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Development of poly(lactic acid) nanostructured membranes for the controlled delivery of progesterone to livestock animals

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ABSTRACT

Solution blow spinning (SBS) is a novel technology feasible to produce nanostructured polymeric membranes loaded with active agents. In the present study, nanofibrous mats of poly(lactic acid) (PLA) loaded with progesterone (P4) were produced by SBS at different P4 concentrations. The spun membranes were characterized by scanning electron microscopy (SEM), differential scanning calorimetry (DSC) and Fourier-transform infrared spectroscopy (FTIR). The in vitro releasing of P4 was evaluated using high-performance liquid chromatography (HPLC). Interactions between progesterone and PLA were confirmed by rheological measurements of the PLA/P4 solutions and in the spun mats by microscopy (SEM), thermal (DSC) and spectral (FTIR) analyses. SEM micrographs provided evidences of a smooth and homogeneous structure for nanostructured membranes without progesterone crystals on fiber surface. FTIR spectroscopy indicated miscibility and interaction between the ester of PLA and the ketone groups of the P4 in the nanofibers. X-ray analysis indicated that the size of PLA crystallites increased with progesterone content. Finally, by in vitro release experiments it was possible to observe that the progesterone releasing follows nearly first-order kinetics, probably due to the diffusion of hormone into PLA nanofibers.

1. Introduction

Addition of active agents into nanofibers has already been reported in the literature [1,2], in which, agents encapsulated directly into fibers and nanostructured membranes present a nearly zero or first-order kinetics releasing [3,4]. The use of nanofibers as carrier is promising for biomedical and agricultural applications [5,6]. In general, the active chemicals are dissolved in the polymer solution and incorporated into nanofibers by using a one-step method like electrospinning [7,8]. Compared to the traditional bulk materials [9], these fibrous nanostructured membranes largely facilitated the drug delivery processes in many aspects of pharmacology [10]. The fibrous structure possesses the advantages of having a three-dimensional open porous medium with high surface area, what improves therapeutic efficacy and reduces toxicity [11,12]. Due to the high surface area and the porous structure of these nanofibers applications in drug delivery systems such as the liberation of anti-neoplastic [1], antibiotics [13], and anti-inflammatory agents [11] have been suggested. Other appealing advantages reported to nanofibers obtained by electrospinning are high encapsulation efficiency, high loading capacity, cost-effectiveness, and ease of operation [1]. Electrospinning is a method for fabrication of nanofibers that have intense research activity in recent years because of its wide range of polymeric materials used and its consistency in producing very fine fibers. Although several variations of electrospinning have been investigated, the low production rate is still a great challenge that limits its large scale commercial application [14–16].

Solution blow spinning (SBS) is an alternative method to obtain polymeric nanofibers [14,15]. This technique produces fibers in the same size range as electrospinning, although with a greater potential for commercial scale-up [14]. Compared to the electrospinning, SBS is faster and operates at higher injection rates. Furthermore, SBS process does not use high voltage or electrically conductive collector to generate nanofibrous membranes [16,17]. Like electrospinning, SBS of active agents in polymer solutions is expected to result in nanofibers with high surface area, fundamental to enhance the water solubility and the dissolution rate of chemicals [18]. Moreover, these can be designed to provide rapid, immediate, or delayed dissolution with sustained release behavior. The aforementioned features along with the ability to spin several polymers without altering the basic setup make the SBS fibers an attractive technology for application in delivery systems.

Biopolymers have been extensively used as material for drug encapsulation due to their biocompatibility [19]. Among these polymers, poly(lactic acid) is widely used in medical applications such

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as drug delivery devices, absorbable sutures, and as materials for medical implants [20] and other related applications [21–23]. Poly(lactic acid) has received great attention because its monomer, L-lactic acid, can be obtained by fermentation from renewable resources such as starches and sugars [21]. One of the main advantages of PLA is that it may be melt-processed to receive different dosages for oral, injection or transdermal administration with predictable releasing behavior [2].

The release of hydrophobic active agents from electrospun PLA fibers is reported to follow a nearly zero-order kinetics due to the degradation mode of the fibers [18]. Moreover Zeng et al. [24] registered a burst release for hydrophilic active agents in PLA fibers via diffusion of the drug on or nearby the fiber's surfaces.

