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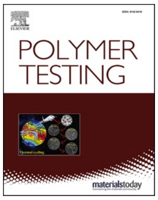

Glycerol-based additives of poly(3-hydroxybutyrate) films

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ABSTRACT

Glycerol (G), polyglycerols (PGs), glycerol triacetate (GTA), and glycerol tributyrate (GTB) were studied as additives of poly(3-hydroxybutyrate) (PHB). The effect of different concentrations (5–30%) of these compounds on microstructure and final properties of PHB films were evaluated. Microscopic studies revealed that samples with GTA and GTB presented uniform microstructure, meanwhile those containing G and PGs evidenced additives exudation. PHB exhibited a ring banded spherulitic structure and these additives, mainly G and PGs, induced modifications during polymer isothermal crystallization. All studied additives decreased PHB melting temperature, facilitating its thermal processability. GTA and GTB addition induced a reduction in PHB glass transition temperature, demonstrating their plasticizing effect. PHB films with GTA and GTB showed the best mechanical performance since they presented the highest deformation at break and the lowest maximum tensile strength, especially those with 30% GTB. Otherwise, G and PGs addition decreased films mechanical properties due to a prodegradative effect induced by them. GTB and PGs increased films water vapor permeability, meanwhile G and GTA decreased this barrier property.

1. Introduction

In the plastic industry, it is essential to incorporate additives, which are physically dispersed in the polymeric matrix, in order to modify the final properties and to obtain materials with different functionalities and useful products [1]. Additives are classified according to their function and not in relation to their chemical structure and composition [2]. Stabilizers and lubricants facilitate polymers processing. If the purpose is to modify mechanical properties, plasticizers, reinforcing fillers, or impact modifiers can be incorporated [3]. On the other hand, the cost of formulations can be reduced by adding loads, diluents, and extenders [4]. Surface properties can be modified using anti-static agents, anti-slip additives, anti-wear additives, and adhesion promoters [5]. Pigments, dyes, and nucleating agents are usually incorporated to modify optical properties [6]. Polymer aging can be controlled by stabilizers [7], while other additives can be foaming agents and flame retardants [8]. All additives must meet some technical requirements. In general, they are expected to be highly effective so that the proposed objectives are achieved at an economically acceptable concentration [9]. Besides, high polymer-additive miscibility and compatibility at molecular level are expected [10]. In addition, they should not be volatile under processing conditions, because it would form a thin layer onto the polymer surface. Another important requirement is that they should not phase separate during the material shelf life, as it would lead to aesthetic problems and loss of effectiveness. Insoluble inorganic additives such as pigments and fillers do not give rise to exudation phenomena, while low molecular weight plasticizers have more tendencies to migrate to the surface and exudate during processing and subsequent aging [7].

For several bio-based polymers and biopolymers, glycerol is the most common additive [11] being widely used in processing of poly(lactic acid) (PLA) [12] and thermoplastic starch (TPS) [13], among others. Glycerol combines the advantages of water (low toxicity, low price, large availability, and renewability) and ionic liquids (high boiling point and low vapor pressure) [14]. It has been recently proposed as a valuable green solvent because it is an organic waste generated by the biodiesel industry [15]. It can be obtained from fats and oils in biodiesel plants by transesterification, a chemical reaction whereby triglycerides react with alcohol in the presence of a catalyst to produce fatty acid

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methyl esters with glycerol as a by-product in abundant quantities [15]. Glycerol triesters (GTs) are another interesting option as plasticizers for biopolymers. These additives present the empirical formula $CH_2(OOR_1)CH(OOR_2)CH_2(OOR_3)$ where R_1, R_2, and R_3 are different or equal alkyl or aryl groups [16]. They are synthesized by esterifying glycerol with a mixture of acids in the presence of a catalyst. These compounds have excellent thermostability and low volatility and are used to modify the properties of a wide variety of polymers [17]. On the other hand, polyglycerols (PGs) are a new class of polymer additives, although they have been known since the early 2002. They represent the first hyperbranched polymers that can be prepared in a controlled synthesis [18]. PGs present a globular structure conformed by highly branched macromolecules with many functional groups. They are characterized by the combination of stability, biocompatibility, high-end groups functionality, and a compact well-defined dendrimer-like architecture [19]. These characteristics can be used to generate materials with new properties, especially for biomedical applications [20,21]. Regarding processing, PGs have considerably higher thermal stability compared to glycerol which allows higher polymer processing temperatures, for example in starch-based biodegradable thermoplastic composites [21].

