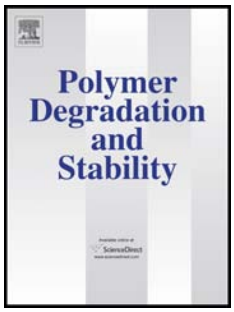
Arrieta, MP.; López Martínez, J.; Rayón Encinas, E.; Jiménez, A. (2014). Disintegrability under composting conditions of plasticized PLA-PHB blends. Polymer Degradation and Stability. 108:307-318. doi:10.1016/j.polymdegradstab.2014.01.034.

Accepted Manuscript



Disintegrability under composting conditions of plasticized PLA-PHB blends

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PII: S0141-3910(14)00045-7

DOI: 10.1016/j.polymdegradstab.2014.01.034

Reference: PDST 7237

To appear in: *Polymer Degradation and Stability*

Received Date: 26 November 2013

Revised Date: 20 January 2014

Accepted Date: 26 January 2014

Please cite this article as: Arrieta MP, López J, Rayón E, Jiménez A, Disintegrability under composting conditions of plasticized PLA-PHB blends, *Polymer Degradation and Stability* (2014), doi: 10.1016/j.polymdegradstab.2014.01.034.

Disintegrability under composting conditions of plasticized PLA-PHB blends

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The disintegration under composting conditions of films based on poly(lactic acid)-poly(hydroxybutyrate) (PLA-PHB) blends and intended for food packaging was studied. Two different plasticizers, poly(ethylene glycol) (PEG) and acetyl-tri-n-butyl citrate (ATBC), were used to limit the inherent brittleness of both biopolymers. Neat PLA, plasticized PLA and PLA-PHB films were processed by melt-blending and compression moulding and they were further treated under composting conditions in a laboratory-scale test at 58 ± 2 ºC. Disintegration levels were evaluated by monitoring their weight loss at different times: 0, 7, 14, 21 and 28 days. Morphological changes in all formulations were followed by optical and scanning electron microscopy (SEM). The influence of plasticizers on the disintegration of PLA and PLA-PHB blends was studied by evaluating their thermal and nanomechanical properties by thermogravimetric analysis (TGA) and the nanoindentation technique, respectively. Meanwhile, structural changes were followed by Fourier transformed infrared spectroscopy (FTIR). The ability of PHB to act as nucleating agent in PLA-PHB blends slowed down the PLA disintegration, while plasticizers speeded it up. The relationship between the mesolactide to lactide forms of PLA was calculated with a Pyrolysis-Gas Chromatography-Mass Spectrometry device (Py-GC/MS), revealing that the mesolactide form increased during composting.

Keywords: Poly(lactic acid); Poly(hydroxybutyrate); blend; biodegradable; plasticizers.

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1- Introduction

Poly(lactic acid), PLA, and poly-hydroxybutyrate (PHB) are two of the bio-based and biodegradable polymers which have focused some attention by their possibilities as environmentally-friendly food packaging materials. In this sense, PLA is currently the most used biopolymer in the food packaging sector for short shelf-life products [1-4], owing to its high mechanical strength, easy processability, superior transparency, availability and low cost. Poly(hydroxybutyrate) (PHB) is the most common representative of poly(hydroxyalkanoates) (PHA) [5], and it has been also proposed for short-term food packaging applications [6]. Conversely, there are not many commercial products of PHB by its narrow processing window, high brittleness, [5] and price [7].

A considerable number of research work has been reported on the misicibility between PLA and PHB and possible applications in food packaging [8-11] It is known that PLA shows limited or partial miscibility with low molar mass PHB [10, 12]. The temperature used during the blend preparation has also significant influence in the miscibility between both polymers. In this sense Zhang et al. (1996) reported that PLA-PHB blends prepared at high temperature exhibited greater miscibility than those prepared by solvent casting at room temperature [13] since PLA-PHB systems are fully miscible in the melt state [11, 14]. This effect could be due to the transesterification reaction between PLA and PHB chains [13]. In addition, the miscibility between PLA and PHB is strongly dependent on their ratio in the blend. For instance, Furukawa et al. (2005) studied PLA/PHB films prepared by solvent casting in chloroform with blending ratios (w/w) 20/80, 40/60, 60/40, and 80/20 (PLA/PHB). They reported that PHB crystallized as very small spherulites that may act as nucleation sites of PLA in the 20/80 blend [8]. Similarly, Zhang and Thomas (2011) studied PLA/PHB blends in different proportions (100/0, 75/25, 50/50, 25/75 and 0/100, w/w) prepared by melt blending followed by compression moulding [9]. They found that PLA/PHB 75/25 films showed interesting properties for specific applications, with increased crystallinity and optimal miscibility between both polymers, resulting in improved tensile properties compared with neat PLA. More recently, Bartczak et al. (2013) proposed the modification of PLA by the addition of PHB up to 20 wt% for food packaging applications and they concluded that PHB can be considered as an effective impact modifier for PLA, increasing its impact resistance [10]. PLA-PHB blends (75:25, w/w) prepared by melt-blending and compression moulding have been proposed for films intended for food packaging [3, 5] and it was observed that the addition of 25 wt% of PHB improved PLA mechanical and barrier properties due to the ability of PHB to act as a nucleating agent at this PLA/PHB ratio [5].

Films processed from PLA/PHB blends are still rigid and brittle, while their processing for films manufacturing remains an issue to avoid fractures [5]. This drawback can be overcome by plasticization to improve processability and ductility of these films [3, 5]. But not all plasticizers could be adequate for such application. It should be taken into account that plasticizers should satisfy the strict requirements applied to materials intended to be in contact with food. They should be also miscible with the polymer matrix [1, 15, 16] and stable at the high temperatures used during processing [16], providing suitable mechanical and barrier properties [15]. In this sense, poly(ethylene glycol) (PEG) and citrate esters have been proposed as efficient plasticizers for PLA [16, 17], while PHB has been successfully plasticized with PEG [18] and acetyl-tri-n-butyl citrate (ATBC) [19]. PEG is water-soluble [20] and ATBC is obtained from naturally occurring citric acid. Both are non-toxic plasticizers [21].

These blends are promising candidates for sustainable post-use waste treatments, such as composting [22]. Disintegration in compost is governed by aerobic fermentation that mostly results in humus-rich soil, while landfill disposal is mediated by anaerobic fermentation producing hazardous methane. Even if methane produced in landfills could be used as an energy source [22], it is known that the huge amount of plastic waste disposal in landfills must be reduced [23, 24]. Thus, composting would be adequate for short-term food packaging plastics as end-life option.

Biodegradation in composting conditions of PLA [25-27] and PHB [5, 6] has been already reported. It is known that PLA degradation in compost takes place in two main and consecutive stages, i.e. the hydrolytic and enzymatic degradation [23]. PLA disintegration starts by surface hydrolysis [28] leading to polymer random decomposition [4], while PHB disintegration is firstly caused by microorganisms that erode the polymer surface and gradually spreading to the bulk [29]. Lemmouchi et al. (2009) reported that the disintegration of PLA in composting conditions was enhanced by the presence of plasticizers [5].

The influence of PHB and plasticizers on the PLA disintegrability in composting conditions was evaluated in this work. Plasticized PLA/PHB films were prepared by melt-blending followed by compression moulding. The disintegration patterns during composting of plasticized PLA/PHB films were investigated and compared with their plasticized PLA counterparts. Disintegrability was followed by morphological, structural, nanomechanical and thermal analysis, with the main objective to obtain information on the compostability of plasticized PLA-PHB blends as end-life option for food packaging applications.

2. Experimental

2.1. Materials

Poly(lactic acid) (PLA Ingeo™ 4032D, M_n= 217 kDa, 2 wt% D-isomer, M_w/ M_n = 2) was supplied in pellets by NatureWorks LLC (Minnetonka, MIN, USA), Poly(hydroxybutyrate) pellets (PHB, P226, M_w = 426 kDa) was purchased from Biomer (Krailling, Germany). Poly(ethylene glycol) (PEG, M_n= 300 g mol^{-1}) and acetyl-tri-n-butyl citrate (ATBC, M = 402 g mol^{-1}, 98% purity) were purchased from Sigma-Aldrich (Madrid, Spain).

2.2. Films preparation

PLA-PHB blends were processed by mixing PLA (previously dried overnight at 80 ºC in a vacuum oven) and PHB pellets (treated for 4 hours at 40 ºC), in 75:25 wt% ratio in a Haake PolyLab QC mixer (Thermo Fischer Scientific Inc., Waltham, MA, USA) at 180 ºC and a rotation speed of 50 rpm for 4 min. ATBC and PEG were further added (15 wt%) after 3 min once PLA or PLA-PHB blends had achieved the melt state. Each blend was then processed into films by compression moulding at 180 ºC in a hot press (Mini C 3850, Carver, Inc., Wabash, IN, USA). Blends were kept between the plates at atmospheric pressure for 2 min until melting and they were further submitted to a pressure cycle of 3 MPa for 1 min, 5 MPa for 1 min and finally 10 MPa for 2 min, with the aim to eliminate the trapped air bubbles [30]. These films were then quenched to room temperature. Five formulations were obtained: control neat PLA film, PLA plasticized with PEG or ATBC (PLA-PEG and PLA-ATBC), and plasticized PLA-PHB blends (PLA/PHB-PEG and PLA/PHB-ATBC). The films average thickness, measured with a Digimatic Micrometer Series 293 MDC-Lite (Mitutoyo, Japan) ± 0.001 mm, was 200 ± 50 µm. Control films were stored at 25 ºC and 30% relative humidity (RH) in an acrylic desiccator cabinet before testing.

