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Formation and Characterization of Oriented Micro- and Nanofibers Containing Poly (ethylene oxide) and Pectin

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Formation of oriented or aligned micro- and nanofibers using biocompatible materials opens the possibility to obtain engineered tissues that can be used in medicine, environmental engineering, security and defense, among other applications. Pectin, a heteropolysaccharide, is a promising material to be incorporated into the fibers because, besides being biocompatible, this material is also biodegradable and bioactive. In this work, the formation of oriented fibers using solutions containing pectin and polyethylene oxide (biocompatible polymers), and chloroform (as the solvent) is investigated. The injection of solution into an intense electric field defined between two parallel electrodes was used to obtain oriented fibers. This novel approach is a modification of the conventional electrospinning process. The presence of pectin in the fibers was confirmed by FTIR analysis. Fibers with diameters of hundreds of nanometers and several centimeters long can be collected. The incorporation of pectin leads to a higher variation of the diameter of the fibers, and a trend to larger fiber diameters. This behavior can be related to the presence of pectin clusters in the fibers.

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Electrospinning is a very flexible process that is able to produce fibers in the nanometric range. The use of this deposition technique allows to electrospun fibers using a wide variety of polymers and others spinnable materials, including metals and ceramics.[1–3] These fibers have potential for a range of useful applications such as nanoelectronics, sensing, biomedical scaffolds, wound dressing materials, drug delivery, nanocomposites, filters, and textiles, among others.[3,4] The obtained structures can be improved if the fibers can be oriented or aligned.[3,5]

Synthetic polymers, such as poly (ethylene oxide), are frequently used in fibers due to their good biocompatibility, and recently polysaccharides are receiving a lot of attention for the same reason. Besides being biocompatible, polysaccharides are also biodegradable and bioactive.[6] Among them, pectin (a heteropolysaccharide) is a very promising material to be incorporated in fibers, as it is both inexpensive and abundantly available. It has being used for applications that include enzyme entrapment and stabilization in biosensors,[7] controlled drug release, and implantable cell carriers.[8] The possibility of obtaining oriented nanofibers containing pectin opens opportunities for application in nano-biosensors and engineered tissues.

For the typical electrospinning setup[1–3] depicted in Figure 1, when a critical voltage applied at the needle of the syringe is reached, the electrical forces at the surface of the drop (at the output of the polarized capillary) overcome the surface tension. Then, an electrically charged stream of polymer is ejected. This stream is stable near the tip but it soon undergoes a process of instability and elongation. The solvent evaporates as the stream travels. This approach, which can be categorized as conventional electrospinning, allows obtaining micro- and nanofibers that are randomly deposited on a grounded target.

Several modifications of the typical electrospinning setup have been considered[3–5,9–11] aiming at obtaining: oriented or aligned fibers, the control of the area covered by fibers, mixing of different fiber materials, high fiber production, definition of the fiber pattern, coaxial fibers, and smooth fibers. These configurations employ additional electrodes, rotating mechanisms and/or the use of multiple capillaries for solution injection. Thus, they represent more complicated setups.

In this work, the formation of oriented fibers using solutions containing pectin and polyethylene oxide (biocompatible polymers), and chloroform (as the solvent) is investigated by using a novel variant configuration of the conventional electrospinning process. This process, which consists of injection of the polymeric solution into an intense electric field defined by two parallel electrodes, is discussed in detail.

Experimental

Solutions without pectin were prepared using different amounts of poly (ethylene oxide) (PEO, molecular weight: 2,000,000, Sigma Aldrich) dissolved in 10 mL of chloroform (1% of ethanol, J. T. Baker). The following concentrations were adopted: 0.25 wt %, 0.5 wt %, 1 wt %, and 2 wt %. Solutions with pectin were prepared using 0.15 g of PEO, 10 mL of chloroform, and different amounts of pectin from apple (molecular weight: 30,000 – 100,000, degree of esterification: 70%–75%, Sigma Aldrich). It is worthy to note that, due to the constraints of forming pectin fibers,[12] PEO is used as a vehicle for pectin. In other words, the easiness of electrospinning PEO solutions and the good solubility of PEO in chloroform (solvent) were used to allow the formation of fibers containing pectin. The following pectin/PEO mass ratios (w/w) were adopted: 0.2, 0.4, 0.6, 1.0, 1.5, 1.8 and 2. The solutions were not filtered. All solutions were stirred (1,000 rpm) during 24 hours at room temperature before deposition and it could be seen that pectin was completely dispersed. In order to assure the reproducibility of fiber composition and behavior we prepared fresh dispersions any time we attempted to form fibers.

