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STSM REPORT

Preparation of active packaging films based on biopolymers and bioactive extracts from plants and fungi by electrospinning technique

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Introduction

Food packaging industry presents one of the fastest growing industries nowadays. New trends of reducing food waste as well as packaging waste, preservation of food and prolonging their shelf-life and substitution of petrochemical sources with renewable ones are leading to development of this industrial area in diverse directions.

Antimicrobial food packaging, as part of active packaging concept has been researched in the last decade. Electrospinning technique has drawn a great attention for preparation of different antimicrobial food packaging materials especially when the natural compounds are involved, because of their preservation and release. In food packaging materials electrospun fibers are used for the encapsulation of plant extract, with the aim to preserve its integrity and control the release of the active substances. By controlling the material properties and processing parameters the ES technique offers possibility to produce fibers that possess the desired physicochemical properties (e.g. fibers with pores, knots…) and are able to encapsulate and release functional chemical compounds. Crude plant extract can be easily obtained by extraction in organic solvents from the fresh plants or from milled dried plant. On the other hand, it's very important to use polymers derived from natural sources. Polylactide (PLA) is one of the most promissing biopolymers due to similar properties with polyolefins with potential use in various fields, such as food packaging, drug delivery systems, medical applications etc. Also, PHB among aliphatic polyesters has great potential in food packaging application, especially because of its barrier properties.

The aim of this STSM was to prepare different active films based on biopolymers and crude extracts from plants by electrospinning technique and their characterisation.

Materials and methods

Materials

Semi-crystalline PLA used in this study was provided from Esun (China), characterized by a number-average molecular weight M_n=60520 g mol^{-1}, weight-average molecular weight M_w=160780 g mol^{-1}, and polydispersity Q=2.6 (from GPC).

Bacterial aliphatic homopolyester PHB was supplied by Biomer (Krailling Germany) as P226F. According to the manufacturer, this is certified both as compostable and food contact, presenting a density of 1. 25 g/cm^3 and a melt flow rate (MFR) of 10 g/10 min at 180°C and 5 kg.

Solvents used in this study were purchased and used as received.

Allium Ursinum extract used in this research was obtained using ultrasound assisted extraction in order to enhance the isolation of bioactive compounds with antimicrobial activity. The extraction process was thus carried out using a 20.06 W/L ultrasonic power at 80 °C for 80 min, using a 70% ethanol in water as solvent. Drug to solvent ratio was 1:5. After extraction, the solvent was evaporated using a rotary evaporator and dried extract was used in further research steps in various concentrations.

Crude plant extract from, *Calendula* and *Helichrysum* were prepared by simple extraction process in 70% ethanol. The dried and milled plants were mixed in 70% ethanol solvent for 24h, filtered and evaporated on rotavapor device to the dry metter. They were stored in the glass vials on the 4-8◦C prior to use.

Film Preparation

The biopolymers solutions for electrospinning were prepared by dissolving 10 wt.-% PHB in TFE and 10 wt.% of PLA in DCM and DMF (70:30) under continuous stirring conditions, at room temperature. Prior to dissolving the polymers extract was added to the solvent and mixture for 1h.

Electrospinning was then performed using a Fluidnatek® LE10 lab line from Bioinicia S.L. (Valencia, Spain) with a variable high-voltage 0-30 kV power supply. The biopolymer solutions were electrospun at room temperature, *i.e.* 25°C, for a given processing time and in optimal conditions to achieve steady fiber formation.

Figure 1.Electrospinning machine LE-10

The obtained electrospun mats were subjected to annealing process using a hydraulic press 4122-model from Carver, Inc. (Indiana, USA). This was optimally performed at 145-160°C, without pressure, for 5 ± 1 s, based on previously studies. The resultant films were air cooled at room temperature. Prior to annealing treatment, the electrospun films were equilibrated in a desiccator at 0% RH and 25°C for at least 1 week.

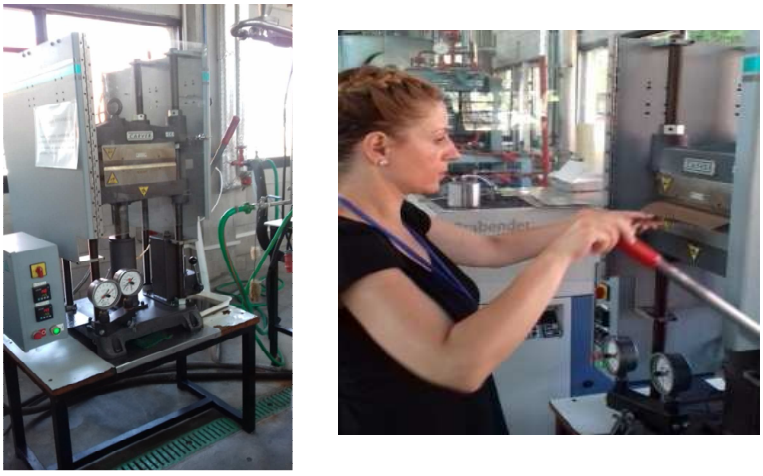

Figure 2. Prepreation of the films of PLA and PLA+AU from the electrospun mats on hydraulic press 4122-model