2.3. Disintegration under composting conditions

Disintegration under composting conditions was performed by following the ISO-20200 standard [31]. Solid synthetic waste was prepared by mixing 10% of compost at pH 6.5 (supplied by Mantillo, Spain), 30% rabbit food, 10% starch, 5% sugar, 1% urea, 4% corn oil and 40% sawdust and it was mixed with water in 45:55 ratio. Water was added periodically to the reaction container to maintain the relative humidity in the compost medium. Films were prepared (30 x 30 x 0.2 mm^3) and they were buried 6 cm depth in plastic reactors containing

the solid synthetic wet waste. Each sample was contained in an iron mesh to allow their easy removal after treatment, but allowing the access of microorganisms and moisture [32]. Reactors were introduced in an air circulation oven (DO/200 Carbolite, Hope Valley, UK) at 58 ºC for 35 days. The aerobic conditions were guaranteed by periodical gentle mixing of the solid synthetic wet waste [18, 25]. Films were recovered from the disintegration container at different times (7, 14, 21 and 28 days), washed with distilled water, dried in an oven at 37 ºC for 24 h, and weighed. Disintegrability was calculated by normalizing the sample weight at each time to the initial value [33], while photographs were taken to all samples once extracted from the composting medium.

2.4. Characterization techniques

2.4.1. *Color properties*

Color properties of plasticized PLA and plasticized PLA/PHB films before and after 7 days of incubation were studied by measuring CIELab colour coordinates *L* (lightness), *a** (red-green) and *b** (yellow-blue), with a KONICA CM-3600d COLORFLEX-DIFF2, HunterLab (Hunter Associates Laboratory, Inc, Reston, VA, USA) colorimeter. The yellowness index (*YI*) was also determined. The instrument was calibrated with a white standard tile. Measurements were carried out in quintuplicate at random positions over the films surface and average values were calculated. Total color differences (Δ*E*) induced by disintegration in samples after 7 days in composting conditions with the control films were calculated by using Equation 1:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^{*2} + \Delta b^{*2}} \quad (1)$$

2.4.2. *Surface microstructure*

Differences in surface microstructures of plasticized PLA and plasticized PLA/PHB films before and after 21 days of treatment were evaluated by using a LV-100 Nikon Eclips optical microscope equipped with a Nikon sight camera at 20X magnification (Tokyo, Japan). The extended depth of field (EDF-z) imaging technique was used to improve resolution. Furthermore, surface microstructure of films before and after 14 and 21 days of disintegration was studied by Scanning Electron Microscopy (SEM) with a Phenom (FEI Company, Eindhoven, The Netherlands) operated at 10 kV.

2.4.3. *Fourier transformed infrared spectroscopy (FTIR)*

FTIR analysis of films was carried out in the 600-4000 cm^{-1} range in attenuated total reflection (ATR) mode with a Perkin-Elmer BX IR spectrometer (Perkin Elmer Spain, S.L., Madrid Spain). Tests were performed at room temperature using 128 scans and 4 cm^{-1} resolution. A background spectrum was obtained before each test to compensate by spectra subtraction the humidity effect and the presence of carbon dioxide.

2.4.4. *Thermogravimetric analysis (TGA)*

Thermogravimetric analysis was performed with a thermogravimetric analyzer TGA/SDTA-851e Mettler Toledo (Schwarzenbach, Switzerland). Tests were run under dynamic mode from 30 to 600 ºC at 10 ºC min^{-1} in nitrogen flow (50 mL min^{-1}) to avoid thermo-oxidative degradation. The initial degradation temperatures (T_0) were determined at 5% mass loss while temperatures at the maximum degradation rate (T_{max}) were calculated from the first derivative of the TG curves (DTG).

2.4.5. *Pyrolysis/ Gas Chromatography- Mass Spectrometry (Py/ GC-MS)*

The relationship between mesolactide and (D, L)-lactide forms in the PLA structure before and after 21 days under composting conditions was evaluated with a Pyrolysis-Gas Chromatography-Mass Spectrometry device (Py-GC/MS). Film samples were pyrolyzed at 1000 ºC for 0.5 s with a Pyroprobe 1000 (CDS Analytical, Oxford, PA, USA), coupled to a gas chromatograph (6890N, Agilent Technologies, Spain S.L., Madrid, Spain) equipped with a 30 m long HP-5 (0.25 mm thickness) column and using helium as carrier gas with a 50:1 split ratio. The GC oven was programmed as previously reported [34], the column program started at 40 ºC for 2 min, followed by a stepped increase of 5 ºC min^{-1} to 200 ºC (15 min hold), and further increase at 20 ºC min^{-1} to 300 ºC (5 min hold). Detection was carried out with an Agilent 5973N mass selective instrument. The transfer temperature from the GC to the MS was set at 180 ºC. The mass selective detector was programmed to detect masses between 30 and 650 amu. The identification of PLA and PHB degradation products was carried out by the characteristic fragmentation patterns observed in Py-GC/MS spectra.

2.4.6. Nanomechanical properties

Nanomechanical properties were measured with a nanoindenter machine G-200 (Agilent Technologies, Santa Clara, CA, USA) with a previously calibrated Berkovich diamond tip. Experiments were carried out by Continuous Stiffness Measurement (CSM) [35, 36] under a 70 Hz harmonic oscillation frequency and 2 nm of harmonic amplitude [36]

maintaining a constant 0.05 s^{-1} indentation rate. An array of 5 x 5 indentations distanced 50 µm were programmed at a constant 2000 nm depth, calculating the average values between the 400 nm and 600 nm depth to avoid the roughness effect at the initial penetration depth. The reduced elastic modulus (E_r) was calculated instead of the Young's Modulus since the Poisson's coefficient is unknown for these blends and plasticized polymers.

3. Results and discussion

Fig. 1 shows the visual appearance of samples recovered at different testing times. In general terms, all materials increased their opacity during composting even at the first tested time (7 days). It is noticeable that PHB slowed down the PLA disintegration rate, since formulations with PHB were still visible after 28 days, while those with no PHB were completely disintegrated at that time. It was observed that disintegrability under composting started in the polymers amorphous phase and this was mostly attacked by microorganisms at the initial stage of this process. This effect was apparent by the loss of transparency in films after treatment. The increase in crystallinity in all these materials decreased their degradation rate since the ordered structure in the crystalline fractions could retain the action of microorganisms. Thus, the addition of mostly crystalline PHB slowed down the disintegration of the PLA matrix. In fact, in a previous work we studied the disintegrability of neat PHB films in composting conditions and it was observed that it only reached 1.5% after 35 days [5]. On the other hand, the addition of plasticizers resulted in a clear increase in the disintegration phenomenon.

Fig. 2 shows the colorimetric results obtained after 7 days of exposition to composting conditions. The characteristic high brightness of neat PLA decreased after 7 days in composting as evidenced by the decrease in the lightness value (L) (Fig. 2a). A similar trend was observed for PLA and PLA-PHB blends plasticized with ATBC. However, a different behaviour was observed for films plasticized with PEG. These samples showed some increase in lightness with testing time. This effect could be due to some plasticizer losses and the consequent compression of macromolecular chains. The addition of PHB to the PLA matrix produced the increase in clear amber tone in PLA-PHB blends. The yellowness index (YI) was measured and results are shown in Fig. 2b. No significant differences in YI were observed between neat and plasticized PLA films before and after 7 testing days. Plasticized PLA/PHB blends showed a clear decrease in YI values, being this effect more evident in the PLA/PHB-PEG film. In addition, positive values of b*, indicative of a deviation towards

yellow, as well as negative values of a*, indicative of a deviation towards green, corroborated this tendency to color change in PLA/PHB-PEG films (Fig. 2c).

As can be seen in Fig. 1 some differences in visual appearance after 7 days of composting with respect to the corresponding formulation before the test start were observed. Total color difference (ΔE) values indicated that neat PLA was the only sample with no apparent visual changes (ΔE = 1.7), since ΔE values higher than 2.0 represent the threshold of the perceptible color difference for the human eye [37]. The ΔE values for PLA-PEG and PLA-ATBC films were 2.9 and 2.5, respectively, while they were 5.3 and 2.1 for PLA/PHB-PEG and PLA/PHB-ATBC, correspondingly. In summary, all plasticized materials showed perceptible color changes after only 7 days of composting treatment. Changes in films color at the first stages of the composting test were related to the beginning of the hydrolytic degradation process, inducing some changes in the films refraction index as a consequence of water absorption and/or the presence of hydrolysis products [33]. At higher testing times, films color could not be determined in the same way due to the samples rupture into small pieces and their irregular surfaces. Nevertheless, some qualitative observations could be drawn, since it was clearly noticeable that apparent color changes were related to the degradation stage. PLA/PHB blends tended to yellow at high testing times (Fig. 1), due to the PLA disintegration and the consequent increase in the PHB proportion in these formulations. Finally, at 28 days, samples showed a clear yellowness pattern due to the total disintegration of the PLA matrix (Fig. 1).

