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Processing, structure, property relationships and release kinetics of electrospun PLA/Carvacrol membranes

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ABSTRACT

In this work, polylactic acid (PLA) membranes at two different carvacrol (CRV) nominal concentration (i.e. 14 wt % and 28 wt%) were prepared via electrospinning technology. The membranes were characterized by scanning electron microscopy, ATR-FTIR and calorimetric measurements as well as tensile tests. Moreover, the release kinetics of CRV in phosphate buffered solution at 37 °C was monitored through UV–Vis measurements and the data were fitted with a power law model. Results indicated that the successful incorporation of CRV in the polymer matrix damaged the fibers morphology but increased all the mechanical parameters investigated (i.e. elastic modulus, tensile strength and elongation). The power law model highlighted that the mechanism of CRV release changed with time from an anomalous release in the first 6 h to a Fickian diffusion mechanism for both the PLA/CRV systems.

1. Introduction

Electrospinning process permits to fabricate non-woven mats composed of continuous fibers ranging from micro- to nano-meter diameters [1–6]. Electrospun micro/nanofibrous membranes show large high specific surface, high porosity as well as tunable mechanical properties and topological features [7–13]. For these reasons, these materials are often chosen as scaffolds potentially useful for widespread applications in traumatic states requiring effective and sustained drug action such as in skin regeneration or treatment of cancer [14]. In fact, electrospun polymeric micro/nanofibrous scaffolds are able to incorporate and release several therapeutic agents including both synthetic and natural products. Over the recent years there was a growing interest in plant-derived essential oils as an effective way fight bacterial infections [14–16].

Several study regarded the incorporation of essential oil in electrospun membranes for wound healing. Within the past two years, several polymer/essential oils including Carvacrol (CRV) [17,18], *Cinnamomum* [19,20], *Thymus vulgaris* [21], *Chamomilla recutita* [22], *Cymbopogon* [23], *Mentha piperita* [23], *Acidum tannicum* [24], *Eremanthus erythropappus* [25], and *Centella asiatica* [26] were proposed in form of thin films or nanofiber mats as promising controlled drug delivery devices.

Among the several synthetic polymer used for electrospinning processing, such as polyvinyl alcohol [27,28], polyglycolic acid, poly-ε-caprolactone [2,22,29–31], poly(ethylene oxide) [19,32], polylactic acid (PLA) was extensively investigated to fabricate electrospun fibers with desired properties for tissue engineering and drug delivery applications [33–36]. PLA is a semicrystalline thermoplastic polymer that presents a fragile behavior with relative high elastic modulus and low elongation at break [37–45].

The effectiveness of the antimicrobial activity over time is mainly determined by the release rate of the antimicrobial agents [46–48]. The release kinetic is influenced by several factor such as the matrix/drug interactions, the amount of drug incorporated in the matrix, the surface exposed by the polymer matrix and/or the presence of nanoparticles [17,18,47,49–54]. Therefore, the goal of this study was to fabricate PLA/CRV electrospun nanofiber, in order to evaluate the effect of the amount of CRV on the morphological and mechanical properties of the electrospun membranes as well as the kinetic release of the essential oil with time in PBS at 37 °C.

2. Experimental section

2.1. Materials and method

PLA 2002D was purchased from NatureWorks. Carvacrol (CRV), Chloroforms (TCM), Acetone (Ac) and Phosphate Buffer Solution (PBS) were purchased from Sigma Aldrich. All the reactants were ACS grade (purity > 99%). In order to avoid hydrolytic scission during the process, PLA was first vacuum dried overnight, at 90 °C.

PLA was added (10 wt%) to 20 ml of TCM:Ac (2:1 vol), and

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completely dissolved by stirring overnight to obtain homogeneous PLA/TCM:Ac solution.

In order to prepare PLA/CRV membranes, CRV was added to the PLA solution (14 and 28 wt% with respect to PLA in agreement with another scientific work [22]).

A conventional electrospinning equipment (Linari Engineering-Biomedical Division, Italy) was used to prepare PLA and PLA/CRV nanofibers membranes. The polymeric solution was filled in a 10-ml glass syringe fitted with a 19-gauge stainless steel needle. The electrospinning was then performed using the following constant parameters: flow rate, 2 ml/h; distance between the needle tip and the collector, 15 cm; supplied high voltage, 15 kV; temperature, 25 °C and relative humidity, 40%. The nanofibers obtained were collected on a grounded rotary drum (diameter = 100 mm, speed = 5 rpm) wrapped in an aluminum foil for 30 min in order to obtain membranes of around 25 μm thickness. The collected PLA and PLA/CRV electrospun membranes were subsequently dried overnight under fume hood in order to remove any residual solvents.

2.2. Morphological analysis

The morphology of the nanofiber mats was evaluated by scanning electron microscopy, (Phenom ProX, Phenom-World, The Netherlands). The samples were attached on an aluminum stub using an adhesive carbon tape and then sputter coated with gold (Sputtering Scancoat Six, Edwards) for 90 s under argon atmosphere before imaging to avoid electrostatic discharge during the test.