One interesting agent to be tested in such delivery system is the progesterone that consists in a steroid hormone naturally produced by the *corpus luteum* on the ovaries of mammals involved in their pregnancy [25]. In veterinary medicine, exogenous progesterone is used as a potent drug for suppression of estrous and ovulation, making possible the synchronization of the estrous and ovulation cycles in livestock animals [26]. Currently, the estrous control of livestock animals is conducted by the insertion of homogeneous dispersions of progesterone embeds in silicone bands. However, the disadvantages of these inserts, according to Rathbone et al. [26] lie in the use of nondegradable polymers and the requirement for a disposal of these bands after their use. Thus, the association of progesterone and poly(lactic acid) matrix can be an excellent option for a bioabsorption material, such as for the manufacturing of intravaginal drug delivery systems [21].

In this sense, the present study aims to investigate the release characteristics of progesterone encapsulated in poly(lactic acid) fibers obtained by solution blow spinning, considering the effect of P4 addition on the morphology and structural properties of nanostructured PLA membranes and their relationship with hormone release kinetic.

2. Materials and methods

2.1. Materials

Poly(lactic acid) (PLA, Mn = 75,000 g/mol) was obtained from Biomater Co. (Brazil), and the progesterone (P4, 99%) from Sigma-Aldrich (USA). The generic chemical structures of both polymer and hormone are shown in Fig. 1. Chloroform and acetone, used as solvent, were purchased from Synth (Brazil). Dialysis membranes (cut-off 12,000 Da) were purchased from Sigma-Aldrich (USA).

2.2. Experimental

2.2.1. Preparation of polymer solutions

The solution formulations for SBS were prepared by weighting 6% wt of PLA and 0, 2, 4 and 8% w/w of P4. Dissolution was carried out in chloroform:acetone at 3:1 (v/v) under vigorous stirring for several hours at room temperature, until complete dissolution.

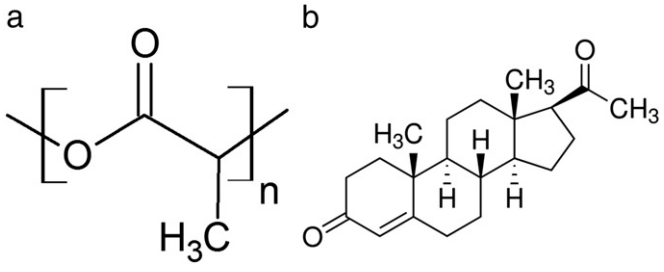

Fig. 1. Chemical structure of (a) poly(lactic acid) and (b) progesterone.

2.2.2. Characterization of the solutions

The intrinsic viscosity of solution (η) was determined for all polymer concentrations at 25 °C in a range of 10–100 s^{-1} shear rate, using an Anton Paar Physica MCR rheometer. Concentric cylinder geometry (R = 24 and 28 mm diameters) was used. All measurements were performed in triplicate and values expressed as the mean.

2.2.3. Fiber spinning

The SBS setup consisted of a syringe pump (KD Scientific, Model 781100) used to feed the polymer solution (120 μl min^{-1}) through a central nozzle. Pressurized air was admitted through a concentric outer nozzle at constant pressure (0.4 MPa). The inner nozzle was positioned in such a way that it protruded 2 mm beyond the outer nozzle. The distance between the concentric nozzle walls was 0.5 mm and the working distance was 12 cm. The SBS overall apparatus parameters were kept constant along all experiments. Experimental operation details can be found elsewhere [14,15]. The generated nanofibers were collected on a rotating drum (200 rpm), wrapped with aluminum foil, generating nonwoven fiber mats. The fiber mats were then collected by peeling off and stored in a desiccator.

2.2.4. Fiber characterization

Fiber morphology was observed using a JEOL model JSM-6510/GS scanning electron microscope (SEM). Samples were prepared by cutting samples of the mats with a blade and fixed on aluminum stubs using double-sided adhesive tape and coated with gold using a sputtering (Balzers, SCD 050). The diameter of the fiber was measured with the aid of image analysis software (Image J, National Institutes of Health, USA). For each experiment, the average fiber diameter and distribution were determined from approximately 100 measurements randomly taken from representative fiber morphology.