Film thickness and conditioning

Before the tests, the whole thickness of all structures was measured using a digital micrometer series S00014, having ±0.001 mm accuracy, from Mitutoyo Corporation (Kawasaki, Japan). Measurements were performed at three random positions and values were averaged. Films were stored in a desiccator at 0% RH and 25°C for further characterization.

Scanning electron microscopy

The morphology of the electrospun fibers was observed by scanning electron microscopy (SEM) using an S-4800 from Hitachi (Tokyo, Japan). Prior to examination, all samples were fixed to beveled holders using a conductive double-sided adhesive tape, sputtered with a mixture of gold-palladium under vacuum, and observed using an accelerating voltage of 5 kV. Fiber sizes were determined by means of the Image J software version 1.50E using the SEM micrographs in their original magnification. At least 25 micrographs were used for the measurements.

Infrared Spectroscopy

Fourier transform infrared (FTIR) spectra were collected coupling the attenuated total reflection (ATR) accessory Golden Gate of Specac, Ltd. (Orpington, U.K.) to a Bruker Tensor 37 FTIR equipment (Rheinstetten, Germany). Single spectra were collected by averaging 20 scans at 4 cm^{-1} resolution of the materials in the wavelength range from 4000 to 400 cm^{-1}. To analyze the extract release, the PHB solution containing the extract was electrospun on the ATR crystal to reach approximately a 30 μm thickness mat and repeated measurements were performed over time. Experiments were performed in duplicate.

Thermal properties

Thermal analyses of electrospun PLA fibers and films were carried out on a DSC 7 analyzer from PerkinElmer, Inc (Waltham, MA, USA) from room temperature to 200°C and for PHB from -30 to 200°C in a nitrogen atmosphere using a refrigerating cooling accessory Intracooler 2 also from PerkinElmer, Inc. The scanning rate was 10 °C /min in order to minimize the influence of this parameter in the thermal

properties. An empty aluminum cup was used as a reference. Calibration was performed using an indium sample. All tests were carried out, at least, in triplicate.

The thermal stability of the electrospun films was investigated by means of thermogravimetric analysis (TGA) using a TG-STDA Mettler Toledo model TGA/ SDTA851e/LF/1600. The samples were heated from 50°C to 900°C at a heating rate of 10°C/min under nitrogen flow. The characteristic temperatures $T_{5\%}$ and T_{max} corresponded, respectively, to the initial decomposition temperature (5% of weight loss) and to the maximum degradation rate temperature measured at the derivative thermogravimetric (DTG) peak maximum.

Wide angle X-ray diffraction

Wide angle X-ray diffraction (WAXS) measurements were performed using a Bruker AXS D4 ENDEAVOR diffractometer (Billerica, Massachusetts, USA). The films were scanned at room temperature in reflection mode using incident Cu K-alpha radiation (k = 1.54 Å), while the generator was set up at 40 kV and 40 mA. The data was collected over a range of scattering angles (2 θ) comprised in the of 2–40° range.

Water vapor permeability

The water vapor permeability (WVP) was determined using the ASTM 2011 gravimetric method. To this end, 5 mL of distilled water was placed inside a Payne permeability cup (∅=3.5 cm) from Elcometer Sprl (Hermalle-sous-Argenteau, Belgium). The films were placed in the cups so that on one side they were exposed to 100% RH, avoiding direct contact with water. The cups containing the films were then secured with silicon rings and stored in a desiccator at 0% RH using dried silica gel at 25°C. Identical cups with aluminum films were used as control samples to estimate water loss through the sealing.