2.3. Fibers diameter determination

Fiber diameter distribution was determined using an image processing software [55]. The plugin (DiameterJ) is able to analyze an image obtained by SEM and to find the diameter of nanofibers at every pixel along a fiber axis. The software produces a histogram of these diameters and summarizes statistics such as mean fiber diameter [56]. In order to better highlight the differences due to the addition of CRV in the polymer matrix, the diameter size distribution is shown as the relative frequency versus the diameter size. The relative frequency was evaluated as the frequency of fiber presenting a certain diameter range divided the whole number of fibers analyzed.

2.4. Mechanical properties

Tensile mechanical measurements were carried out by using a dynamometer (Instron model 3365, UK) on rectangular shaped specimens (10 × 90 mm) cut off from films and average thickness of approximately 50 μm. The tests were performed at a crosshead speed of 1 mm min^{-1} for 2 min and 50 mm min^{-1} thereafter until fracture occurred. The distance between the jaws was 30 mm, whereas the thickness was measured before each measurement was taken.

The representative nominal stress-strain curves were reported for each material. The nominal stress was calculated as the ratio of the tensile force to the initial perpendicular cross section of the sample while the strain was evaluated as the ratio between the change in laws distance and the initial jaws distance. The elastic modulus was calculated from the initial part of the slope from nominal stress–strain curves.

Seven samples were tested for each material and the average values of the mechanical parameters and standard deviations were reported.

2.5. FT-IR/ATR analysis

The chemical surface properties of the samples were assessed by spectroscopic analysis. FT-IR/ATR analysis was carried out by using a Perkin-Elmer FT-IR/NIR Spectrum 400 spectrophotometer, the spectra were recorded in the range 4000–400 cm^{-1}.

2.6. Kinetics of release of the essential oil constituents from the polymeric films

A series of CRV/PBS solutions containing 1–50 mg/l of CRV were used to obtain a calibration curve correlating the absorbance peak intensity and the essential oil concentration using a UV/vis spectrophotometer (model UVPC 2401, Shimadzu Italia s.r.l., Milan, Italy). In the concentration range here investigated, the calibration curves were found to be lines. The maximum absorbance peaks were detected at 272 nm. The release of the essential oils from the films was investigated by immersing a preweighed sample (a rectangular of 10 × 40 mm, approximately 30 mg) in 10 ml of PBS. At specific time intervals, the absorbance peak intensity of the storage solutions was measured and converted to the quantities of essential oil released based on the calibration line. After each measurement, the samples were immersed in 10 ml of fresh PBS, and the cumulative release of oil here reported was calculated by sequentially adding the oil released after each step. Each measurement was performed in triplicate.

2.7. Calorimetric analysis

The thermal properties of the electrospun PLA and PLA/CRV systems were studied by using a differential scanning calorimeter (DSC), Shimadzu (model DSC-60 Italia s.r.l., Milan, Italy). Specimens, of approximately 10 mg weight, were sealed in aluminum pans. The experiments were performed under nitrogen atmosphere with a double cycle of heating from room temperature to 190 °C at 5 °C/min separated by a single cooling run at 5 °C/min.

The degree of crystallinity (χ) of PLA and its composites (calculated considering the actual amount of PLA according to its weight percentage in the PLA/CRV system) was calculated according to Eq. (1):

$$\chi = \frac{\Delta H_m - \Delta H_{cc}}{\Delta H_m^0} \cdot 100 \quad (1)$$

where ΔHcc and ΔHm are, respectively, the cold crystallization enthalpy and the melting enthalpy of the sample. ΔHm_0 is the melting enthalpy of 100% crystalline PLA (93.7 J/g) [57].

3. Results and discussion

3.1. Morphology and ATR-FTIR of electrospun PLA and PLA/CRV systems

The SEM images of electrospun PLA and PLA/CRV nanofiber mats at different CRV concentration are shown in Fig. 1(a)–(c).

The micrographs reveal that all the fibers are randomly oriented and their diameters are in the nanoscale range. More in detail, in Fig. 1(a) it is possible to observe that the membrane fibers present homogeneous diameter all over the surface. Differently, the morphology of all the other PLA/CRV fibers is less smooth if compared with neat PLA electrospun mats, in particular the system PLA/CRV 28% (Fig. 1(c)).

Image processing analysis was carried out on the SEM micrographs in order to quantify the diameter size distribution as well as the average diameter of the fibers. The results showed in Fig. 1(a')–(c'), revealed that PLA and PLA/CRV systems are characterized by the highest frequency fiber diameter at 1.4 μm and an average diameter size of 1.38 ± 0.83 μm for PLA, 1.44 ± 1.15 μm for PLA/CRV 14% and 1.54 ± 1.07 μm for PLA/CRV 28%.

SEM micrographs revealed that the presence of CRV in the PLA electrospun membranes affect the fibers morphology inducing a slight increase of their diameter. According to our previous work studying similar systems [2], this phenomenon can be likely ascribed to the effect of CRV on the solution properties that can affect the fiber morphology during electrospinning.

In Fig. 2 are shown ATR-FTIR measurements carried out on neat PLA and on all PLA/CRV mats.

The spectra of PLA showed the typical peaks of the polymer, as