

**Impact of thermal processing or solvent casting upon crystallization of PLA nanocellulose and/or nanoclay composites**

TRIFOL, J, VAN DRONGELEN, M., CLEGG, Francis <<http://orcid.org/0000-0002-9566-5739>>, PLACKETT, D., SZABO, P. and DAUGAARD, A.E.

Available from Sheffield Hallam University Research Archive (SHURA) at:

<http://shura.shu.ac.uk/23747/>

---

This document is the author deposited version. You are advised to consult the publisher's version if you wish to cite from it.

**Published version**

TRIFOL, J, VAN DRONGELEN, M., CLEGG, Francis, PLACKETT, D., SZABO, P. and DAUGAARD, A.E. (2019). Impact of thermal processing or solvent casting upon crystallization of PLA nanocellulose and/or nanoclay composites. *Journal of Applied Polymer Science*, p. 47486.

---

**Copyright and re-use policy**

See <http://shura.shu.ac.uk/information.html>

# Impact of thermal processing or solvent casting upon crystallization of PLA nanocellulose and/or nanoclay composites

J. Trifol<sup>a</sup>, M. van Drongelen<sup>a</sup>, F.Clegg<sup>b</sup>, D. Plackett<sup>c</sup>, P. Szabo<sup>a</sup>, A. E. Daugaard<sup>a</sup>

<sup>a</sup> Danish Polymer Centre, Department of Chemical and Biochemical Engineering, Søtofts Plads, Technical University of Denmark, Building 229, DK – 2800, Kgs. Lyngby, Denmark

<sup>b</sup> Materials and Engineering Research Institute, Sheffield Hallam University, Howard Street, Sheffield, S1 1WB, U.K.

<sup>c</sup> Faculty of Pharmaceutical Sciences, University of British Columbia, 2405 Westbrook Mall, Vancouver, BC V6T 1Z3, Canada

## Abstract:

Here we present how processing (solvent casting or isothermal crystallization) impacts crystallinity of poly(lactic acid) (PLA) and its nanocomposites (PLA/1wt% cellulose nanofibers (CNF), PLA/1wt% nanoclay (C30B) or PLA/1wt% CNF/1wt% C30B). Polarized optical microscopy demonstrated a heterogeneous nucleation process during isothermal crystallization leading to smaller homogeneously distributed spherulites. With solvent casting, no effect on morphology was observed with respect to the nanoparticles, but an increased spherulite size was observed at higher temperatures. This fact raises significant concerns regarding the suitability of solvent casting as a lab-scale procedure to investigate materials. Additionally, combining the reinforcing agents, CNF and C30B, did not increase nucleation rate, in contrast with the general tendency, where the incorporation of both particles led to improved properties (e.g., thermomechanical and barrier properties). However, a combination of C30B and CNF did lead to an overall increase in the rigid amorphous fraction (RAF) and a reduced mobile amorphous fraction (MAF).

## 1. INTRODUCTION

The extensive use of non-renewable resources is a well-known and serious global issue. Petroleum reserves are continually being depleted and since most plastics made from these reserves are non-biodegradable, their disposal is a problem. In this context, substituting petroleum-based plastics with bio-based and biodegradable alternatives is an attractive proposition for sustainable development. Nevertheless, bio-based polymers such as poly (lactic acid) (PLA) still suffer from practical drawbacks such as brittleness, poor thermomechanical properties, and slow crystallization leading to longer processing times and modest gas barrier properties<sup>1,2</sup> when compared to currently used petrochemical-derived plastics. Consequently, considerable efforts have been focused towards improving the properties of bio-based polymers in general, and PLA in particular. These include the reinforcement of PLA with different types of nanoparticles such as nanocellulose<sup>3</sup>, nanoclay<sup>4</sup>, or a combination of both<sup>5,6</sup>.

Briefly, PLA is a biodegradable, thermoplastic aliphatic polyester obtained from the ring-opening polymerization of lactide<sup>7</sup>, which is produced from the dehydration of lactic acid obtained from different

40 renewable resources. Nanoclays are composed of several layers of inorganic platelets stacked on top of each  
41 other, with platelet diameters in the range of 100 to 1000 nm and thickness <1 nm. In this study, commercially  
42 available, organically modified clay (Cloisite<sup>®</sup> 30B abbreviated to C30B) was used. In this clay the platelets are  
43 surface modified with hydrophobic bis (2-hydroxy-ethyl)-methyl tallow alkyl ammonium cations<sup>8</sup>. Cellulose  
44 nanofibers (CNF) have lengths close to or in the microscale and diameters in the nanoscale. CNF typically  
45 exhibit hydroxyl (or carboxylate<sup>9</sup>) groups on the nanofiber surfaces, but the CNF used in this work was partially  
46 acetylated during the CNF extraction procedure, presenting a small amount of acetyl groups on the surface  
47 (degree of substitution or DS ~ 10%) and slightly decreasing the hydrophilic character of the CNF<sup>10</sup>.

48

49 In our previous studies<sup>2</sup>, it was found that the incorporation of 1% CNF in solvent-cast PLA led to a reduction of  
50 63% in Oxygen Transmission Rate (OTR), whereas with the equivalent composite containing 1% C30B only a  
51 26% decrease was observed. Nanoclay has a higher surface-to-volume ratio than CNF, where a fully exfoliated  
52 platelet of dimensions 400 x 300 x 1 nm<sup>11</sup> has an approximate surface to volume ratio of  $2 \times 10^9 \text{ m}^{-1}$ , while fibers  
53 of 25 nm diameter and 650 nm length exhibit only a surface to volume ratio of  $1.6 \times 10^8 \text{ m}^{-1}$ . Platelet-shaped  
54 morphologies therefore have 12.5 times higher surface-to-volume ratio, and would therefore be expected to  
55 improve the barrier properties more efficiently than the CNF. However, the CNF-based composites were  
56 showing better barrier properties than C30B. Considering this factor, it would be reasonable to suggest that part  
57 of the improvement in barrier properties for CNF composites, could be due to crystallinity effects originating  
58 from the fibers that is not happening on the C30B. Crystallinity is well known to have a positive impact on  
59 properties of materials such as gas barrier<sup>12,13</sup> or mechanical properties<sup>14-18</sup>. The crystallization properties of  
60 PLA<sup>14,19,20</sup> and PLA-based nanocomposites, such as PLA/CNF<sup>18</sup>, PLA/C30B<sup>13</sup>, and even PLA-based  
61 composites with banana fibers and clay<sup>21</sup> have been the subject of earlier studies. Differential scanning  
62 calorimetry (DSC) has revealed that both CNF- and C30B-containing PLA composites show a similar degree of  
63 crystallinity, while still exhibiting significantly different barrier properties. Therefore, this study was conducted  
64 to investigate the differences in crystallinity and morphology induced by the different types of reinforcing  
65 agents.

66

67 Crystallinity is a broad topic that, especially in composite literature, has typically been described using only the  
68 degree of crystallinity, albeit other crystallinity-related parameters, such as polymorphism and the rigid  
69 amorphous fraction (RAF) could affect polymer properties. Crystalline PLA exhibits multiple polymorphic  
70 phases, namely  $\alpha$ ,  $\beta$  and  $\gamma$ , (and  $\eta$  stereocomplex<sup>22</sup>) and it has been found that the prevailing  $\alpha$  form exists as  
71 two different polymorphs ( $\alpha$  = ordered and  $\alpha'$  = disordered) which have different chain packing<sup>23</sup>. In addition,  
72 it has been widely accepted that the amorphous region of semi-crystalline polymers is itself composed of two  
73 fractions—the mobile amorphous fraction (MAF), which shows chain mobility, and the rigid amorphous  
74 fraction (RAF), an intermediate confined nanophase<sup>24</sup>. Classically, it has been considered that the RAF is  
75 present at the interface between the crystals and the surrounding amorphous phase; however, nanoparticles can  
76 also induce a confined nanophase wherein conformational rearrangements may occur, the so-called “cooperative  
77 rearrangement region” (CRR)<sup>25</sup>, for this reason some authors distinguish the RAF generated by crystallinity  
78 ( $\text{RAF}_{\text{CRYST}}$ ) from the RAF created by nanoparticles ( $\text{RAF}_{\text{NANO}}$ ). Despite the complexity of crystallinity,  
79 understanding the critical parameters that affect materials properties can prove helpful towards achieving an

80 optimal material performance.

81

82 One of the major potential applications of bio-based polymers is in bio-based packaging materials. There are  
83 three critical parameters for such applications to be realized that can be affected by the crystallinity of the  
84 materials. The first parameter, mass transport/barrier properties, controls the quality of the contained food and  
85 the impact of crystalline morphology on water vapor transport is currently under investigation<sup>26</sup>. The second  
86 parameter, transparency, is evaluated in the present work and is highly relevant in films for food packaging  
87 applications<sup>27</sup> since customers may want to clearly see the food before purchasing. Generally, an increased  
88 crystallinity can improve certain properties, but it can also decrease optical transparency<sup>2</sup> as a result of  
89 scattering of light due to the different densities between crystalline and amorphous domains. The third  
90 parameter, thermal transitions (i.e.  $T_g$  and  $T_m$ ), is also evaluated herein and can be influenced by crystallinity,  
91 which significantly affects material processing.

92

93 Here we investigate the differences in crystallinity induced by nanocellulose and nanoclay as well as the  
94 influence of combining both types of particles, while providing an insight into differences in crystallinity  
95 induced by solvent casting and isothermal crystallization. Solvent casting is widely used to investigate the  
96 impact of nanoparticles on polymer properties. However, traditionally, except for some recent techniques such  
97 as electrospinning<sup>28</sup>, or the use of 3D printed sacrificial molds<sup>29</sup>, industrially relevant techniques usually relies  
98 on higher processing temperatures. Depending on processing and crystallization behavior, different  
99 nanoparticles can have a different impact on crystallization, and solvent casting might therefore not accurately  
100 predict the performance of nanocomposites processed in more conventional processes at high temperature.

101

## 102 **2. EXPERIMENTAL**

103

### 104 **2.1 Materials and methods**

105 L-PLA (Ingeo 2003D) was supplied by Natureworks (Minnesota, USA). The clay used for this study was  
106 Cloisite<sup>®</sup> C30B and was supplied by Southern Clay Products (Texas, USA). C30B is a commercially available  
107 clay comprehensively described in the literature<sup>30-32</sup>. The extraction of nanocellulose CNF<sup>10</sup> and the  
108 preparation of nanocomposites have been described in more detail elsewhere<sup>2,5</sup> though a short summary is  
109 presented below.