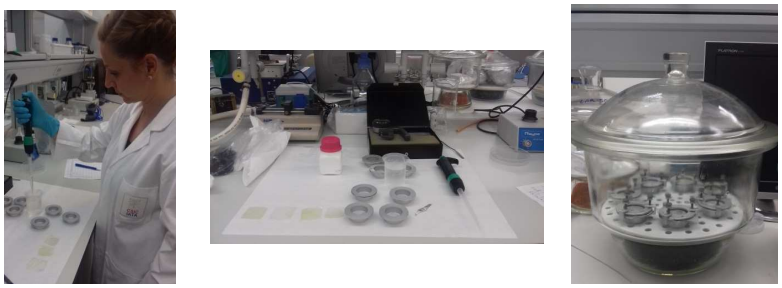

Figure 3. Determination of WVTR

The cups were weighed periodically using an analytical balance of ±0.0001 g accuracy. Water vapor permeation rate (WVRT), also called water permeance when corrected for permeant partial pressure, was determined from the steady-state permeation slope obtained from the

regression analysis of weight loss data per unit area *vs.* time, in which the weight loss was calculated as the total cell loss minus the loss through the sealing. Permeability was obtained by correcting the permeance by the average film thicknesses. Measurements were performed in triplicate.

Antimicrobial properties

Antimicrobial effect of the electrospun films was evaluated for one gram positive and one gram negative bacterial culture. Film's with dimensions of 2 x 2 cm^2 were washed with distilled water to remove impurities on the surface and sterilized by UV radiation. Before use, all pieces were moisturized with Triton X-100 from Sigma Aldrich (Madrid, Spain). Antibacterial activity was determined based on the guidelines of the Japanese Industrial Standard JIS Z 2801:2006. This method is designed to evaluate the efficiency against bacteria on the surface of finished polymer products, including films and pieces (Reference attached paper). Count of each bacterial colony was compared on inoculated TSA plates containing the films without extract, that is, the test samples, with a control sample with no film. Assays were performed in triplicate. The antibacterial activity was taken as the test surface reduction (R) using the expression:

Where A is the mean of bacterial counts of the control sample immediately after inoculation, B is the mean of bacterial counts of the control sample after 24 hr, and C is the mean of bacterial counts of the test sample after 24 hr. Antimicrobial activity was evaluated with the following assessment: Nonsignificant ($R<0.5$), slight ($R\geq0.5$ and<1), significant ($R\geq1$ and<3), and strong ($R\geq3$).

Results and discussion

Two different polymer systems have been used for this study. Polylactic acid (PLA), and Polyhidroxybutirate (PHB). Also three different plant extract were prepared as active substances -*Allium ursinum* (known as wild garlic), *Helihrysum* and *Calendula.* For both polymeric systems all three extracts were incorporated by electrospinning technique. According to the amount of material and preparation of the solvent the best results were obtained for PLA with AU extract and PHB with all three extracts, and further characterisation has been obtained only for those samples. Samples of PLA with *Calendula* were not compatible because of the polarity of the solvents and significant phase separation, so the electrospinning process was not possible.

I system

PLA loaded with 10% *Allium Ursinum* extract

Electrospinning of neat PLA and PLA with 10 wt. % of AU extract has been processed on Fluidnatek LA-10 electrospinning device (Figure 1.) Electrospining conditions were adjusted to the solution.

Morphology of the mats of PLA and PLA with10 wt.% of AU has been measured on SEM and presented in Figure 5 (a-PLA and b-PLA+AU).

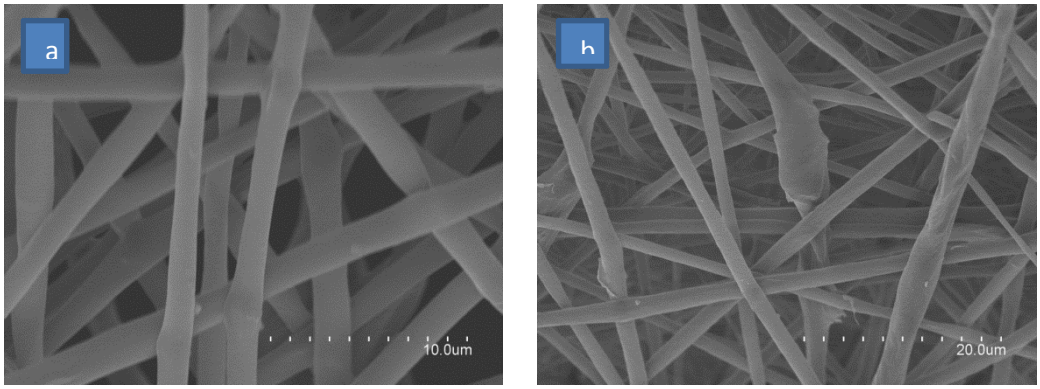

Figure 4. SEM (a) PLA (b) PLA+10wt.% AU extract

Produced samples were cut in smaller pieces and films were prepared on the hot press (PLA samples on 155◦C for 3 seconds, and PLA with AU on 145◦C for 3 sec). Obtained films had good transparency, while samples with AU extract showed slight change in color (Figure 5.)