Fourier transform infrared spectroscopy (FTIR) data were recorded on a Nicolet 470 Nexus FTIR spectrometer. The FTIR spectrometer was purged continuously with nitrogen. A total of 64 scans were considered with a resolution of 2 cm^{-1}. The infrared spectra were recorded in transmission mode on thick blow spinning samples deposited on a silicon wafer.

X-ray diffraction (XRD) patterns were generated from non-woven fibrous mats using a Shimadzu XRD-6000 Diffractometer with a Ni filtered CuKα radiation (1.54 Å) at 50 kV and 20 mA. Scans were carried out from 5 to 30° (2θ) at a scan rate of 2°/min. The size of crystallites of PLA was measured by Scherrer equation [17].

Thermal analysis was conducted by differential scanning calorimetry (DSC) in a Q100 TA Instruments. Samples were crimped in aluminum pans and heated at a rate of 10 °C/min, from 0 °C to 180 °C in a nitrogen atmosphere.

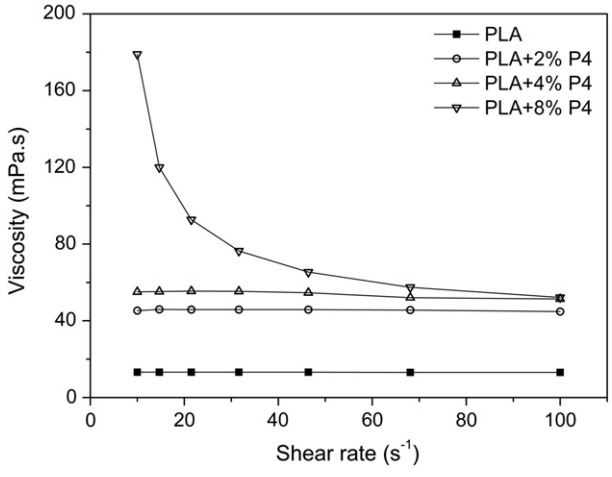

Fig. 2. Variation in viscosity of PLA and PLA–progesterone solutions as a function of the shear rate.

2.2.5. Assessment of progesterone in vitro release

The release in vitro of the progesterone inserted in all membranes was measured by a minor adaptation of the published method [27].

Briefly, approximately 50 mg of progesterone-loaded membranes and 30 ml of a hydro-alcoholic solution (ethanol:water 62.5:37.5 v/v) were transferred to a dialysis membrane. The dialysis membrane was then allocated into a 300 ml jacket glass reactor containing 220 ml of the release media (hydro-alcoholic solution), at 37 °C and stirred at 100 rpm. The amount of released drug was assessed by intermittent aliquot sampling (around 3 ml) taken from the media. The samples were returned to the release media after each analysis to maintain the system volume constant. The amount of progesterone release was assessed by a UV–visible spectrophotometer (Shimadzu, model UV-2601) at 237 nm.