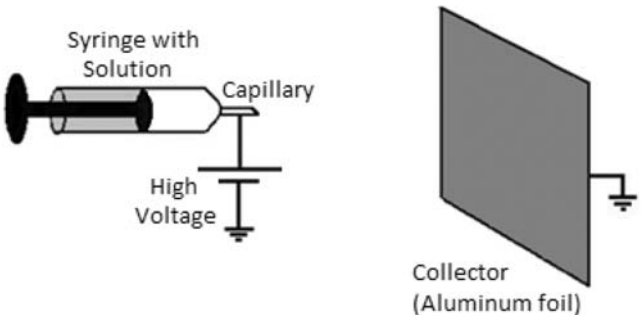

Figure 1. Schematic representation of a typical electrospinning setup.

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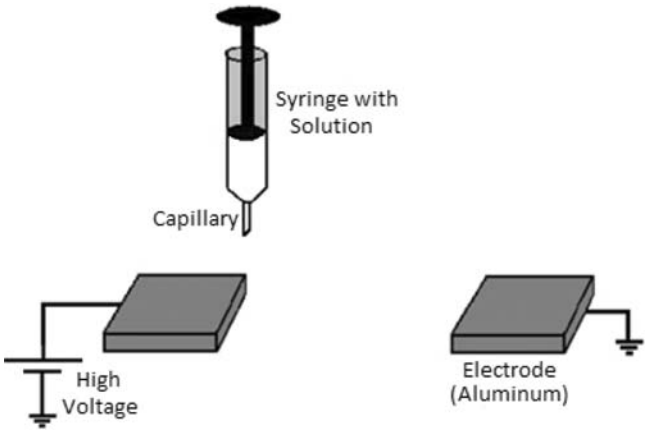

Figure 2. Schematic representation of the experimental setup with parallel macro electrodes.

The experimental setup is presented in Figure 2. The electrodes were made of aluminum with dimensions of 5.0 cm × 2.5 cm × 1.2 cm. Voltages applied between electrodes ranged from 10 kV to 30 kV and the distance between electrodes was 14 cm. Polymer was injected inside the electric field by positioning the tip of a tuberculin syringe 1.5 cm or 2 cm above and 0.5 cm far from the middle of the upper extremity of the positive electrode. This condition, i.e., the relative position of the positive electrode and the needle of the syringe, was determined experimentally aiming at optimizing fiber formation between electrodes.

Images of the deposition process were taken with a Sony DSC-T100 camera and analyzed frame by frame using appropriate software (Roxio Easy Media Creator). The negatives of the images are presented to facilitate the observation of details.

After deposition, fibers were manually collected on top of silicon substrates and analyzed using a metallurgical optical microscope (MT8520, Meiji Techno) and a Scanning Electron Microscope (JEOL JSM-6360). Fiber diameters were measured using the software Infinity Analyze. For each concentration of pectin, 20 fibers were collected and analyzed. An average diameter was obtained using 20 measurements performed along the fibers.

FTIR analysis was performed using a Thermo Nicolet Nexus 870 system. For this purpose fibers were removed from the silicon substrate and placed on a diamond crystal.

Results and Discussion

The results are presented in two sections, to facilitate comprehension. Section "A" deals with the setup development and characterization, using only PEO fibers. Section "B" describes the formation of oriented fibers containing pectin, using PEO as a vehicle.

Development and characterization of a novel electrospinning setup:.— When polymer is injected into the setup of Figure 2, the shape of the drop is influenced by the intense electric field, as depicted in Figure 3, which presents sequential images. This occurs because electrical forces at the surface of the drop overcome the surface tension. Such behavior is typical for precursor solutions that present effective charge, such as the ones observed on polar compounds immersed on a polar solvent. As the electric field is intense, the drop will be deformed and elongated (Figure 3a–3c) even if just a small amount of charge is present. At a certain point, a stream of polymer or fiber will be formed between the needle of the syringe and the positive electrode, as presented in Figure 3d. On such condition, the electric field distribution will change near the positive electrode and the highest electrical potential will be applied on the recently formed polymeric stream/fiber.