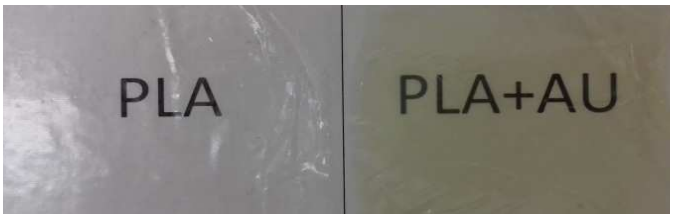

Figure 5. PLA films and PLA loaded with AU extract

Changes in spectrum of the PLA loaded with AU extarcts was monitored during time to understand the release of the AU extract and shown in figure 6.

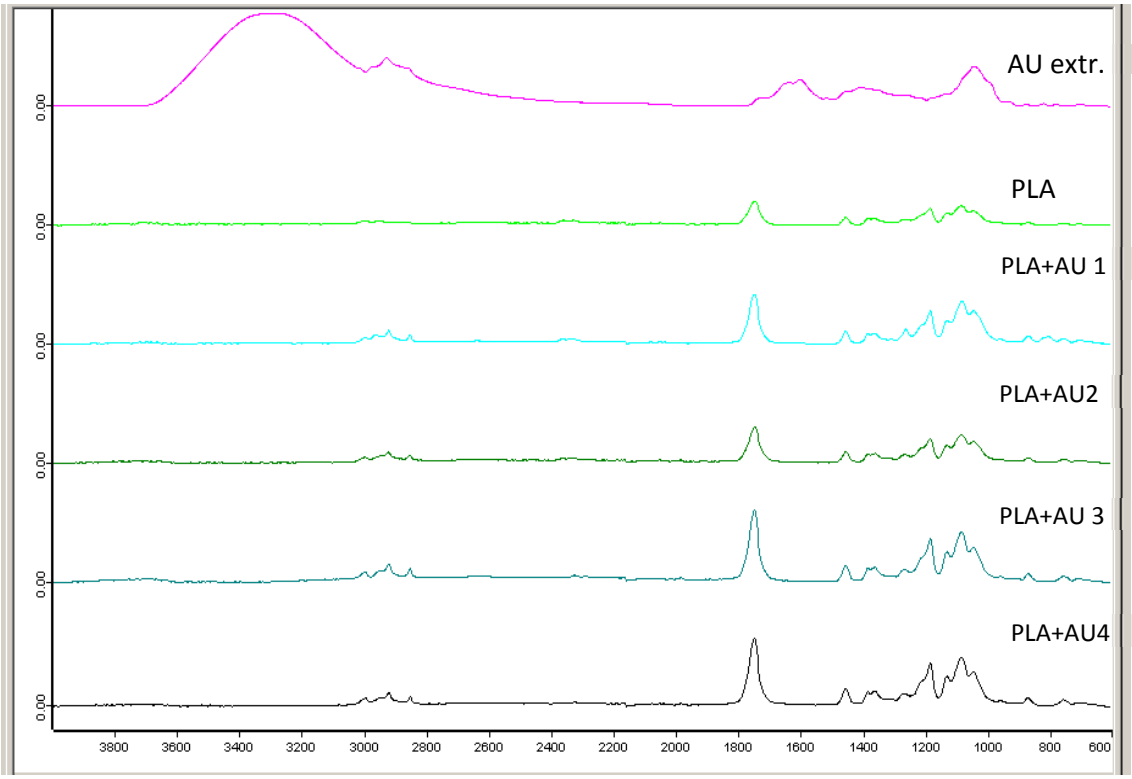

Figure 6. FTIR scans of PLA mats with *Allium ursinum* extract

System II

PHB loaded with 10 wt.% of *Allium Ursinum* extract; PHB loaded with 10 wt.% of *Calendula* extract; PHB loaded with 10 wt.% of *Helichrysum* extract. All samples were produced on electrospinning device LE-10. Morphology of samples is presented in figure 7.

