FABRICATION AND CHARACTERIZATION OF ELECTROSPUN ANTHOCYANIN-LOADED POLYLACTIDE NANOFIBERS

PALAK HANDAN* AND KAYAOGLU BURÇAK KARAGÜZEL

Istanbul Technical University, Faculty of Textile Technologies and Design, Department of Textile Engineering, Gumussuyu Campus, Inonu Cad., No. 65, Istanbul, Turkey

ABSTRACT

In this study, morphological, chemical and thermal characteristics of biobased and biodegradable anthocyanin-loaded polylactide (PLA) nanofibrous membranes were investigated. To prepare electrospinning solutions, PLA was dissolved at a concentration of 10% (wv^{-1}) in a solvent system of chloroform/dimethylformamide (75/25% vv^{-1}), and anthocyanin at different concentrations (1, 2, and 3% wv^{-1}) was added into the polymer solutions. The prepared solutions were electrospun by using a single syringe electrospinning setup. The morphological, chemical and thermal structure of the neat and anthocyanin-loaded PLA nanofibrous membranes were characterized via Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FT-IR), and Differential Scanning Calorimetry (DSC), respectively. The FT-IR spectra proved the incorporation of anthocyanin into nanofibrous membranes successfully. It was observed that when anthocyanin was added into the polymer solution; bead-free nanofibers were produced, and when the concentration of anthocyanin was increased, mean fiber diameter increased as well. In addition, anthocyanin loading did not affect the crystallization behavior of PLA; however, the glass transition temperature (T_g) of the nanofibrous membranes including no anthocyanin in the structure was higher than those of the other membranes including anthocyanin.

KEYWORDS

Polylactide (PLA); Anthocyanin; Nanofibers; Electrospinning; Bio-based.

* **Corresponding author:** Palak H., e-mail: palakh@itu.edu.tr

INTRODUCTION

Electrospinning is a practical technique to fabricate ultrafine polymer fibers in different diameters ranging from nanometers to micrometers by applying an electric field on the polymer solution. Electrospun nanofibrous membranes (ENMs) show unique characteristics, i.e., high specific surface area, high porosity, small pore size and high absorbance capacity [1]. ENMs can also be produced by incorporating various compounds such as pigments, nanoparticles, antimicrobials and drugs into the structure to improve their properties for use in different application areas.

The need of using sustainable and biobased polymers increases globally due to environmental concerns. PLA is a biobased, biodegradable and biocompatible aliphatic polyester, which is derived from renewable resources, i.e., corn starch and sugar cane. Due to its good mechanical and thermal properties, PLA is used in various engineering applications instead of petroleum-based polymers i.e., polyethylene terephthalate (PET) and polystyrene (PS) [12]. PLA was also used in the development of electrospun nanofibrous mats for biosensors [2], active food packaging [3,4,6,10] and pH indicator [7,9] applications.

Anthocyanin from natural sources, i.e., black carrots, red cabbage, grape, blueberry, etc., is a water-soluble, non-toxic and commercially available natural pigment. It is stable against photodegradation and color resistant at higher temperatures. In addition, anthocyanin has strong bioactivities, including antioxidant, anti-inflammatory, antibacterial activities [14]. It is mostly used for developing halochromic/pH-sensitive materials since it is able to change its color with the change of pH [8,14].

In this study, it is aimed to develop biobased-biodegradable anthocyanin-loaded PLA nanofibrous membranes, and reveal the effect of anthocyanin loading on the morphological, chemical and thermal characteristics of the membranes. The proposed anthocyanin-loaded nanofibrous membranes have a potential to be further developed as pH-sensitive ENMs for protective clothing, filtration, wound dressings, and food packaging applications.

Table 1. Properties of the PLA grade [12].

Grade	D-content (mol%)	Melt flow rate g/10min (210°C)	Molecular weight (kg/mol)	Polydispersity index
4060D (Amorphous)	12	7-10	190	1.9

Table 2. Properties of the solvents.

Solvent	Boiling Point (°C)	Dielectric constant (ε)	Hansen solubility parameter, δ ($MPa^{1/2}$)
DMF	153	36.70	24.2
CHL	61	4.80	18.7

EXPERIMENTAL

Materials

Commercial grade of PLA (4060D) was supplied from NatureWorks LLC (USA) (Table 1). Chloroform (CHL, molecular weight: 119.38 g/mol, 99% purity, Sigma-Aldrich) and N,N-dimethylformamide (DMF, molecular weight: 73.09 g/mol, 99% purity, Sigma-Aldrich) were used as solvents (Table 2). Black carrot anthocyanin in powder form was supplied from GemmaNatural (Turkey).