110

### 111 **2.2 Nanocomposite preparation**

#### 112 **2.2.1 CNF extraction<sup>10</sup>**

113 The CNF extraction procedure as well as its characterization has been already published<sup>10</sup>. Briefly, 50 g of sisal  
114 fibers were cleaned overnight with 1.5 L of aqueous NaOH solution (2 wt%) at room temperature, and after  
115 filtration the fibers were alkali-treated three times with 1.5 L of aqueous NaOH solution (10 wt%) at boiling  
116 point over 1.5 h. The mercerized fibers were bleached with 1.25 mL of water at 70°C, 8 mL of acetic acid and  
117 40 mL of NaClO<sub>2</sub> added every hour for 7 hours. Subsequently, the fibers were acetylated using 900 mL of a  
118 solution of HNO<sub>3</sub>/acetic acid (1:6 v/v) at boiling point for 90 minutes. After that, the pulp was solvent  
119 exchanged in dimethylformamide (DMF). A 1 wt% solution of acetylated pulp in DMF was then dispersed for

120 72 hours with vigorous magnetic stirring followed by centrifugation at 2500 rpm for 10 mins to remove any  
121 remaining aggregates, the supernatant was used to prepare the nanocomposites.

122  
123

## 124 2.2.2 Nanocomposite preparation via solvent casting

125 Briefly, PLA and nanoparticles were dissolved/dispersed separately in a solvent, and subsequently the solution  
126 of PLA and the suspension of nanoparticles were mixed and cast in a Teflon mold, and the solvent was removed  
127 by evaporation. Due to the different nature of the particles, an optimized protocol was used for each  
128 combination.

129

### 130 2.2.2.1 PLA and PLA/C30B nanocomposites

131 Neat PLA and PLA/C30B (1wt% of clay) nanocomposites were prepared using dichloromethane (DCM) as a  
132 solvent. In total, 10 g of PLA was dissolved in 200 mL of DCM, and the mixture was kept under magnetic  
133 stirring in a sealed flask overnight. Separately, 3 g of C30B was mixed with 300 mL of DCM and kept under  
134 magnetic stirring for 24 hours. Subsequently, the clay suspension was ultrasonicated for 3 hours at 200W and  
135 then homogenized for 90 minutes with an Ultra-Turrax homogenizer (Jonke & Kunnel IKA Ultra-Turrax T25)  
136 at 20500 rpm. The PLA solution and nanoclay suspension were mixed to obtain the desired concentration of  
137 C30B in PLA and a final volume of 250 mL obtained by dilution with DCM. Thereafter the mixture was  
138 magnetically stirred, ultrasonicated for 90 minutes, homogenized for 30 minutes and finally degassed by  
139 ultrasonication for 5 minutes. Next, 80 mL of the suspension was poured slowly into a Teflon mold covered by a  
140 5-13  $\mu\text{m}$  filter paper into a Climacell climatic chamber, which was kept at 23°C for 16 h. Finally, the films were  
141 removed from the Teflon mold and further dried at 50°C under vacuum for at least 24 hours to remove the  
142 remaining traces of DCM.

143

### 144 2.2.2.2 PLA/CNF and PLA/CNF/C30B nanocomposites

145 The PLA/CNF and PLA/CNF/C30B nanocomposites (1wt% of each nanoparticle) were prepared using  
146 dimethylformamide (DMF) as a solvent. A total of 3.3 g of PLA was dissolved in 66 mL of DMF, whilst kept  
147 for 2 h at 70°C under vigorous magnetic stirring. Separately, 2 g of C30B was mixed with 200 mL of DMF and  
148 the mixture was kept under strong magnetic stirring for 24 h at room temperature. Next, the nanoclay suspension  
149 was ultrasonicated for 3 hours at 200W and thereafter homogenized for 90 min at 20500 rpm using an Ultra-  
150 Turrax homogenizer. Finally, the required amounts of the PLA, CNF, and C30B solutions or suspensions were  
151 mixed by magnetic stirring and poured slowly into a Teflon mold, where they were dried for 15 h at 80°C and  
152 subsequently for 24 h at 50°C under vacuum.

153

### 154 2.2.2.3 Thermal treatments

155 Solvent-cast materials were treated to obtain amorphous and isothermally crystallized products. In order to  
156 obtain fully amorphous specimens for UV-Vis spectroscopy and room temperature X-ray diffraction (XRD)  
157 measurements, solvent-cast products were placed between two aluminum foils and then subsequently hot  
158 pressed for 5 mins at 170°C, followed by fast cooling with water (at 10°C) (quenching). Thereafter, the fully

159 amorphous materials were isothermally crystallized for two hours at the corresponding crystallization  
160 temperature in an oven to achieve crystallized materials.

161

162 For other measurements including dynamic differential scanning calorimetry (DSC), temperature-modulated  
163 differential scanning calorimetry (MDSC), polarized optical microscopy (POM) and X-ray diffraction (XRD),  
164 the formation of fully amorphous materials and their isothermal crystallization at specified temperatures was  
165 performed in the instruments themselves as described in the corresponding sections.

## 166 **2.3 Characterization**

167

### 168 2.3.1 Polarized Optical Microscopy

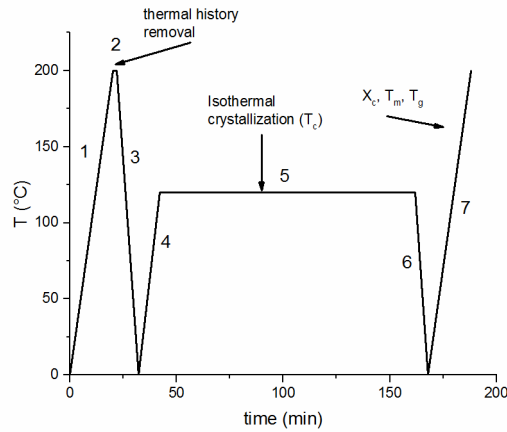
169 Isothermal crystallization of the PLA and its nanocomposites was evaluated by polarized optical microscopy  
170 (POM) using a Nikon Eclipse E100 microscope with a Mettler Toledo FP82HT hot-stage at 140°C, 120°C, and  
171 100°C. Fully amorphous samples were obtained from solvent-cast materials by inserting them between two  
172 microscope slides and placing them on the microscope hot stage, which was preheated to 200°C. After 2  
173 minutes the samples were removed and cooled as quickly as possible with a tissue impregnated with ethanol.  
174 The sample was replaced on the hot stage after it reached the desired isothermal crystallization temperature and  
175 photographs were taken at regular intervals.

176

### 177 2.3.2 DSC

178 Isothermal crystallization kinetics were evaluated using a TA Instruments DSC Q1000 and the protocol  
179 illustrated in Figure 1. First, any crystallinity in the solvent cast materials dictated by the thermal history of the  
180 nanocomposites was erased by heating the samples at 10°C/min up to 200°C (1), maintaining the sample for two  
181 mins at 200°C (2), and then cooling at 20°C/min to 0°C (3). Thereafter, the samples were raised to the desired  
182 isothermal crystallization temperature (140°C, 120°C, 100°C or 80°C) at 10°C/min (4), and kept for 2 hours at  
183 that temperature (5). Finally, the samples were cooled to 0°C at 20°C/min (6) and then heated to 200°C at  
184 10°C/min (7). The glass transition,  $T_g$ , and melting temperature,  $T_m$  (from the maximum of the melting peak),  
185 were determined during this final heating run. No crystallization peaks were observed during any of the cooling  
186 cycles indicating that no significant crystallization occurred during this part of the treatment.

187



188

189 **Figure 1** Schematic of the procedure used for the  $X_c$  (degree of crystallinity),  $T_m$  (melting temperature),  $T_g$  (glass transition)  
 190 determination as well as for the evaluation of the crystallization kinetics.

191

192 The areas under the crystallization peaks obtained during the isothermal crystallization cycles (5) were  
 193 integrated using TA Instruments Universal Analysis Software. The baseline was extrapolated from the heat flow  
 194 signal after crystallization of the material was complete. The resulting area was integrated and the half  
 195 crystallization time was considered as the time when 50% of maximum crystallinity was reached.

196

197 The heat capacity variation ( $\Delta C_p$ ) values of PLA and its nanocomposites when in a fully amorphous state at  
 198 their corresponding  $T_g$  was determined in triplicate using a TA Instruments Discovery DSC. The samples were  
 199 submitted to a thermal treatment similar to stages (1-4) described in Figure 1, but cycle 4 heating was done until  
 200 200°C. This was made to erase all of the thermal story of the samples and therefore measure the heat capacity  
 201 variation ( $\Delta C_p$ ) when fully amorphous.

202

### 203 2.3.3 MDSC

204 The advantage of utilizing MDSC over DSC is that heat flow due to crystallization (termed reversible enthalpy)  
 205 can be separated from that due to melting (termed non-reversible enthalpy), enabling a more accurate  
 206 determination of the degree of crystallinity. MDSC was performed using a TA Instruments DSC Q1000 and a  
 207 cycle similar to the protocol described in Figure 1, albeit with a modulation cycle (Amplitude =  $\pm 0.50^\circ\text{C}$ , period  
 208 = 30 s) during the final heating stage (stage 7).

209

210 To determine the degree of crystallinity ( $X_c$ ), Eq 1 was used:

211

$$X_c = \frac{\Delta H_{\text{nonrev}} - \Delta H_{\text{rev}}}{\Delta H_0} \quad (\text{Eq 1})$$

212

213 where  $X_c$  is the degree of crystallinity of the composite,  $\Delta H_{\text{nonrev}}$  is the non-reversible enthalpy, and  $\Delta H_{\text{rev}}$  is the  
 214 reversible enthalpy. A melting enthalpy,  $\Delta H_0$ , of  $106 \text{ J g}^{-1}$  for 100% crystalline  $\alpha$ -poly (L-lactide) was used as  
 215 reported <sup>33</sup>, while an enthalpy  $25 \text{ J g}^{-1}$  lower ( $81 \text{ J g}^{-1}$ ) was considered for the  $\alpha'$  phase, as suggested in the  
 216 literature <sup>34</sup>. Again, no crystallization peaks were observed during the cooling cycles.