Visual observations were confirmed by calculating the disintegration degree (weight loss) as a function of time (Fig. 3) where 90% of disintegration was considered as the goal of samples disintegrability [33], as indicated in the current legislation for biodegradable materials [31]. No significant differences in weight loss were observed between samples after 7 days, but after 14 days the disintegration rate clearly increased for all formulations. SEM micrographs (Fig. 4) showed deep fractures on the films surfaces after 7 testing days, and they were particularly notorious in plasticized materials. No significant differences were observed in plasticized PLA/PHB blends, but PLA-PEG showed higher disintegration rate than PLA-ATBC. This significant difference in plasticized PLA films could be explained by the hydrophilic nature of PEG, in contrast with the hydrophobic character of ATBC. Water absorption and diffusion through the polymer bulk in the initial phase of disintegration in PLA-PEG films was faster than in PLA-ATBC, resulting in higher hydrolysis in the polymer chain leading to small molecules (monomers and short-chain oligomers) that are available for the microorganisms attack [26]. However, PLA/PHB-ATBC blends showed higher

disintegration rate (up to 65%) after 21 days of composting than PLA/PHB-PEG (below 50%). This different behaviour could be explained by the formation of acid groups during the plasticizer release in PLA/PHB-ATBC films, which are able to promote the hydrolysis of polymer chains [33] and consequently accelerating the disintegration process. This result was confirmed by FTIR analysis where the formation of hydroxyl groups (3200-3600 cm^{-1}) in plasticized PLA-PHB blends showed higher intensity for the PLA/PHB-ATBC blend, as discussed below. Moreover, SEM micrographs of the PLA/PHB-ATBC blend after 21 days in composting conditions also showed evident signs of surface erosion with deep fractures, while the PLA/PHB-PEG blend showed a more regular surface. As observed in Fig.3, plasticized PLA films achieved more than 85% of disintegration after 21 days and all materials showed weight losses higher than 90% after 28 days, indicating the compostable nature of all these blends.

The film surfaces were also investigated by optical microscopy and their roughness profiles were determined with the EDF-z technique (Fig. 5). In a previous work we observed that neat PLA films showed smooth surfaces which were rough in neat PHB films [5]. When applying this technique to PLA and plasticized PLA films, smooth surfaces were obtained (Fig. 5) in both cases, although plasticized PLA/PHB films showed some roughness. The more irregular surface profiles showed by films with PHB could be attributed to its higher crystallinity. After 21 days under composting conditions, all samples showed some increase in roughness when compared to the same formulation before the beginning of the test.

Fig. 6 shows the FTIR spectra of each film at different testing times. All PLA based samples revealed the typical band at 1750cm^{-1} assigned to the asymmetric stretching of the carbonyl group (-C=O) by lactide [1, 25]. At 1180 cm^{-1} the -C-O- bond stretching in the -CH-O- group of PLA was also observed [25]. The 1450 cm^{-1} band was assigned to the $-CH_3$ group [1, 38]. It was reported that the intensity of the -C=O band increased with the composting time due to the hydrolytic degradation, resulting in some increase in the number of carboxylic end groups in the polymer chains [25]. In these materials, the -C=O band intensity increased with the composting time in both, PLA and plasticized PLA samples, while it showed broader absorption in films containing PHB. This result was related to the crystalline carbonyl group stretching in PHB at 1735 cm^{-1} [29]. The good miscibility between PLA and PHB was related to the observation of a unique narrow band corresponding to the carbonyl group [9]. Two bands were clearly observed in the PLA/PHB-ATBC film at this wavenumber range at early disintegration stages (Fig. 6e). This observation could be related to some loss of interaction between both polymers with the increase in disintegration time.

Another important band was observed around 1600 cm^{-1}, corresponding to the formation of carboxylate ions by the action of microorganisms, able to consume lactic acid and PLA oligomers on the film surface leaving carboxylate ions at the chain ends [33]. This behaviour was particularly noticeable in PLA-ATBC films after 21 days under composting (Fig. 6c). It should be also highlighted that this band appeared after 7 days in PLA and plasticized PLA samples and after 14 days in plasticized PLA/PHB films, confirming that PHB helps to slow down the PLA disintegration rate under composting conditions. Two more bands corresponding to the amorphous and crystalline phases of PLA were observed at 866 cm^{-1} and 756 cm^{-1}, respectively [38]. These bands seemed to be unmodified in all samples during the whole composting test with the exception of the PLA-ATBC film, in which the band at 866 cm^{-1} clearly increased in intensity after 21 days (Fig. 6c).

TG and DTG curves (not shown) revealed the complete weight loss of PLA and plasticized PLA films in a single degradation step, while plasticized PLA/PHB blends were degraded in two steps, where the first one was assigned to the PHB decomposition and the second one, at higher temperatures, was related to the PLA thermal degradation. The initial degradation temperature (T_0) was calculated for decomposition degree (α) 0.05 and the maximum degradation temperatures (T_{max}) were calculated from TG and DTG curves. The main results are summarized in Table 1. It was observed that both degradation temperatures decreased significantly with the disintegration time. While a slight decrease (around 0.3%) was observed for T_0 of neat PLA after 7 days, plasticized PLA films showed higher reductions (around 11% in PLA-PEG and 10% in PLA-ATBC). Lower reductions were observed for PLA/PHB-PEG and PLA/PHB-ATBC blends (5% and 1% in T_0, respectively). The decrease in T_0 after 7 testing days could be related with the high plasticizer loss caused by hydrolysis during the initial disintegration stages. It was also observed that plasticizers were more efficiently retained by PLA/PHB blends than by neat PLA. After 7 days the reduction of T_{max} in PLA did not show large differences between PLA, PLA-PEG and plasticized PLA/PHB blends (around 3-5%) but PLA-ATBC showed a considerable reduction in this value, close to 20%. The T_{max} of PLA reached the maximum reduction (18-23%) after 21 days in plasticized PLA films. The corresponding peak of DTG associated with the PLA thermal degradation almost disappeared in the PLA/PHB-PEG film after 21 days, suggesting the gradual disappearance of PLA in the blend by being preferentially attacked by microorganisms during composting. Conversely, the reductions of T_{max} corresponding to the PHB thermal degradation only reached 8% after 21 days of disintegration.

Fig. 7a shows the chromatogram obtained after pyrolysis of the PLA/PHB-PEG film before composting. Py-GC/MS analysis of all films showed the typical thermal degradation products of PLA with the characteristic series of signals at m/z = 56 + (n x 72) attributed to the PLA degradation products [34]. Peaks at retention times 17.5 min and 18.5 min showed highly similar mass spectra for m/z = 56 + 72 (n=1) where the main fragments were those with m/z = 32, 43, 45 and 56 being assigned to mesolactide and (L,D)-lactide [34]. For PLA/PHB blends, the broad peak of crotonic acid showing the main fragmentation at m/z = 39, 41, 68, 69, and 86, was observed [5]. The peak at 23 min retention time was only observed in films plasticized with PEG with m/z = 32, 41, 68, 87, 103, 154, corresponding to the thermal degradation products of PEG. On the other hand, blends plasticized with ATBC showed two main peaks corresponding to tributyl propene-1,2,3-tricarboxylate (m/z = 41, 57, 112, 139, 156, 157, 168, 213 and 269) at 40.5 min and the characteristic peak of tributyl acetylcitrate (m/z = 29, 41, 57, 129, 139, 157, 185 and 259) at 42.5 min (Fig 7b and 7c).

It was observed that PLA/PHB-ATBC samples before and after 21 days under composting conditions showed the same ratio for these two peaks, while the PLA-ATBC film showed some decrease in the intensity of the tributyl propene-1,2,3-tricarboxylate peak after 21 days, suggesting that ATBC was easily released from the PLA matrix. This result is in agreement with the higher degradation rate in PLA-ATBC samples (Fig. 3). This behaviour could be explained by the preferential microorganisms attack to low molar mass fragments, which are more easily consumed when ATBC is available while these molecules are more difficult to reach in the non-plasticized PLA/PHB blend. Furthermore, smaller peaks at higher retention times also showed the characteristic series of signals of PLA (m/z = 56 + n x 72) with n = 2 and 3. Table 2 also shows the mesolactide to (D,L)-lactide ratio obtained from the Py-GC/MS chromatograms. These results showed the increase in the mesolactide fraction of all materials during composting and they could be related to the preferential selection of microorganisms to attack the L-lactide fraction of the polymer structure [39].