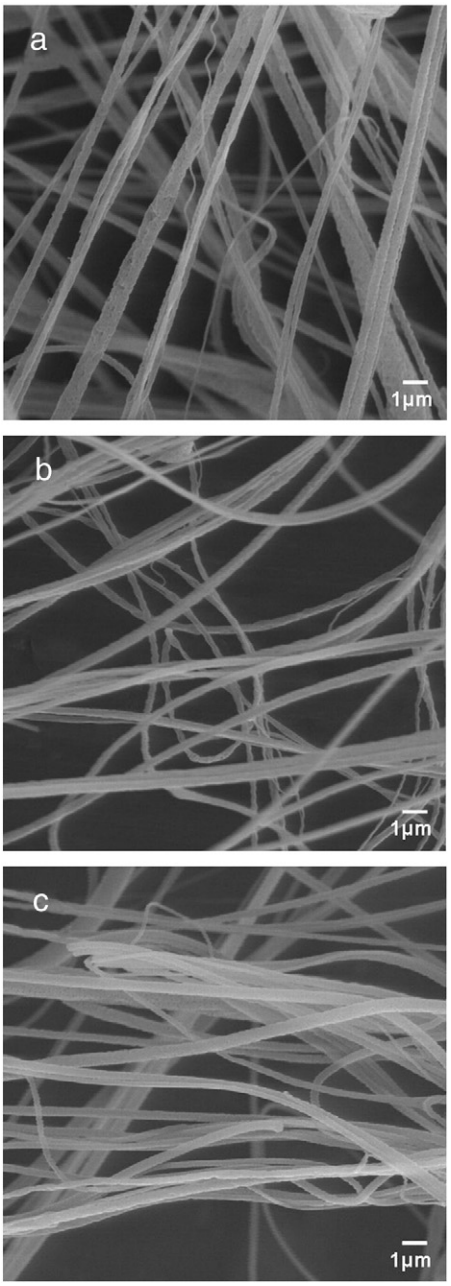

Fig. 3. SEM examples of SBS fibrous structures generated from PLA solutions (6% wt) in chloroform:acetone solvent at different P4 concentrations: (a) 2%; (b) 4% and (c) 8% by weight.

To analyze the in vitro release data kinetics, a first order rate model was used (Eq. (1)).

$$\frac{C_r}{C_0} = \exp(-K \cdot t) \tag{1}$$

where, C_0 is the initial concentration of P4, K is the first order constant, and C_r is the remaining P4 concentration.

First order constant K was obtained by the minimization of the following objective function:

$$fob = \left[\left(\frac{C_r}{C_0}\right)^{exp} - \left(\frac{C_r}{C_0}\right)^{calc}\right]^2 \tag{2}$$

where *exp* refers to the experimental concentration values and *calc* refers to the calculated concentration values. The constant K value for each system has been estimated using the software Excel (Microsoft, USA).

The fitted K constants, the calculated half-life and the percentage root-mean-square deviation (or *rmsd* (%)) for each system can be calculated from Eq. (3), where C is the released P4 concentration, C_0 is the initial P4 concentration and *NOBS* is the number of experimental observations.

$$rmsd\,(\%) = 100\sqrt{\frac{\sum_{1}^{NOBS}\left(\left(\frac{C_r}{C_0}\right)^{exp} - \left(\frac{C_r}{C_0}\right)^{calc}\right)^2}{NOBS}}. \tag{3}$$

3. Results and discussion

The viscosity data for each of the polymeric solutions is presented in Fig. 2, revealing differences in their properties as a function of hormone concentration.

Viscosity curves for polymer solutions of neat PLA in chloroform: acetone at 3:1 (v/v), revealed a Newtonian behavior, as well as for samples with 2 and 4% of progesterone added. In general, the viscosity of the PLA solution increased as the concentrations of progesterone increased. Moreover, a pseudoplastic behavior was observed to PLA + 8% P4 samples (Fig. 2). The effect of the presence of progesterone in increasing PLA solution viscosity can be explained in terms of the interaction forces taking place between progesterone and poly(lactic acid). Depending on the type and intensity of these interactions, rheological properties of polymer solutions may change. These

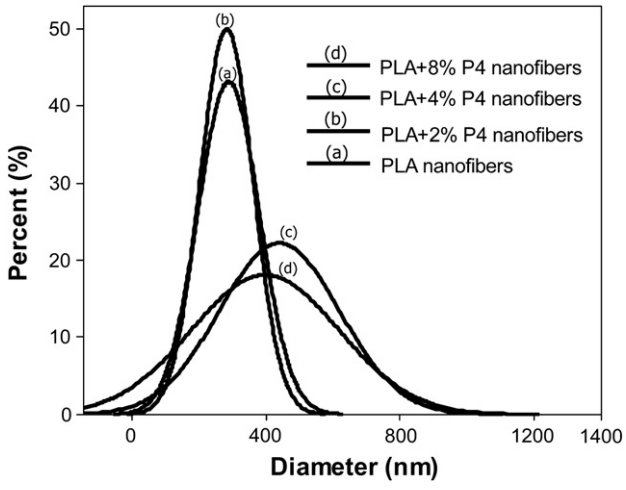

Fig. 4. Effect of progesterone in diameter size distribution of PLA fibers.

Table 1
Solution blow spinning fiber diameter.

Fiber	Progesterone added (% wt)	Resulting fiber diameter (nm)
PLA	0	289 ± 92
PLA	2	283 ± 80
PLA	4	441 ± 180
PLA	8	398 ± 220

changes, however, may result in different structural arrangements of the polymer chains in the nanofibers that ultimately may also affect the progesterone releasing behavior [28]. Similar interactions can be observed in plasticizing or antiplasticizing phenomenon in polymers i.e., the glass transition temperature of poly(lactic acid) can increase or decrease according to added plasticizer, which may result in a drastic change in the releasing kinetics [29]. The increase in the viscosity of PLA solutions by the addition of progesterone indicates that interactions between the polymer and progesterone are taking place.

