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Transcrystallization in Mcl-PHAs/Cellulose Whiskers Composites

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ABSTRACT: Nanocomposite materials were prepared from an elastomeric medium-chain-length poly(hydroxyalkanoate) (Mcl-PHA) latex as semicrystalline matrix using a colloidal suspension of hydrolyzed cellulose whiskers as natural and biodegradable filler. After stirring, the preparations were cast and evaporated. High-performance materials were obtained from this system, preserving the natural character of PHA. However, differences were reported by comparison with amorphous PHA filled systems. These differences were ascribed to a transcrystallization phenomenon of semicrystalline PHA on cellulose whiskers, evidenced by dynamic mechanical analysis. Transcrystallization hindered the mechanical percolation of cellulose whiskers and the formation of a rigid network within the polymer matrix during the film formation by evaporation. The whiskers network can reorganize under thermal aging.

Introduction

Previous works performed at CERMAV deal with the processing and the characterization of nanocomposite materials with a synthetic^{1–4} or natural⁵ amorphous polymeric latex as matrix reinforced by cellulose whiskers. When homogeneously dispersed into polymer matrices, they give a remarkable reinforcing effect, even at concentrations of a few percent. The resulting properties were strongly related to the aspect ratio of the filler and to the geometric and mechanical percolation effects.^{1–5} It was also shown that, during the processing of the cellulose whiskers reinforced thermoplastic composite by film-casting techniques (water evaporation), geometrical constraints due to the particle size of the latex have a direct effect on the percolation phenomenon and therefore on the resulting mechanical reinforcement effect of cellulose whiskers.⁵

In addition, it is well established that the stress transfer from matrix to filler is strongly dependent on the degree of intimate contact between the two phases. The efficiency of this transfer will depend on the structure of the matrix in the vicinity of the filler, referred to as an interphase. In particular, a highly oriented layer can develop at the filler/matrix interface under appropriate conditions, when semicrystalline polymers are used as the matrix. The development of this surface layer has been termed transcrystallization.⁶ The formation of this transcrystalline layer in contact with a foreign surface is associated with the consequence of a high nucleating ability of the fillers compared to that of the bulk matrix. Most of the experimental results in the literature were obtained with polypropylene (PP), and several different types of fiber surfaces have been shown to induce transcrystallinity in PP. These include Kevlar,^{7–9} nylon,^{8–10} PET,^{7,10,11} PTFE,^{7,12} carbon,^{7,9,13} cellulose,^{14–16} and glass.^{17,18} A review paper has been published by Ishida and Bussi.¹⁹

Several theories have been suggested to account for the development of transcrystalline layer.^{8,9,17,19–22} These

include epitaxial growth based on lattice matching, wettability and surface energy of the substrates, adsorption of small molecules, stress-induced crystallization by local flow, residual stress caused by mismatch in coefficients of thermal expansion, topography of the substrates, and residual crystals at the surface of the foreign particles.

The aim of the present paper is to process and characterize natural nanocomposite materials using an elastomeric medium-chain-length poly(hydroxyalkanoate) (mcl-PHA) latex as the semicrystalline matrix and cellulose whiskers as the reinforcing phase. The presence of functional groups in PHA is expected to allow favorable interactions with the hydroxyl groups of cellulose, like hydrogen bonds. The cellulose whiskers used in this study, prepared from tunicin, an animal cellulose, consist of parallelepiped rods with lengths ranging from 100 nm to several micrometers for widths on the order of 10–20 nm. The interfacial phenomena are therefore assumed to be noticeable owing to the high specific area of tunicin whiskers (~170 m²/g). For a 6 wt % tunicin whiskers filled composite, there are of the order of 100 000 cm² of filler surfaces/cm³ of material. However, the dimensions of tunicin whiskers prevent any experimental evidence of transcrystallinity by classical means, such as optical microscopy.

