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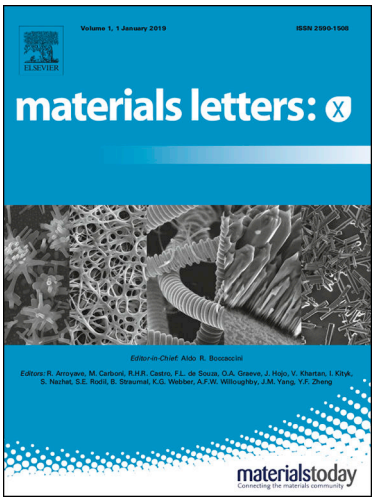

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Photo-crosslinked soy protein-based electrospun scaffolds

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Abstract: Among the plant-derived proteins, soy protein isolate (SPI) has been used in the development of different electrospun systems for biomedical applications, food technology and filtration systems. Despite the great potential of SPI, its processability and stability in physiological conditions are usually poor, limiting its applications. In this work, we explored the methacrylation of SPI to develop a photo-crosslinkable polymer (SPIMA) for electrospinning applications. After parameter optimization, SPIMA fibrous mats were successfully obtained by electrospinning. The resulting scaffolds were then photo-crosslinked by UV-light irradiation (λ = 365 nm) during different times. Interestingly, our findings highlighted that the developed photo-crosslinkable soy protein showed better processability and stability than unmodified protein. This procedure provides a feasible method for obtaining SPI crosslinked scaffolds.

Keywords: electrospinning; electrospun mats; soy protein; photo-crosslinking.

1 Introduction

Plant-derived proteins continue drawing attention due to their high availability, tailorable properties, hydrophilicity, biodegradability, biocompatibility, and biological activities [1]. Due to the advantages of plant-derived proteins, significant efforts are being devoted to exploring their use in a variety of applications [2]. Among the techniques explored to obtain micro- to nanofibrous morphology, electrospinning has been intensively used. Electrospun nanofibrous structures can be obtained from polymeric solutions, emulsions, suspensions, or molten polymers. Soy protein isolate (SPI)-based electrospun mats have been studied for different biomedical applications [3], food technology [4] and filtration systems [5].

Electrospinnability of protein solutions is not easy to achieve, and different strategies have been explored so far. Particularly, pure SPI solution could not be processed by electrospinning. Proteins have been blended with synthetic and natural polymers to improve mechanical properties, thermal stability, morphology, and degradability [6,7]. The stability in physiological conditions is a key factor for biomedical applications. So far, the erosion of SPI-based electrospun scaffolds is around 24 h [3]. In addition, highly toxic organic solvents (e.g., trifluoroacetic acid) are commonly used for preparing electrospinnable solutions. Recently, the utilization of green solvents (Class 3, according to ICH guidelines) has become a key requirement for eco-friendly electrospinning [8].

On the other hand, the hydrophilicity of protein-based scaffolds increases both dissolution and degradation rates, leading to low stability in aqueous solutions. Several strategies to tailor the SPI stability, such as chain crosslinking or blending, have been explored [7,9,10]. In a previous work, we investigated the methacrylation of gelatin (GelMA) as a chemical strategy to modulate protein stability through photo-crosslinking [11]. Thus, we fabricated uniform

bead-free electrospun GelMA nanofibers with well-defined micro- and nanotopographic features.

In this work, we aim to explore the synthesis and fabrication of photo-crosslinked SPI nanofibers. As far as we aware this is the first time that SPIMA is synthesized and electrospun, leading to nanofibrous soy-based scaffolds.

2 Materials and Methods

2.1. Materials

Soy protein isolate (SPI) was purchased from My Supps GmbH, Germany. Methacrylic anhydride (MA) and poly(ethylene oxide) (PEO, Mw=1000 kDa) were purchased from Sigma Aldrich, Germany. Glacial acetic acid and anhydrous ethanol were acquired from Cicarelli®, Argentina. Potassium hydroxide was purchased from Biopack, Argentina. The photoinitiator 1-[4-(2-hydroxyethoxy) phenyl]-2-hydroxy-2-methyl-1-propan-1-one (Irgacure-2959) was kindly provided by BASF (Germany).