Fig. 3 shows the SEM images of solution blow spinning nanofibers obtained from PLA plus progesterone additions of 2, 4 and 8% wt as collected on the rotating drum.

Solution blow spun fibrous membranes with uniform morphology and a narrow size distribution were produced. The hormone-free and the hormone loaded PLA fibers both have smooth surfaces and no progesterone crystals were observed on the fiber surface. This suggested that progesterone was dispersed homogeneously in the solution and into the spun fibers.

Fiber diameter distributions are shown in Fig. 4. For PLA fibers and PLA with small amount of P4 (2%), a narrow distribution was obtained. Conversely, when the solution is blown with 4 and 8% of P4, the distribution of fiber diameters broadened (Fig. 4). A possible reason for this behavior is that the addition of progesterone leads to an increase in the polymer solution viscosity.

The average diameters ranged from 289 nm for PLA fibers to about 440 nm for fibers with progesterone (Table 1). The increase in the average fiber diameter due to the addition of active agents is similar to results found in the literature [24], and can be interpreted as a success in the incorporation of P4 in the PLA matrix.

The recorded FTIR transmittance spectra of PLA solution blow spun fibers and PLA/progesterone are shown in Fig. 5 in the 600–3000 cm^{-1} region. The assigned peaks for PLA spectra are in agreement to the bands previously reported in literature for poly(lactic acid) fibers [30]. Three characteristic peaks at 1045, 1086, and 1130 cm^{-1}, for neat PLA, correspond to the symmetrical stretching of C-CH_3, to the symmetrical stretching of COC, and to the asymmetrical rocking of CH_3, respectively. The intense absorption at 1750 cm^{-1} is due to the existence of abundant carbonyl groups in the bulk PLA.

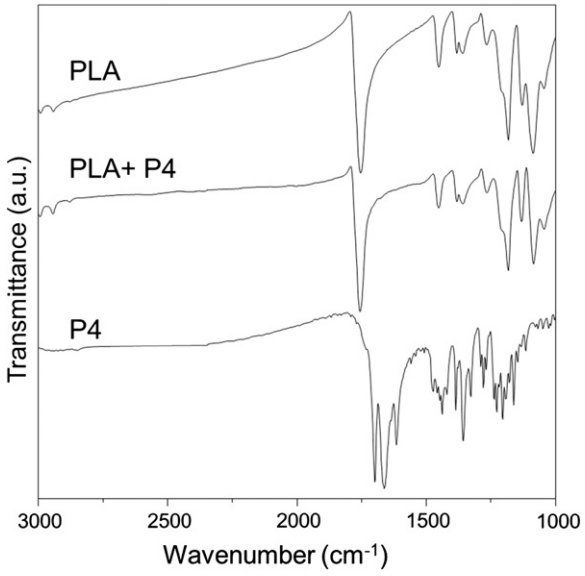

Fig. 5. Infrared spectra of PLA and PLA–progesterone fibers in the region 3200–900 cm^{-1}.

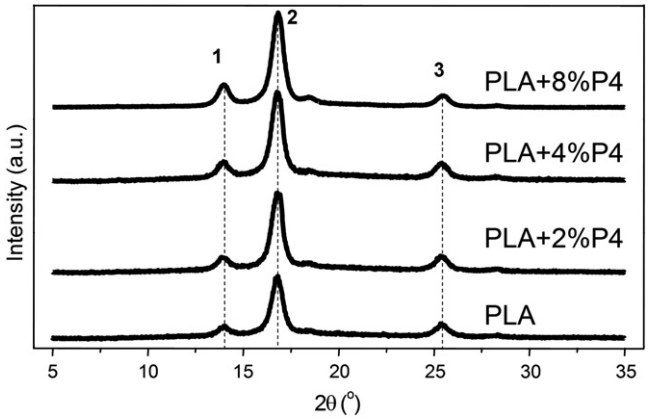

Fig. 6. X-ray diffraction patterns of PLA and PLA–progesterone fibers obtained by SBS.