Due to charge distribution on the electrode on such condition, the drop deposited on the powered electrode will be deformed through the electrode edge; this will occur due to the high polar components of the polymeric molecule and also will be enhanced due to the solvent polarity. In fact, the chloroform molecule accommodation is highly influenced by the electric field.[13] Therefore, some solution can even flow on the vertical surface of the powered electrode. Then, this deformed drop will be responsible for the oriented fibers obtained between electrodes (Figure 3e). Furthermore, the stream also connects needle and electrode. Thus, the polymeric solution can flow on this tinny surface and produce new drops which also lead to fiber formation. However, these fibers will be formed between stream and grounded electrode.

Figure 4 shows a sequence of what occurs when a large amount of polymer is injected into the intense electric field. After the formation of the polymer connection between the positive electrode and the needle of the syringe (Figure 4a), a rearrangement of charge occurs and polymer streams are ejected from the positive electrode and from the needle of the syringe (Figure 4b). These streams travel toward the grounded electrode (Figure 4c), following electric field lines, and fibers can be formed near the syringe and electrodes, or in the space between electrodes.

The appearance of the powered electrode after fiber formation reveals the main regions where the drop was formed and flowed. As can be seen in Figure 5, the drop on the horizontal surface is not regular and tends to the grounded electrode direction; furthermore, some polymer can also be seen on the vertical surface of the positive electrode that faces the grounded electrode. Figure 6 shows that streams/fibers are ejected from the vertical surface. Finally, if a drop

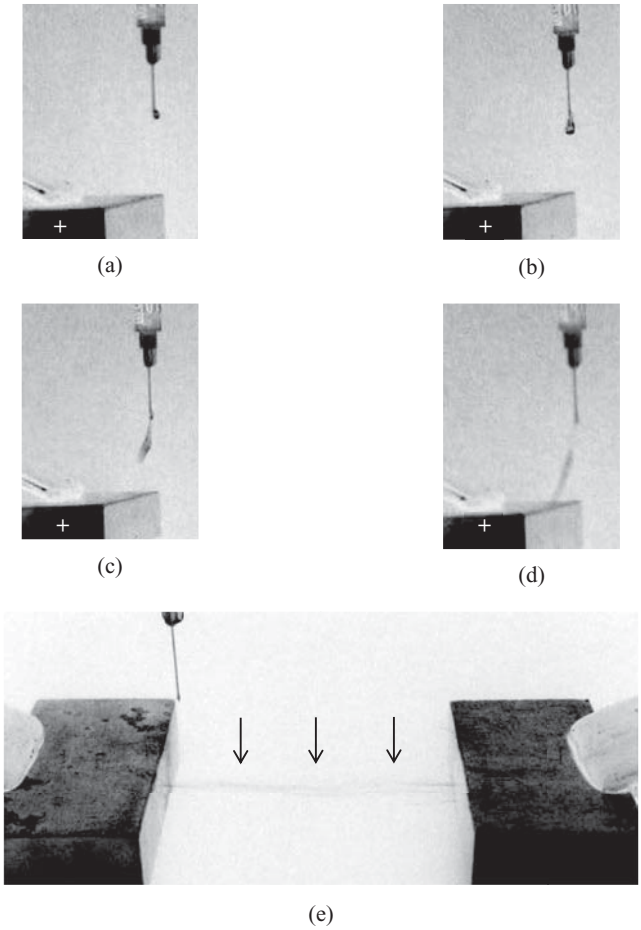

Figure 3. Sequential images illustrating: (a)–(d) the distortion of the polymer drop, that leads to a fiber/stream connected between syringe needle and positive electrode, and (e) fibers connected between electrodes at the end of the formation process. Solutions with PEO and chloroform (1 wt %), applied voltage of 20 KV, and height of the syringe needle of 2 cm.

