Development of Bacterial Cellulose Nanowhiskers Reinforced EVOH Composites by Electrospinning

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ABSTRACT: In the current study, hybrid electrospun ethylene vinyl alcohol (EVOH) fibers reinforced with bacterial cellulose nanowhiskers (BCNW) were developed and characterized. Additionally, electrospinning was suggested as a method for the incorporation of well-dispersed BCNW into an EVOH matrix by melt compounding. With the aim of maximizing the BCNW's loading in the electrospun fibers, an optimized method was applied for generating fibers from solutions containing up to 40 wt % BCNW. As demonstrated by FTIR spectroscopy, it was possible to incorporate BCNW concentrations up to ~ 24 wt %, although a complete incorporation of the nanofiller into the fibers was only achieved with solutions containing up to 20 wt % of the filler, DSC analyses suggested that the incorporation of the nanofiller reduced the crystallinity of the as-obtained EVOH fibers and produced an increase in the glass transition temperature of these during the second heating run. Thermogravimetric analyses showed that even though EVOH protects the nanowhiskers from thermal degradation, the electrospun hybrid fibers present a relatively lower thermal stability than the pure EVOH fibers. FTIR analyses of the samples subjected to different thermal treatments confirmed that the stiffening effect observed by DSC only occurs after melting of the EVOH phase and is cooperative with a partial acid chemical development in the BCNW, which promotes strong chemical interactions between the polymeric matrix and the nanofiller. Finally, the hybrid electrospun fibers were incorporated into pure EVOH by melt compounding to produce composite films. This methodology showed higher stability and dispersion of the BCNW than direct addition of the freeze-dried nanofiller to EVOH. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: 1398–1408, 2012

Key words: bacterial cellulose; electrospinning; nanocomposites; EVOH; melt compounding

INTRODUCTION

The electrospinning technique has recently attracted considerable interest since it is a versatile method to produce continuous polymer fibers with diameters in the submicron range with applicability as textiles,[1] wound dressings,[2] tissue engineering scaffolds,[3] electronic devices,[4] and, more recently, in the nanocomposites field.[5–7] Since recently, great efforts are being focused on the use of nanofillers from renewable resources and, specifically, cellulose nanowhiskers (CNW) have gained great interest for their use as nanoreinforcement in electrospun fibers. Electrospinning has recently been applied to obtain cellulose fibers[8,9] as well as hybrid fibers reinforced by cellulose whiskers.

Cellulose is one of the most abundant biopolymers found in nature and it is commonly extracted from vegetal resources such as wood, cotton, and linter. However, it can also be synthesized by some bacterial species, which in a culture medium rich in polysaccharides are able to produce a layer of bacterial cellulose (BC). With the aim of removing amorphous regions, cellulosic materials are usually subjected to acid hydrolysis, breaking down the hierarchical structure of the material into crystalline nanocrystals, commonly known as CNW. As a result of their remarkable mechanical properties, as well as their fully degradable and renewable character, CNW have been widely used as reinforcing filler for polymeric matrices such as poly(lactic acid) (PLA),[10,11] poly(hydroxybutyrate-valerate) (PHBV),[12,13] starch,[14] and polyvinyl acetate.[15]

CNW have been used to reinforce electrospun polystyrene nanofibres.[16] The material was added as a freeze-dried product and the dispersion of the nanoreinforcement in tetrahydrofuran (THF) was improved by adding a nonionic surfactant. Cellulose nanocrystals were successfully incorporated from aqueous suspensions into electrospun poly(vinyl alcohol) fibers

with CNW loadings up to 15%, improving the thermomechanical properties of the material.[6] FTIR analyses confirmed the presence of the nanofiller within the matrix. Nevertheless, visualization of the nanowhiskers inside the electrospun fibers was not possible by means of transmission electron microscopy (TEM), likely because of the lack of contrast between the two components of the fibers. Cryo-SEM and field emission scanning eelectron microscopy (FE-SEM) of ultrathin cross-sections of the electrospun mats were developed[6] but did not lead to a clear identification of the cellulose nanocrystals.

BC whiskers have been recently incorporated into electrospun nanofibres of poly(ethylene oxide) from aqueous dispersions, showing a partial aggregation of the nanofiller inside the fibers as observed by TEM.[17] Additionally, BC microfibrils' solutions in dimethylformamide (DMF)/THF obtained through the solvent exchange method were used to generate electrospun reinforced poly(methyl methacrylate) fibers showing a high degree of dispersion of the cellulose microbrils.[7]

The polymeric matrix chosen in this case is an ethylene vinyl alcohol copolymer (EVOH), which is a semicrystalline polymer with excellent barrier properties to gases and food aroma compounds. The main reason for choosing this matrix is its hydrophilicity, which potentially makes it compatible with the BCNW without the need for surface modification of the whiskers. Electrospinning of EVOH copolymers from 2-propanol-water solutions[18] as well as EVOH membranes end-capped with sulfonic acid[19] have been previously developed. In any case, hybrid electrospun EVOH fibers incorporating nanofillers have not been previously reported.

In a previous study,[20] electrospun EVOH fibers loaded with bacterial cellulose nanowhiskers (BCNW) were produced and the method was optimized with the aim of improving the incorporation degree and dispersion of the nanofiller. It was demonstrated that partially hydrated BCNW were more efficiently incorporated into the fibers than the freeze-dried ones.

The present work reports on the characterization of EVOH electrospun fibers obtained from polymeric solutions containing concentrations of BCNW up to 40 wt % by means of the previously optimized method. The incorporation degree of the filler and the corresponding thermal properties of the composite fibers were studied to determine the highest BCNWs' concentration which allowed an efficient incorporation. With the aim of understanding the surprising increase observed in the glass transition temperature (T_g) of the fibers after being subjected to one heating step beyond the EVOH melting point, the thermal stability and chemical changes suffered by the hybrid fibers after heating were also monitored by FTIR spectroscopy. Finally, the effectiveness of electrospinning as a vehicle for incorporating well-dispersed BCNW into an EVOH matrix by means of melt compounding was investigated.