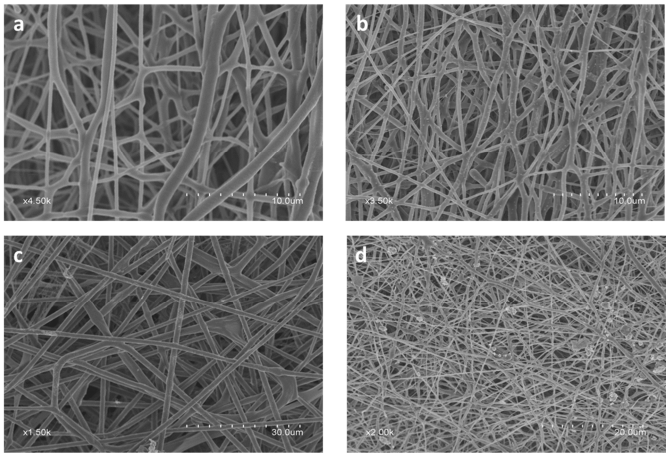

Figure 7. SEM (a) PHB (b) PHB+HEL. (c) PHB+CAL (d) PHB+AU

Produced samples were cut in smaller pieces and films were prepared on the hot press (PHB samples on 155°C for 3 seconds, and PHB with AU, CAL and HEL on 145◦C for 3 sec). Obtained films had good transparency, while samples with extracts showed slight change in color (Figure 8). Characterisation was conducted on mats and films for all samples.

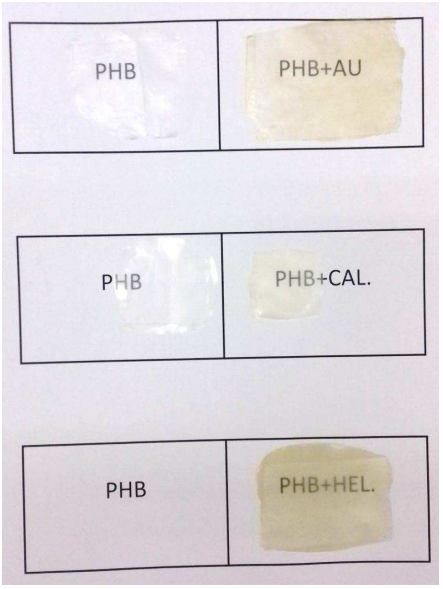

Figure 8. PHB film and PHB loaded with AU, CAL and HEL extratcs respectively

Changes in spectrum of the PHB loaded with AU extracts was monitored during time to understand the release of the AU extract and shown in figure 9.

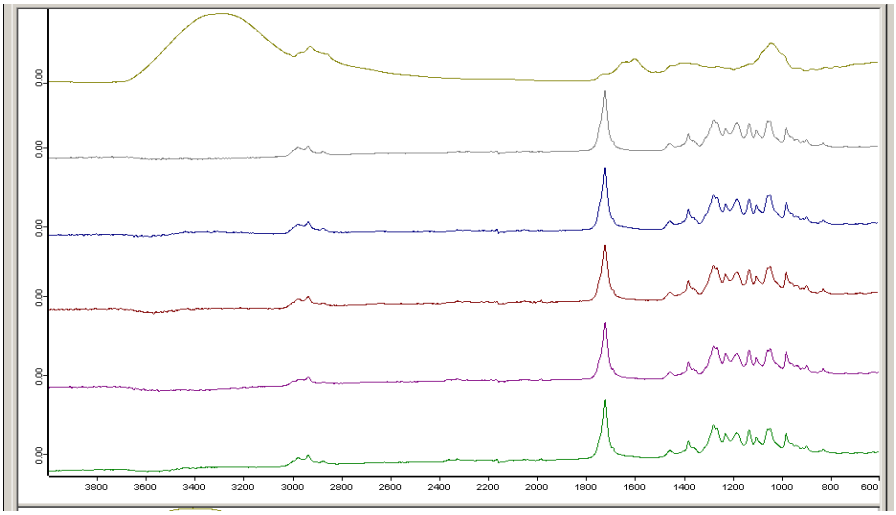

Figure 9. FTIR scans of PHB mats with *Allium ursinum* extract

Characterisation of the both systems has been performed, but still there are some results that are not yet ready for discussion. Further on, the publication of results is intended till the end of the 2017, when they will be publicaly available.

Conclusion

The use of natural extracts in food packaging application has been limited because of processing of polymer matrix on the high temperature, thus disrupting the structure and activity of bioactive compunds. Electrospinning techniqe is appropriate method for protection of natural compaunds, as well as controlled realease from the polymer matrix in active packaging solutions. Aim of this research was development of active packaging soulution based on biobased materials filled with different plant extaracts by electrospinning technique and its characterisation. Besides the polymer used as matrix, it has been shown that the different extract have different influence on prepared solution for ES, influencing the processing paramethers and shape of the fibers. This research presents one part of the research from both institutions, and indicates future collaboration on these topics.

Dr Tanja Radusin, 25.07.2017