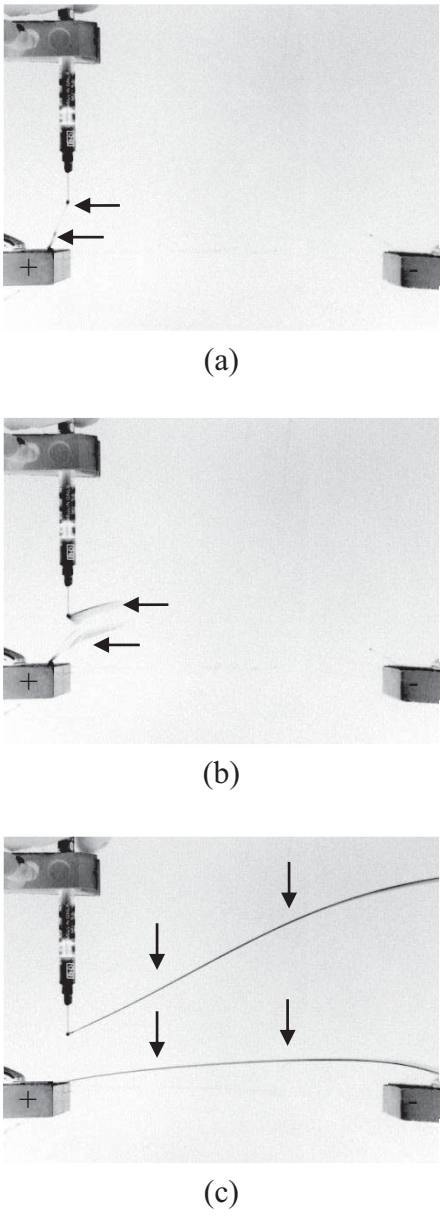

Figure 4. Sequential images for injection of large amounts of solutions with PEO and chloroform (1 wt %), applied voltage of 20 KV, and height of the syringe needle of 2 cm. Arrows indicate the position of the polymer streams.

of the polymeric solution is primarily deposited on the electrode and in sequence the voltage is applied at once, it is possible to observe the drop deformation on the onset of 12 KV, but only if the drop is near the edge of the powered electrode. On the other hand, if viscosity is increased (being higher than the ones used in this work) the drop will not flow. These simple experiments reinforce the need of using the syringe with a floating potential. The syringe main functions are: (a) provide drops sufficiently small to assure the formation of nanofibers, and (b) allow the constant flow of polymer solution on the first stream formed. Moreover, the floating potential on the syringe needle means a high potential on the drop (at the output of the needle), which allows the stream formation.

Using the injection of a small amount of polymeric solution, fibers with diameters of the order of micrometers are easily observed by eye. After collection, if a 1 wt % solution of PEO in chloroform was used, nanofibers can be observed with a microscope. The range of average diameters is presented in Figure 7. These micro- and nanofibers

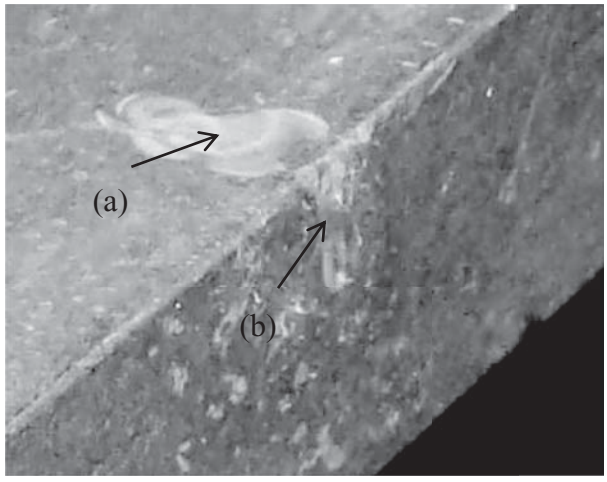

Figure 5. Photograph of the electrode after fiber formation, illustrating deposition of polymer on top, near the edge (a), and polymer flow on the edge (b).

have lengths of several centimeters (separation between electrodes) that represent an advantage over oriented fibers obtained with the use of conventional electrospinning with additional electrodes.[9] For solutions with a PEO concentration of 0.25 wt % the initial movement of the polymer stream toward the positive electrode is not observed, indicating an insufficiency of effective charge. Using a concentration of 0.5 wt %, several injections are needed in order to obtain microfibers connected between electrodes, and thus it is more difficult to obtain the nanofibers. With a concentration of 2 wt %, the higher viscosity favors the formation of fibers with diameters of micrometers.