Experimental Section

Cultivation. Mcl-PHAs were produced by open-loop controlled fed-batch fermentation of *Pseudomonas putida* KT2442 in a 200 L R200 bioreactor (New MBR, Switzerland).²³ Cultivations were performed at 30 °C, pH = 7.0, and sufficient air throughput to prevent an oxygen limitation. Sodium octanoate was used as the sole carbon source, and its concentration was monitored using a Biospectra on-line GC. The resulting signal was used to adapt the rate of a feed pump supplying a 0.5 M sodium octanoate solution to maintain a constant octanoate concentration. Final biomass concentration and PHA amount per cell of 23.6 g L⁻¹ and 18.5 wt % were obtained, respectively.

Recovery Procedure. Solubilization was performed according to the procedure described in a previous paper.²³ Cells were subjected to a heat shock in a steam autoclave at 121 °C for 1 min, cooled to 60 °C, and adjusted to a concentration of approximately 100 g L⁻¹. The acidity was adjusted to and

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maintained at pH = 8.5. Solubilization of non-PHA biomass was achieved by the addition of ethylenediaminetetraacetic acid (EDTA), sodium dodecyl sulfate (SDS), and protease (Alcalase). Immediately after solubilization, cell material was recirculated through a cross-flow-filtration loop containing a Rhone-Poulenc KERASEP filter (filter area, 0.024 m²; pore size, 0.1 μ m; channel diameter, 2.5 mm). After each diafiltration run, the membrane was rinsed with CH₂Cl₂ and with 5 N NaOH for 4 h. A final latex concentration to about 10–15% solids (w/v) was achieved by closing the incoming water supply and subsequent filtration of the latex suspension.

Cellulose Whiskers. Cellulose microcrystals, or whiskers, were obtained from tunicate, a sea animal. Mantles of tunicates are made of cellulosic microfibrils particularly well organized and therefore highly crystalline. Colloidal suspensions of whiskers in water were prepared as described elsewhere.^{1,2} Mantles were first cut into small fragments that were deproteinized by three successive bleaching treatments, following the method of Wise et al.²⁴ The bleached mantles (the tunicin) were then disintegrated in water with a Waring blender (at a concentration of 5 wt %). The resulting aqueous tunicin suspension was mixed with H₂SO₄ to reach a final acid/water concentration of 55 wt %. Hydrolysis conditions were 60 °C for 20 min under strong stirring. A dispersion of cellulose whiskers resulted. After sonication, the suspension was neutralized and washed by dialysis. It did not sediment or flocculate as a consequence of surface sulfate groups created during the sulfuric acid treatment.²⁵ Tunicin whiskers consist of parallelepiped rods with length ranging from 100 nm to several micrometers (average value around 1 μ m) for widths on the order of 10–20 nm.^{1,2} The aspect ratio L/d (L being the length and d the diameter of the filler) of these whiskers was estimated from transmission electron microscopy and was around 67.

Film Processing. The colloidal cellulose whiskers suspension was mixed with the mcl-PHA latex at different ratio in order to obtain composite films with a good level of dispersion and with different compositions. The cellulose whiskers loading was varied from 0 to 6 wt %. After stirring, the air in the suspension was removed by vacuum prior to casting in a Teflon mold (2 × 7 mm). Films were obtained by storing the casting at 35 °C to allow water evaporation and polymer particle coalescence.

Sieve Analysis. Particle size and particle size distributions of PHA granules were obtained from dynamic light scattering (quasi-elastic light scattering) with a Malvern Autosizer Lo-Ci instrument. The diluted polymer suspension was set in a cell and exposed to a monochromatic beam (λ = 670 nm). Particles shift under the Brownian motion and oscillate around an equilibrium position. Intensity fluctuations scattered at 90° were amplified and used to determine the diffusion coefficient. The particle mean diameter was calculated from the Stokes–Einstein equation. The values reported in this work are the average of at least five measurements. The distribution of the particle size was characterized by the variance. A latex was considered monodisperse if the variance was lower than 0.02, of narrow distribution if the variance was between 0.02 and 0.1, and of broad distribution if the variance was beyond 0.1.

Molecular Weight Measurements. Number-average molecular weight, \bar{M}_n , weight-average molecular weight, \bar{M}_w , and polydispersity index \bar{M}_w/\bar{M}_n were determined by size exclusion chromatography (SEC) with a Waters apparatus (510 pump), using a set of four columns (Styragel HR0.5, HR1, HR2, and HR5). The mobile phase was tetrahydrofuran, with an eluent flow rate of 1.1 mL min⁻¹. A refractive index detector (Waters 410) was used for detection. For calibration, polystyrene standard solutions were injected.