Nanomechanical properties were investigated with the nanoindentation technique. Only fresh samples (stored at 25ºC and 30% RH) and those after 7 disintegration days could be tested, since those after 14 and 21 days were too fragile. The calculated hardness (*H*) and reduced modulus (E_r) in depth profiles are shown in Fig. 8 Neat PLA showed the highest *H* and E_r values (around 200 MPa and 3500 MPa, respectively). The presence of either plasticizers or PHB in PLA formulations clearly reduced these parameters, even before the disintegration test, due to the plasticizer effect which reduced the inherent brittleness of both biopolymers [5]. All films after 7 testing days showed lower values than the fresh materials.

These results demonstrated that nanoindentation is a powerful tool to evaluate the loss in mechanical properties of biopolymers submitted to composting [40, 41]. The lowest values corresponded to plasticized films after 7 days, corroborating the observation of the higher degradation rate in plasticized materials. Although the reduction on mechanical properties is one of the main consequences of the degradation process occurring during composting, this process also favoured the microorganism's action. This effect could be explained since the loss in mechanical properties after 7 composting days produced a brittle material consisting in broken pieces with a high defects density, such as cracks and porous structure, permitting the easy access of microorganisms to the polymer bulk. The optical inspection of nanoindented samples (Fig. 9) revealed these defects.

Fig. 10 shows the H and E_r values averaged between 400 and 600 nm depth for plasticized and unplasticized materials. The reduction in both parameters after 7 composting days was more evident in films plasticized with PEG. While neat PLA lost around 20% in H and Er, all other materials lost approximately 50%, with the exception of the PLA/PHB-ATBC film that showed a similar behaviour than neat PLA. This result confirmed that ATBC increased the interaction between PLA and PHB and this blend showed higher mechanical resistance.

Conclusions

Formulations based on plasticized PLA/PHB blends were successfully disintegrated under composting conditions in less than one month, stating their biodegradable character. The ability of PHB to act as nucleating agent in PLA/PHB blends slowed down the PLA disintegration, while plasticizers speeded it up. TGA analysis revealed that plasticizers were mainly lost during the initial disintegration stages, while substantial losses in mechanical properties for all blends were also observed. The presence of plasticizers favoured the surface hydrolysis leading to the loss in mechanical properties, which also made disintegration easier. Py-GC/MS studies demonstrated the increase in the mesolactide form for all blends due to the high microorganism's activity during composting. In summary, plasticized PLA/PHB blends may offer good perspective for biodegradable food packaging industry by improving the polymer performance in films manufacturing and use.

Acknowledgements

Authors thank Spanish Ministry of Economy and Competitiveness by financial support (MAT2011-28468-C02-01 and MAT2011-28468-C02-02). M.P. Arrieta thanks Generalitat

Valenciana for Santiago Grisolía Fellowship (2011/007). Authors gratefully acknowledge Prof. Mª Dolores Salvador (Polytechnic University of Valencia) for her assistance with nanomechanical and optical microscope analysis.

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Figure captions

Fig. 1. Visual aspect of plasticized PLA and PLA-PHB films at different disintegration times

Fig. 2. Colorimetric parameters of plasticized PLA and PLA-PHB films before and after 7 days (7d) under composting conditions from the CIELab space: **a)** Lightness (*L*) values, **b)** yellowness index (*YI*) and **c)** a* and b* coordinates.

Fig. 3. Degree of disintegration of: control PLA film, plasticized PLA and plasticized PLA-PHB films under composting conditions as a function of time.

Fig. 4. SEM observations of neat PLA, plasticized PLA and plasticized PLA-PHB films at different degradation time under composting conditions.

Fig. 5. EDF-z profiles of films before and after 21 days under composting.

Fig. 6. Infrared spectra (2000-700 cm^{-1}) of: a) PLA, b) PLA-PEG, c) PLA-ATBC, d) PLA-PHB-PEG and e) PLA-PHB-ATBC at different disintegration times under composting conditions. f) Infrared spectra (4000-2500 cm^{-1}) of plasticized PLA-PHB blends at day 0 and 21.

Fig. 7. a) Chromatogram obtained after pyrolysis of PLA-PHB-PEG.
ATBC degradation products obtained after pyrolysis of: **b)** PLA-ATBC and **c)** PLA-PHB-ATBC before and after 21 days composting test.

Fig. 8. a) Hardness and **b)** modulus curves obtained by nanoindentation of fresh films and those after 7 days in composting conditions.

Fig. 9. Optical micrographs of the films surface of fresh films and after 7 composting days. Fresh PLA film shows the imprints of the Berckovich indenter.

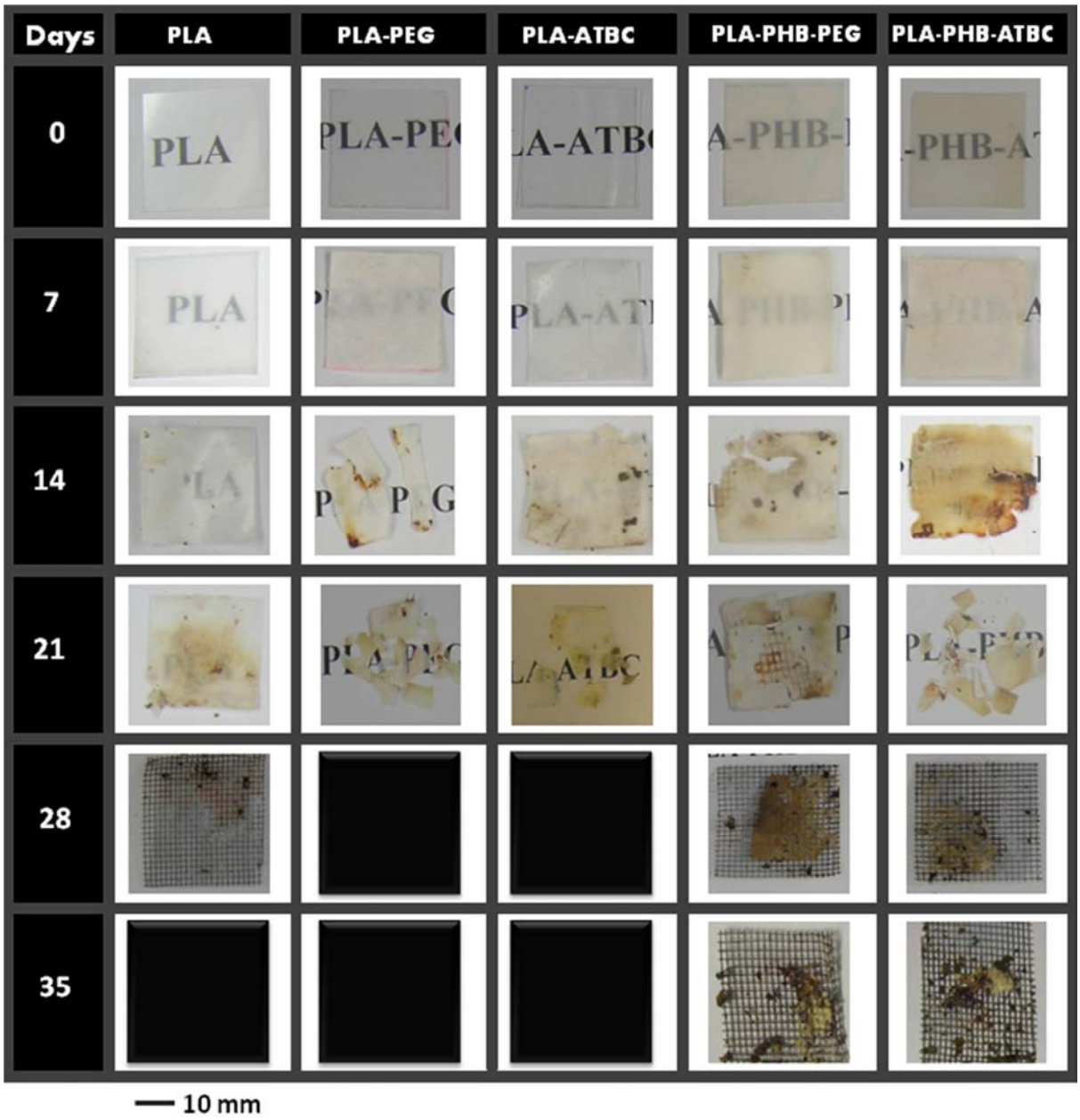
Fig. 10. Summary of H and E_r results for each film calculated (400-600 nm) in depth