For voltages lower than 20 kV the behavior presented in Figure 4 is not observed, what can be associated with mechanisms of effective charge formation/rearrangement. For polymeric solution concentrations of 1 wt % and 2 wt %, as seen in Figure 7, the higher applied voltage leads to thinner fibers. For voltages higher than 25 kV it was more difficult to obtain oriented fibers. On such high voltage, the polymer can be easily deformed on all direction, and the phenomenon observed in Figure 4 does not occur.

Figure 8 shows nanofibers that were collected forming a perpendicular configuration on top of a silicon substrate. It can be observed that the nanofibers do not have a uniform diameter. The insert shows an array of freestanding fibers obtained sequentially after moving later-

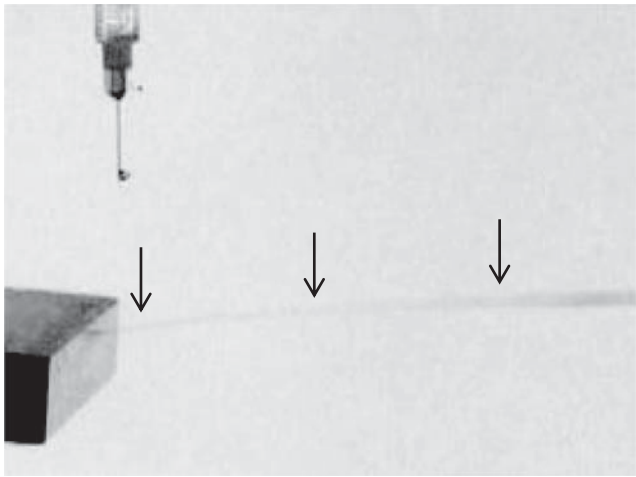

Figure 6. Stream/fibers ejected from the vertical surface of the positive electrode that faces the grounded electrode.

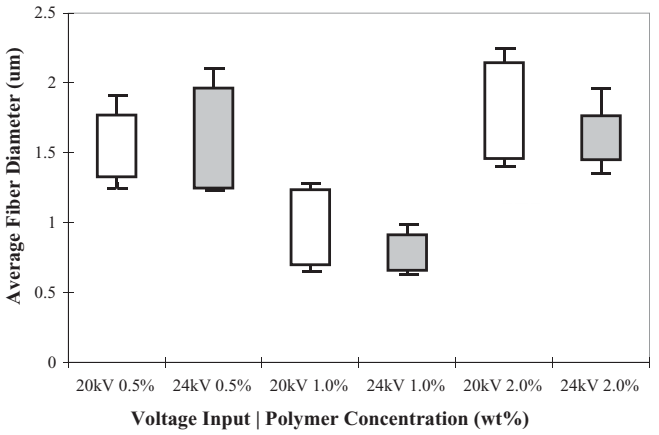

Figure 7. Average fiber diameter as a function of concentration (solutions containing PEO and chloroform) and applied voltage, using a height of the syringe needle of 2 cm.

ally the glass substrate. Then the substrate was rotated 90° to deposit a sequence of transversal fibers. This approach can be adopted to obtain scaffolds with defined shapes for application in tissue engineering.

The fibers can be shaped after deposition using, as an example, the stylus of a profilometer. Figure 9 shows the image of a zigzag shape defined in the middle of a microfiber. These results show that the obtained fibers are flexible and can be manipulated without rupture.

Oriented fibers containing pectin:.— For solutions containing pectin the general behavior of fiber formation, presented in Figure 4, is also observed. These solutions could be easily injected into the electric field, contrary to the results reported by Seo et al.[6] that used aqueous solutions, requiring heating of the solutions to a temperature of 70°C to decrease the viscosity. Thus, the PEO-pectin dispersion does not impose a severe decrease on charge density, i.e., the drop can be deformed and produces the first stream.