By comparing both spectra, it can be concluded that the presence of progesterone did not significantly affect the backbone structure of PLA. However, an alteration is observed in the ester related bands (1045–1130 cm^{-1}) where a closer investigation reveals a PLA/P4 interaction. The relative intensity of carbonyl band (COC) increases to PLA/P4 combination and can be numerically assessed as the COH/(C-CH_3 + CH_3) ratio which can be calculated as the absorbance values at 1086/(1045 + 1130). This ratio was found to be 0.3 and 0.5, respectively, to PLA and PLA/P4. This increase reflects the new distribution of 31 helix of poly(lactic acid) resulting from the competition between ester group of PLA and ketone groups in progesterone.

Fig. 6 shows the X-ray diffraction patterns of the evaluated samples. All samples show similar diffraction patterns, clearly exhibiting two reflection peaks (near 13 and 16°), ascribed to α crystals, and a small peak (near 24.8°) associated with the β phase of poly(lactic acid) [31]. Formation of β crystals is caused by the different extent of deformation of the polymer molecules during fiber formation by solution blow spinning [17].

In order to characterize fiber crystallinity, crystallite sizes (D) were measured and plotted as a function of progesterone content as shown in Fig. 7. The identified peaks correspond to diffraction bands depicted in Fig. 6. Electrospun PLA nanofibers have been reported to form pseudo-orthorhombic crystallites, with the following parameters: a =

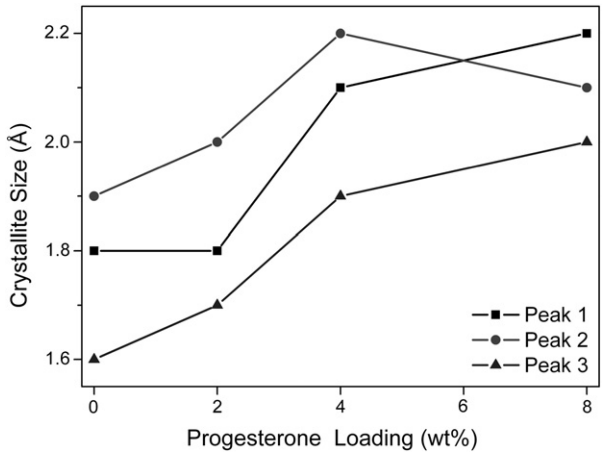

Fig. 7. Effect of progesterone in crystallite size of PLA nanofibers.

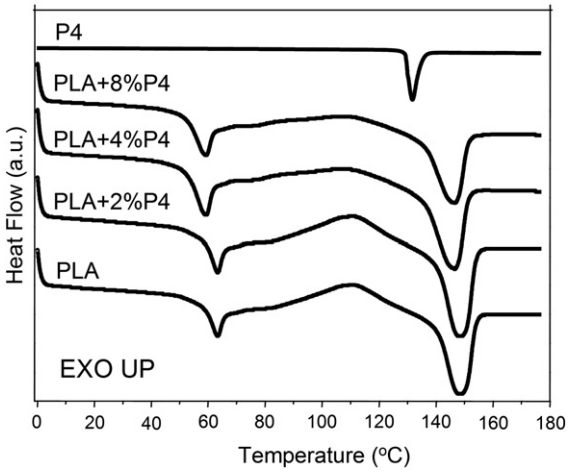

Fig. 8. Differential scanning calorimetry curves for PLA and PLA–progesterone fibers obtained by SBS.

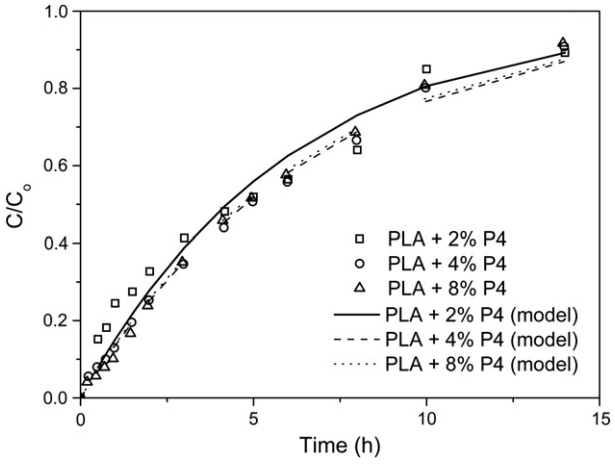

Fig. 9. In vitro release profile of progesterone from PLA membranes.