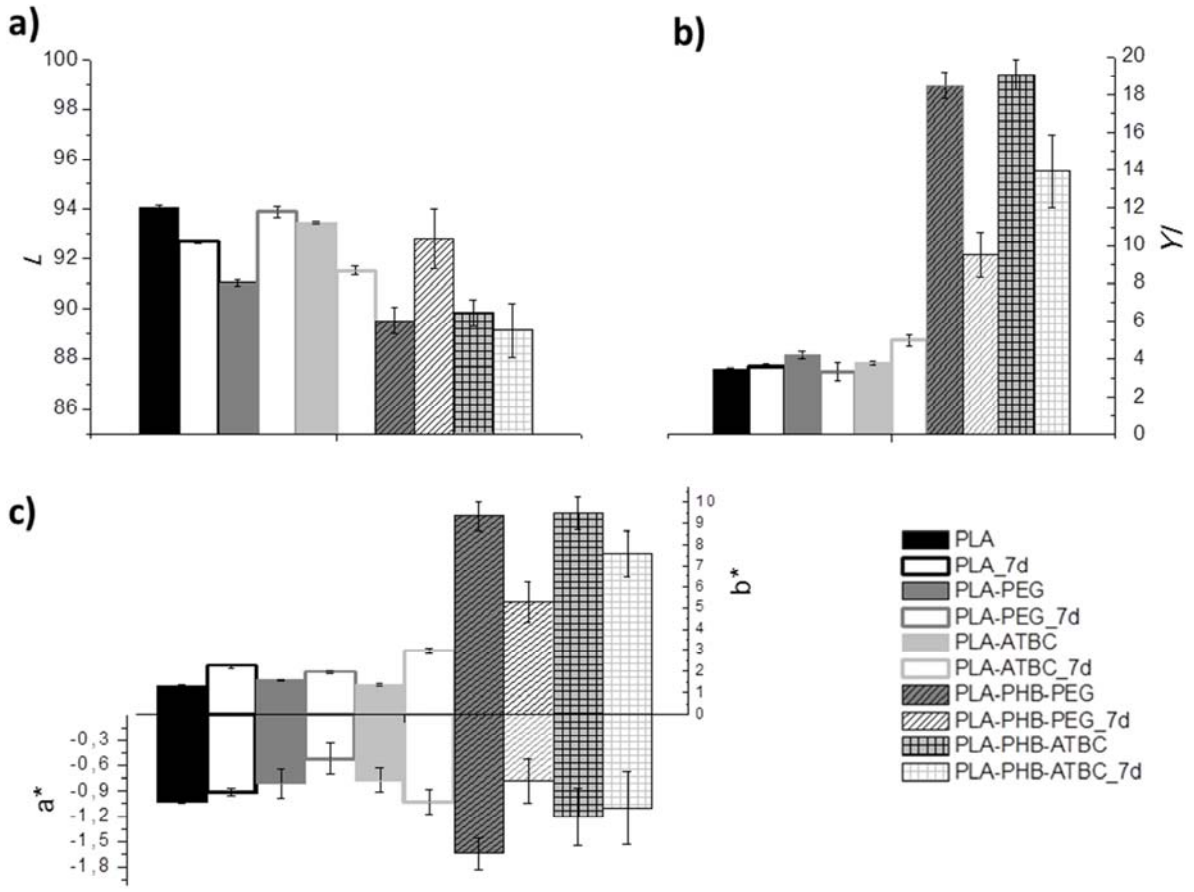
Table 1. TG and DTG parameters for films at different disintegration times

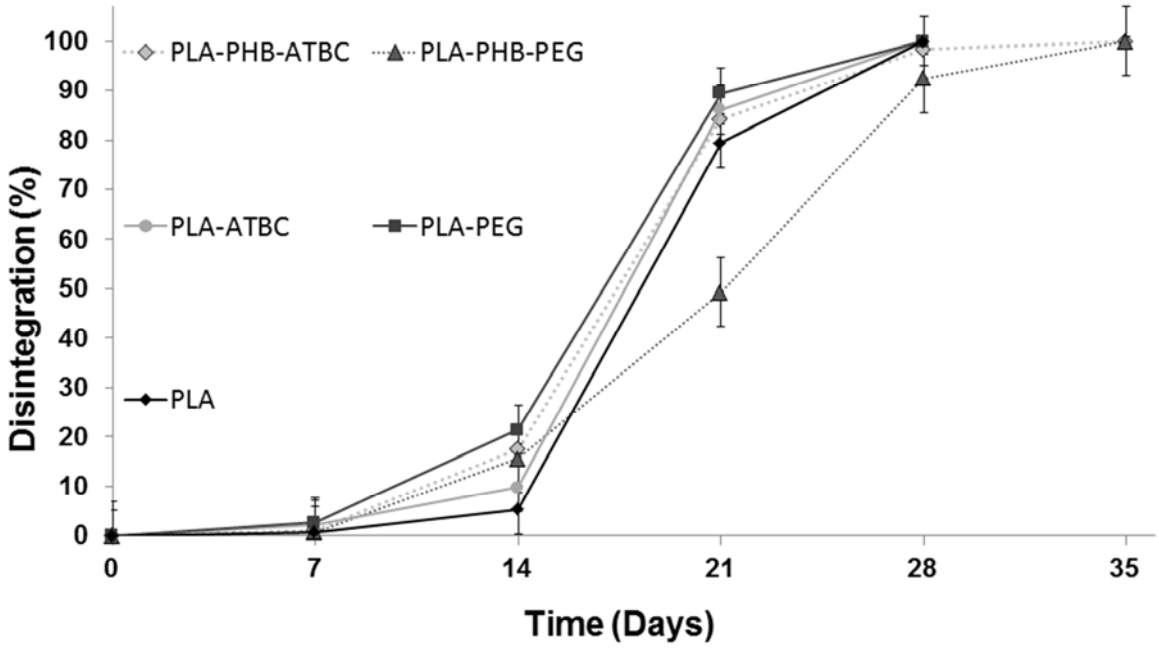
Formulation	Disintegration time (days)	T_0(ºC)	T_{max} PHB (ºC)	T_{max} PLA (ºC)
PLA	0	332	-	366
	7	331	-	363
	14	261	-	346
	21	240	-	341
PLA-PEG	0	271		366
	7	240		350
	14	233		325
	21	218		298
PLA-ATBC	0	280		364
	7	253		278
	14	228		291
	21	224		278
PLA-PHB-PEG	0	269	285	324
	7	255	280	314
	14	224	282	337
	21	221	262	-
PLA-PHB-ATBC	0	272	290	361
	7	269	290	346
	14	220	271	332
	21	220	268	285

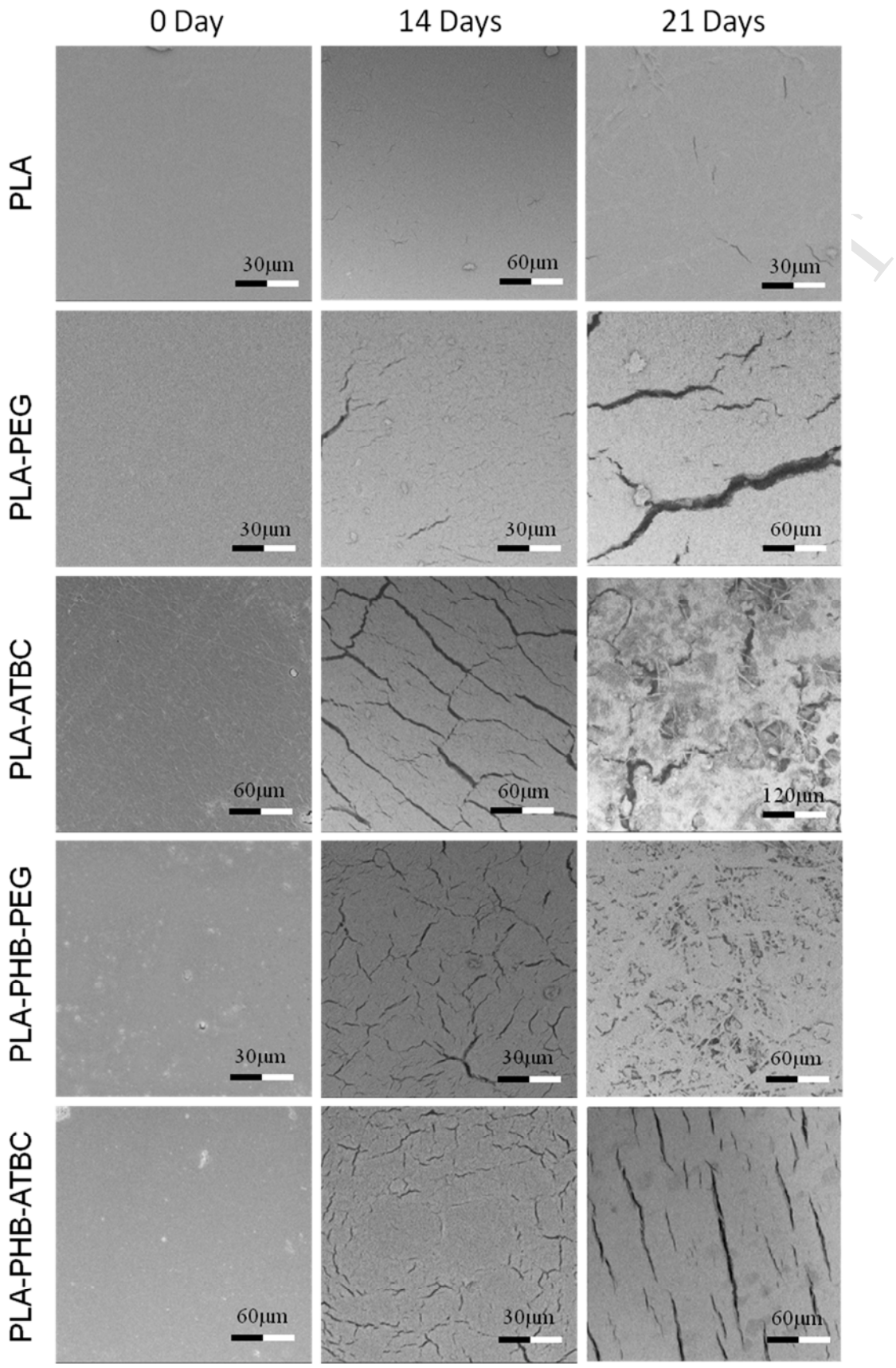
Table 2. Ratio between mesolactide and D,L-Lactide Py-GC/MS areas