Methods

The polymer solutions were prepared by dissolving PLA at a concentration of 10% (wv^{-1}) in a binary solvent system (CHL/DMF: 75/25% vv^{-1}) for 4h at 50°C [4]. Anthocyanin at different concentrations (1, 2, and 3% wv^{-1}) was magnetically stirred in DMF for 2h. Then, it was added into the polymer solutions, and final polymer solutions including anthocyanin were ultrasonicated for 1h, then magnetically stirred for 16h at room temperature.

The prepared polymer solutions were used in an electrospinning device of Nanospinner24 (Inovenso) for producing nanofibrous mats. The applied voltage was in a range of 10-12 kV and tip-to-collector distance and feed rate were fixed at 12-13 cm and 2.5 ml/h, respectively. The nanofibrous mats were electrospun and deposited on a cylindrical rotary collector rotating at 60 rpm. Electrospun nanofibrous mats were produced at room temperature with a relative humidity (RH) of ~40-50%.

The morphology of nanofibrous membranes was examined with a Tescan Vega3 scanning electron microscope (SEM). Before imaging, samples were placed into a Quorum Sputter Coater to be coated with a thin layer of Au/Pd for 2 min. To measure the diameter of the nanofibers, SEM images were analyzed with ImageJ software. 100 measurements were taken on each sample, and average nanofiber diameters were calculated. In order to identify the bonds and functional groups of nanofibrous mats, Perkin Elmer Spectrum 65 FTIR-ATR spectrometer was used. In order to analyze the thermal and crystallization behavior of nanofibrous membranes, a differential scanning calorimetry (DSC), Perkin Elmer DSC400, was used. The samples were heated from 25°C to 200°C at a heating rate of 10°C/min and then cooled to 25°C at a rate of 10°C/min.

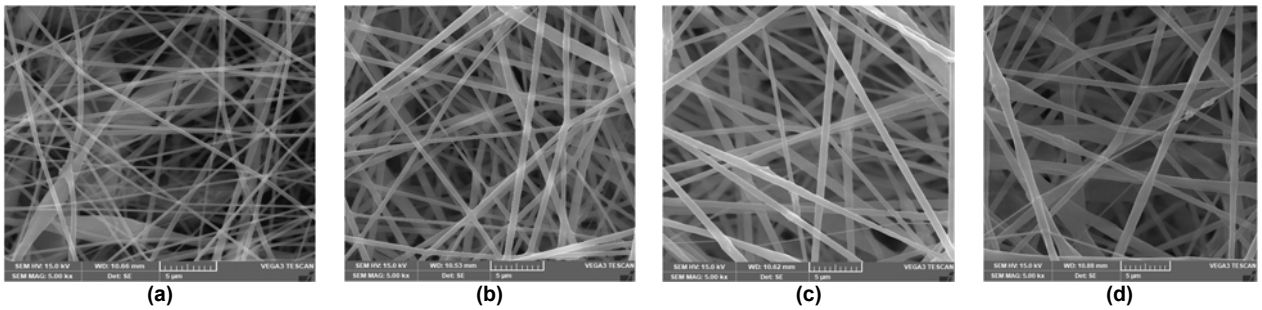
RESULTS AND DISCUSSION

Morphological analysis

SEM images of electrospun nanofibers are shown in Fig. 1(a–d). Bead formation was observed on the PLA nanofibers (Fig. 1a) since an inherent amorphous structure led to lower levels of polymer chain entanglement [13]. On the other hand, the viscosity increased after 1% wv^{-1} anthocyanin was added into the polymer solution; thus, bead-free uniform nanofibers were produced (Fig. 1b), and the mean fiber diameter increased from 327 ± 101 nm to 481 ± 90 nm. Similarly, once the concentration of anthocyanin was increased to 2% (Fig. 1c), and 3% (wv^{-1}) (Fig. 1d), uniform fibers having a larger mean diameter, i.e., 590± 130 nm and 629± 86 nm respectively, were produced.