MATERIALS AND METHODS

Materials

EVOH copolymer grade (Soarnol) EVOH29 (containing 29 mol % of ethylene) was supplied by The Nippon Synthetic Chemical Industry Co., Ltd. (NIPPON GOHSEI) (Japan). Sulfuric acid 96% and 2-propanol were purchased from Panreac (Barcelona, Spain).

Preparation of bacterial cellulose mats

BC mats were obtained by following the same procedure as described in a previous work.[20] Briefly, the bacterial strain *Gluconacetobacter xylinum* 7351 was incubated in a modified Hestrin/Shramm medium at 30°C. All of the cells were precultured in a test tube containing 5 mL of medium and re-inoculated into larger volumes until obtaining the final 20-L culture medium. The synthesized BC pellicles, about 5 cm thick, were cut into small pieces (approximately 2 × 2 cm²) and subsequently sterilized and cleaned in boiling water and then in a 10% (v/v) NaOH aqueous solution to remove bacteria cells and the absorbed culture media. Finally, the pH was lowered to 7 by boiling in distilled water several times.

Preparation of bacterial cellulose nanowhiskers

BCNW were obtained as a partially hydrated precipitate ("centrifuged BCNW") after subjecting the BC mats to hydrolysis with sulfuric acid. The material (ca. 1 kg of partially hydrated BC) was treated with 1 L of 50% (v/v) sulfuric acid/water, in a cellulose/acid ratio of approximately 7 g/L, at 50°C for 5 days (~ 120 h) until a homogeneous solution was obtained. This solution was subjected to 10 centrifugation and washing cycles at 12,500 rpm and 15°C for 20 min and BCNW were obtained as a white precipitate. The humidity of the material was determined and the yield of the extraction process was estimated to be 79.64% respect to the dried native BC. The material was kept refrigerated until use.

Electrospinning

BCNW were incorporated into 4.1%, 4.6%, 4.8%, 5%, and 5.5% (w/v) EVOH solutions in 70/30 (v/v) 2-propanol/water and hybrid fibers were generated by electrospinning. CNW were dispersed in the solvent by intense homogenization (Ultra-turrax). Concentration of CNW was varied from 5 to 40% (w/w)

regarding the EVOH weight to obtain solutions with a total solids concentration of 5.75% (w/w).

The electrospinning equipment was the same as described in a previous work.[20] Solutions were transferred to 5-mL plastic syringes and connected through polytetrafluoroethylene (PTFE) tubes to a stainless steel needle (Ø 0.9 mm). An electrode was clamped to the needle tip and connected to a high-voltage 0–30 kV power supply operating at 10–12 kV, and polymer solution was fed into the needle at a rate of 0.6 mL/h by a syringe pump (KD Scientific Inc., Holliston, MA). The counter electrode was connected to a rectangular plate covered by aluminum foil, which was placed parallel to the needle to collect the electrospun fibers. The distance between the needle and the plate was 12 cm and experiments were carried out at ambient temperature.

Preparation of films

The BCNW were incorporated into EVOH fibers by electrospinning with the aim of improving the dispersion of the nanofiller within the matrix during a subsequent melt compounding step. This proprietary procedure[21] is thought to be a solution to resolve the problem of the difficulties in dispersion of the direct addition of nanofillers using melt compounding routes. Thus, after optimizing the amount of BCNW incorporated into the hybrid fibers, electrospun EVOH fibers containing 15% (w/w) of BCNW were melt compounded in an internal mixer with EVOH29 pellets to obtain a blend having a final concentration of 3 wt % BCNW (sample code: 3% BCNW ES). One fraction of this blend was ground to powder and reprocessed to asses the effect of further processing on the dispersion and stability of the composite (sample code: 3% BCNW ES2). In addition, pure EVOH and EVOH blended with freeze-dried BCNW (sample code: EVOH29 and 3% BCNW FD, respectively) were melt compounded in a similar manner for comparison purposes.

Adequate amounts of EVOH pellets were melt mixed with the freeze-dried BCNW or the fiber's powder (i.e., 11.64 g of EVOH pellets with 0.36 g of freeze-dried BCNW, and 9.24 g of EVOH pellets with 2.76 g of electrospun EVOH-BCNW fibers). All the blends were prepared in a Brabender Plastograph double screw mini-mixer with 16 g capacity, for 3 min at 190°C and using 100 rpm as the screw speed. The materials were then converted into films by compression molding using a hot-plate hydraulic press at 180°C and 2 MPa for 3 min, followed by fast cooling with water.

Scanning electron microscopy

SEM was conducted on a Hitachi microscope (Hitachi S-4100) at an accelerating voltage of 10–15 kV

and a working distance of 12–16 mm. The electrospun mats were sputtered with a gold–palladium mixture under vacuum before their morphology was examined using SEM.

Fiber diameters of the electrospun fibers were measured by means of the Adobe Photoshop CS3 extended software from the SEM micrographs in their original magnification.

Optical microscopy

Polarized light microscopy (PLM) examinations were performed using an ECLIPSE E800-Nikon optical microscope (Kanagawa, Japan) equipped with a capture camera DXM1200F-Nikon.

Transmission and attenuated total reflectance FTIR analysis

Transmission FTIR experiments were recorded in a controlled chamber at 21°C and 40% RH using a Bruker (Rheinstetten, Germany) FTIR Tensor 37 equipment. The spectra were taken at 1 cm^{-1} resolution averaging a minimum of 10 scans. Analysis of the spectra was performed using Grams/AI 7.02 (Galactic Industries, Salem, NH) software. Samples of ~ 2 mg of electrospun fibers were ground and dispersed in 200 mg of spectroscopic grade KBr. A pellet was then formed by compressing the sample at ~ 150 MPa. A calibration curve was obtained by recording the IR spectra of pellets containing 6 mg of EVOH-BC nanowhiskers samples with nanowhiskers' concentrations ranging from 10 wt % to 40 wt %. The detailed procedure can be found elsewhere.[20] Briefly, the intensity of the band at 1165 cm^{-1}, which was chosen as characteristic for cellulose, was divided by the intensity of the band at 838 cm^{-1}, which was assigned to the EVOH contribution. Subsequently, the IR spectra of the electrospun fibers were analyzed and the percentages of BCNW incorporated into the fibers from the solutions were estimated.