217 The mobile amorphous fraction (MAF) and rigid amorphous fraction (RAF) in the nanocomposites were  
 218 determined by adapting an existing protocol for PLA<sup>35</sup>. Instead of only comparing the heat capacity of a  
 219 specimen to the heat capacity of neat amorphous PLA (therefore different specimens), accuracy was improved  
 220 by using the fully amorphous specimen (PLA or its nanocomposites) as reference to evaluate the relative impact  
 221 of each crystalline morphology in the same treated material. Thereafter, these values were normalized using the  
 222 heat capacity of PLA and nanocomposites. The reason for this was that PLA and its nanocomposites when in  
 223 their fully amorphous phase showed different heat capacities due to the presence of nanoparticles affecting the  
 224 amount of RAF.

225 The MAF was therefore determined by means of Eq 2.

226

$$\% \text{ MAF} = \frac{\Delta C_p \Delta C_{p_{\text{MAT}}}}{\Delta C_{p'} \Delta C_{p_{\text{PLA}}}} * 100 \text{ (Eq 2)}$$

227 where  $\Delta C_{p_{\text{PLA}}}$  is the heat capacity variation of fully amorphous PLA, while  $\Delta C_{p_{\text{MAT}}}$  is the heat capacity  
 228 variation of the fully amorphous tested material (which could be PLA or any of its nanocomposites). These  
 229 values were obtained as described above in Section 2.3.2 by DSC.  $\Delta C_{p'}$  is the specific heat change of the  
 230 specimen (PLA or any of its composites) in a completely amorphous state at its particular  $T_g$ , while  $\Delta C_p$  is the  
 231 specific heat change of the same specimen under test conditions (solvent-cast or isothermal crystallization) at its  
 232 corresponding  $T_g$ . In order to acquire an accurate value for  $C_p$ , a representative point and a representative slope  
 233 were considered before and after the  $T_g$ . From these values two equations were obtained, one that extrapolates  
 234 the value of  $C_p$  before the glass transition and the other, which extrapolates the value after the glass transition.  
 235 The  $\Delta C_p$  was calculated as the difference between both values (using the corresponding  $T_g$  for each crystalline  
 236 morphology).

237 The RAF was calculated by means of Eq 3:

$$\% \text{ RAF} = 100 - \% \text{ MAF} - \% X_c - X_{\text{nano}} \text{ (Eq 3)}$$

238 where RAF is the rigid amorphous fraction, MAF the mobile amorphous fraction,  $X_c$  the degree of crystallinity,  
 239 and  $X_{\text{nano}}$ , nanoparticle content (wt%).

240 Finally, the RAF was separated between the  $\text{RAF}_{\text{NANO}}$  (induced by nanoparticles) and  $\text{RAF}_{\text{CRYST}}$  (induced by  
 241 crystallinity, as has also been done for PLA/nanoclay nanocomposites<sup>36</sup>. First, the  $\text{RAF}_{\text{NANO}}$  was calculated by  
 242 means of Eq 4 (the heat capacity variation among fully amorphous PLA and fully amorphous composites is due  
 243 to RAF and nanoparticles) and afterwards the value of  $\text{RAF}_{\text{CRYST}}$  was calculated by means of Eq 5.

$$\% \text{ RAF}_{\text{NANO}} = \left( 1 - \frac{\Delta C_{p_{\text{MAT}}}}{\Delta C_{p_{\text{PLA}}}} - X_{\text{nano}} \right) * 100 \text{ (Eq 4)}$$

244

$$\% \text{ RAF} = \% \text{ RAF}_{\text{NANO}} + \% \text{ RAF}_{\text{CRYST}} \text{ (Eq 5)}$$

245

246 In Eq 5,  $\Delta C_{p_{\text{PLA}}}$  is the heat capacity variation of fully amorphous PLA, while  $\Delta C_{p_{\text{MAT}}}$  is the heat capacity  
 247 variation of the fully amorphous tested material.

248 Data from isothermal crystallization experiments within the range 10-70% relative crystallinity were fitted to the  
 249 Avrami kinetic model<sup>37</sup> (Eq 6):

250

$$\ln(-\ln(1-X_c)) = \ln k + n \times \ln t \text{ (Eq 6)}$$



251

252 In Eq 6,  $X_c$  is the relative degree of crystallinity,  $k$  is the overall kinetic constant,  $n$  is the Avrami index, and  $t$  is  
253 crystallization time. The start time ( $t_0$ ) was considered the time at which crystallization was noticeable (therefore  
254 right after  $t_{\text{delay}}$ ). The Avrami indices are based on two factors (Eq 7), namely the growth directions of the  
255 spherulites ( $n_D$ ), which is a value ranging from 1-3 that is dependent on the growth directions of the spherulites,  
256 and the time-dependent crystallization ( $n_N$ ), which has a value between 0 for instantaneous nucleation and 1 for  
257 sporadic nucleation<sup>37</sup>.

$$258 \quad n = n_D + n_N \text{ (Eq 7)}$$

259

#### 260 2.3.4 XRD

261 Isothermal crystallization was also measured by XRD using a Philips X'Pert Pro diffraction system fitted with  
262 an Anton Paar HTK 1200N oven chamber and utilizing a Cu-tube ( $\lambda = 1.542 \text{ \AA}$ ) operating at 40 kV and 40 mA.  
263 A heating/cooling profile similar to that described in Figure 1 for DSC was used; however, due to cooling  
264 restrictions, the samples were only cooled to 50°C instead of 0°C. During the isothermal crystallization stage,  
265 diffraction patterns were acquired with a scan range of 10-30 °2 $\theta$ , step size of 0.067° and acquisition time of 1  
266 min. After completion of the isothermal crystallization procedure the samples were cooled to 50°C, at which  
267 point an XRD pattern was acquired over the same scan range, but with a smaller step size of 0.017 and longer  
268 acquisition time of 10 min to enhance the signal/noise ratio.

269

270 The solvent-cast materials, the amorphous materials (after hot pressing/quenching) and isothermally crystallized  
271 materials (hot pressed and crystallized in an oven) were analyzed by the same XRD instrument at room  
272 temperature, but without the oven chamber and using scan range of 10-30 °2 $\theta$ , step size 0.04° and 4 s/step.

273

#### 274 2.3.5 Optical properties by UV-Vis spectroscopy

275 Optical properties were measured from at least three different points within the same sample under investigation  
276 using a UV-Vis spectrometer (Polar Star Omega) in the range of 200-1000 nm. An average of the measurements  
277 is presented.

278

### 279 3. RESULTS AND DISCUSSION

280

#### 281 3.1 Discussion on material preparation

282 One of the key parameters for the successful improvement of properties in nanocomposites is to achieve good  
283 nanoparticle dispersion. In the present work, PLA was reinforced with two different nanoparticles of different  
284 nature (one hydrophilic, CNF, and one hydrophobic, C30B) and thus each behaves differently in the same  
285 solvent. It was not possible to find a common solvent/procedure, which led to both particles being well-  
286 dispersed and possessing suitable film properties. Therefore, to ensure a good dispersion of nanoparticles in the  
287 composites and thus allow a fair comparison, the best solvent and processing procedure (the ones that they were  
288 leasing to better nanoparticle dispersion) for each nanoparticle was used in each case. For C30B it was found  
289 that use of DCM as solvent followed by drying at room temperature led to well- dispersed composites with good  
290 film quality, whereas the same procedure applied to CNF led to poorly dispersed composites. After evaluation  
291 of different solvents, it was found that DMF was the most suitable solvent for this particular CNF, and that 80°C

292 was the best drying temperature. As summarized in Table 1, all composites were prepared with 1 wt% of  
293 nanoparticles, in order to strike a balance between a good dispersion and a significant increase in material  
294 performance, while minimizing the chance of clay platelet/nanofiber aggregates being present.

295 **Table 1 Composition of the samples; PLA and nanocomposites in wt%.**

	PLA	CNF	C30B
PLA	100%	-	-
PLA/C30B	99%	-	1%
PLA/CNF	99%	1%	-
PLA/CNF/C30B	98%	1%	1%

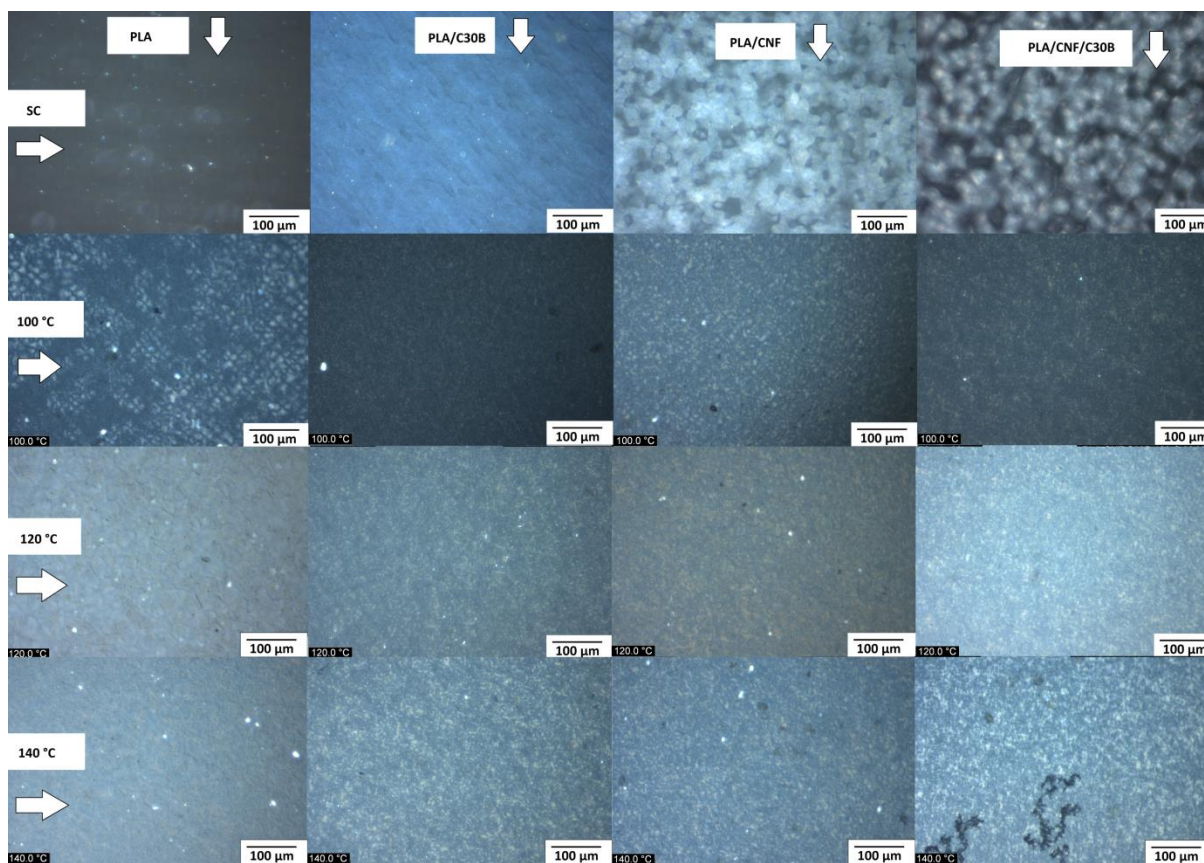
296 The CNF shows a diameter of 27 +/-13 nm and a length of 658 +/- 290 nm and is well dispersed in the  
297 nanocomposites as reported elsewhere.<sup>10,2</sup> The montmorionite-based C30B in the PLA/C30B and hybrid  
298 composites is also well dispersed, near to full-exfoliation<sup>5</sup> and is therefore approximated to have individual  
299 layers of 400 x 300 x 1 nm<sup>11</sup>.