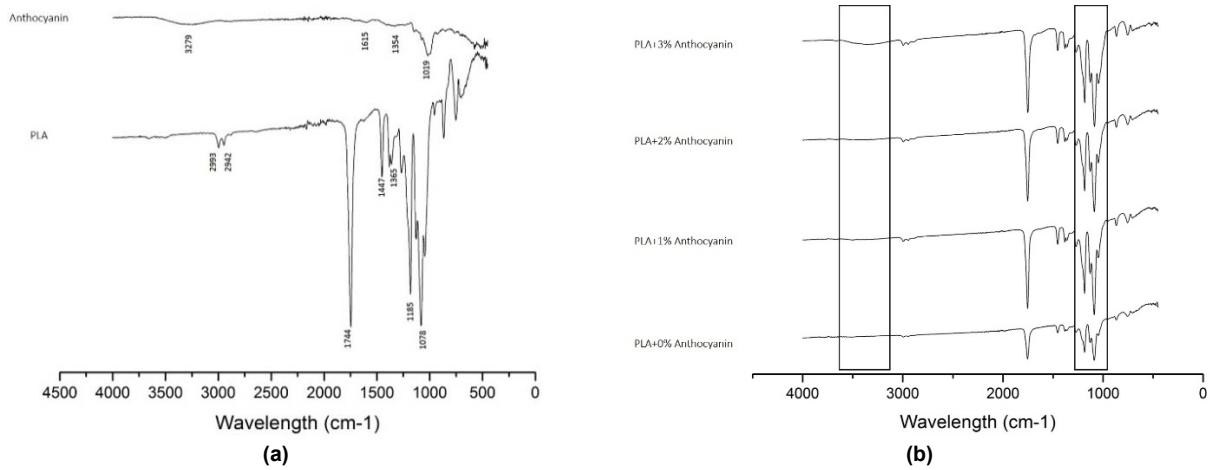
FT-IR spectrum analysis

Figure 2a shows the FT-IR spectra of PLA and anthocyanin, whereas Figure 2b shows the FT-IR spectra of nanofibrous PLA membranes without any additive, and with anthocyanin. The spectra of nanofibrous membranes were similar to the spectrum of PLA since it is the dominant component of the nanofibrous membranes. Since the anthocyanin concentration was the highest, the specific band of anthocyanin at 3279 cm^{-1} was mainly remarkable in the FT-IR spectrum of PLA+3% anthocyanin membrane. Relatively strong bands were observed in the region of 1400 cm^{-1} to 1080 cm^{-1} for the nanofibrous membranes containing anthocyanin, proving the formation of C-O asymmetric stretching, C-O-C and CH_2 vibrations groups in the structure [5].



(a) (b) (c) (d)

Figure 1. SEM images and the fiber diameter distribution of nanofibers produced with 10% PLA including 0% (a), 1% (b), 2% (c) and 3% (d) (wv^{-1}) anthocyanin.



(a) (b)

Figure 2. (a) FT-IR spectra of PLA and anthocyanin, (b) FT-IR spectra of nanofibrous PLA mats with and without anthocyanin loading.

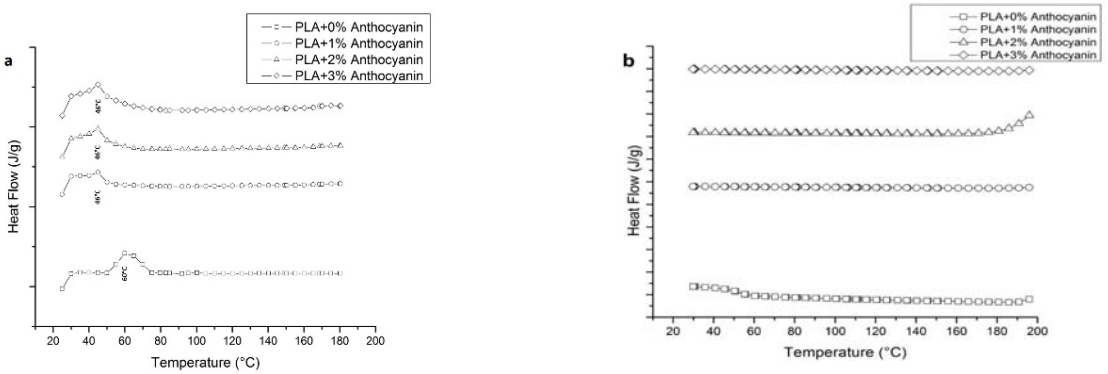


Figure 3. Differential scanning calorimetry heating (a) and cooling (b) thermograms of nanofibrous mats.

CONCLUSIONS

In this study, anthocyanin-loaded PLA based nanofibrous membranes were successfully produced by electrospinning method. SEM analysis indicated that PLA fibers were obtained at nanoscale. When the concentration of anthocyanin was increased, uniform fibers having a larger mean diameter were produced. FTIR analysis indicated that the spectra are mainly dominated by the characteristic peaks of PLA. The main peak of anthocyanin was also observed in the spectra, which means that it was successfully loaded into the nanofibrous membranes. The addition of anthocyanin did not affect the crystallization behaviour of PLA, and nanofibrous membranes did not show any crystallinity. The T_g of nanofibrous membrane which has no anthocyanin in the structure was higher than those of the other membranes containing anthocyanin due to plasticizing effect of the anthocyanin pigment. It was concluded that anthocyanin loading did not have a negative effect on the characteristic properties of PLA based nanofibrous membranes.

For further studies, the proposed anthocyanin-loaded nanofibrous membranes can be developed as pH-sensitive ENMs for the applications areas of tissue engineering, protective clothing, filtration, and food packaging.

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