Figure 10 shows two parallel fibers collected simultaneously that were formed using a solution with a pectin/PEO mass ratio of 1.8, applied voltage of 25 kV, and height of the syringe needle of 1.5 cm. Since the fibers containing pectin present more irregularities compared

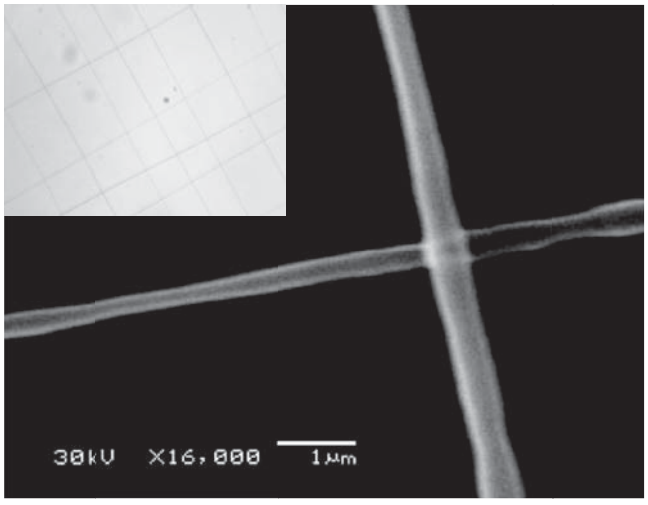

Figure 8. SEM images of nanofibers deposited on a silicon substrate using solutions with PEO and chloroform with a concentration of 1 wt %, applied voltage of 25 KV, and height of the syringe needle of 2 cm. The insert shows an array of freestanding fibers collected after injecting a single drop of polymeric solution multiple times.

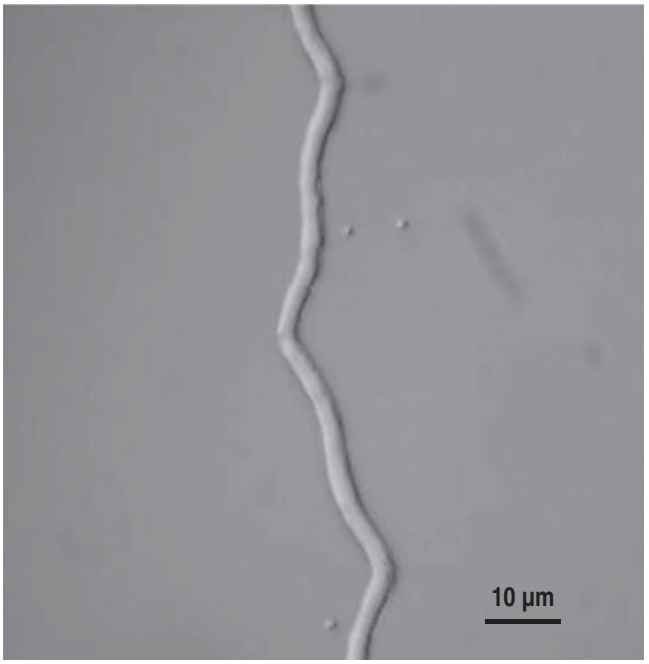

Figure 9. Zigzap shape defined in the middle of a microfiber using the stylus of a profilometer.

to the ones without pectin (Figure 8), it is speculated that the dispersed pectin produces small clusters inside the PEO fiber when solvent evaporates during the formation of the fiber (see FTIR analysis).

Figure 11 shows FTIR spectra of pure films (on silicon substrate) of pectin (a) and PEO (b). The absorption peak at 1625 cm^{-1} is related to the asymmetric carboxylate stretch modes of pectin[14] and the peak at 1730 cm^{-1} is induced by stretching of carboxyl groups of COOH in pectin.[15] The peak at 1730 cm^{-1} can be used to follow the presence of pectin in the fibers, while the peak at 842 cm^{-1} (CH_2 bending) is characteristic of the PEO polymer. Some FTIR spectra for fibers formed from solutions containing pectin are also presented; the spectrum related to pectin with pectin/PEO mass ratios of 0.0, 0.4, and 2.0, respectively, are represented as Figure 11c–11e. In Figure 11d and 11e, the absorption peaks at 1625 cm^{-1} and 1730 cm^{-1} gave results very well defined.