Currently, processing of bio-based polymers could contribute to lowering the sustainability issues and environmental challenges posed by the production and disposal of synthetic plastics. However, large-scale commercial deployments of bio-based materials to replace conventional polymers remain challenged by several factors. Some of the challenges are attributed to the relatively poor performance, variability of properties with the feedstock, high production cost, and lack of infrastructure. In recent years, numerous research works have been focused on the valorization of biodegradable polymers and significant advances have been made on the obtaining of eco-friendly materials for several applications. Sustainability, industrial ecology, eco-efficiency, and green chemistry are guiding the development of the next generation of materials, products, and processes. Nowadays, there is a rapidly growing interest in biomaterials for a variety of biological and medical applications. Among biopolymers, poly(hydroxyalkanoate)s, PHAs, are very interesting candidates. Particularly, poly(3-hydroxybutyrate) (PHB) is the most extensively studied polymer among the isolated PHAs. Despite PHB is an ideal candidate to produce biodegradable materials, its thermal processing is difficult because of the polymer's relatively low degradation temperature near its melting point, pronounced brittleness, very low deformability, and susceptibility to a rapid thermal degradation [22,23]. For those reasons, the incorporation of different additives compatible with this biopolymer are being studied in order to evaluate the most adequate option from both economical and industrial point of view.

The main objective of this work was to study the efficiency of glycerol-based compounds, such as glycerol, polyglycerols, and glycerol triesters, as PHB additives. It was evaluated the effect of these additives on morphology and crystalline structure of PHB films as well as on thermal, mechanical, and barrier properties by different characterization techniques.

2. Materials

Poly(3-hydroxybutyrate) powder (PHB) was purchased from Biomer (Germany). Additives used as PHB additives were: glycerol (G - 99.5% purity - Ciccarelli, Argentina), glycerol tributyrate (GTB – 98.5% purity - Sigma - Aldrich, Switzerland), and glycerol triacetate (GTA - 99.5% purity - Sigma-Aldrich, Switzerland). Two polyglycerols (PGs) with different number average molecular weight (M_n): PG_{1174} (1174 g/mol) and PG_{5040} (5040 g/mol) were also studied. PGs were synthesized at the Laboratoire de Photochimie et d'Ingénierie Macromoléculaires (LPIM), Equipe Chimie et Physico-Chimie des Polymères Institut Jean-Baptiste Donnet, Mulhouse, France and they were obtained by a strategy based on the Ring-Opening Multibranching Polymerization (ROMBP) of glycidol using slow monomer-addition conditions [24]. Hyperbranched aliphatic polyethers with controlled molecular weights and narrow molecular weight distribution were prepared via anionic polymerization of glycidol with rapid anion-exchange equilibrium [24].

IUPAC nomenclature, molecular formula, molecular weight (M_w), and boiling temperature (T_b) of all additives employed in this study are shown in Table 1.

3. Experimental

3.1. Materials processing

PHB powder and additives were manually mixed in order to well-impregnate the polymer. Studied formulations and their names are described in Table 2. Mixtures were melt-processed in a Bradender Plastograph (Germany) at 180 °C and 60 rpm, for 15 min.

3.2. Films preparation

Films of melt-processed samples were obtained by thermo-compression using a hydraulic press at 190 °C and 5 kg cm^{-2}, for 15 min. Thickness of films was measured using an electronic digital caliber MAX-CAL (Fowler & NSK, Japan). At least 10 measurements were taken for each specimen and obtained values correspond to the average thickness.

3.3. Films characterization

3.3.1. Structural aspects

Reflected Optical Microscopy (ROM) was carried out to evaluate additives exudation from PHB matrix. For this study, an optical microscope Karls Zeiss (Germany) in the reflection mode was employed. Transparent specimens, generally 0.1 mm thick, were observed at a magnification of 264× and images were acquired using a microscope equipped with digital camera Leica DFC280.