ATR-FTIR spectra of BCNW and electrospun EVOH/BCNW exposed to different thermal treatments were collected in the same environmental conditions as the transmission experiments, coupling the ATR accessory GoldenGate of Specac Ltd. (Orpington, UK) to the above-mentioned FTIR equipment. All spectra were recorded within the wavenumber range of 4000–600 cm^{-1} by averaging 20 scans at 4 cm^{-1} resolution.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) experiments were carried out in a Perkin–Elmer DSC-7 calorimeter. Samples of ~ 3 mg were subjected to the

same treatment applied in the first part of this study, i.e., an initial conditioning step from 30 to 120°C and two heating steps up to 220°C. The heating and cooling rates for the runs were 10 °C/min.

Thermogravimetric analysis

Thermogravimetric (TG) curves were recorded with a TA Instruments model Q500 TGA. The samples ($\sim$ 20 mg) were heated from 50 to 600°C with a heating rate of 10 °C/min under nitrogen atmosphere.

RESULTS AND DISCUSSION

Fiber morphology and degree of incorporation of the BCNW

In the present study, BCNW extracted from BC by sulfuric acid digestion were incorporated into EVOH through electrospinning. These BCNW present a morphology of nanofibrils with cross-sections smaller than 30 nm, having a crystallinity index of 82.2%, as previously reported.[20] The production of electrospun EVOH fibers containing BCNW has been previously reported and the method was optimized in terms of efficient nanofiller incorporation for a maximum loading of 8 wt % BCNW.[20]

With the purpose of further increasing the amount of nanowhiskers incorporated into the fibers, several hybrid materials were developed adding various amounts of centrifuged BCNW, but fixing the total solids concentration of the electrospinning solutions at 5.75 wt %, i.e., the maximum solids concentration which provided stable electrospinning conditions for the composites. This was done by varying both the nanowhiskers and the EVOH concentration, and aimed at obtaining less viscous solutions to incorporate higher loadings of the reinforcing BC filler.

Solutions containing a fixed solids concentration of 5.75 wt % and the following concentrations of BCNW (with respect to the EVOH weight) were prepared and incorporated into fibers by electrospinning: 5 wt % BCNW (5.5 wt % EVOH in the solution), 15 wt % BCNW (5 wt % EVOH in the solution), 20 wt % BCNW (4.8 wt % EVOH in the solution), 25 wt % BCNW (4.6 wt % EVOH in the solution), and 40 wt % BCNW (4.1 wt % EVOH in the solution). Increasing the amount of BCNW in the solution up to 50 wt % did not lead to fibrillar morphologies, because electrospraying phenomena occurred; thus, the maximum concentration of nanowhiskers in the electrospinning solution that yielded fibers was 40 wt %. Additionally, control electrospun fibers were prepared with the EVOH concentrations previously listed.

Figure 1 shows the SEM micrograph and PLM image of fibers obtained from solutions containing
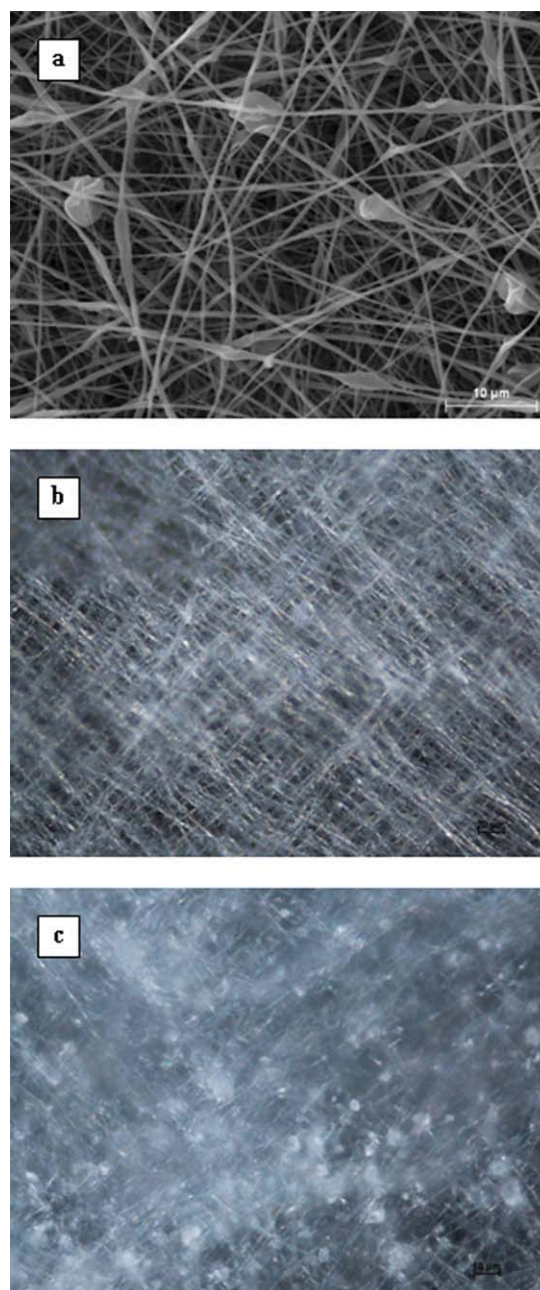

Figure 1 SEM micrograph (a) and polarized light optical microscopy image of electrospun fibers obtained from EVOH + 15 wt % BCNW (b), and from EVOH + 40 wt % BCNW (c). The scale markers correspond to 10 microns in picture (a) and (c), and to 20 microns in picture (b). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