2.2. Synthesis of SPIMA

SPIMA was designed considering previous works [11,12]. Briefly, a predetermined amount of SPI was dissolved in distilled water to prepare a 16% w/v solution. Separately, a KOH solution 1.6 mol/L was prepared. Both solutions were mixed, and it was placed in a preheated oil bath at 50°C and stirred for 1.5 h. Then, 150 mg of MA was added into the mixture and stirred for 2 hours at 50°C. The reaction was neutralized to pH 7.0 with 0.05 mol/L HCl solution and purified by dialysis using a dialysis tube (12-14 kDa cut-off) against distilled water for 72 h, with the water being refreshed every 12 h. After the dialysis, SPIMA was freeze-dried.

2.3. Obtaining electrospun SPIMA scaffolds

Solution containing 3% w/v of SPIMA, 1% w/v of PEO, and photoinitiator in 10 mol/L acetic acid were processed using an electrospinning equipment (Yflow S.D., Spain) with an 18-gauge stainless steel needle and an aluminum collector plate placed at 20 cm away. A flow rate of 0.2 mL/h and voltage of 12 kV were used. All experiments were carried out at room temperature, while the relative humidity (RH) was varied.

All electrospun mats were moistened in anhydrous ethanol and then exposed to UV light (UVL-28, λ = 365 nm) during different time periods (0, 2.5, 5, and 10 min), and the samples were named S1, S2, S3, and S4, respectively. The UV-light source was placed 2.5 cm away from the samples.

2.4. Characterization

The methacrylation degree, calculated as the amount of amine groups converted to methacrylamide groups expressed as a percentage, was obtained by Habeeb's assay [13]. Fourier transform infrared spectroscopy with attenuated total reflectance mode (ATR-FTIR) (Nicolet 6700, Thermo Scientific Inc., USA) was conducted in the wavenumber range from 600 cm^{-1} to 3900 cm^{-1}. SEM micrographs were obtained using a scanning electron microscope (Jeol Inc., JSM-6460LV, USA) and analyzed using ImageJ® software. The statistical analysis was carried out using InfoStat® software.

3. Results and Discussion

3.1. Synthesis of SPIMA

SPIMA with a methacrylation degree of 54.8 ± 3.7% was successfully synthesized (Figure SI-1 of Supporting Information). ATR-FTIR spectrum of SPI shows peaks at 1240, 1526, and 1645 cm^{-1}, related to C-N stretching plus N-H bending (Amide III), N-H bending (Amide II), and C=O stretching (amide I), respectively. Moreover, an N-H stretching (Amide A) can be observed at 3284 cm^{-1} [14,15]. The same peaks were observed in the SPIMA spectrum because of the chemical modification did not add new vibrational bonds (Figure SI-2 of Supporting Information).

3.2. Obtaining electrospun SPIMA scaffolds

In the last decade, the electrospinning of natural polymers has emerged as powerful strategy to create biocompatible scaffolds for mimicking cellular environments. However, most natural polymers are either difficult or impossible to be electrospun using green solvents. A minimum amount of water-soluble high molecular weight synthetic polymers (e.g., PEO) could enhance biopolymers processability. Based on previous experiments, we chose PEO (1% w/w) for improving the fiber formation process.

Relative humidity has an important effect on protein fiber morphology, especially when using water-containing solvent systems [16,17]. Therefore, we studied the effect of varying the RH from 60 to 40% on fiber formation (Figure 1). By decreasing RH, the processability of SPIMA protein chains was improved, changing from fibers with high number of beads (60% RH, Figure 1-A) to better defined defect-free fibers (40% RH, Figure 1-C). In addition, we observed that a RH higher than 60% made their processability completely unstable, as reported for hydrophilic polymers [16,18].