The effect of additives on PHB morphology was studied by Polarized Optical Microscopy (POM) using a polarized optical microscope Karl Zeiss (Germany), equipped with crossed polarizers, a hot stage (Mettler FP5), and a temperature controller (Mettler). A similar procedure was employed by Gunaratne and Shanks [28]. Thin films, mounted onto glass slides and covered with coverslips, were heated from room temperature to 195 °C at 10 °C/min. Samples were kept at 195 °C for 5 min and then cooled to 25 °C at 10 °C/min. Isothermal crystallization behavior was observed at magnifications of 160×, 400X, and 640X by maintaining samples at 25 °C for 1 h. Micrographs were taken using a digital camera Leica DFC280 attached to the microscope.

Surfaces homogeneity and appearance of PHB films, as well as the effect of studied additives on materials microstructure were evaluated by Scanning Electron Microscopy (SEM). This study was performed

Table 1
Physical and molecular properties of glycerol, glycerol tributyrate, glycerol triacetate, and polyglycerols used as PHB additives.

Additive	IUPAC nomenclature	Molecular formula	Molecular weight (g/mol)	PDI	T_b (°C)
Glycerol (G)	Propane-1,2,3-triol	C_3H_8O	92.09 (M_w)	NA	290 [25]
Glycerol tributyrate (GTB)	2,3-di (butanoyloxy) propyl butanoate	$C_{15}H_{26}O_6$	302.3 (M_w)	NA	288 [26]
Glycerol triacetate (GTA)	2,3-diacetyloxypropyl acetate	$C_9H_{14}O_6$	218.2 (M_w)	NA	258 [27]
Polyglycerol (PG)	NA	NA	1174 (M_n) 5040 (M_n)	1,2 1,6	NA

NA: not available, M_w: Molecular weight., M_n: Average molecular weight in number.

Table 2

Formulations based on poly(3-hydroxybutyrate) (PHB) and additives: glycerol (G), glycerol tributyrate (GTB), glycerol triacetate (GTA), and polyglycerols (PG_{1174} and PG_{5040}).

Sample	PHB (g)	G (g)	GTA (g)	GTB (g)	PG_{1174} (g)	PG_{5040} (g)	Concentration (%, w/w)
PHB	45.0	–	–	–	–	–	0
PHB-5G	42.5	2.25	–	–	–	–	5
PHB-10G	40.5	4.50	–	–	–	–	10
PHB-5GTA	42.5	–	2.25	–	–	–	5
PHB-10GTA	40.5	–	4.50	–	–	–	10
PHB-20GTA	36.0	–	9.00	–	–	–	20
PHB-30GTA	31.5	–	13.5	–	–	–	30
PHB-5GTB	42.5	–	–	2.25	–	–	5
PHB-10GTB	40.5	–	–	4.50	–	–	10
PHB-20GTB	36.0	–	–	9.00	–	–	20
PHB-30GTB	31.5	–	–	13.5	–	–	30
PHB-5PG_{1174}	42.5	–	–	–	2.25	–	5
PHB-10PG_{1174}	40.5	–	–	–	4.50	–	10
PHB-5PG_{5040}	42.5	–	–	–	–	2.25	5
PHB-10PG_{5040}	40.5	–	–	–	–	4.50	10

using an electron microscope JEOL JSM-35 CF (Japan), with a secondary electron detector. Films were mounted onto bronze stubs, coated with a gold layer using an argon plasma metallizer (sputter coater PELCO 91000), and observed at a magnification of 20000×.

Diffractograms were obtained with an X-ray diffractometer Philips PW1710 (Holland), provided with a tube, a copper anode, and a detector operating at 45 kV and 30 mA within 2Θ from 5 to 60°.

3.3.2. *Thermal properties*

Thermal analysis was performed by Differential Scanning Calorimetry (DSC) using a calorimeter TA Instrument Discovery Series (USA), under nitrogen atmosphere. Samples (~10 mg) were first heated from −90 to 195 °C at a heating rate of 10 °C/min (run 1). After being kept at 195 °C for 2 min, samples were cooled to-90 °C at 10 °C/min and kept at this temperature for 2 min. They were again heated at a rate of 10 °C/min to 195 °C (run 2). DSC curves were recorded and from those thermograms the following parameters were obtained: melting temperature (T_m), enthalpy of melting (ΔH_m), and glass transition temperature (T_g). Degree of crystallinity of PHB ($X_{C\text{-}DSC}$) was determined using Equation (1), according to the procedure described by Wellen et al. [29].

$$X_{C-DSC}(\%) = \frac{\Delta H_m}{\Delta H_m^0 \times w_{PHB}} \times 100 \qquad [1]$$

where ΔH_m is the melting enthalpy of the sample, $\Delta H^0{}_m$ is the melting enthalpy of a 100% crystalline PHB (146 J/g) [30] and w_{PHB} is the weight fraction of PHB in the sample.