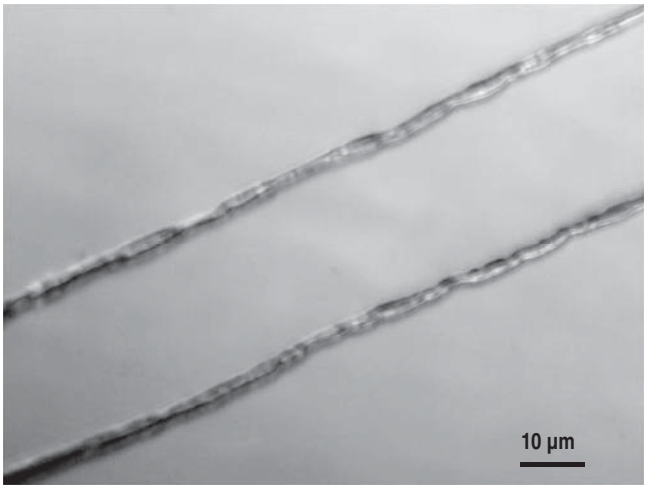

Figure 10. Parallel fibers collected simultaneously on a silicon substrate that were formed using a solution with pectin/PEO mass ratio of 1.8, applied voltage of 25 kV, and height of the syringe needle of 1.5 cm.

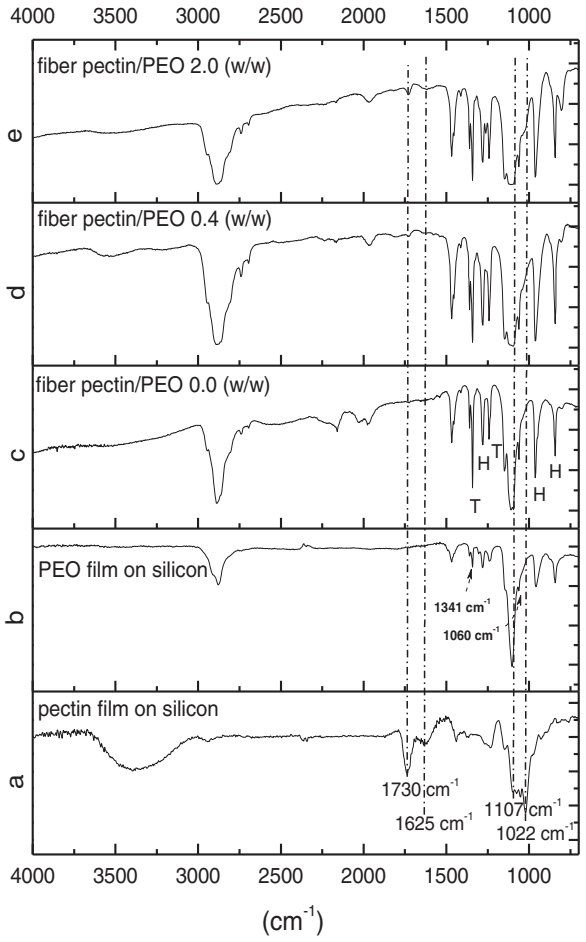

Figure 11. FTIR spectra for: (a) pectin film on silicon; (b) PEO film on silicon; and fibers from solutions with pectin: pectin/PEO mass ratio (w/w) of (c) 0.0; (d) 0.4 and (e) 2.0. The H and T correspond to helical and transplanar PEO structures, respectively.

The 1300 cm^{-1} to 800 cm^{-1} region presents characteristic bands that are unique to a compound, despite being usually difficult to interpret, and also correspond to the "fingerprint" of the polysaccharide molecules.[16] Moreover, as stated by Kim and co-workers:[17]

- PEO is a semicrystalline polymer whose more thermodynamically stable structure at room temperature is a helical conformation. However, this structure can change under tension (resulting mostly likely in a planar zigzag conformation) or if water is added, which produces a triclinic crystalline structure.
- Helical structures are characterized by 1358, 1278, 1235, 1060, 947, and 842 cm^{-1} bands and the transplanar conformation by 1341, 1240, and 961 cm^{-1} bands.
- For fibers, the 1060 cm^{-1} PEO band tends to disappear due to the PEO chain alignment on fiber direction.
- Whereas semicrystalline PEO presents doublets bands, the amorphous one leads to singlet bands.