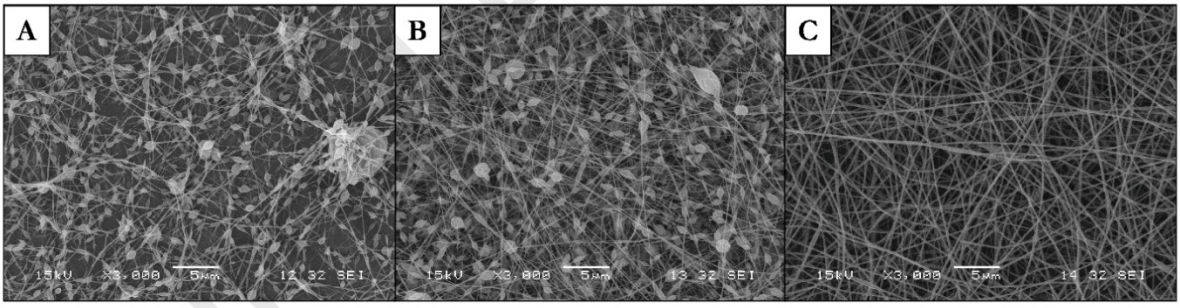

Figure 1. SEM micrographs showing the influence of RH on SPIMA electrospun mats morphology: 60% (A); 50% (B). and 40% RH (C).

Then, photo-crosslinking of SPIMA mats (40% RH) was performed and their fibrous morphology analyzed by SEM (Figure 2). As Figure 2 shows, photo-crosslinking did not affect the fibrous structure of the scaffolds, however their shape became flatten and connected between them. This change was clearly attributed to the ethanol spraying previous UV-irradiation. The behavior of these swollen-like aspect fibrous mats was reported for SPI crosslinked by using other methods [19]. The irradiation time affects both average fiber diameter and fiber diameter distribution. The mean fiber diameters were 219 ± 24, 284 ± 56, 299 ± 52, and 308 ± 47 nm for S1, S2, S3 and S4, respectively. The larger the irradiation time, the more the sample was heated. Hence, fiber diameter shows a tendency to increase with prolonged irradiation times. However, the result of statistical Tukey's test showed that the mean values for S2, S3 and S4 have no significant difference between them and the S1 is the only statistically different sample (Table SI-2 and Table SI-3 of Supporting Information).