15 wt % of BCNW. For all the electrospun materials, morphologies containing some beads, similar to the ones observed in a previous work with lower BCNW's loadings,[20] were obtained. It was also confirmed by polarized light optical microscopy that these fibers presented a highly homogeneous dispersion of highly crystalline BCNW, since fibers of pure EVOH did not show birefringence under crossed
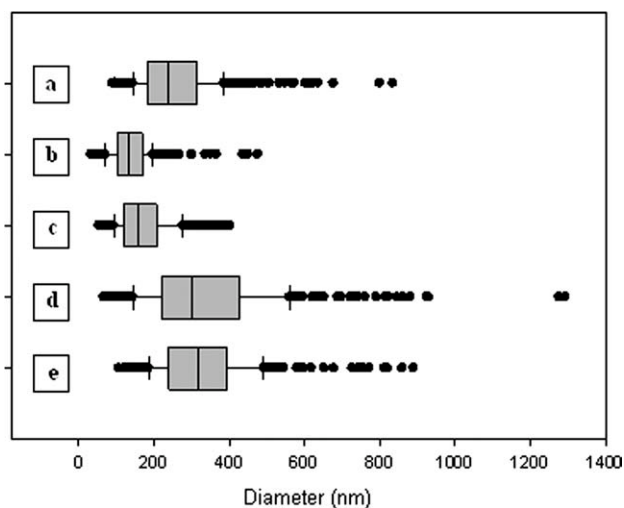
Figure 2 Box plot of size distribution of electrospun fibers obtained from (a) EVOH + 40 wt % BCNW; (b) EVOH + 25 wt % BCNW; (c) EVOH + 20 wt % BCNW; (d) EVOH + 15 wt % BCNW; and (e) EVOH + 5 wt % BCNW. The division within the boxes represents the median of the data, whereas the vertical limits represent the lowest and highest data within 1.5× interquartile range. Data not included within this range are plotted as dots and correspond to outliers (anomalous data) which in this case are most likely a consequence of the appearance of beads.

polarizers. As observed in Figure 1(c), fibers' beads appear as brighter areas when observed through polarized light. This birefringence suggests that BCNW may be agglomerated in those areas and thus, beads have a BCNW concentration higher than equilibrium. By varying the EVOH/BCNW ratio, uniformity and diameters of fibers were affected, as seen in Figure 2. From this figure, it is observed that increasing the amount of whiskers in the solutions leads to a decrease in the attained fiber diameter and to a more uniform size distribution. Nevertheless, this was not the case for the materials obtained from solutions containing 40 wt % of the filler, as in these materials an increase in the diameter of the fibers was obtained, as well as a more beaded morphology. A decrease in the fibers' diameter when increasing the concentration of CNW incorporated into electrospun fibers has been previously discussed in several works[6,16,20] and it was attributed to an increase in the fibers' conductivity when incorporating negatively charged CNW. This may suggest, among other things, that the incorporation of BCNW in the case of fibers generated from 40 wt % BCNW solutions is lower than expected. Probably, high concentrations of BCNW lead to agglomeration of BCNW in the electrospinning solution and thus, it is likely that a significant amount of the nanofiller remains as a residue in the electrospinning syringe.

The FTIR technique was used in the different electrospun fibers to check the degree of filler incorpora-

tion into EVOH across the different materials. Figure 3 shows the IR spectra in the range of interest of electrospun fibers with different nanowhiskers' concentrations. By applying a calibration curve developed in a previous work,[20] which relates the ratio between the intensities of the bands at 1165 cm^{-1} and at 838 cm^{-1} with the BCNWs' concentration, the degree of nanowhiskers' incorporation was estimated. Figure 3 displays the estimated amount of BCNW retained in the fibers for the compositions used. The highest concentration of BCNW was obtained for the 25 wt % BNNW solution, however only solutions containing up to 20 wt % BCNW were seen to achieve a complete incorporation of the nanofiller present in solution into the electrospun fibers. This result confirms that the incorporation degree of BCNW is significantly lower than expected in the case of fibers generated from 40 wt % BCNW solutions, just as previously suggested by morphological observations.

Thermal properties

DSC analysis was carried out to study the thermal properties of electrospun fibers. Table I shows the melting point, melting enthalpy, and the glass transition temperature of the electrospun fibers. From the first heating run it is deduced, in agreement with previous results,[20] that by increasing the nanowhiskers content, the melting point and melting enthalpy of the fibers decrease. CNW, when added as a partially hydrated precipitate, seem to interfere in the crystallization of EVOH during the formation of the fibers giving rise to a more amorphous structure with smaller or more defective crystallites. It is
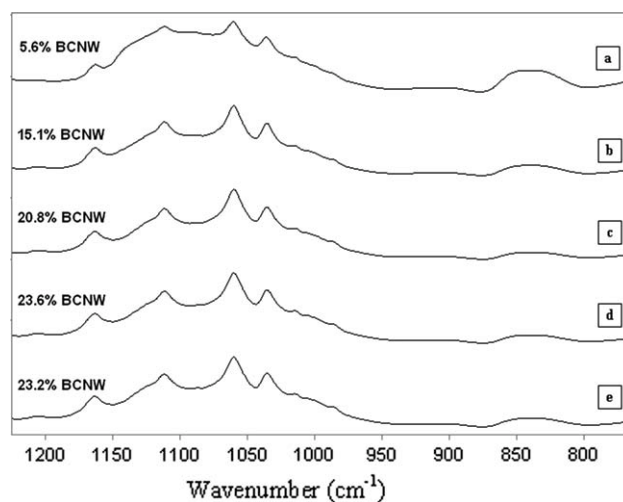

Figure 3 FTIR spectra and estimated degree of BCNW incorporated into electrospun fibers obtained from EVOH solutions containing: (a) 5% BCNW; (b) 15% BCNW; (c) 20% BCNW; (d) 25% BCNW; and (e) 40% BCNW. Data have been offset for clarity.