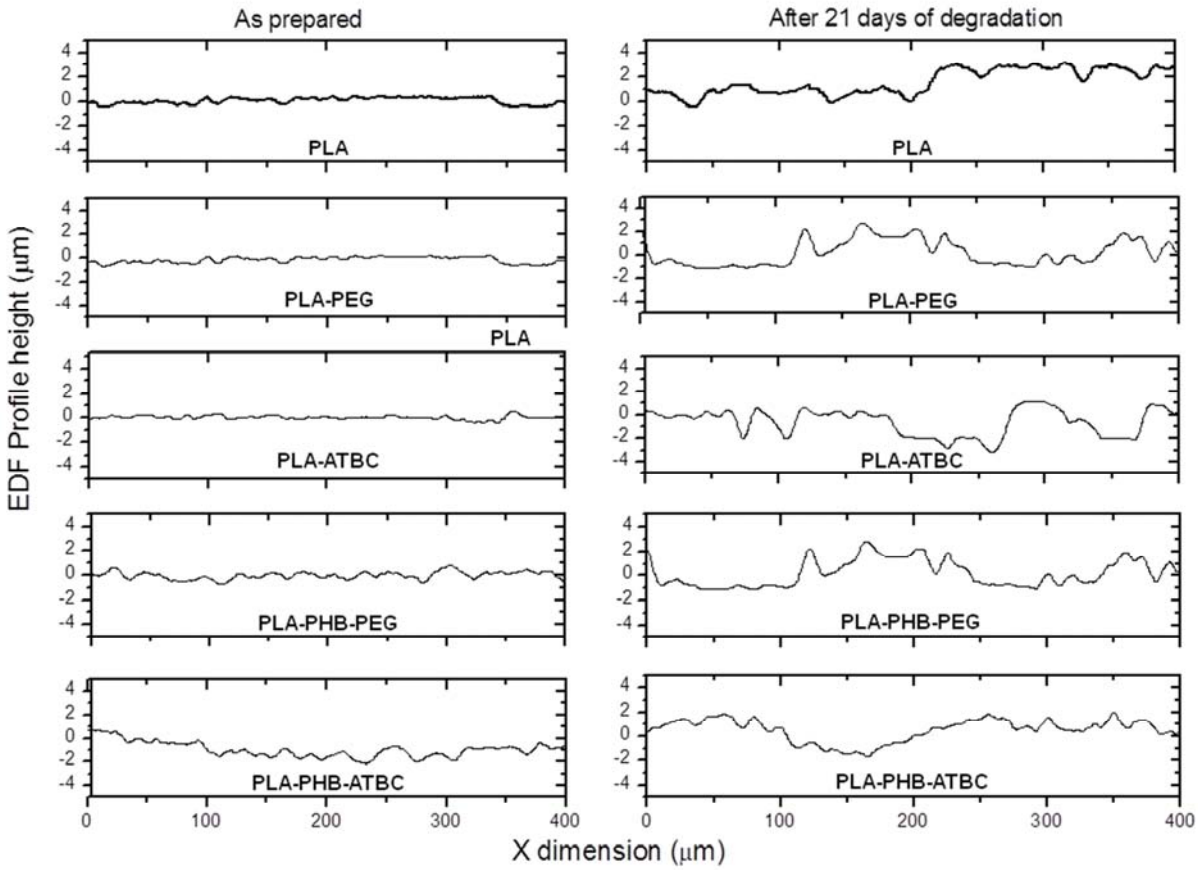
Formulation	Disintegrability day	mesolactide : D,L-Lactide
PLA	0	1 : 3.08
	21	1 : 1.66
PLA-PEG	0	1 : 7.15
	21	1 : 5.61
PLA-ATBC	0	1 : 6.33
	21	1 : 2.47
PLA-PHB	0	1 : 7.86
	21	1 : 2.62
PLA-PHB-PEG	0	1 : 7.36
	21	1 : 5.43
PLA-PHB-ATBC	0	1 : 4.43
	21	1 : 3.39