Thermal degradation was evaluated by Thermogravimetric Analysis (TGA) using a thermogravimetric balance TA Instrument Discovery Series (USA). Samples (~10 mg) were heated from 25 to 350 °C at 10 °C/min, under nitrogen atmosphere (20 mL/min). Curves of mass percentage as function of temperature were recorded, and the maximum decomposition temperature was obtained from the first derivative curve (DTGA), in accordance with the methodology proposed by Seoane et al. [31].

3.3.3. *Tensile properties*

Tensile tests were carried out with an Instron Tensile Testing Instrument model 3369 (UK), using a crosshead speed of 5 mm/min and a load cell of 1 kN. At least ten probes of 13 mm × 100 mm of each film formulation were assayed and stress-strain curves were obtained from load-displacement data. Maximum tensile strength (σ_m), Young's modulus (E), and elongation at break (ϵ_B) were calculated, according to ASTM D882-91 standard method.

3.3.4. *Barrier properties*

A Permatran W3/33 (Mocon Inc., USA) was used to determine Water Vapor Permeability (WVP), according to the methodology established in the ASTM F-1249 standard method. Film samples were placed in a test cell, which is divided into two chambers separated by the specimen. The inner chamber is filled with nitrogen (carrier gas) and the outer chamber with water vapor (test gas). Water molecules diffuse through the film to the inside chamber and WVP is registered. Measurements were carried out at 25 °C and films were subjected to a partial water vapor pressure gradient. Masking specimens with precut aluminum foil were used to avoid saturation leaving an uncovered film area of only 5 cm^2.

4. Results and discussion

4.1. *Macroscopic observation*

All studied formulations could be processed by melt mixing and thermo-compression, obtaining films with different macroscopic appearance. Pure PHB samples were difficult to manipulate due to the fragile nature of this biopolymer. Other authors reported similar appearance of PHB samples obtained by different processing methods [32,33]. Additives incorporation to the PHB matrix allowed obtaining specimens with different features at macroscopic level, depending not only on the type of additive but also on its concentration. Films based on PHB with 20 and 30% of G and PGs exhibited a clear phase separation due to a significant additive exudation from the polymer matrix. This phenomenon led to sticky and difficult to handle materials. For this reason, these samples were not characterized not only because it is complicated to assay materials with these characteristics but also due to the possibilities of applying them are scarce.

4.2. *Microstructural characterization*

ROM micrographs and SEM images of PHB samples and films with the studied additives are shown in Fig. 1. ROM and SEM micrographs of pure PHB revealed that films had a very smooth and uniform surface morphology, without the occurrence of porous (Fig. 1a), similar to previous results reported for PHAs films [33]. Alike, Rastogi and Samyn [34] reported that PHB films presented a clean, smooth, and compact surface. ROM micrographs of samples containing G revealed the presence of drops on film surfaces, even in the formulation with the lowest studied concentration (5%, w/w). Accordingly, SEM images showed numerous defects, mostly holes of diverse shapes and sizes (Fig. 1b). Jost and Langowski [35] described a similar morphology for extruded poly (3-hydroxybutyrate-co-3hydroxyvalerate), PHBV, films plasticized with glycerol. These authors attributed the observation to additive drops evaporation by the electron beam during sample observation. Other additives also cause the occurrence of holes or pores in PHB films. For