Differential Scanning Calorimetry. Differential scanning calorimetry (DSC) was performed with a Perkin-Elmer DSC7 equipment, fitted with a cooler system using liquid nitrogen. It was calibrated with an indium standard and operated at a heating rate of 15 K/min. Scans were performed from -60 to +80 °C on samples (~10 mg) at least 2 weeks after film formation to ensure stabilization of the degree of crystallinity and perfect reliability of measurements.

X-ray Diffraction. Wide-angle X-ray scattering (WAXS) patterns were measured in reflection with a diffractometer using a static detector (Siemens D500). Samples were mounted on a support and exposed for a period of 10 s for each angle of incidence using a Cu K α_1 X-ray source with a wavelength of 1.5406 Å operating at 40 kV and 20 mA. The angle of incidence was varied between 3° and 40° by steps of 0.04°. WAXS patterns were recorded at several time intervals after film formation to ensure perfect reliability of measurements and to detect any possible evolution of the material with time.

Dynamic Mechanical Analysis. Dynamic mechanical tests were carried out with a Rheometrics RSA2 spectrometer in the tensile mode. Test conditions were chosen in such a way that the measurements were in the linear viscoelasticity region (the maximum strain ϵ was around 10⁻⁴). The specimen was a thin rectangular strip with dimensions of 30 × 3 × 1 mm. The setup measured the complex tensile modulus E^* , i.e., the storage component E' and the loss component E'' . In the present work, results are displayed through E' and $\tan \phi$ ($=E''/E'$). Measurements were performed in isochronal conditions at 1 Hz, and the temperature was varied by steps of 3 K.

Results and Discussion

Characterization of the PHA Matrix. The particle size of treated mcl-PHA granules extracted from the biomass was obtained from dynamic light scattering measurements. It was observed that the average particle diameter of PHA granules in the latex was around 0.92 μ m. A similar size was reported for poly(hydroxy-octanoate) (PHO) granules produced by *Pseudomonas oleovorans* and extracted from bacteria by a chemical digestion procedure, using sodium hypochlorite.²⁶ This value corresponds to the in vivo dimension of the native granule in inclusion. The variance was around 0.12. This value is much lower than what was observed for the PHO latex.²⁶ Compared to this last latex, for which the variance was equal to 1, the PHA granules produced by *Pseudomonas putida* displayed therefore a much lower size distribution.

Size exclusion chromatography (SEC) was performed to characterize the molecular weight of the polymer obtained from *Pseudomonas putida*. The number-average molecular weight, \bar{M}_n , was found around 43 × 10³ g mol⁻¹, and the weight-average molecular weight, \bar{M}_w , was equal to 78 × 10³ g mol⁻¹. These values are much lower than those (90 × 10³ and 140 × 10³ g mol⁻¹, respectively) measured for PHO.²⁶ The polydispersity index, \bar{M}_w/\bar{M}_n , was therefore 1.81, which is close to what was reported for PHO granules purified from *Pseudomonas oleovorans* (polydispersity index ranging from 1.66 to 2.07 was observed depending on the hypochlorite concentration used during the purification step).

The PHA film resulting from the coalescence of the mcl-PHA latex was characterized by wide-angle X-ray scattering (WAXS) (Figure 1). The amorphous part of the polymer is characterized by a broad hump located around 2θ = 20°, whereas the crystalline zones display four diffraction peaks near 5°, 20°, 18°, and 22°. Unit cell and interpretation of these data were reported from a fiber diagram elsewhere.²⁷ The melting and glass transition temperatures were determined from DSC experiments and were found around 55 and -36 °C, respectively. The melting and glass transition temperatures were determined from differential scanning calorimetry (DSC) experiments and were found around T_m = 57 °C and T_g = -34 °C, respectively. These values agree with what was reported for poly(hydroxyoctanoate) (PHO) in the literature.^{26,28} These two experi-