300

### 301 3.2 Spherulite morphology and distribution by POM

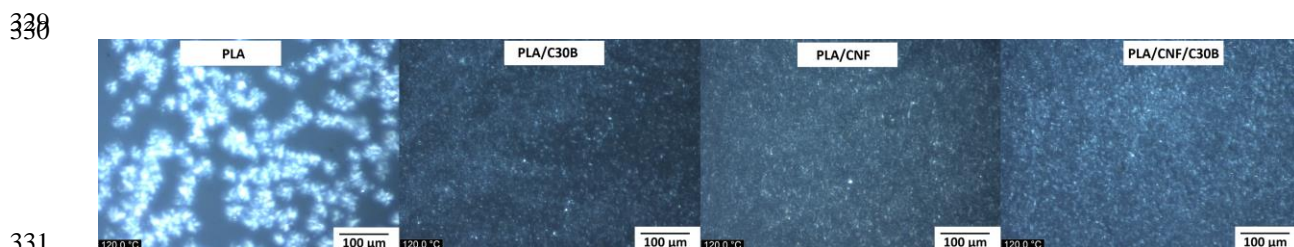
302 The spherulite morphology and distribution in PLA and PLA nanocomposites prepared by solvent casting,  
303 followed by complete isothermal crystallization at 100°C, 120°C and 140°C, were evaluated by POM (see  
304 **Error! Reference source not found.**). First, the differences between solvent casting and isothermal  
305 crystallization were investigated. Solvent-cast samples (Figure 2, first row) of PLA/CNF and PLA/CNF/C30B  
306 show micron-sized (around 40 μm) spherulites, while any spherulites present in PLA and PLA/C30B are too  
307 small to be observed by POM, though DSC shows a crystallinity of 7 and 32% respectively in these samples.  
308 Furthermore, the presence of C30B does not influence the spherulite nature when combined with CNF. Though  
309 crystallization has occurred in the CNF-containing samples (34 and 35%, respectively, according to DSC) the  
310 spherulites do not cover the whole area.

311 It is clear that spherulite size is not only a result of the type of reinforcing agent, but is also highly dependent on  
312 the processing conditions, illustrating that processing temperature is a key factor for spherulite size and  
313 distribution.



314 **Figure 2** POM of PLA and nanocomposites (1wt% per nanoparticle type) with different crystalline morphologies. 1<sup>st</sup> row)  
 315 Solvent cast, 2<sup>nd</sup> row) crystallized at 100°C, 3<sup>rd</sup> row) crystallized at 120°C) and 4<sup>th</sup> row) crystallized at 140°C.  
 316

317 When comparing the fully crystallized morphologies between the isothermally crystallized nanocomposites at  
 318 different crystallization temperatures (Figure 2, rows 2-4 and columns 3-4) no clear differences are observed,  
 319 but there is a large difference when comparing these with the respective PLA samples (Figure 2, rows 2-4,  
 320 column 1, inclusively). The nanocomposites, at all crystallization temperatures, show a greater amount of much  
 321 smaller and more evenly distributed spherulites resulting from nucleation caused by the CNF and C30B that are  
 322 homogeneously distributed, whereas the PLA contains larger spherulite sizes (up to ~65 μm) that are  
 323 heterogeneously distributed. Evaluating the crystalline morphology of the fully crystallized samples is  
 324 complicated due to the high amount and close packing of spherulites. But POM photographs of PLA and its  
 325 nanocomposites taken at an earlier stage of the isothermal crystallization (approximately 20-30% of the total  
 326 crystallization at 120°C, Figure 3) allows some differentiation. Here, it can again be seen that PLA contains  
 327 much larger spherulites, whereas all of the nanocomposites contain substantially smaller homogeneously  
 328 distributed spherulites because of the nucleating properties of the nanoparticles.



331 **Figure 3** POM of PLA and its nanocomposites (1 wt% per nanoparticle type) during crystallization at 120°C.  
 332

### 333 3.3 XRD studies

334 In order to elucidate which crystalline phase is generated by different crystallization procedures, and whether  
335 the nanoparticles could induce the formation of one phase over another, XRD patterns were collected. There are  
336 two thermally induced phases,  $\alpha$  (ordered) and  $\alpha'$  (disordered) and it has already been reported that lower  
337 processing temperatures tend to favor the creation of the disordered phase<sup>23</sup>. To aid investigation and for  
338 comparison, XRD patterns were also collected at 80°C, since this was likely to be a relevant temperature to  
339 observe the formation of the  $\alpha'$  phase. The  $\alpha$  and  $\alpha'$  phases can be distinguished, since the latter does not  
340 exhibit any characteristic peaks, such as those corresponding to reflection plane 210, which is located at  $2\theta \sim$   
341  $22.5^\circ$ , while the peaks that are present in both phases, such as 200/110 and 203/113, are shifted to lower  $2\theta$   
342 angles in the  $\alpha'$  phase<sup>23,34</sup>.

343

344

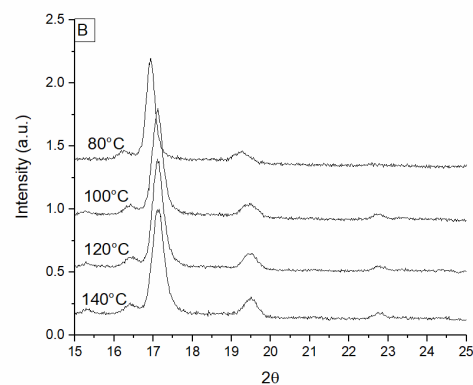
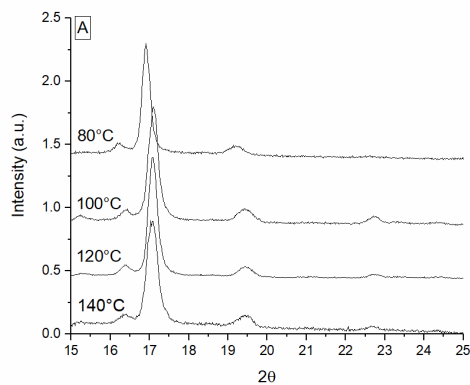
345

346

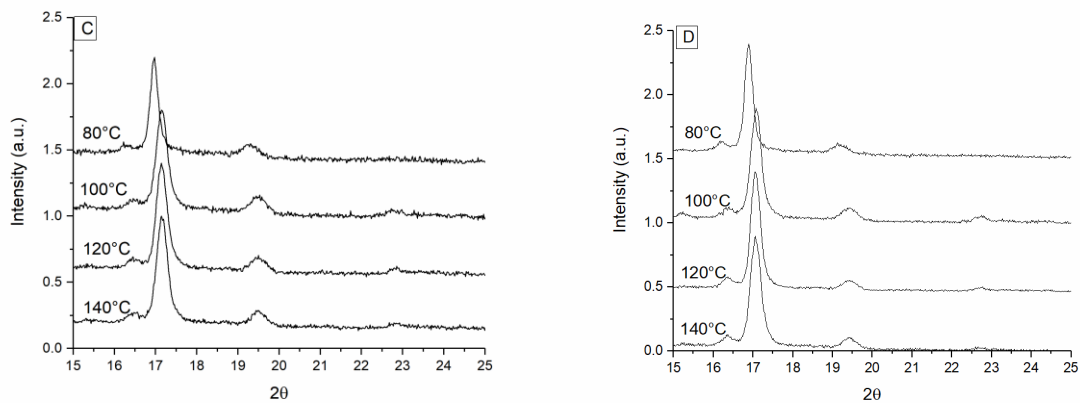
347

348

349



350



351

352

353

354

**Figure 4** XRD patterns of PLA and nanocomposites crystallized at different temperatures. A) PLA, B) PLA/C30B, C) PLA/CNF, D) PLA/CNF/C30B. All of the composites have a 1wt% of each nanoparticle type.

355

356

357

358

359

360

361

362

363

364

365

366

Figure 4 presents the XRD patterns collected from isothermally crystallized PLA and its nanocomposites. In the left column are the two main crystallinity peaks of PLA at  $2\theta \sim 17^\circ$ , which corresponds to the reflection planes 200/110, and  $2\theta \sim 19.5^\circ$ , which corresponds to plane 203/113. While no significant variations in the relative positions of these peaks were observed in the solvent-cast nanocomposites due to the addition of different nanoparticles (Figure 4B-C), it was found that the crystallization temperature was clearly affecting peak position. There is a shift to lower  $2\theta$  ( $\sim 0.2^\circ$ ) when comparing samples crystallized at  $>100^\circ\text{C}$  with samples crystallized at  $80^\circ\text{C}$ , this shift, as mentioned above, has been associated to  $\alpha'$  phase. It can be also seen that the peak at  $2\theta \sim 22.5^\circ$ , corresponding to reflection plane 201, and which is associated with the  $\alpha$  phase, is present in the PLA and nanocomposites crystallized at  $140^\circ\text{C}$ ,  $120^\circ\text{C}$ , and  $100^\circ\text{C}$ , while it is completely absent in the PLA and nanocomposites crystallized at  $80^\circ\text{C}$ . This supports the conclusion that PLA and nanocomposites crystallized at  $80^\circ\text{C}$  contain predominantly the  $\alpha'$  phase, while nanocomposites crystallized at higher temperatures predominantly contain the  $\alpha$  phase.