TABLE I
DSC Maximum of Melting (T_m), Melting Enthalpy (ΔH), and Glass Transition Temperature (T_{g1}), of Electrospun Fibres Obtained During the First Heating Run and Glass Transition Temperature (T_{g2}), Obtained During the Second Heating Run

	T_m (°C)	ΔH (J/g EVOH)	T_{g1} (°C)	T_{g2} (°C)
5.48% EVOH	190.6 (0.8)	79.1 (1.6)	66.6 (1.0)	63.0 (0.6)
5.48% EVOH + 5% BCNW	188.8 (0.4)	69.1 (0.8)	66.2 (2.2)	63.9 (0.4)
5% EVOH	192.5 (0.8)	79.2 (0.1)	67.3 (1.8)	62.7 (0.1)
5% EVOH + 15% BCNW	188.2 (2.0)	43.9 (0.3)	68.7 (0.2)	67.8 (0.1)
4.79% EVOH	191.9 (0.9)	78.8 (3.5)	67.2 (2.8)	63.1 (1.3)
4.79% EVOH + 20% BCNW	185.5 (0.1)	37.9 (0.9)	69.5 (0.3)	75.0 (0.2)
4.6% EVOH	190.9 (1.4)	77.3 (1.2)	69.1 (0.4)	63.3 (0.6)
4.6% EVOH + 25% BCNW	187.2 (0.8)	33.8 (1.5)	68.0 (0.4)	76.2 (0.3)
4.1% EVOH	192.4 (1.4)	70.7 (1.1)	63.6 (2.2)	62.6 (0.3)
4.1% EVOH + 40% BCNW	184.4 (0.2)	49.7 (2.0)	66.3 (2.6)	76.5 (0.2)

also observed that during the first heating run, no significant changes in the glass transition temperature (T_g) are produced when incorporating BCNW into the fibers as compared to the corresponding pure EVOH fibers.

Just as observed in a previous work,[20] no melting endotherm was detected for the fibers incorporating BCNW, thus indicating that the BCNW incorporated into the EVOH matrices prevent the material from re-crystallizing during the spinning process.

On the other hand, there is a significant increase in the T_g during the second heating run with increasing the BCNW content (see Fig. 4). Just as observed in Figure 4, this increase is more obvious when increasing the concentration up to 20 wt %, being less significant when increasing the concentration from 20 wt % to 25 wt % in agreement with the FTIR incorporation results which indicated that in the latter case the actual amount of BCNW incorporated into the fibers is below 25 wt %. Therefore, the
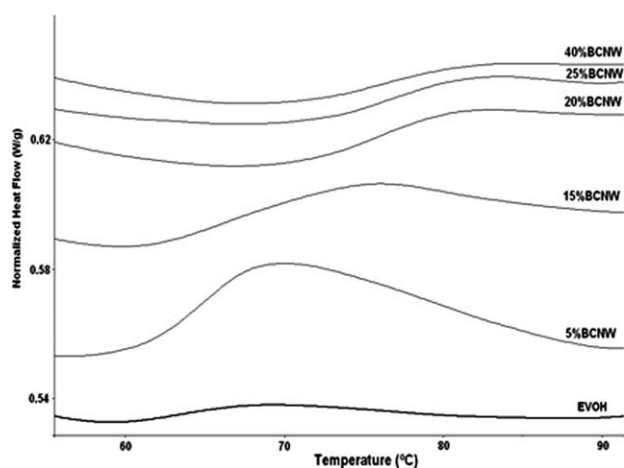
lower relative increase in the T_g observed is related to the fact that no complete incorporation occurs for concentrations of BCNW higher than 20 wt %. From these results it is deduced that incorporating BCNW in relatively low concentrations does significantly increase the rigidity of the amorphous phase (as estimated during the second DSC heating run) of the hybrid composite.

Thermogravimetric analyses showed previously that BCNW extracted by sulfuric acid treatment have a relatively low thermal stability.[20] With the aim of studying the effect of incorporating BCNW on the thermal stability of the electrospun hybrid fibers, TGAs were carried out. Figure 5 shows the experimental results for pure EVOH electrospun fibers and hybrid EVOH-BCNW fiber mats. The mass loss below 100°C is ascribed to water loss. In the case of pure EVOH, the main weight loss occurs within the temperature range 300–430°C. At 430°C a loss of 95% is observed and the loss above this temperature is ascribed to char. On the other hand,
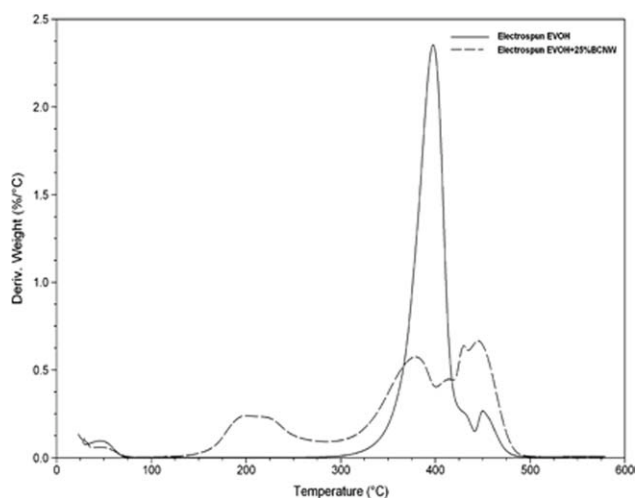

Figure 4 DSC curves obtained during the second heating run. Curves show the glass transition temperature of electrospun fibers obtained from solutions containing pure EVOH, 5% BCNW, 15% BCNW, 20% BCNW, 25% BCNW, and 40% BCNW.

Figure 5 DTG curves of electrospun fibers obtained from EVOH and from EVOH + 25 wt % BCNW.
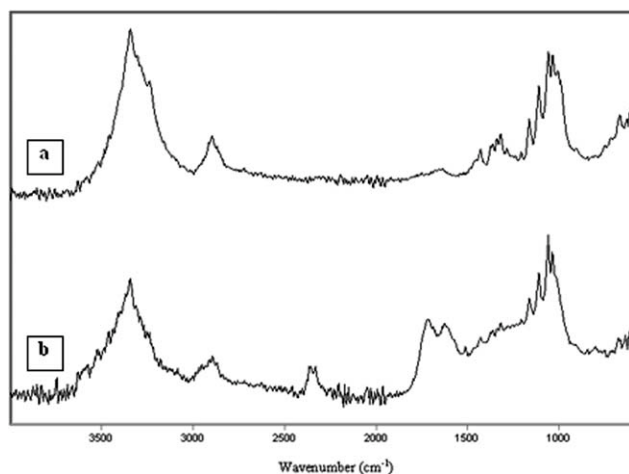
Figure 6 ATR-FTIR spectra of (a) freeze-dried BCNW and (b) freeze-dried BCNW after being subjected to the DSC heating-cooling program.