The evaluation of PEO FTIR spectra in Figures 11b and 11c show that both structures, helical (H) and transplanar (T), are present; this result is similar to data reported by Kim. For these spectra, the main differences rely on the relative intensity of these bands, with fiber PEO (Figure 11c) showing a higher relative intensity for transplanar structure bands, such as the one at 1341 cm^{-1}. However, the addiction of pectin (Figures 11d and 11e) increases the 1060 cm^{-1} band, which means a decrease on chain alignment, a result that is opposite to ones reported by Kim, obtained from PEO fibers containing gold nanoparticles. It is also possible to observe the 1022 cm^{-1} band overlapping with the one at 1060 cm^{-1}, which means that the pectin characteristic band for a high degree of esterification (DE)[16] remains detectable and at the same wavenumber, i.e., pectin clusters are probably formed. Furthermore, the bands in the 1100 cm^{-1} to 1000 cm^{-1} region that are correlated with PEO are not only broader for pectin-PEO fibers but also present a small shift (∼10 cm^{-1}) toward higher wavenumbers. Finally, the peak at 1730 cm^{-1} usually increases when the amount of expected pectin in the fiber increases but there was no linearity between the w/w proportion and the height of the characteristic FTIR peaks for each compound. Therefore, all the obtained results point out to a high degree of interaction between pectin and PEO molecules, such as with hydrogen bridge formation. Every band in Figure 11 spectra could be identified considering only helical and transplanar structures, which means that water probably does not interfere on the dispersion or is not present.

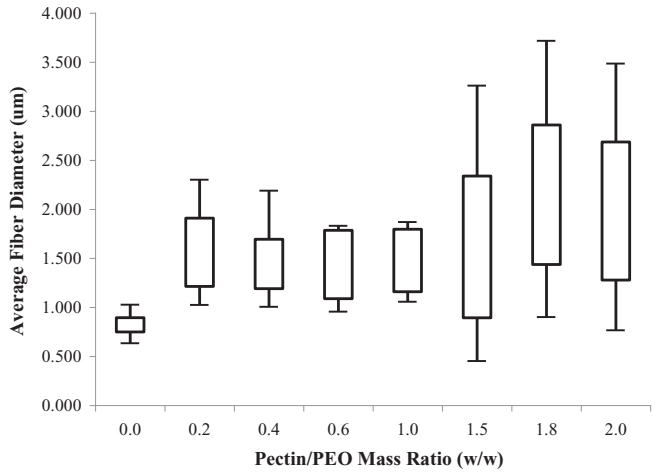

Figure 12. Average fiber diameter as a function of pectin/PEO mass ratio, using an applied voltage of 25 kV and height of the syringe needle of 1.5 cm.

Few FTIR spectra did not show significant presence of pectin, even for high pectin/PEO mass ratios. This suggests that segregation of the less dispersed pectin could be occurring during the solvent evaporation and fiber formation. This phenomenon would result in non-uniformity in fiber diameter and pectin/PEO mass ratio, and consequently FTIR spectra changes as previously related, which is compatible with microscopy images interpretation.

Figure 12 presents the range of average diameters of the fibers as a function of the amount of pectin in the solution. It can be observed that the incorporation of pectin leads to higher variation of the fiber diameter and a trend to larger fiber diameters. Larger amounts of fibers connected between electrodes also could be observed when solutions with pectin were used, what is favored by the larger diameter of the fibers. However, fibers with diameters in the range of hundreds of nanometers were also collected. These trends can be related to the presence of pectin clusters in the fibers, what may cause the increase of the diameter, but also opening the possibility to use the proposed procedure to the formation of nanostructures.

Conclusions

A process to obtain polymeric oriented fibers containing pectin was defined. The incorporation of pectin in the fibers was confirmed by FTIR analysis. The resultant fibers are flexible and can be handled. Also, the proposed experimental setup is capable of producing long fibers with a length of 14 cm or higher. Fibers with diameters in the range of hundreds of nanometers could be produced. The incorporation of pectin leads to a higher variation of the fiber diameter, due to cluster formation.

Acknowledgments

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