367

368

369

370

371

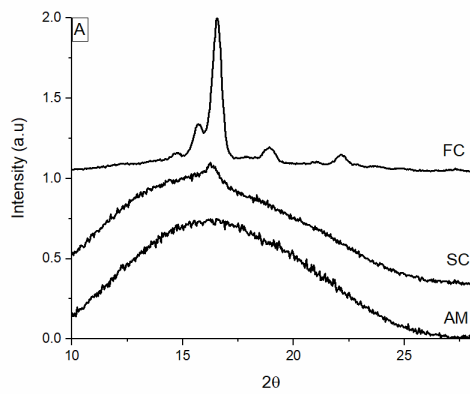
372

373

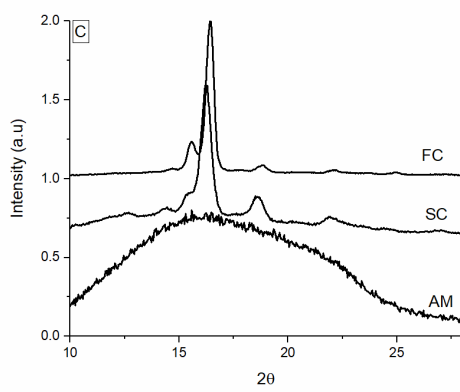
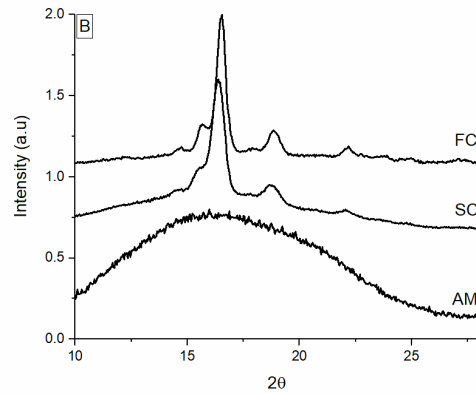
374

375

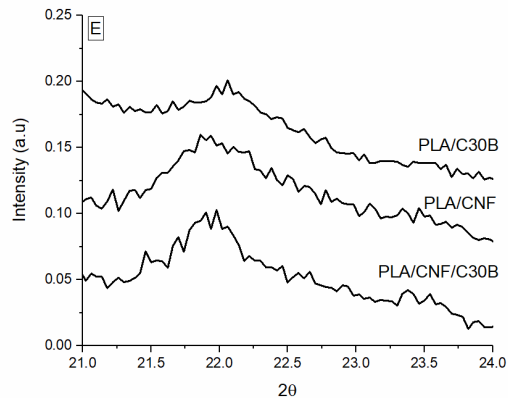
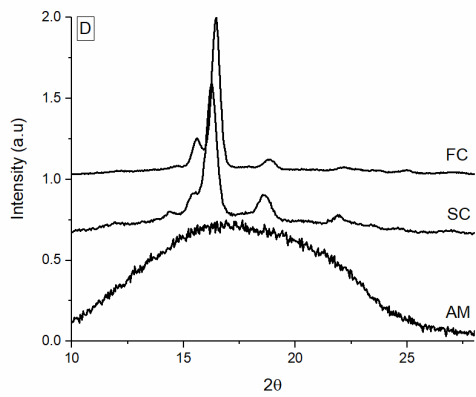
In order to evaluate whether the solvent casting procedure also induces the  $\alpha'$  phase, the XRD patterns (Figure 5) of the solvent-cast (SC) PLA and nanocomposites were compared with those of the respective fully amorphous (AM - obtained from hot pressing at  $170^\circ\text{C}$  followed by fast quenching) and fully crystallized (FC ( $120^\circ\text{C}$ )) - fully amorphous composites that were thereafter crystallized for 120 mins at  $120^\circ\text{C}$  in an oven) samples. Note that the XRD patterns in Figures 4 and 5 are not directly comparable because the former were collected during a crystallization monitoring experiment at  $50^\circ\text{C}$  (due to cooling restrictions) while the patterns in Figure 5 were collected at room temperature. Although the intensities may differ, the peak positions remain comparable.



376



377



378

379 **Figure 5** XRD patterns of solvent cast, amorphous and fully crystallized materials. From A) to D) same material under  
 380 different crystalline morphologies; A) PLA, B) PLA/C30B, C) PLA/CNF, D) PLA/CNF/C30B. E) Zoom-in of all of the  
 381 solvent cast materials in the range of  $2\theta = 21-24$ . All of the composites have a 1wt% of each nanoparticle type.

382 It is noticed that there is also the same shift to lower  $2\theta$  for all of the two main crystallinity peaks for the  
 383 solvent-cast samples when compared with those crystallized at  $120^\circ\text{C}$  which contained mainly the  $\alpha$  phase;  
 384 therefore, it can also be concluded that the solvent-cast samples contained mainly the  $\alpha'$  phase. However, in  
 385 Figure 5E it can be seen that all of the diffraction traces from the solvent-cast nanocomposite samples,  
 386 especially the PLA/CNF and PLA/CNF/C30B, also showed a small peak at  $2\theta \sim 22^\circ$ , which is ascribed to the  $\alpha$

387 phase. In fact, other researchers<sup>38</sup> have discussed the formation of the  $\alpha$ -phase in solvent-cast samples even at  
 388 low temperature, due to an increase on chain mobility arising from the solvent. However, in the present work  
 389 there is apparently a coexistence of both phases. The  $\alpha$ -phase is attributed to the solvent casting itself, while the  
 390  $\alpha'$ -phase is attributed to the drying of the materials after solvent casting, at temperatures below the  $\alpha$ -phase  
 391 formation temperature ( $< 80^\circ\text{C}$ ).

392 It is reassuring to note that no crystalline peaks were found for the fully amorphous (AM) quenched composites,  
 393 but only a broad characteristic amorphous halo, thereby proving that the procedure used to prepare hot-pressed  
 394 amorphous samples (hot pressing at  $170^\circ\text{C}$  for 5 minutes) followed by fast cooling was successful.

### 395 3.4 Evaluation of the impact of nanoparticles on isothermal crystallization kinetics

396  
 397 The isothermal crystallization kinetics of PLA and its nanocomposites were monitored at  $140^\circ\text{C}$ ,  $120^\circ\text{C}$ ,  $100^\circ\text{C}$   
 398 and  $80^\circ\text{C}$ , the results are presented in Table 1 except for PLA at  $140^\circ\text{C}$ , since crystallization was not complete,  
 399 and for all of the samples at  $80^\circ\text{C}$  since the rate was too slow to determine.

400 **Table 2** Half-crystallization and crystallization detection times of PLA and composites.

T ( $^\circ\text{C}$ )	PLA		PLA/C30B	
	$t_{\text{delay}}^{\text{a}}$	$t_{1/2}(\text{exp})^{\text{b}}$	$t_{\text{delay}}^{\text{a}}$	$t_{1/2}(\text{exp})^{\text{b}}$
140			0.5	11.5
120	2.4	26	0.4	2
100	4.9	52.1	1.8	3.7
T ( $^\circ\text{C}$ )	PLA/CNF		PLA/CNF 1%/C30B	
	$t_{\text{delay}}^{\text{a}}$	$t_{1/2}(\text{exp})^{\text{b}}$	$t_{\text{delay}}^{\text{a}}$	$t_{1/2}(\text{exp})^{\text{b}}$
140	2.7	30.8	2.2	25.2
120	1.8	10.3	0.8	4.8
100	2.9	24.5	3.4	15.2

- 401 a)  $t_{\text{delay}}$  (delay from arrival to the crystallization temperature and the start of the peak in DSC,  
 402 b)  $t_{1/2}$  half-crystallization time (since the sample reached crystallization temperature)

403  
 404 The crystallization rate reach a maximum at  $120^\circ\text{C}$  for all samples, and it is evident that the nanocomposites  
 405 crystallize faster than neat PLA at the investigated temperatures. Among the nanocomposites, PLA/C30B  
 406 showed faster nucleation than PLA/CNF or PLA/CNF/C30B, which was even more pronounced at  $140^\circ\text{C}$ . The  
 407 better performance of C30B as a nucleating agent compared with CNF could be explained by the fact that clay is  
 408 more likely to have a higher specific surface area, resulting in an increasing number of nucleation sites. In  
 409 general, it was also found that  $t_{\text{delay}}$  (the time between the material reaching the crystallization temperature and  
 410 onset of the crystallization peak) was proportional to the half-crystallization time.

411  
 412 In order to retrieve more information regarding crystallization kinetics, data from isothermal crystallization  
 413 experiments within the range of 10-70% relative crystallinity were fitted to the Avrami kinetic model as  
 414 described in materials and methods, resulting in the Avrami indices shown in Table 3.

415 **Table 3** Summary of the values of Avrami indices (n) for neat PLA and nanocomposites crystallized at 140°C, 120°C, and  
 416 100°C.

T (°C)	PLA	PLA/C30B	PLA/CNF	PLA/CNF/C30B
140		1.52	1.94	2.12
120	1.97	1.73	1.91	2.43
100	2.27	2.36	1.99	2.49

417 PLA/C30B nanocomposite showed a strong tendency to decrease the Avrami indices, in line with increased  
 418 crystallization temperature reflecting decreased growing directions. A similar trend has also been reported by  
 419 other authors for PLA<sup>39</sup>, and PLA with 10% and 15% of C30B<sup>40</sup> and PLA with other organically modified clays  
 420 at 1% and 3% loading<sup>41</sup>. There are also other reports claiming a more moderate trend<sup>42</sup>, which might be  
 421 attributed to differences in clay dispersion. Notwithstanding this point, the PLA/CNF nanocomposite had  
 422 constant values regardless of crystallization temperature, which is in agreement with some reports in the  
 423 literature for low bacterial cellulose content in PLA<sup>43</sup> and for non-modified nanocrystalline cellulose in PLA<sup>44</sup>.  
 424 Interestingly, it was found that the hybrid nanocomposite PLA/CNF 1%/C30B 1% showed moderate variation  
 425 in Avrami indices in line with increasing temperature between that of CNF and C30B, thereby suggesting the  
 426 simultaneous growth of both clay- and nanocellulose-nucleated spherulites. In any case, all of the composites  
 427 showed no major dissimilarities in values of n suggesting that there is no strong effect from the nanoparticles on  
 428 the spherulite growing direction. Avrami indices for the neat PLA (n=1.97 for crystallization at 120°C and  
 429 n=2.27 for crystallization at 100°C) were not considered because, as can be seen in **Error! Reference source**  
 430 **not found.**, after complete crystallization at 120°C, the PLA spherulites reached a diameter of approximately 65  
 431 μm, while PLA film showed a thickness of 75 μm. Consequently, a spherulite growing in PLA could be affected  
 432 by the physical dimensions of the film.