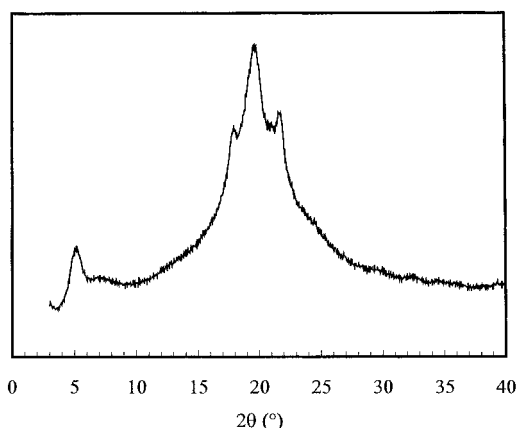


Figure 1. Wide-angle X-ray diffraction pattern for a PHA film obtained from the coalescence of the PHA latex produced by *Pseudomonas putida*.

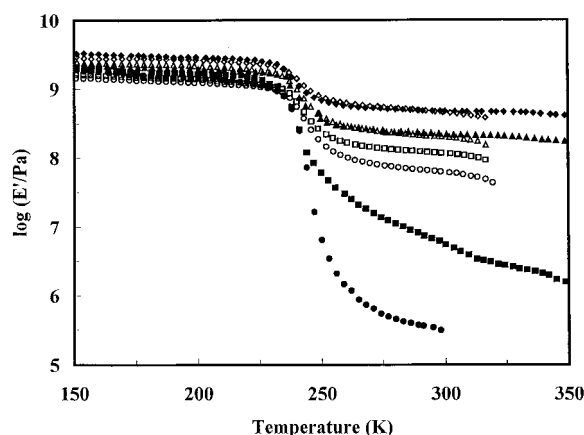


Figure 2. Logarithm of the storage tensile modulus E' vs temperature at 1 Hz for amorphous (filled symbols) and semicrystalline (open symbols) PHA composites filled with 0 (●, ○), 1 (■, □), 3 (▲, △) and 6 wt % (◆, ◇) of tunicin whiskers.

ments shown that the mcl-PHA used in this study is a semicrystalline polymer.

However, it is worth noting that the degree of crystallinity of PHA increased with annealing time after film formation at room temperature, which is higher than its T_g and lower than its crystallization temperature. After 2 weeks, the degree of crystallinity did not change further.

Cellulose Whiskers Filled PHA. Figure 2 shows a plot of the tensile storage modulus at 1 Hz as a function of temperature for various whisker/PHA compositions ranging from 0 to 6 wt %. Open symbols correspond to the present mcl-PHA matrix systems and filled symbols to experimental data reported elsewhere⁵ for composites systems obtained from tunicin whiskers and an amorphous PHO latex as matrix.

Unfilled materials display typical behaviors of either fully amorphous (filled circles) or semicrystalline (open circles) polymers. For temperatures below T_g the polymer is in the glassy state, and the modulus slightly decreases with temperature but remains roughly constant (around 2 GPa). Then, a rapid decrease in the elastic tensile modulus, by more than 3 decades, for the amorphous polymer, and by more than 1 decade, for the semicrystalline one, is observed, corresponding to the glass-rubber transition. The rubbery modulus of the amorphous polymer remains roughly constant (around 1 MPa), up to the terminal zone, for which the elastic

tensile modulus becomes lower and lower with temperature and the experimental setup fails to measure it, due to irreversible chain flow. The rubbery modulus is known to depend on the degree of crystallinity of the material (open circles). The crystalline regions of mcl-PHA act as physical cross-links for the elastomer. In this physically cross-linked system, the crystalline regions would also act as filler particles due to their finite size, which would increase the modulus substantially (~80 MPa). The modulus drops irretrievably with the breakup of crystalline domains, around 320 K.