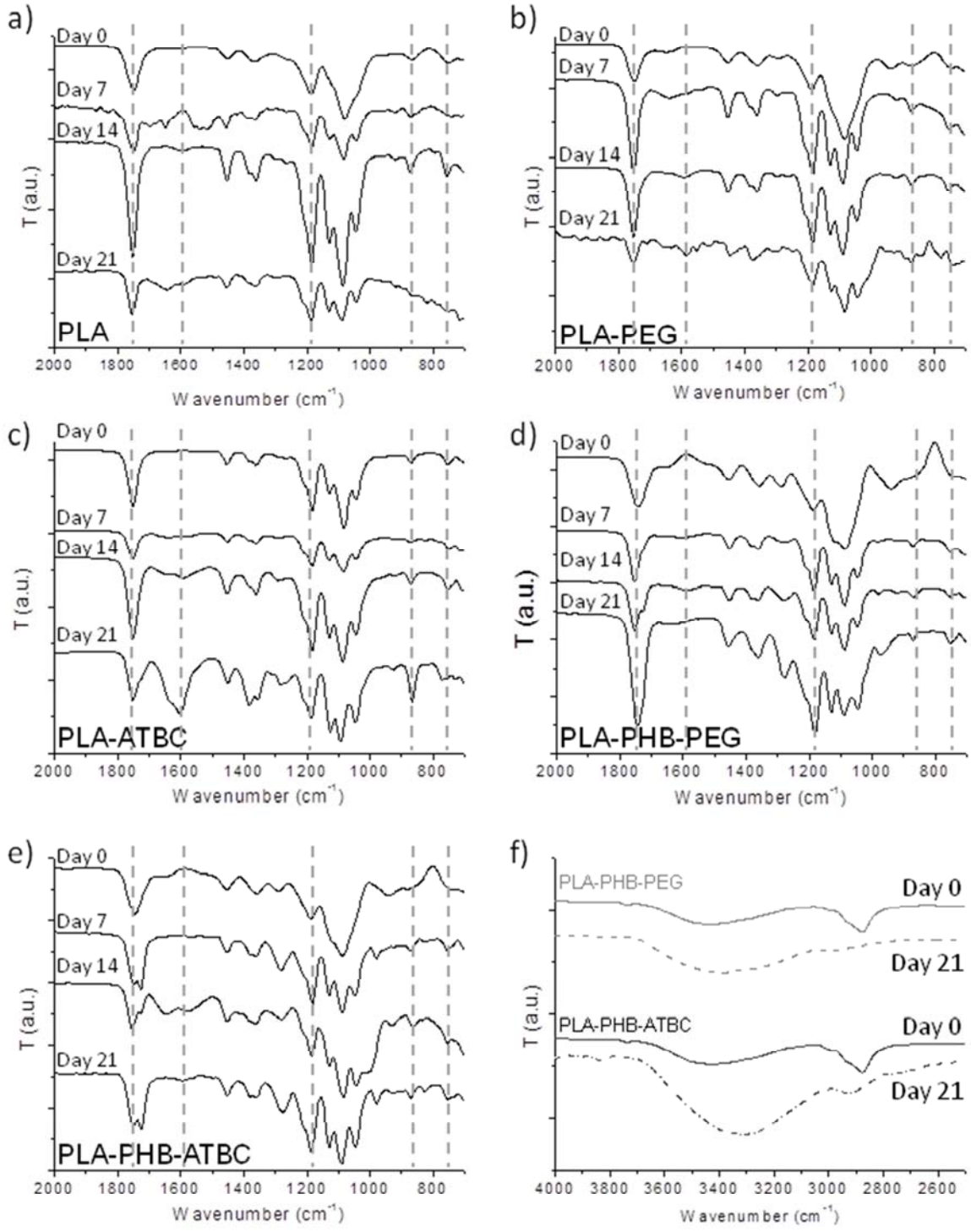

Days
PLA
PLA-PEG
PLA-ATBC
PLA-PHB-PEG
PLA-PHB-ATBC
0
7
14
21
28
35
10 mm

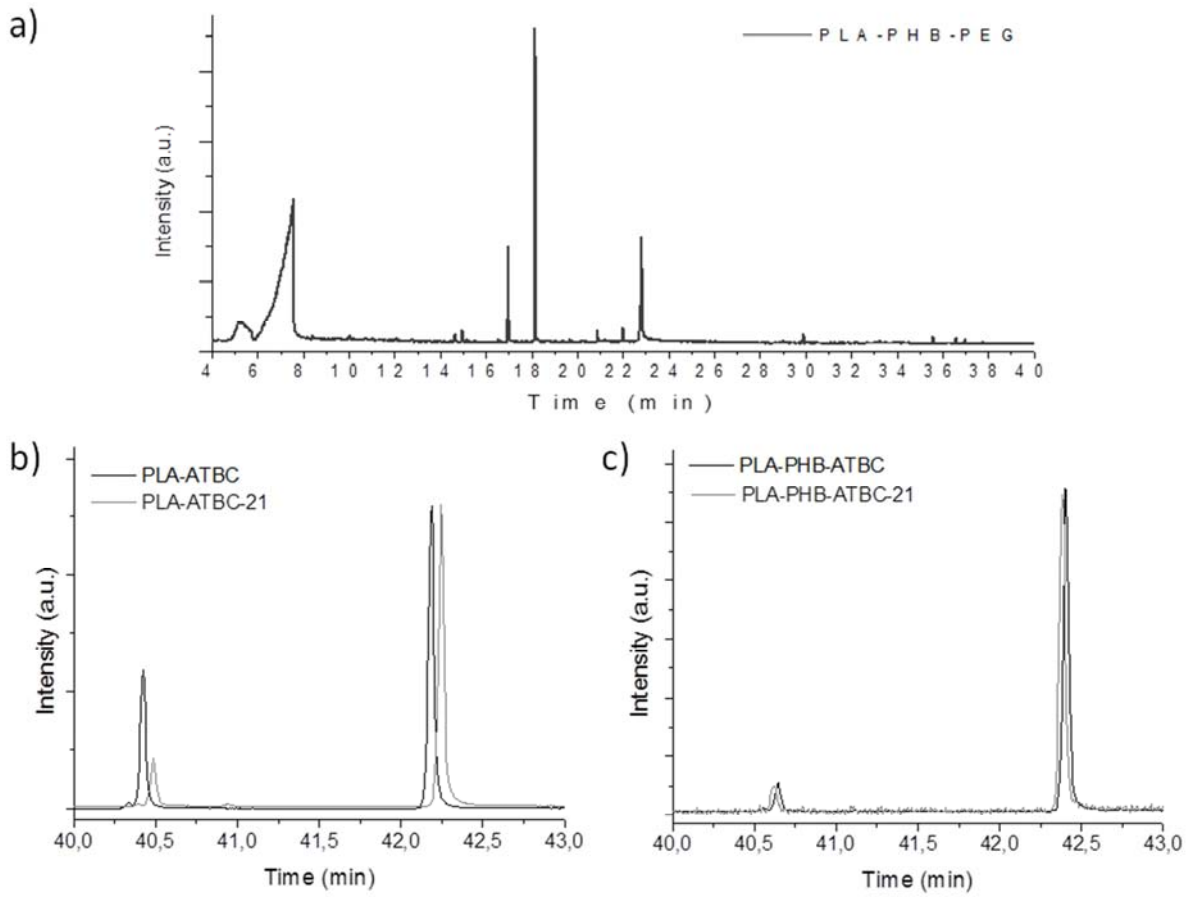




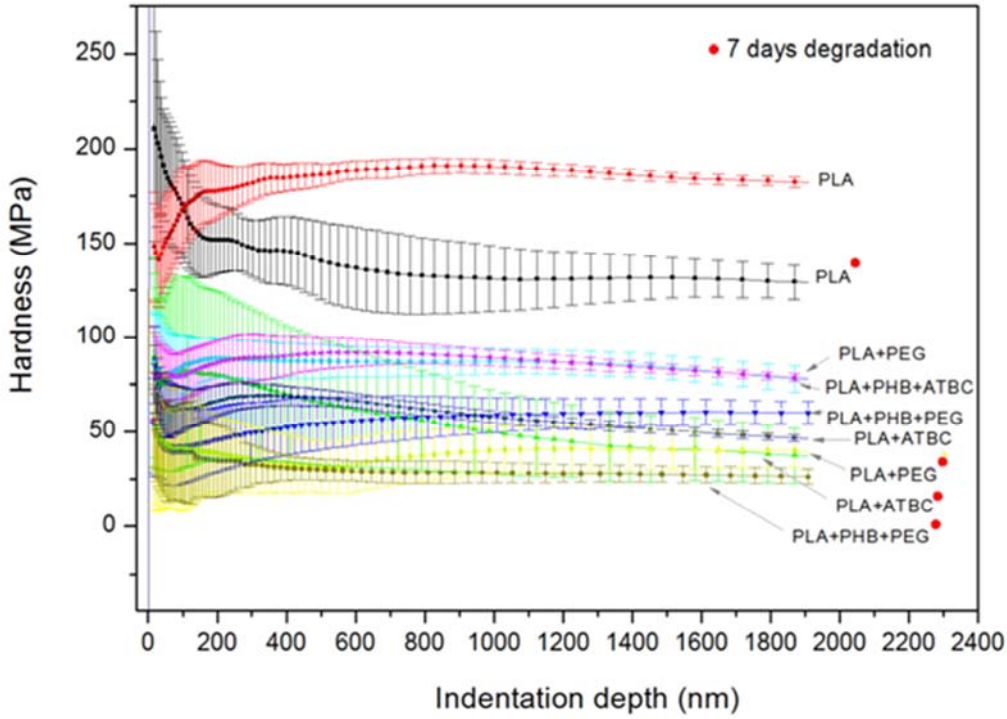

0 Day
14 Days
21 Days
PLA
PLA-PEG
PLA-ATBC
PLA-PHB-PEG
PLA-PHB-ATBC
30μm
60μm
30μm
30μm
30μm
60μm
60μm
60μm
120μm
30μm
30μm
60μm
60μm
30μm
60μm



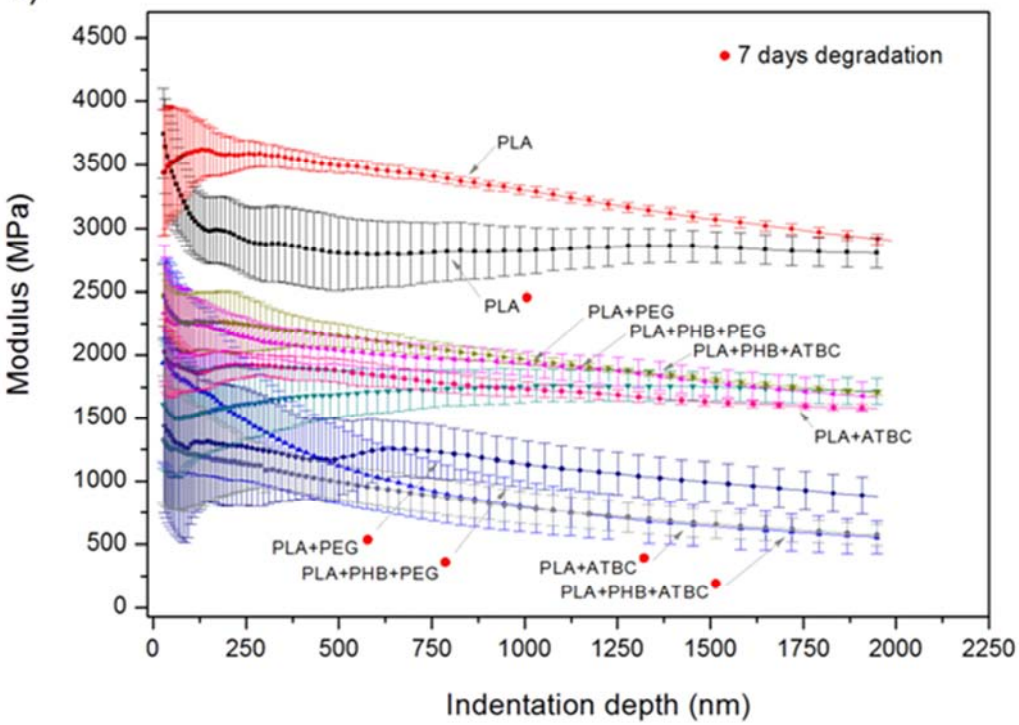

a)
Day 0
Day 7
Day 14
Day 21
PLA
T (a.u.)
2000 1800 1600 1400 1200 1000 800
W avenumber (cm-1)
b)
Day 0
Day 7
Day 14
Day 21
PLA-PEG
T (a.u.)
2000 1800 1600 1400 1200 1000 800
W avenumber (cm-1)
c)
Day 0
Day 7
Day 14
Day 21
PLA-ATBC
T (a.u.)
2000 1800 1600 1400 1200 1000 800
W avenumber (cm-1)
d)
Day 0
Day 7
Day 14
Day 21
PLA-PHB-PEG
T (a.u.)
2000 1800 1600 1400 1200 1000 800
W avenumber (cm-1)
e)
Day 0
Day 7
Day 14
Day 21
PLA-PHB-ATBC
T (a.u.)
2000 1800 1600 1400 1200 1000 800
W avenumber (cm-1)
f)
PLA-PHB-PEG
Day 0
Day 21
PLA-PHB-ATBC
Day 0
Day 21
T (a.u.)
4000 3800 3600 3400 3200 3000 2800 2600
W avenumber (cm-1)