433

### 434 3.5 Influence of crystallization temperature and nanoparticles on thermal transitions

435 The effect of the crystallization temperature and the presence of nanoparticles on  $T_g$  and  $T_m$  was studied by  
 436 DSC, and the results are shown in Table 4. PLA is known to have a very low  $T_g$ , which limits its performance in  
 437 some areas such as packaging of microwave-heated food or hot tea-coffee cups among others. Additionally, a  
 438 large variation on  $T_m$  will certainly affect the optimal PLA processing temperature in an extruder during  
 439 thermoforming. This is relevant for PLA, but it is critical for PLA/CNF composites since cellulose and CNF  
 440 suffer from degradation at high temperature. Therefore, an evaluation of the impact of the nanoparticles and  
 441 nanoparticle-induced crystallization is fundamental to optimize the performance of the composites.

442

443 **Table 4** Thermal properties ( $T_g$  and  $T_m$ ) (°C) of the PLA and nanocomposites after full crystallization at 140°C, 120°C, and  
 444 100°C, solvent-cast and in an amorphous state.

Crystallization	PLA		PLA/C30B		PLA/CNF		PLA/CNF/C30B	
	$T_g$	$T_m$	$T_g$	$T_m$	$T_g$	$T_m$	$T_g$	$T_m$
140°C <sup>a</sup>	58.8	162.3	57.6	161.9	56.5	163.2	57.9	162.6
120°C <sup>a</sup>	61.7	154.9	58.8	154.4	60.0	155.3	59.6	154.4
100°C <sup>a</sup>	60.5	149.4	61.9	149.3	61.2	150.6	62.0	150.3



Amorphous <sup>c</sup>	55.5	-	56.2	-	54.9	-	55.2	-
SC <sup>b</sup>	60.4	155.0	59.8	154.5	61.7	151.1	61.4	153.7

445 a) Isothermal crystallization at the corresponding crystallization temperature.

446 b) Solvent-cast nanocomposites: PLA and PLA/C30B at 23°C; PLA/CNF and PLA/CNF/C30B at 80°C.

447 c) Amorphous nanocomposites obtained from a fast melt-quenching.

448 In general, the incorporation of CNF and/or C30B did not have a significant impact on the  $T_g$  or  $T_m$  of PLA  
449 within the respective isothermally crystallized samples; however, the  $T_m$  of all of the PLA and nanocomposite  
450 samples did increase in line with increased crystallization temperature. For example, when increasing the  
451 isothermal crystallization temperature of PLA from 100 to 140°C, the  $T_m$  increased from 149.4 to 162.3°C,  
452 whereas for PLA/CNF 1% it increased from 150.6 to 163.2°C. The reason of this increased melting temperature  
453 with increases crystallization temperature is that at high temperatures larger, more stable, lamellae are formed  
454 within the spherulites which melt at higher temperatures It can also be seen that all of the isothermally  
455 crystallized materials showed higher  $T_g$  values when compared with the respective amorphous materials, which  
456 is attributed to a constriction of the MAF occurring after the material is crystallized. However, this might not be  
457 a direct effect of the crystallinity, but is probably due to induced changes on the amorphous region by the  
458 growing spherulites. The MAF trapped between spherulites has also been shown to have different relaxation  
459 values when compared with the amorphous matrix <sup>36</sup>. In this context, the presence of nanoparticles, unlike  
460 crystallization, does not constrain the amorphous phase as much as the presence of spherulites otherwise a larger  
461 variation would be observed between the  $T_g$  values of neat PLA and PLA nanocomposites. Finally, it was found  
462 that the  $T_m$  values of the solvent-cast PLA/CNF (151.1°C) and PLA/CNF/C30B (153.7°C) were slightly below  
463 the values of solvent-cast PLA (155.0°C) and PLA/C30B (154.5°C), suggesting that the different crystalline  
464 morphology for these two groups of samples, as observed in **Error! Reference source not found.**, have a small  
465 impact on  $T_m$ ; however, variations among the materials are close to the accuracy of the DSC.

### 466 3.6 Crystallinity and mobile/rigid amorphous fraction

467 It is widely accepted that crystallinity plays a very important role in materials properties, which makes  
468 crystallinity analysis (usually by DSC) essential for evaluation of the properties of materials and composites.  
469 Recently, evaluation of the so-called Rigid Amorphous Fraction (RAF) and its impact on material properties has  
470 been receiving additional attention. Apart from reducing the Mobile Amorphous Fraction, (MAF), which are the  
471 only “mobile” domains, and therefore essential for the extensibility of the material, it has been speculated that  
472 the RAF could be linked to specific material properties. Among other factors, the RAF is suspected of having a  
473 larger free volume than the MAF, which would have a significant impact on the sorption properties of the  
474 material. Therefore, understanding how crystallization affects those properties could be key to understanding,  
475 foreseeing and optimizing material properties.

476 The melting enthalpy of 100%  $\alpha$ -crystalline PLA was considered to be 106 J g<sup>-1</sup>, while that of 100%  $\alpha'$ -  
477 crystalline PLA was considered to be 25 J g<sup>-1</sup> lower at 81 J g<sup>-1</sup> <sup>34</sup>, while for solvent-cast materials an  
478 intermediate value of 93 J g<sup>-1</sup> was used (average of the enthalpy of the  $\alpha$  and  $\alpha'$  phases). The heat capacity  
479 change at the  $T_g$  of fully amorphous PLA was found in this study to be 0.55 J g<sup>-1</sup> K<sup>-1</sup>, which is similar to what  
480 has been found by others, between 0.48 and 0.628 J g<sup>-1</sup> K<sup>-1</sup> <sup>34,35,45,46</sup>. As expected, The heat capacity variation at

482 the glass transition temperature of the fully amorphous nanocomposites are slightly lower than the neat PLA and  
 483 are shown in [Error! Reference source not found.](#). Additionally, in the same Table, the values of RAF induced by  
 484 nanoparticles ( $RAF_{NANO}$ ) are presented as calculated by means of Eq 4. The values of the heat capacity of PLA  
 485 and nanocomposites at the  $T_g$  can be found in the supplementary information (Table S1).

486 Furthermore, the MAF and RAF values of the PLA and its nanocomposites alongside their degrees of  
 487 crystallinity are summarized in Table 5. RAF values in Table 4 have been split according to RAF induced by  
 488 the crystalline morphology ( $RAF_{CRYST}$ ) and the total RAF (i.e.  $RAF_{CRYST} + RAF_{NANO}$ ) by means of Eq 4. The  
 489 completely amorphous PLA/C30B 1% sample has a RAF of 4%, which therefore defines the  $RAF_{NANO}$  as 4%,  
 490 since there is no impact from crystallinity. The solvent-cast PLA/C30B 1% sample has a RAF (i.e. total CRYST  
 491 + NANO) of 16%, while 4% of this is the  $RAF_{NANO}$  (same as the amorphous sample) and the remaining 12% is  
 492 attributed to  $RAF_{CRYST}$ .

494  
 495  
 496  
 497

**Table 5** Degree of crystallinity ( $X_c$ ), mobile amorphous fraction (MAF), and rigid amorphous fraction (RAF) of PLA and nanocomposites under different crystalline morphologies.

Isothermal		PLA			PLA/C30B		
Crystallization	$X_c$	MAF	RAF/ $RAF_{CRYST}$ / $RAF_{NANO}^d$	$X_c$	MAF	RAF/ $RAF_{CRYST}$ / $RAF_{NANO}^d$	
140°C <sup>a</sup>	--	--	--	36%	43%	20%/16%/4%	
120°C	35%	42%	22%	39%	36%	24%/20%/4%	
100°C	33%	45%	23%	36%	36%	27%/23%/4%	
Amorphous <sup>c</sup>	0%	100%	0%	0%	95%	4%/0%/4%	
SC <sup>b</sup>	6%	90%	4%	29%	54%	16%/12%/4%	

Isothermal		PLA/CNF			PLA/CNF/C30B		
Crystallization	$X_c$	MAF	RAF/ $RAF_{CRYST}$ / $RAF_{NANO}^d$	$X_c$	MAF	RAF/ $RAF_{CRYST}$ / $RAF_{NANO}^d$	
140°C	36%	43%	20%/16%/4%	37%	37%	24%/15%/9%	
120°C	37%	38%	24%/20%/4%	40%	32%	25%/16%/9%	
100°C	36%	37%	26%/22%/4%	37%	33%	28%/19%/9%	
Amorphous <sup>c</sup>	0%	95%	4%/0%/4%	0%	89%	9%/0%/9%	
SC <sup>b</sup>	34%	41%	29%/25%/4%	35%	35%	32%/23%/9%	

- 498 a) The data for PLA at 140°C are not presented, due to incomplete crystallization.  
 499 b) Solvent-cast nanocomposites: PLA and PLA/C30B at 23°C; PLA/CNF and PLA/CNF/C30B at 80°C.  
 500 c) The amorphous nanocomposites were obtained from a fast melt-quenching.  
 501 d) Rigid amorphous fraction: Overall RAF%/% / $RAF_{CRYST}$ / $RAF_{NANO}$ ).

502  
 503

504 Regarding the presence of nanoparticles, seemingly both CNF and C30B showed a similar impact on the  
505 formation of  $RAF_{NANO}$  in amorphous samples (each at 4%), despite the fact that C30B is likely to show a higher  
506 specific area than CNF. This is attributed to the presence of the long chain hydrophobic modifier present on the  
507 C30B surfaces apparently minimizing the creation of  $RAF_{NANO}$  by enhancing compatibility between polymer  
508 matrix and nanoparticles. Hybrid CNF/C30B showed an  $RAF_{NANO}$  (9%) close to the combination of the  
509  $RAF_{NANO}$  induced by the two types of particles individually. A corresponding decrease in MAF is observed  
510 when C30B or CNF is added and suggests the nanoparticles are well dispersed and do not interact with each  
511 other, since interaction among nanoparticles is likely to decrease the overall surface area of nanoparticles in the  
512 polymer matrix and lead to a decreased RAF.