fibers containing EVOH and BCNW present a degradation profile composed of three steps. The first step takes place between 125 and 290°C with a weight loss of 21%. This step is ascribed to cellulose degradation processes such as depolymerization, dehydration, and decomposition of glycosyl units.[16] It has been previously reported that in the case of isolated nanowhiskers, this process takes place in the range of 100–225°C.[20] This slight increase in the degradation temperature suggests that the EVOH matrix partially protects the cellulose whiskers in the composite from thermal degradation. The second step, with a mass loss of 30%, occurs between 290 and 400°C and most likely corresponds to EVOH degradation. Finally, the third step takes place above 400°C and corresponds to the oxidation and breakdown of the charred residue. Increasing the concentration of nanowhiskers did not affect significantly the TGA profile. These results suggest that BCNW partially decrease the thermal stability of EVOH electrospun fibers, which may be caused by the presence of sulfate groups and residual acidity on the nanowhiskers surface as a consequence of the acid hydrolysis.[22] Specifically, for the fibers containing 40 wt % BCNW, a weight loss of 14.9% was observed below 220°C (maximum temperature reached during the DSC runs), suggesting that some cellulose chemical alterations which may be responsible for the increase in the T_g observed during the second DSC heating run take place during the first heating run.

To study the degradation processes during the DSC heating runs in detail, ATR-FTIR spectra of BCNW subjected to the same DSC program as the electrospun fibers (i.e., two heating runs up to 220°C) and electrospun fibers obtained from solutions containing 40 wt % BCNW subjected to one heating step up to 220°C and to the complete DSC program were analyzed.

The ATR-FTIR spectra in Figure 6 clearly indicate that degradation of the isolated BCNW takes place after two DSC heating runs. The decrease in intensity of the broad band between 3000 and 3600 cm^{-1}, corresponding to -OH stretching intramolecular hydrogen bonds, indicates that dehydration has taken place. Furthermore, the intensity of all the cellulose characteristic bands located between 1035 and 1430 cm^{-1} diminishes as a consequence of heat-induced chemical alterations. Additionally, the degraded sample shows two bands at 1715 cm^{-1} and 1621 cm^{-1}, which have been previously related to the thermo-oxidative degradation of cellulose[22,23] and correspond to carbonyl groups and unsaturated carbon–carbon groups. Visual examination of the sample after the DSC program confirmed that BCNW have experienced a severe thermal degradation, since they presented the typical blackish color associated to the production of char.

Figure 7 shows the ATR-FTIR spectra of electrospun EVOH fibers derived from the solution containing 40 wt % of BCNW before and after being subjected to a heating step up to 220°C, and to the complete DSC program consisting in two consecutive heating runs up to 220°C. As observed for the pure BCNW, just by subjecting the composite fibers to one heating step, there is a significant decrease in the intensity of bands corresponding to -OH stretching intramolecular hydrogen bonds and to -CH stretching. The decrease in the cellulose characteristic bands located between 1035 and 1430 cm^{-1} is clearly less significant for the electrospun fibers than for the pure BCNW, thus indicating that the EVOH matrix is reducing the extent of the chemical alteration processes in the composite. In comparison with
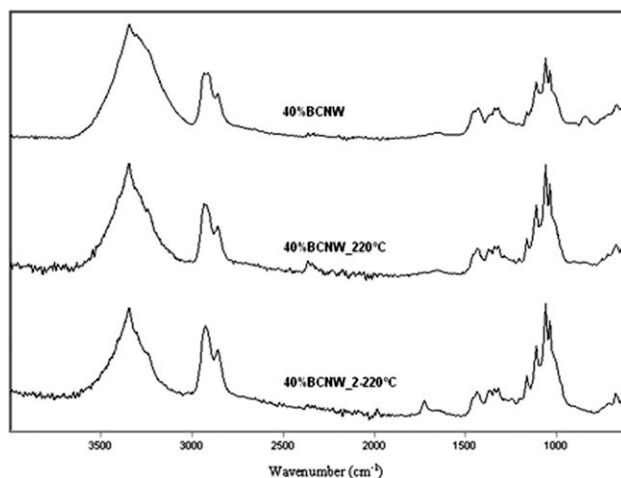

Figure 7 ATR-FTIR spectra of fibers obtained from the solution containing EVOH + 40 wt % BCNW (top spectrum); after one heating step up to 220°C (middle spectrum); and after two heating steps up to 220°C (bottom spectrum).

the pure BCNW sample, visual examination of both electrospun fibers samples presented a light yellow color, suggesting that samples were just partially degraded.

An interesting fact is that, after applying one heating run, the material does not present the bands at 1715 cm^{-1} or 1621 cm^{-1}, whereas when applying two heating steps, the band at 1715 cm^{-1} corresponding to the presence of carbonyl groups and thus, to oxidative degradation, is clearly observed. Previous works proposed a degradation mechanism of cellulose described as follows. At a heating rate higher than 2 °C/min cellulose first undergoes a decrease in its degree of polymerization. In more advanced degradation steps, dehydration, decarboxylation, decarbonylation, and ring scission take place and carbonyl bonds are formed.[24] According to this mechanism, when heating up to 220°C cellulose is partially degraded, as concluded by the decrease in its characteristic bands, but two heating steps up to 220°C are needed to reach more severe degradation for the material.

On the other hand, the band at 838 cm^{-1}, which is ascribed to skeletal vibrations and CH$_2$ rocking of EVOH,[25] disappears after the first heating run. This fact, together with the decrease in bands of -OH stretching intramolecular hydrogen bonds, could be indicating that interactions between the OH groups of cellulose and EVOH have been developed as a consequence of heating, therefore impeding the skeletal vibrations of the matrix and resulting in stiffening.

As a conclusion of the FTIR analyses, when the sample is subjected to one heating step up to 220°C, the cellulose present in the fibers begins to undergo thermal degradation and, as a consequence, strong interactions between EVOH and cellulose are promoted. These developed interactions may cause an increase in the material stiffness and thus, be responsible for the increase in the T_g observed during the second heating run. During the subsequent second heating step, the material suffers a more advanced degradation giving rise to the formation of carbonyl bonds. Finally, these interactions will have a stronger influence on the melting and crystallization behavior of hybrid materials during this second heating.