1.06 nm, b = 0.61 nm and c = 2.88 nm [32]. The increased concentration of progesterone tended to increase the crystallite size. These results also indicate that in the crystallization of PLA and the PLA-blend fibers when produced by SBS, the nucleation and growth step is controlled by interaction of polymers in the blend. Moreover, the degree of miscibility (phase separation) between poly(lactic acid) and progesterone might interfere in cell parameters and thus in the crystallinity of the spun fibers.

DSC was used to determine melting temperature (T_m), glass transition temperature (T_g), and the heat of fusion (ΔH_f) for neat PLA nanofibers and PLA/progesterone nanofibers as shown in Fig. 8.

It can be seen in Fig. 8 that the presence of one intense melting peak indicates the semicrystalline nature of PLA, according to the data reported in Table 2, therefore corroborating the XRD data. Values of ΔH_f were calculated by integrating the area under the endothermic curves, for fibers of poly(lactic acid) without and with progesterone. In general, all values are close to combination PLA/progesterone, though some structural observation can be drawn.

Both melting (T_m) and glass transition temperatures (T_g) decrease slightly as the progesterone content increases. In general, reductions observed in the melting point in a polymeric blend are normally associated to alteration on morphological effects (e.g., a decrease in lamellar thickness) and to weak polymer–polymer interactions [33]. In other words, the progesterone seems to have a plasticizer behavior within the PLA polymer matrix. Similarly, the change in glass transition temperature is generally explained as a consequence of polymer/plasticizer interactions such as due to dipole forces [28]. In particular, a decrease in T_g is also associated to plasticizer effect of the additive.

The plasticizer effect due to the presence of P4 can be also confirmed by the reduction in ΔH_f which is directly associated to the temperature needed to change phases in the polymer's structure. In short, as the melting enthalpy decreases, lower temperature is required to break interchain bonding and change phase. The interactions between poly(lactic acid) and progesterone observed by FTIR and the short timescale for fiber forming were probably responsible for inhibiting progesterone crystallization therefore giving rise to its plasticizing behavior.

Fig. 9 illustrates the release behaviors of progesterone from the nanostructured PLA membranes. It is observed that the difference between the PLA/P4 systems releasing is basically related to the total amount of progesterone released (i.e., a determined amount of PLA + 8% P4 will release more progesterone in the media than the same quantity of PLA + 4% P4, which, consequently, will release more progesterone in the media than the PLA + 2% P4 fiber). However, the release comportment of each system is basically the same, obeying a first order kinetic of liberation with very similar *K* constant values. Moreover, Fig. 9 showed that the half-life for all samples is between 4 and 5 h.

The fitted *K* constants, the calculated half-life and the percentage root-mean-square deviation for each system are shown in Table 3.

Table 2
Characteristic temperatures and heat of fusion (ΔH_f) and crystallization (ΔH_f) for solution blow spinning nanofibers.

System	T_g (°C)	T_c (°C)	T_m (°C)	ΔH_f (J/g)	ΔH_c (J/g)
PLA	59	112	150	23	15
PLA + 2% P4	57	112	145	20	5
PLA + 4% P4	56	111	147	21	5
PLA + 8% P4	56	113	147	21	5
Progesterone	10	–	132	90	–

4. Conclusions

The results of the current study confirm some miscibility of progesterone and poly(lactic acid) according to rheology, thermal, and spectroscopic used methods. Progesterone was found to have the effect as plasticizer when added to PLA nanofibers. The experimental data show a decrease in the T_g values, which is generally attributed to polymer/active agent interactions. This shows that solution blow spinning can be effectively used to encapsulate active agents into biodegradable and biocompatible polymer fibers. Moreover, these nanofibers can be potentially used in the controlled delivery of progesterone, obeying a first-order kinetics release rate, in order to control of the estrus cycle in livestock animals.

Acknowledgments

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Table 3
First order constant, *rmsd* (%) and calculated half-life for the different release systems.

System	*K* (h^{-1})	*rmsd* (%)	Calculated half-life (h)
PLA + 2% P4	0.1592	5.80	4.35
PLA + 4% P4	0.1457	1.92	4.76
PLA + 8% P4	0.1492	2.07	4.65

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