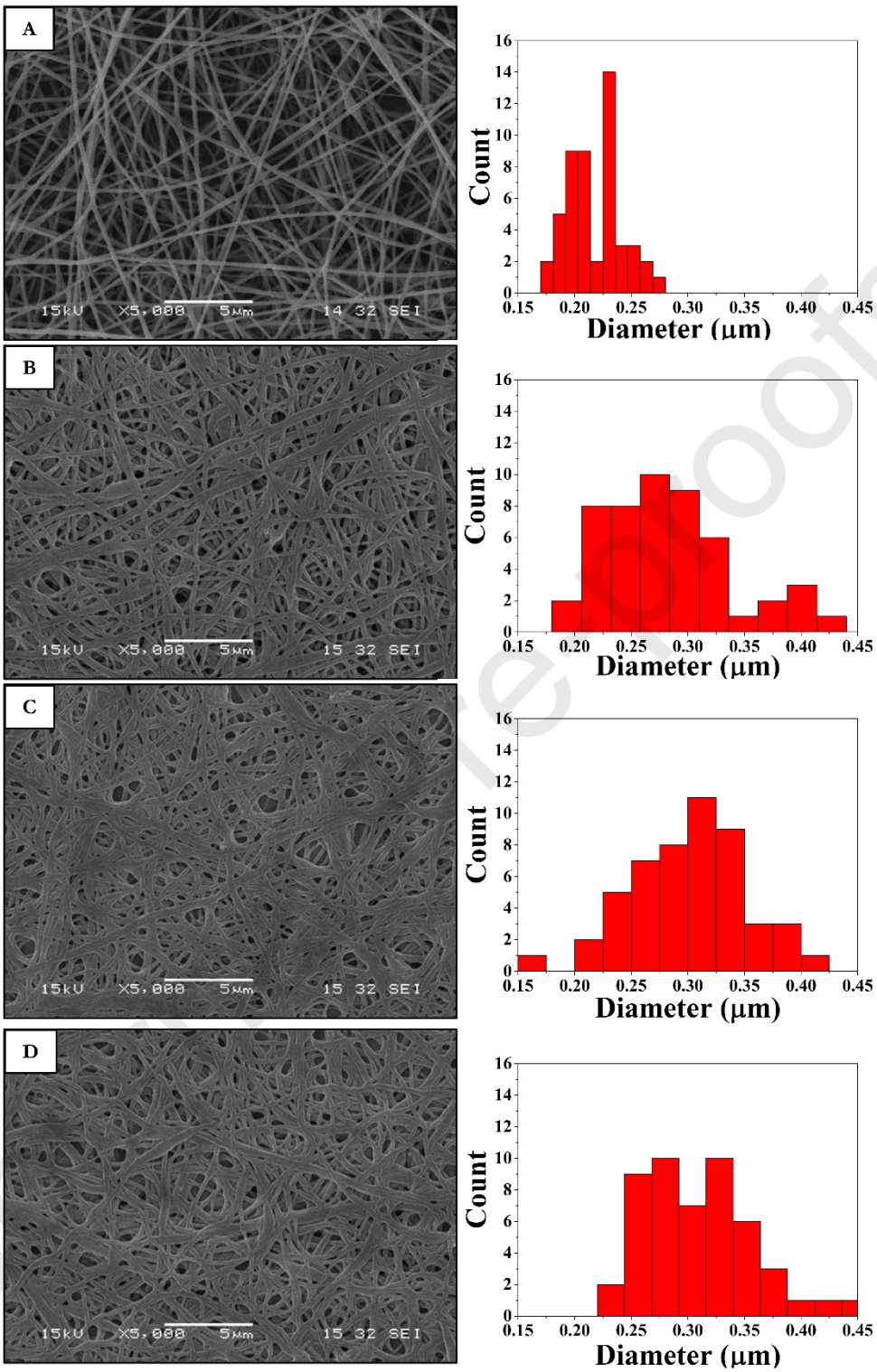

Figure 2. Micrographs of SPIMA electrospun mats after different UV-light exposure time: 0 min (A); 2.5 min (B); 5 min (C); and 10 min (D), and their respective fiber diameter distribution.

ATR-FTIR spectra of photo-crosslinked membrane showed the characteristic bands of both SPIMA and PEO (Figure 3). The peaks at 1146, 1095, and 1059 cm^{-1} (triplet) corresponding to the C-O-C group vibration, and at 2880 cm^{-1} corresponding to the C-H group of PEO [20,21]. Due to crowdy spectrum, we could not identify the peaks related to crosslinking.

Remarkably, the crosslinked SPIMA mats exhibited enlarged water stability (3 days) as compared with SPI electrospun scaffolds. However, even though it seems promising, the overall behavior should be evaluated by following the requirements of each specific application.