Further experiments of preconditioning by annealing of the fiber composites at increasing temperatures were also carried out, to check whether it was possible to enhance the T_g of the hybrid composite material without having to erase the thermal history and consequently eliminate the ultrathin fibrilar morphology. If this was feasible, then it could also be possible to obtain thermally enhanced electrospun fibrils. Thus, preconditioning or annealing of the sample obtained from the solution containing 20 wt

% of BCNW at both 170°C (just before the onset of the EVOH phase melting) and 190°C (corresponding to the endset of the EVOH phase melting) for 10 min was carried out followed by the usual first melting run up to 220°C. From the results, the T_g of the sample annealed at 170°C did not change compared to pure EVOH but it was much higher, i.e., at 73°C, immediately after melting, suggesting that thermal reinforcement does not take place before melting of the EVOH phase occurs in the electrospun composite fiber mats.

As a result, the stronger interfacial interaction between matrix and filler is not only related to chemical alterations produced in the nanofiller during the melting run but it is also caused by the cooperative melting of the EVOH phase and the subsequent enhancement in the interaction between filler and matrix. On the other hand, further studies using neutralized BCNW (results not shown) exhibited a much better thermal stability than the acidic ones as they undergo thermal degradation at temperatures higher than 200°C. Electrospun fibers incorporating those neutralized nanowhiskers were developed and the results will be reported elsewhere. In anticipation to that and just for the sake of the current discussion, it was in fact found out that an increase in the T_g of the hybrid fibers was not observed. This latter result confirms that to obtain a stiffening effect on the composite electrospun fibers, it is required that BCNW remain acid to promote strong interactions with the molten EVOH matrix.

At this point, it becomes clear that the electrospinning process applied succeeds in incorporating highly dispersed fractions of BCNW into the EVOH matrix but *per se* it does not result in a stiffening effect of the material. It is the partial thermally induced interaction of by the acidic nanofiller with the molten matrix what promotes a strong interfacial adhesion between the filler and the matrix. Nevertheless, this reinforcing effect is obtained at the expense of producing fibers which have a relatively low thermal stability and thus, perhaps a limited applicability.

Incorporation of BCNW into EVOH by melt compounding

Once the production of electrospun hybrid fibers was optimized, those fibers were used as a vehicle for the incorporation of well-dispersed BCNW into an EVOH matrix by means of melt mixing. The appearance and thermal properties of the attained material were studied and compared to the blend obtained by direct melt mixing of freeze-dried BCNW with the polymeric matrix.

Figure 8 shows typical optical photographs of the films obtained from the different blends. All the films present a relatively good contact transparency
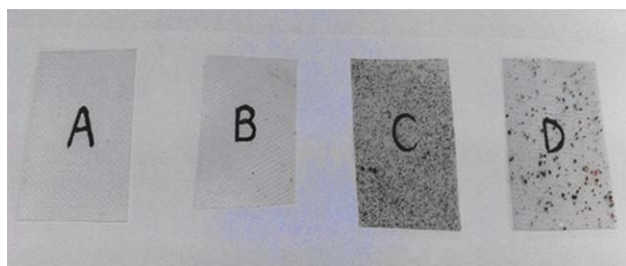
Figure 8 Photographs of films obtained by melt compounding of (A) EVOH29; (B) 3% BCNW ES; (C) 3% BCNW FD, and (D) 3% BCNW ES2. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

although clear thermal degradation of the filler took place by direct incorporation of this into the polymer. Thus, it is clearly observed that freeze-dried BCNW undergoes severe thermal degradation during the melt mixing process and thus, the filler appears as black spots distributed along the film [Fig. 8(C)]. This was not unexpected since the applied processing temperature of 190°C is somewhat above the degradation onset temperature of BCNW (see above). However, the composite incorporating electrospun fibers were highly transparent [Fig. 8(B)], with little spots suggesting that thermal degradation is less relevant and hence that the BCNW within the electrospun fibers are more stable

as suggested above. By subjecting the material to an additional melt mixing step, i.e., by reprocessing, the obtained film presented now large dark spots evenly distributed along the film [Fig. 8(D)]. This observation suggests that the obtained BCNW are rather unstable at the EVOH melt mixing temperature and caution should be taken when processing the material.

The films were also observed with polarized light to assess the birefringence arising from the BCNW within the films and hence the crystalline filler dispersion in the polymeric matrix (see Fig. 9). From the results, it is confirmed that the most efficient procedure for the incorporation, stability, and dispersion of BCNW consists in the production of hybrid electrospun fibers previous to the melt mixing step with virgin EVOH. By reprocessing this material, BCNW tend to degrade and agglomerate to a significant extent. Less agglomeration occurs when incorporating freeze-dried BCNW, but as expected, the bright areas corresponding to crystalline domains of the filler are not seen confirming that the nanofiller has suffered severe thermal degradation and agglomeration.