For both kinds of systems (amorphous and semicrystalline PHA based composites) the films that contain cellulose whiskers have a slight increase in their storage modulus below T_g . In this temperature range, the difference between the elastic tensile modulus of the cellulose whiskers and that of the matrix is not high enough to easily appreciate a reinforcement effect with only 6 wt % of fillers. Above T_g , a greater increase in the composite modulus is observed with increasing weight fraction of cellulose whiskers. This reinforcing effect is more pronounced for the amorphous PHA matrix systems. A simple mixing rule allows to account for this phenomenon. The films reinforced with 1 wt % of whiskers are very sensitive to the crystallinity state of the matrix. The rubbery modulus of the amorphous PHA based system (filled squares) is much lower than the one of the semicrystalline matrix system (open squares). For instance, the relaxed modulus at $T_g + 50$ °C (~290 K) of the 1 wt % tunicin whiskers filled semicrystalline PHA is 10 times higher than that of the 1 wt % filled amorphous matrix. This indicates that the mechanical behavior of the composite is matrix dominated for this filler loading level (1 wt %). At higher whiskers content, the differences between both kinds of matrix shade off. This indicates that the mechanical behavior of the material becomes whiskers dominated.

However, the negative slope of the curve $\log E'$ vs temperature seems to be higher (in absolute value) for the semicrystalline PHA based systems than it is for the amorphous ones. In addition, the rubbery modulus drops with the breakup of crystalline domains, around 320 K, whatever the whiskers content may be. For amorphous based systems, the modulus value stays almost constant over a large temperature range above T_g . It was shown,² using a synthetic poly(styrene-*co*-butyl acrylate) (poly(S-*co*-BuA) as matrix, that it was only when the whiskers start to decompose at around 500 K that this stabilization disappeared, inducing a catastrophic decrease of the mechanical properties. This phenomenon was ascribed to the formation of a rigid whiskers network within the amorphous thermoplastic matrix, the cellulose whiskers being linked through hydrogen bonds.

Figure 3 shows the evolution of the mechanical loss factor $\tan \phi$ as a function of temperature for semicrystalline mcl-PHA composites with various concentrations of tunicin whiskers. We ascertain that it exhibits a maximum in the temperature range of the glass transition of the matrix. This relaxation process, labeled α , is therefore associated with the anelastic manifestation of the glass-rubber transition of the amorphous domains of PHA. This mechanism involves cooperative motions of long-chain sequences. The temperature position of the α peak is not modified by the presence of different amounts of whiskers. No significant broadening of the peak is observed either. Finally, the intensity of $\tan \phi$

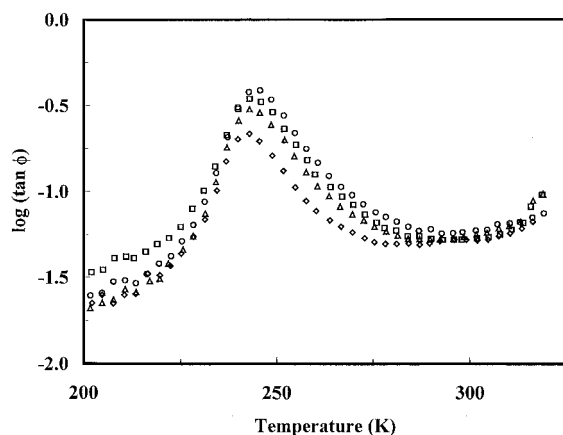


Figure 3. Logarithm of the loss angle tangent $\tan \phi$ vs temperature at 1 Hz for semicrystalline mcl-PHA composites filled with 0 (\circ), 1 (\square), 3 (\triangle), and 6 wt % (\diamond) of tunicin whiskers.

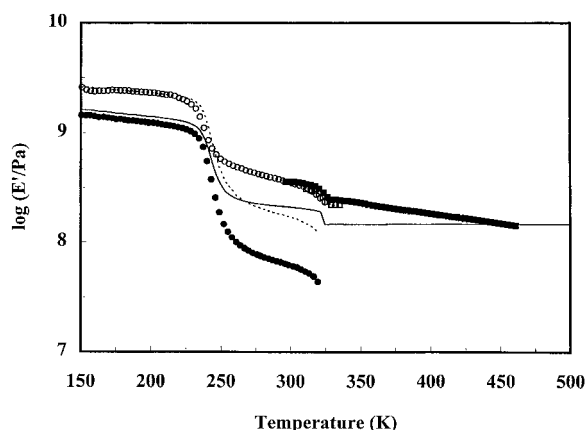


Figure 4. Logarithm of the storage tensile modulus E' vs temperature at 1 Hz: comparison between the experimental data for semicrystalline mcl-PHA filled with 0 (\bullet) and 6 wt % (\circ) of tunicin whiskers and predicted data from the Halpin-Kardos model (---) and from the percolation approach (—) for the composites filled with 6 wt % of tunicin whiskers. (\square) and (\blacksquare) refer to experiments performed with a semicrystalline mcl-PHA filled 6 wt % of tunicin whiskers, using a soak time = 600 and 2000 s, respectively.

peaks, directly linked to the drop of the composite elastic tensile modulus, decreases with increasing filler loading.