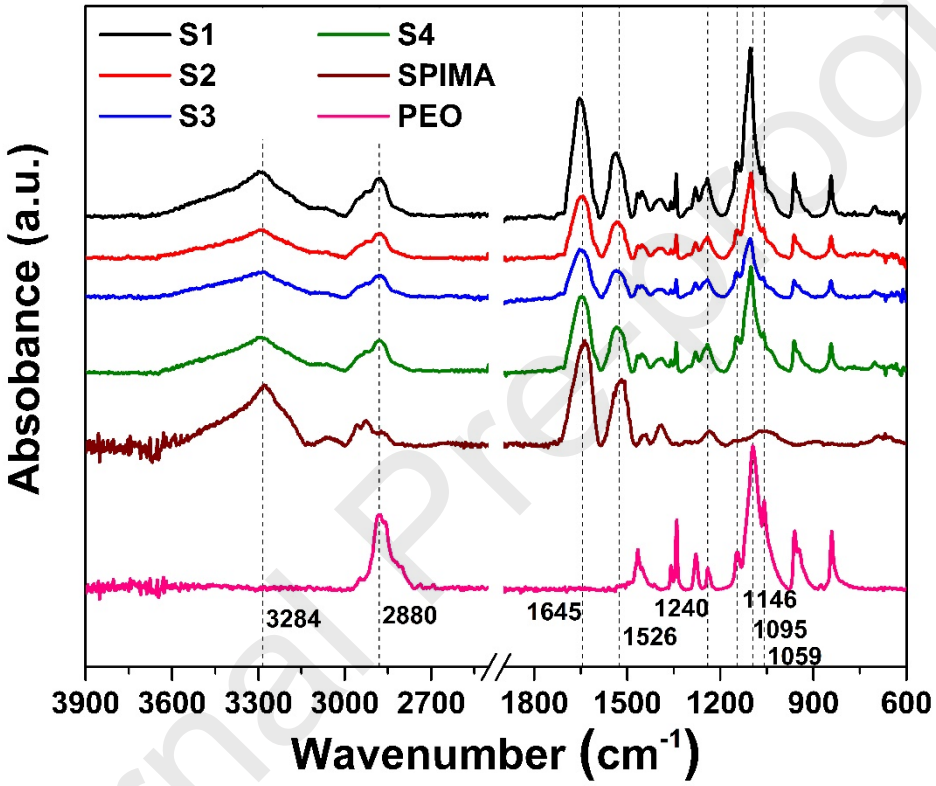

Figure 3. ATR-FTIR spectra of SPIMA electrospun mats.

4. Conclusions

We developed a new SPI derivative, which bears photo-crosslinkable groups (SPIMA). Using both water-soluble polymer (PEO) and green solvent, we demonstrated that it is possible to fabricate SPIMA-based nanofibrous scaffolds successfully through electrospinning technique. Furthermore, we can modulate the SPIMA fiber morphology by controlling RH. Even the swollen-like mats were observed after photo-crosslinking, the crosslinked mats kept their fibrous structure and, remarkably, improved their stability in water. The photo-crosslinkable SPIMA and the eco-friendly processing conditions make these electrospun scaffolds interesting candidates for several bionanotechnological applications, such as biomedical, food packaging, and nutraceuticals, among others.

Acknowledgments

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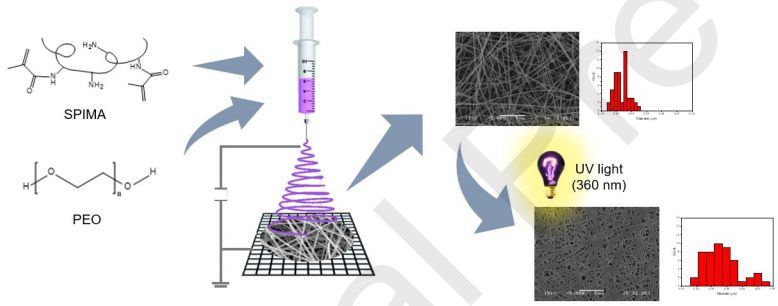

Highlights

- Photo-crosslinkable soy protein was successfully synthesized and electrospun
- The use of glacial acetic acid as electrospinning green solvent enabled fiber formation
- Crosslinked scaffolds displayed improved water stability

Credit Author Statement

Matthäus Davi Popov Pereira da Cunha: Investigation, Formal analysis, Writing - Original Draft, Visualization

Ana Agustina Aldana: Conceptualization, Writing - Review & Editing, Supervision

Gustavo Abel Abraham: Conceptualization, Writing - Review & Editing, Supervision, Project administration, Funding acquisition