Finally, DSC analyses were carried out to study the effect of the incorporation method on the thermal properties of the composite films. In Table II, the melting enthalpy of the EVOH films is lower than that of electrospun EVOH fibers (see Table I)
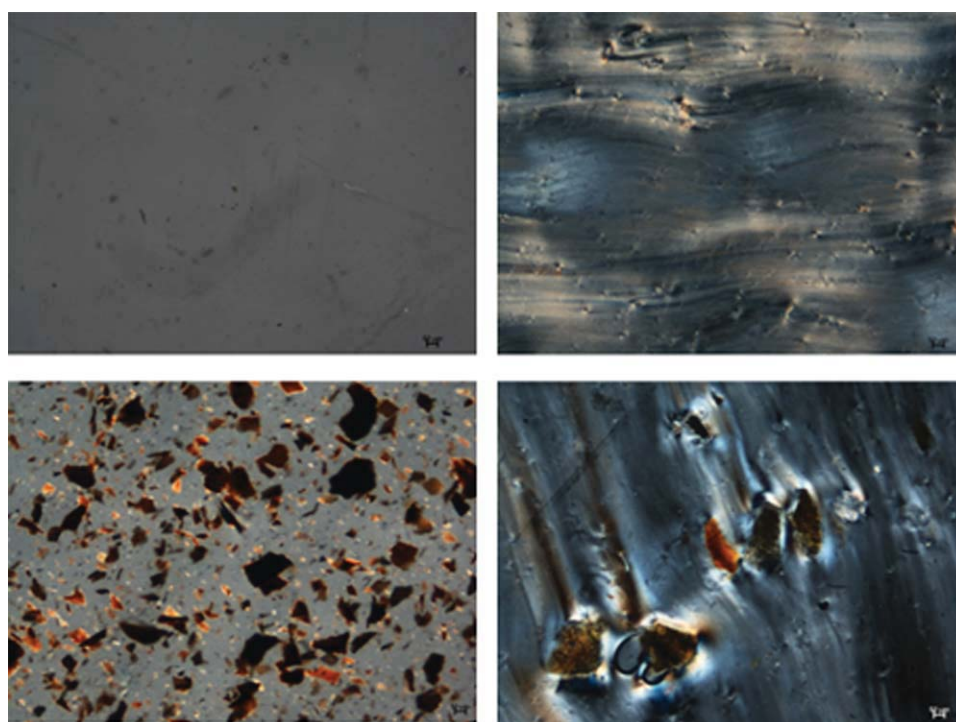

Figure 9 Polarized light optical microscopy images of the (top-left) EVOH29 film; (top-right) 3% BCNW ES film; (bottom-left) 3% BCNW FD film and (bottom-right) 3% BCNW ES2 film. Scale markers are 50 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE II
DSC Maximum of Melting (T_m), Melting Enthalpy (ΔH), and Glass Transition Temperature (T_{g1}), Obtained During the First Heating Run and Glass Transition Temperature (T_{g2}), Obtained During the Second Heating Run, of EVOH-BCNW Films Obtained by Melt Compounding

	T_m (°C)	ΔH (J/g EVOH)	T_{g1} (°C)	T_{g2} (°C)
EVOH29	191.0 (0.2)	62.0 (0.1)	64.3 (0.8)	63.9 (0.3)
3%BCNW ES	188.7 (0.2)	52.3 (3.3)	63.0 (0.2)	62.4 (0.2)
3% BCNW FD	189.2 (0.2)	62.2 (4.7)	63.0 (0.1)	62.9 (0.1)
3%BCNW ES2	188.1 (0.6)	67.5 (9.0)	62.6 (0.1)	62.7 (0.1)
3%BCNW ES[a]	187.2 (2.4)	7.1 (0.3)	66.9 (0.2)	65.9 (0.1)

[a] Refers to areas containing brown spots.

indicating that lower crystallinity is obtained by the compression molding and rapid cooling. It is also clearly observed that when incorporating BCNW the melting temperature decreases as compared to neat EVOH indicating that smaller or more defective crystals are being formed. In the case of the 3%BCNW ES sample, a significant decrease in the melting enthalpy is also observed. It was previously suggested that BCNW hinder the crystallization process of EVOH when incorporating them into electrospun fibers and therefore, a decrease in the melting enthalpy of composites incorporating electrospun fibers was something to expect. Obviously, areas corresponding to BCNW which suffered thermal degradation present a much lower melting enthalpy. On the other hand, when the material is re-processed or when freeze-dried BCNW are directly melt mixed with EVOH, the melting enthalpy is not significantly altered, suggesting that degraded BCNW do not present an obstacle for the EVOH crystallinity. Regarding the glass transition temperature, it was previously observed that for low loadings, such as 5 wt %, no significant improvements were detected and hence, for a loading of 3 wt % the T_g suffers just a slight decrease. Only those areas corresponding to partially degraded BCNW present an increase in the polymer T_g, confirming the fact that the stiffening effect of the nanofiller is related to its acidic character and the subsequent interactions between matrix and filler established when applying a thermal treatment that leads to chemical alterations of the filler.

CONCLUSIONS

By using an optimized method electrospun EVOH-BCNW fibers were generated from solutions with nanowhiskers concentrations up to 40 wt %. As observed by SEM, morphologies of smaller average diameters and more uniform fibers were attained when increasing the nanofiller concentration.

By means of FTIR, it was estimated that an amount of $\sim$ 24 wt % BCNW was the actual maximum concentration incorporated into the fibers. On the other hand, the incorporation of the nanofiller was only proven to be complete in concentrations up to 20 wt % BCNW. Additionally, DSC analyses showed that the incorporation of BCNW hinders the crystallization of EVOH and produces an increase in the T_g of the fibers after one heating step. Furthermore, those effects are directly related to the amount of BCNW effectively incorporated into the fibers, being less pronounced for concentrations higher than 20 wt % nanowhiskers.

The increase in the T_g can be explained by the acidic character of the incorporated BCNW, which is responsible, on the other hand, for the decrease in the thermal stability of the hybrid fibers as compared to pure EVOH fibers. It was observed that, when subjected to thermal treatments beyond the EVOH matrix melting point, the hybrid fibers seem to undergo a chemistry development in which strong interactions are formed between hydroxyl groups from cellulose and EVOH. As a consequence of those established interactions, a significant stiffening of the fibers is produced, reflected in a consistent increase in the T_g of the material. Therefore, there is a compromise between the stiffening effect induced by heating of the material and the thermal stability of the fibers.

Finally, electrospun fibers were employed for the incorporation of BCNW into EVOH via melt compounding. As assessed by optical and polarized optical microscopy, the degree of dispersion achieved and the stability of the filler was found to be much higher than that attained when direct melt mixing of freeze-dried BCNW with the polymeric matrix. No T_g enhancement was found on the composite films at the low filler loadings used, unless clear degradation took place, although the crystallization of EVOH was somewhat hindered, especially when incorporating hybrid electrospun fibers.

Nevertheless, it has been demonstrated that the electrospinning technique can be used as an efficient vehicle for the incorporation of well-dispersed BCNW into an EVOH matrix by melt compounding.

However, the nanofabrication process of BCNW needs to be optimized to obtain a more thermally stable material which can be processed with EVOH by melt compounding without suffering thermal degradation. Further results on this direction will be reported elsewhere.

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