra of the fibers (fig. 7). All solvents were removed from the fibers during the manufacturing process.

As it can be seen from the FT-IR analysis on polystyrene - limonene fiber mats and polystyrene - ethyl acetate fiber mats (fig. 7. a and b, the fibers are very much alike, being very similar with polystyrene spectrum (as row material). This means that polystyrene chemical structure did not change during the electrospinning process. Moreover, the fiber mats spectra reveal the lack of the solvents tracks, meaning there is not any residual solvent in the fibers.

Conclusions

Nonwoven fiber mats could be produced through electrospinning of polystyrene and limonene or, the conventionally used ethyl acetate in order to compare the results. Different concentrations of solutions were tested in order to obtain fine, thin and continuous fiber mats. From the FT-IR spectrum one can observe that polystyrene did not suffer any chemical changes during the electrospinning process. No absorption peaks for other bonds of functional groups were found in the limonene spectrum, leading to the conclusion that the obtained limonene is of high purity (91.06 %). Compared with the electrospun fibers obtained from polystyrene and ethyl acetate, the fibers produced using limonene are very much alike, consisting mainly of polystyrene, with only a few number of beads. Compared to the mixtures of polystyrene and ethyl acetate, in the mixtures of polystyrene and limonene the flow rates were reduced with up to 70 % and the polymer concentration in solution is 50 % lower, leading to much thinner fibers. The PS fibrous mats obtained using limonene may be used as an alternative for wound dressings due to their low-cost materials and fabrication. The future perspective is to test the polystyrene - limonene fiber mats as wound dressing,

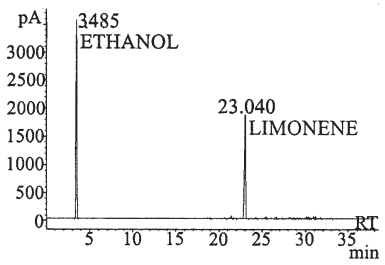

Fig. 6. GC-FID chromatogram of limonene extract

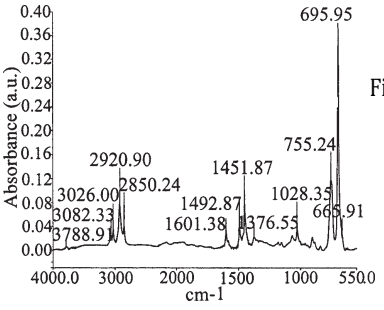

Fig. 7a. FT-IR spectrum of 35% PS-limonene fiber mats

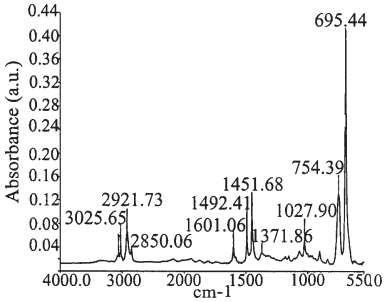

Fig. 7b. FT-IR spectrum of 70% PS-ethyl acetat fiber mats

due to the fact that PS does not trigger cell attachment and is non toxic, meaning the dressing can be easily changed without causing any trauma to the patient.

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