The variation of the storage tensile modulus of the pure semicrystalline mcl-PHA film (filled circles) added as a reference, as well as of the mcl-PHA film containing 6 wt % of cellulose whiskers (open circles), as a function of temperature is plotted in Figure 4. The observed values of the 6 wt % filled composite have to be compared with those (dashed line) predicted from a classical mean-field mechanical model developed for short-fiber composite. In this mean-field approach, following Halpin and Kardos,²⁹ the modulus, the mechanical anisotropy, and the geometry of the fibers are accounted for, but one assumes that there is no interaction between the fibers. Details of the calculations used for this model are reported elsewhere.³ The characteristics of the fiber have the following values: $L/d = 67$ (estimated from transmission electron microscopy), Poisson's ratio, $\nu_f = 0.3$ (cellulose being a rigid crystal in the whole temperature range), stiffness in the fiber direction, $E_{11f} = 150$ GPa (average value from literature),^{30–35} stiffness perpendicular to the fiber direction, $E_{22f} = 15$ GPa, and in-plane shear modulus, $G_f = 5$ GPa.³⁵

We ascertain that the predicted modulus values agree with the experimental data in the glassy state but fail to describe the rubbery modulus. This is an indication that the glassy modulus of the cellulose whiskers filled PHA is well described from a mean-field approach. However, it is worth to note that the slope of the curve $\log E'$ vs temperature is well predicted by the Halpin-Kardos model. In the rubbery zone, the difference between predicted and experimental data can be explained in light of percolation concepts.

The influence of such an effect on the mechanical properties of the films can be calculated following the method of Ouali et al.³⁶ in their adaptation of the percolation concept to the classical phenomenological series-parallel model of Takayanagi et al.³⁷ Details of the calculations can be found elsewhere.^{1,2,4,5} The main advantage of this approach is to account for interactions between the fibers and for the hydrogen-bonding forces that hold a rigid percolating cellulose whiskers network when the percolation threshold is reached. Here, the threshold fraction to reach percolation of whiskers was determined from a numerical calculation and was 1 vol %, which corresponds to 1.5 wt %.^{1,2} The stiffness of the cellulose microcrystals network, different from the one of an isolated whisker, was experimentally measured from a tensile test performed on a tunicin whiskers sheet and was found equal to 15 GPa.^{1,2} The calculated curve based on the percolation theory is reported in Figure 4 as a solid line. This model was found to precisely fit the experimental modulus data when a synthetic poly(S-co-BuA) latex was used as matrix.^{1,2} On the contrary, calculation underestimated the relaxed modulus of amorphous PHO based composites.⁵ This phenomenon was ascribed to the particle size of the latex.⁵ For the present mcl-PHA latex, the particle size is similar to the one of the amorphous PHO produced by *Pseudomonas oleovorans*. The difference between experimental and predicted data from the percolation model in the rubbery zone (Figure 4) can be ascribed to the same phenomenon. However, in the amorphous matrix based system, the percolation model allowed to account for the stabilization of the modulus over a large temperature range above T_g , which is not the case for the semicrystalline mcl-PHA based system. The slope of the experimental $\log E'$ vs temperature curve (open circle) is higher (in absolute value) than the predicted one (solid line). In addition, the percolation theory shows a slight decrease of the modulus at the matrix melting point (320 K), due to the breakup of crystalline domains, and a stabilization at higher temperature, ascribed to the fact that the stiffness of the material should be due to infinite aggregates of tunicin whiskers in this temperature range.