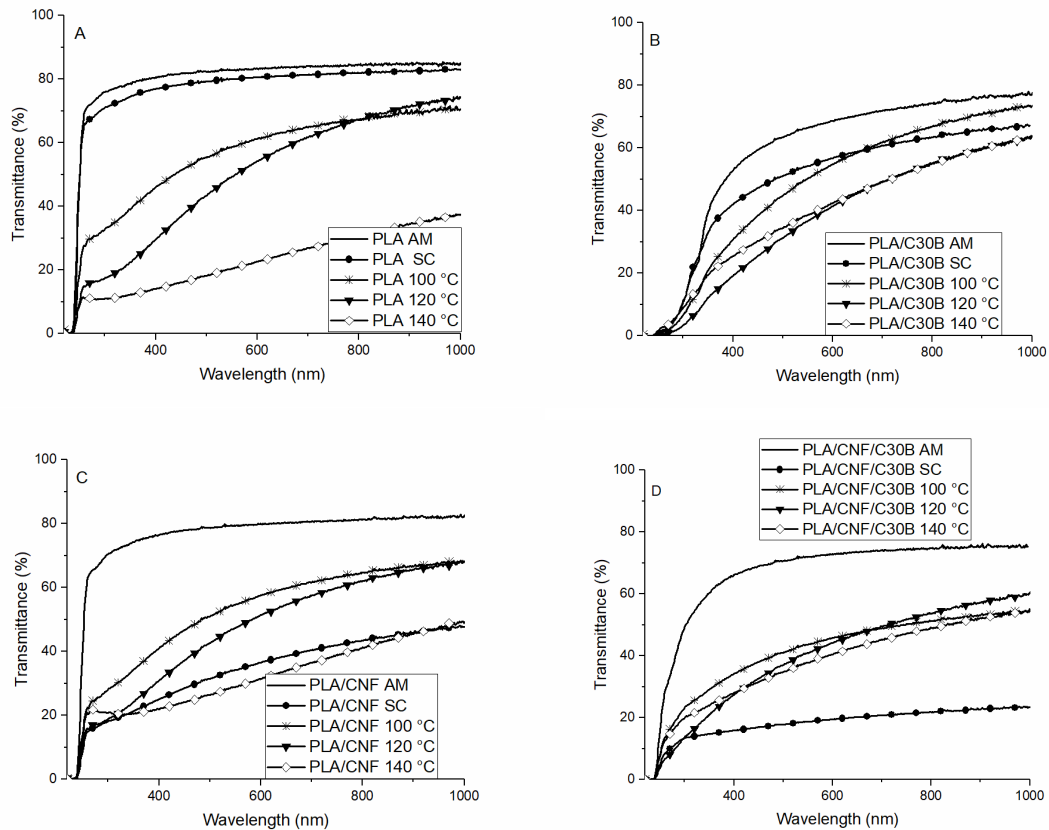
513 The RAF of neat PLA isothermally crystallized at 100°C (23%) and 120°C (22%) is in the higher range of  
514 values presented in the literature (12%-22%)<sup>35,36,47</sup>. When evaluating the effect of isothermal crystallization  
515 temperature on the RAF values for all samples it can be noted that higher crystallization temperature leads to  
516 slightly reduced RAF, which is a trend reported in the literature<sup>48</sup>.

517 It is shown that all of the isothermally crystallized and solvent-cast nanocomposites exhibit increased  
518 crystallinity and a reduced MAF when compared with the respective neat isothermally crystallized PLA  
519 samples. This is considered a result of the smaller spherulite sizes induced in nanocomposites (Figure 2),  
520 allowing for a better packing of the different spherulites within the matrix, which subsequently allowed the  
521 formation of more crystalline domains, thus decreasing the MAF. The isothermally crystallized nanocomposites  
522 showed an increased  $RAF_{CRYST+NANO}$ , compared to neat PLA, which was due to the presence of nanoparticles.  
523 When comparing the values of  $RAF_{CRYST}$  it can be seen that the nanocomposites have lower values. Furthermore,  
524 it can be observed that solvent-cast PLA/C30B 1% shows a much lower amount of RAF (13%) than solvent-cast  
525 PLA/CNF 1% (25%) and solvent-cast PLA/CNF 1% (28%). This combined with the fact that the former has a  
526 much smaller spherulite size (as shown in Figure 2 - top row) and the extent of crystallinity for all three samples  
527 is approximately the same, suggests that spherulite size and RAF are closely related.

### 528 **3.7 Evaluation of the influence of crystalline morphology and nanoparticles on optical transparency**

529 The impact of nanoparticles and crystalline morphology on the transparency of films was investigated, and the  
530 results are summarized in Figure 6. Transparency is a key property in plastics for food packaging applications,  
531 and while nanoparticles are smaller than the wavelength of the visible light and therefore should not influence  
532 the transmission of visible light, their impact on crystallinity might affect transparency.

533



534

535

536 **Figure 6** UV-VIS spectra of neat PLA and its nanocomposites with different crystalline morphologies after isothermal  
 537 crystallization at different temperatures, and for amorphous- and solvent casting-induced crystallinity.

538 When evaluating the impact of crystallization temperature on the transparency of films, it is evident that  
 539 crystallization at 100 °C leads to more transparent materials, although the difference in materials crystallized at  
 540 120 °C is generally small. Surprisingly, crystallization at 140 °C dramatically affects the transparency of PLA  
 541 and CNF-containing nanocomposites, whilst not having such a significant influence on nanocomposites with  
 542 C30B. Assuming that there is no noticeable change in crystalline morphology among PLA/CNF, PLA/C30B and  
 543 PLA/CNF/C30B crystallized at 140 °C, and also that PLA shows incomplete crystallization at this temperature,  
 544 this effect is mostly attributed to a temperature-induced internal deformation (i.e. bubbles or irregularities),  
 545 which can cause light scattering.

546 This observation is interesting, assuming that the decrease in transparency is due to thermal deformation, it is  
 547 anticipated that composites showing better thermomechanical properties would provide better transparency.  
 548 Higher thermomechanical resistance leads to less deformed material. However it could be argued that there is an  
 549 inconsistency with PLA/CNF crystallized at 140 °C, material that in our previous work <sup>2</sup> showed better  
 550 thermomechanical properties than PLA/C30B but still showed reduced transparency without any clear  
 551 difference in crystalline morphology. However, this can be explained based on the different reinforcing  
 552 mechanisms of both nanoparticles. Previously we have observed better reinforcing performance of CNF, which  
 553 was attributed to the establishment of a percolated network of the CNF, which can uphold any stress of the  
 554 polymer chains. Meanwhile, the higher specific area and higher compatibility of C30B should lead to stronger  
 555 interfacial bonding between the polymer matrix and the clay (including the aliphatic chains at its surface) and

556 thus allows a better stress transfer. This anchoring mechanism of the chains by the nanoclays, also reported  
557 elsewhere <sup>36</sup>, prevents any shape degradation during the higher temperature processing and reduces any thermal  
558 deformation as observed with CNF. In the case of CNF, care should therefore be taken during processing, since  
559 too high a processing temperature could lead to reduced performance. Presumably, in this scenario the reduced  
560 surface area of CNF and lower compatibility with the polymer matrix leads to deformations at higher processing  
561 temperatures due to a lack of anchorage between polymer chains and nanoparticles.

562 When comparing respective amorphous samples with nanocomposites isothermally crystallized at 120°C and  
563 100°C, it is evident that, for all of the cases, increased crystallinity leads to reduced transparency in the visible  
564 region of the spectra (>400 nm). This can be explained by the fact that crystalline domains have higher density  
565 than amorphous domains, which leads to light scattering as the light passes between each of the domains. It is  
566 also noteworthy that the presence of nanoparticles does not significantly affect the transparency of films within  
567 the isothermally crystallized samples, which is presumably due to the dispersion of the nanoparticles on a  
568 nanometer scale. A similar observation can be seen for the respective amorphous nanocomposites, although in  
569 this case the PLA/C30B does show a slight decrease in transparency. When evaluating the transparency of  
570 solvent-cast films, it can be seen that solvent-cast PLA/C30B shows increased transparency when compared to  
571 the other nanocomposites, while PLA/CNF/C30B shows a significantly reduced transparency. This is attributed  
572 to smaller or larger spherulite sizes observed in the solvent-casted PLA/C30B or PLA/CNF/C30B  
573 nanocomposites as shown in Figure 2 (row 1). In addition to this, also the amount of RAF could affect the  
574 transparency of the samples. RAF has been reported to have a larger free volume than the MAF,<sup>49</sup> which  
575 ultimately could cause light scattering and effectively the free volume regions of RAF could act as “bubbles” in  
576 a polymer matrix.

577 A UV-blocking effect (reduction of transmittance below 400nm) can be seen for PLA/C30B 1%, which is not  
578 observed for PLA/CNF 1% which shows behavior similar to PLA. Surprisingly, PLA/CNF 1%/C30B 1% shows  
579 less of a UV-blocking behavior when compared with PLA/C30B 1%, even though the same amount of C30B is  
580 present. A better dispersion of clay (i.e. exfoliation) will lead to increased nanoparticle surface area that can  
581 block UV light <sup>50</sup> suggesting this effect may be attributed to small differences in clay dispersion between C30B-  
582 reinforced composites caused by the presence of CNF. It is remarkable that, after full crystallization, at any of  
583 the temperatures studied, the hybrid nanocomposites containing 1% CNF and 1% C30B shows good  
584 transparency, which is often a desirable feature for food packaging films.

#### 585 **4. CONCLUSIONS**

586

587 In the present work, the influence of processing conditions on the crystallinity of solvent-cast and isothermally  
588 crystallized PLA, PLA/CNF 1%, PLA/C30B 1%, and PLA/CNF 1%/C30B 1% was evaluated by DSC, MDSC,  
589 POM, and XRD. It was found that solvent casting at room temperature induces a different crystallinity  
590 compared to isothermal crystallization and therefore leads to materials with different performance compared to  
591 high temperature processed materials. Similarly, composites prepared at high temperature, might not accurately  
592 be used to assess material properties, when the material ultimately is intended to be processed via solvent-based  
593 electrospinning at lower temperatures. However, different solvent casting methods were shown to induce

594 different crystallinity. The combination of both CNF and C30B did not show significant variations in spherulite  
595 size or distribution when compared with single nanoparticle- filled composites.