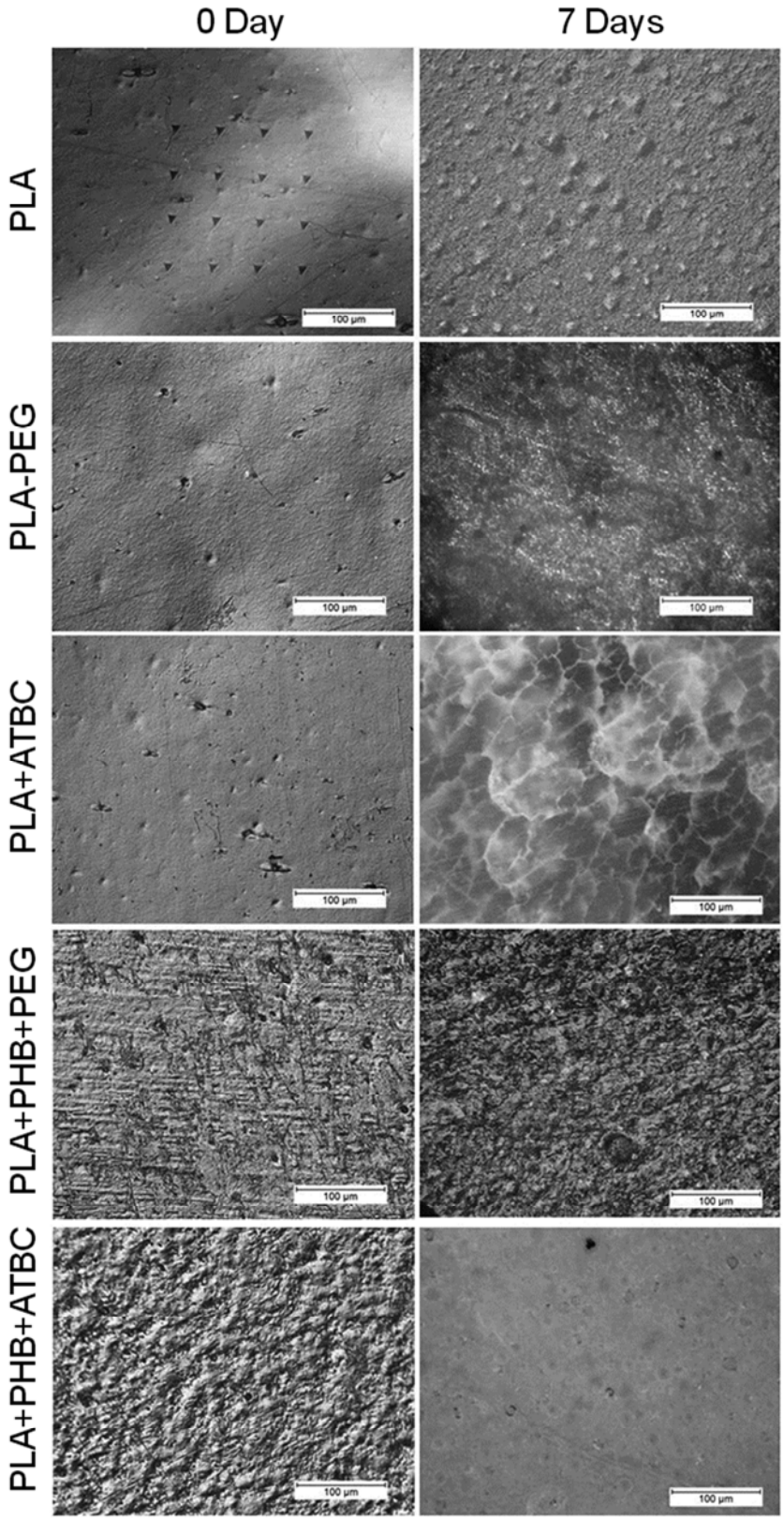


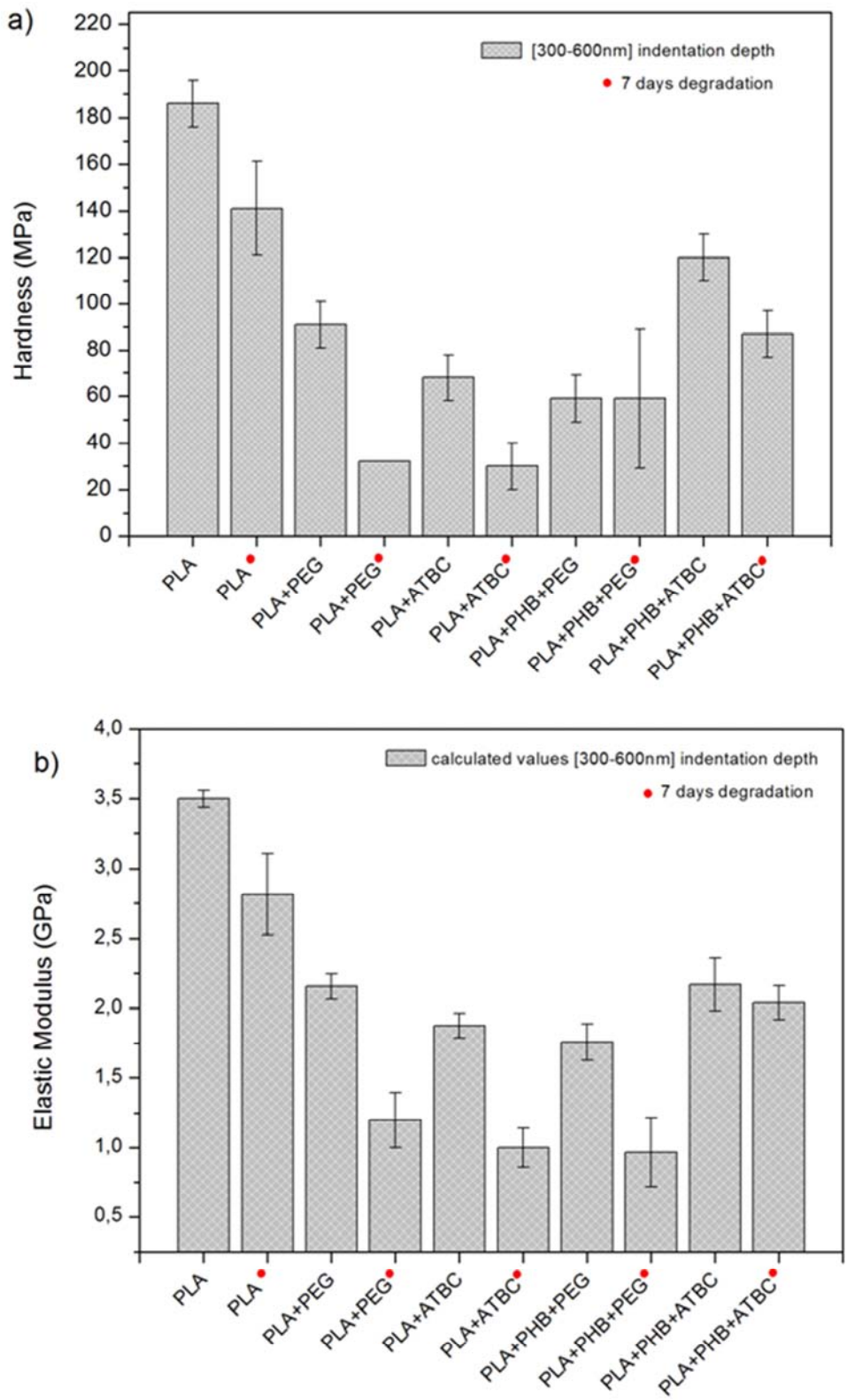
a)


7 days degradation
Hardness (MPa)
Indentation depth (nm)
PLA
PLA
PLA+PEG
PLA+PHB+ATBC
PLA+PHB+PEG
PLA+ATBC
PLA+PEG
PLA+ATBC
PLA+PHB+PEG

b)


7 days degradation
Modulus (MPa)
Indentation depth (nm)
PLA
PLA
PLA+PEG
PLA+PHB+PEG
PLA+PHB+ATBC
PLA+ATBC
PLA+PEG
PLA+PHB+PEG
PLA+ATBC
PLA+PHB+ATBC


0 Day
7 Days
PLA
PLA-PEG
PLA+ATBC
PLA+PHB+PEG
PLA+PHB+ATBC
100 µm


a)
Hardness (MPa)
[300-600nm] indentation depth
7 days degradation
0
20
40
60
80
100
120
140
160
180
200
220
PLA
PLA
PLA+PEG
PLA+PEG
PLA+ATBC
PLA+ATBC
PLA+PHB+PEG
PLA+PHB+PEG
PLA+PHB+ATBC
PLA+PHB+ATBC
b)
Elastic Modulus (GPa)
calculated values [300-600nm] indentation depth
7 days degradation
0,5
1,0
1,5
2,0
2,5
3,0
3,5
4,0
PLA
PLA
PLA+PEG
PLA+PEG
PLA+ATBC
PLA+ATBC
PLA+PHB+PEG
PLA+PHB+PEG
PLA+PHB+ATBC
PLA+PHB+ATBC