The high reinforcing effect observed for the 6 wt % filled semicrystalline mcl-PHA composite can be ascribed to a percolation phenomenon. This is confirmed by the resemblance between the relaxed modulus of the amorphous and semicrystalline matrix based composites in Figure 2. However, the stronger decrease of the rubbery experimental modulus of the 6 wt % filled semicrystalline mcl-PHA composite, compared to predicted data from the percolation theory, in Figure 4, as well as the dramatic drop of the mechanical properties at the melting point of the matrix could display a percolation of cellulose whiskers through crystalline domains of PHA. We believe that the formation of the cellulose network is hindered by the matrix crystalliza-

tion during or after the film formation, when a semicrystalline polymer is used as the matrix. This phenomenon can be ascribed to the presence of a transcrystalline region around the whiskers. Cellulose probably acts as a nucleating agent for PHA, producing a transcrystalline region around the cellulose whisker. This results in a disastrous decrease of the mechanical properties of the semicrystalline mcl-PHA composites filled with tunicin whiskers, as soon as the melting point of the matrix is reached. It is ascribed to the breakup of crystalline domains and, at the same time, to the breakup of the cellulose network formed through transcrystalline domains. This phenomenon occurs, whatever the whiskers content, up to 6 wt %, may be.

This phenomenon can probably be affected by the experimental conditions used for dynamic mechanical experiments. One parameter can especially affect the experimental conditions. This parameter is the time allotted to reach thermal stabilization between each thermal step (3 K). This time, termed "soak time" in the RSA2 software, was stated at 30 s in all the previous experiments. Additional experiments were performed with higher soak time values of 600 and 2000 s, which correspond to isothermal aging times of 10 and about 33 min, respectively, between each measurement. Owing to the long time required for these experiments, isochronal curves at 1 Hz were performed between 290 K up to the temperature at which the modulus drops. The former value corresponds to a temperature at which cellulose whiskers are still linked through transcrystalline domains of mcl-PHA. Results for the 6 wt % tunicin whiskers filled composite are reported in Figure 4 as open squares (soak time = 600 s) and filled squares (soak time = 2000 s). We ascertain that a soak time of 600 s allows the formation of a weak linked cellulose whiskers network, because the modulus drops shortly after the melting point. On the contrary, a higher soak time allows the formation of a stronger rigid network. The mechanical properties stabilize over a large temperature range above the melting point of the mcl-PHA matrix. It will be only at about 460 K that this stabilization will disappear, inducing a catastrophic decrease of the modulus. This temperature is lower than the degradation temperature of cellulose (~500 K), but it is worth noting that the times involved in these experiments are very high and will probably bring about an anticipated degradation temperature.

Further experiments were performed in order to promote the thermal whiskers network formation in semicrystalline mcl-PHA composites. One of these was the thermal aging of 6 wt % cellulose composites at 75 °C (348 K) for 72 h. However, isochronal storage modulus curves, using a soak time of 30 s, displayed again a modulus drop at 320 K. We have no satisfactory explanation, at the moment, for this unexpected phenomenon and for the difference between the behavior of the in situ thermally aged and simply oven thermally aged composite. It can possibly be ascribed to the application of the small strain applied for each measurement during dynamic mechanical tests.

Conclusion

A latex of mcl-PHA obtained from *Pseudomonas putida* grown on sodium octanoate was prepared using a recovery process, where cells are ruptured by thermal treatment and the resultant debris was treated with an enzyme cocktail to solubilize all cell components apart

from PHA. Nanocomposite materials were obtained by reinforcing this mcl-PHA latex with cellulose whiskers from tunicin. The mechanical properties are substantially improved by increasing the filler loading, preserving the natural and degradable nature of PHA. Tunicin whiskers, with a high aspect ratio close to 67 and a percolation threshold close to 1 vol %, induce a mechanical percolation phenomenon which leads to high mechanical properties through the formation of a rigid filler network. However, owing to the semicrystalline nature of the matrix, this whiskers network originates from the formation of interwhiskers links through transcrystalline layers grown on cellulose surface. This results in a disastrous decrease of the mechanical properties of the semicrystalline mcl-PHA composite filled with tunicin whiskers, as soon as the melt temperature of the matrix is reached. However, suitable in situ thermal treatment allows to restore the cellulose whiskers network through hydrogen bonds. In addition to some practical applications, this study can help to understand some physical properties as geometrical and mechanical percolation effects.

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