596

597 The thermal transitions were not significantly influenced by the nanoparticles, but the increasing isothermal  
598 crystallization temperatures did result in decreasing glass transition temperature and increasing melting points  
599 when assessing nanoparticles either individually or in combination. The kinetic investigation of crystallization  
600 demonstrated that combining both types of nanoparticles did not lead to a sum of both crystallization rates but to  
601 an average. A similar effect was also observed with spherulite growth directions. PLA/C30B 1% showed a clear  
602 tendency towards decreased growing directions with increased crystallization temperature, whereas PLA/CNF  
603 1% showed no significant variations and a combination with PLA/CNF 1%/C30B 1% showed only a moderate  
604 intermediate decrease in growing direction. The combination of both nanoparticles within the amorphous matrix  
605 effectively led to a doubling of the  $RAF_{NANO}$ , which was also due to the sum of each individual nanoparticle;  
606 however, no further variations in  $RAF_{NANO}$  due to the effect of each nanoparticle was apparent after full  
607 isothermal crystallization. Within the isothermally crystallized samples, the main difference in the hybrid  
608 composites was a reduced MAF, which was compensated by an increase in extent of crystallinity, probably due  
609 to better spherulite packaging.

610

611 Transparency of the nanocomposites generally depended on the spherulite size, which was clearly seen for the  
612 solvent casted samples. For isothermally crystallized samples, a similar degree of crystallinity and spherulite  
613 size were determined, though higher crystallization temperatures resulted in lower transparency, which was  
614 attributed to defects in the materials rather than crystallization phenomena.

615

## 616 **5. ACKNOWLEDGEMENTS**

617

618 Jon Trifol Guzman is grateful for financial support from the FP7 - People - 2011, ITN Marie Curie Initial  
619 Training Network (ITN) under grant agreement no. 290098 "NewGenPak".

## 620 **6. Data availability**

621 The raw and processed data required to reproduce these findings cannot be shared at this time due to  
622 technical or time limitations

623

## 624 **7. REFERENCES**

- 625 1. Madhavan Nampoothiri, K.; Nair, N. R.; John, R. P. *Bioresour. Technol.* **2010**, *101*, 8493.
- 626 2. Trifol, J.; Plackett, D.; Sillard, C.; Hassager, O.; Daugaard, A. E.; Bras, J.; Szabo, P. *J. Appl. Polym. Sci.*  
627 **2016**, *133*, 1.
- 628 3. Khalil, H. A.; Bhat, A. H.; Yusra, A. I. *Carbohydr. Polym.* **2012**, *87*, 963.
- 629 4. Krishnamachari, P.; Zhang, J.; Lou, J.; Yan, J.; Uitenham, L. *Int. J. Polym. Anal. Charact.* **2009**, *14*,  
630 336.
- 631 5. Trifol, J.; Plackett, D.; Sillard, C.; Szabo, P.; Bras, J.; Daugaard, A. E. *Polym. Int.* **2016**, *65*, 988.
- 632 6. Wu, C. N.; Saito, T.; Yang, Q.; Fukuzumi, H.; Isogai, A. *ACS Appl. Mater. Interfaces* **2014**, *6*, 12707.
- 633 7. Jamshidian, M.; Tehrani, E. A.; Imran, M.; Jacquot, M.; Desobry, S. *Compr. Rev. Food Sci. Food Saf.*

- 634 **2010**, 9, 552.
- 635 8. Bitinis, N.; Verdejo, R.; Maya, E. M.; Espuche, E.; Cassagnau, P.; Lopez-Manchado, M. A. *Compos. Sci. Technol.* **2012**, 72, 305.
- 636
- 637 9. Fukuzumi, H.; Saito, T.; Isogai, A. *Carbohydr. Polym.* **2013**, 93, 172.
- 638 10. Trifol, J.; Sillard, C.; Plackett, D.; Szabo, P.; Bras, J.; Daugaard, A. E. *Cellulose* **2016**.
- 639 11. Ploehn, H. J.; Liu, C. *Ind. Eng. Chem. Res.* **2006**, 45, 7025.
- 640 12. Gorrasi, G.; Anastasio, R.; Bassi, L.; Pantani, R. *Macromol. Res.* **2013**, 21, 1110.
- 641 13. Picard, E.; Espuche, E.; Fulchiron, R. *Appl. Clay Sci.* **2011**, 53, 58.
- 642 14. Sarasua, J. R.; Arraiza, A. L.; Balerdi, P.; Maiza, I. *Polym. Eng. Sci.* **2005**, 45, 745.
- 643 15. Garlotta, D. *J. Polym. Environ.* **2001**, 9, 63.
- 644 16. Guinault, A.; Sollogoub, C.; Domenek, S.; Grandmontagne, A.; Ducruet, V. *Int. J. Mater. Form.* **2010**,
- 645 3, 603.
- 646 17. Perego, G.; Cella, G. D.; Bastioli, C. *J. Appl. Polym. Sci.* **1996**, 59, 37.
- 647 18. Suryanegara, L.; Nakagaito, A. N.; Yano, H. *Compos. Sci. Technol.* **2009**, 69, 1187.
- 648 19. Naga, N.; Yoshida, Y.; Noguchi, K.; Murase, S. *Open J. Polym. Chem.* **2013**, 03, 29.
- 649 20. Miyata, T.; Masuko, T. *Polymer.* **1998**, 39, 5515.
- 650 21. Jandas, P. J.; Mohanty, S.; Nayak, S. K. *J. Therm. Anal. Calorim.* **2013**, 114, 1265.
- 651 22. Sarasua, J. R.; Rodriguez, N. L.; Arraiza, A. L.; Meaurio, E. *Macromolecules* **2005**, 38, 8362.
- 652 23. Zhang, J.; Tashiro, K.; Tsuji, H.; Domb, A. J. *Macromolecules* **2008**, 41, 1352.
- 653 24. Wunderlich, B. *Prog. Polym. Sci.* **2003**, 28, 383.
- 654 25. Adam, G.; Gibbs, J. H. *J. Chem. Phys.* **1965**, 43, 139.
- 655 26. Trifol, J.; Mericer, C.; Plackett, D.; Sillard, C.; Minelli, M.; Hassager, O.; Daugaard, a E.; Bras, J.;
- 656 Giacinti, M.; Szabo, P. *20th Int. Conf. Compos. Mater. Copenhagen* **2015**, 19.
- 657 27. López-Rubio, A.; Lagarón, J. M.; Hernández-Muñoz, P.; Almenar, E.; Catalá, R.; Gavara, R.; Pascall,
- 658 M. A. *Innov. Food Sci. Emerg. Technol.* **2005**, 6, 51.
- 659 28. Martínez-Sanz, M.; Olsson, R. T.; Lopez-Rubio, A.; Lagaron, J. M. *J. Appl. Polym. Sci.* **2012**, 124,
- 660 1398.
- 661 29. Mohanty, S.; Larsen, L. B.; Trifol, J.; Szabo, P.; Burri, H. V. R.; Canali, C.; Dufva, M.; Emnéus, J.;
- 662 Wolff, A. *Mater. Sci. Eng. C* **2015**, 55, 569.
- 663 30. Mohapatra, A. K.; Mohanty, S.; Nayak, S. K. *Polym. Compos.* **2012**, 33, 2095.
- 664 31. Katiyar, V.; Gerds, N.; Koch, C. B.; Risbo, J.; Hansen, H. C. B.; Plackett, D. *J. Appl. Polym. Sci.* **2011**,
- 665 122, 112.
- 666 32. Rhim, J. W.; Hong, S. I.; Ha, C. S. *LWT - Food Sci. Technol.* **2009**, 42, 612.
- 667 33. López-Rodríguez, N.; Martínez De Arenaza, I.; Meaurio, E.; Sarasua, J. R. *RSC Adv.* **2015**, 5, 34525.
- 668 34. Righetti, M. C.; Gazzano, M.; Di Lorenzo, M. L.; Androsch, R. Enthalpy of melting of  $\alpha'$ - and  $\alpha$ -crystals
- 669 of poly(L-lactic acid). *Eur. Polym. J.* **2015**, 70, 215–220.
- 670 35. Zuza, E.; Ugartemendia, J. M.; Lopez, A.; Meaurio, E.; Lejardi, A.; Sarasua, J. R. *Polymer.* **2008**, 49,
- 671 4427.
- 672 36. Saiter, A.; Delpouve, N.; Dargent, E.; Oberhauser, W.; Conzatti, L.; Cicogna, F.; Passaglia, E. *Eur.*
- 673 *Polym. J.* **2016**, 78, 274.

- 674 37. Lorenzo, A. T.; Arnal, M. L.; Albuerne, J.; Müller, A. J. *Polym. Test.* **2007**, *26*, 222.
- 675 38. Pan, P.; Han, L.; Shan, G.; Bao, Y. *Macromolecules* **2014**, *47*, 8126.
- 676 39. Lohmeijer, P. J. A.; Goossens, J. G. P.; Peters, G. W. M. *J. Appl. Polym. Sci.* **2017**, *134*, 2.
- 677 40. Krikorian, V.; Pochan, D. J. *Macromolecules* **2004**, *37*, 6480.
- 678 41. Li, X.; Yin, J.; Yu, Z.; Yan, S.; Lu, X.; Wang, Y.; Cao, B.; Chen, X. *Polym. Compos.* **2009**, *30*, 1338.
- 679 42. Lee, S.-H.; Wang, S.; Teramoto, Y. *J. Appl. Polym. Sci.* **2008**, *108*, 870.
- 680 43. Ambrosio-Martín, J.; Lopez-Rubio, A.; Fabra, M. J.; Gorrasi, G.; Pantani, R.; Lagaron, J. M. *J. Appl.*  
681 *Polym. Sci.* **2015**, *132*, 1.
- 682 44. Pei, A.; Zhou, Q.; Berglund, L. A. *Compos. Sci. Technol.* **2010**, *70*, 815.
- 683 45. Arnoult, M.; Dargent, E.; Mano, J. F. *Polymer.* **2007**, *48*, 1012.
- 684 46. Henricks, J.; Boyum, M.; Zheng, W. *J. Therm. Anal. Calorim.* **2015**, *120*, 1765.
- 685 47. Magoń, A.; Pyda, M. *Polymer.* **2009**, *50*, 3967.
- 686 48. Righetti, M. C.; Tombari, E. *Thermochim. Acta* **2011**, *522*, 118.
- 687 49. Lin, J.; Shenogin, S.; Nazarenko, S. *Polymer.* **2002**, *43*, 4733.
- 688 50. Gao, F. *Mater. Today* **2004**, *7*, 50.
- 689
- 690
- 691
- 692