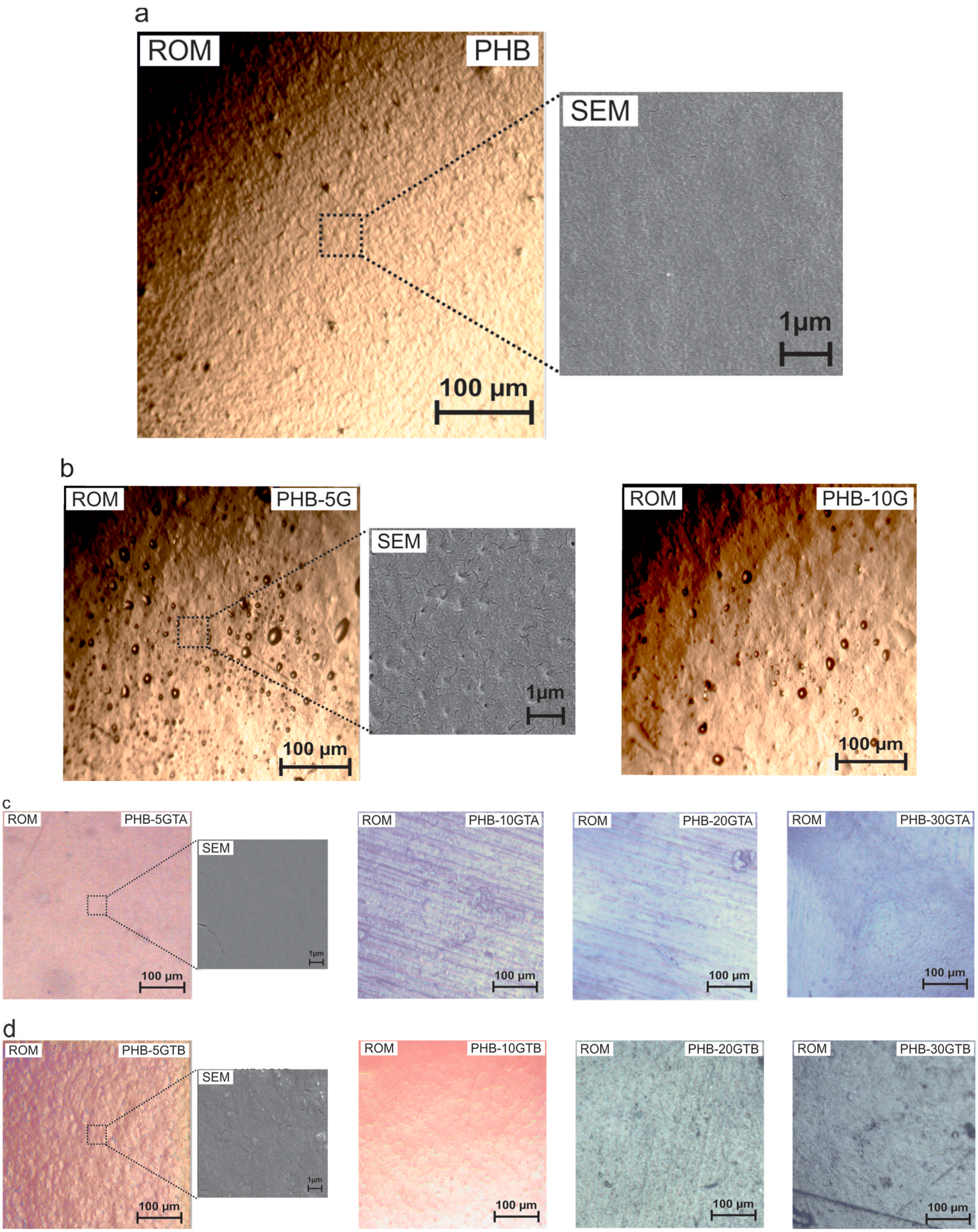

Fig. 1. ROM and SEM micrographs of films surfaces of a) polyhydroxyalcanoate (PHB), b) PHB with glycerol (G), c) PHB with glycerol triacetate (GTA), d) PHB with glycerol tributyrate (GTB), e) PHB with polyglycerol 1174 (PG_{1174}), and f) PHB with polyglycerol 5040 (PG_{5040}).

example, Lópes et al. [36] reported that PHB films containing poly (ethylene glycol), PEG, presented micropores along the cross-section, which became more pronounced as PEG content increases. Besides, films containing G and PGs presented certain roughness. Regarding PHB samples with GTA and GTB, ROM micrographs evidenced the absence of free additive on their surfaces and SEM images were in accordance with these observations since no holes were detected (Fig. 1c and d). The lack of drops was observed in all studied concentrations of GTA and GTB (5,

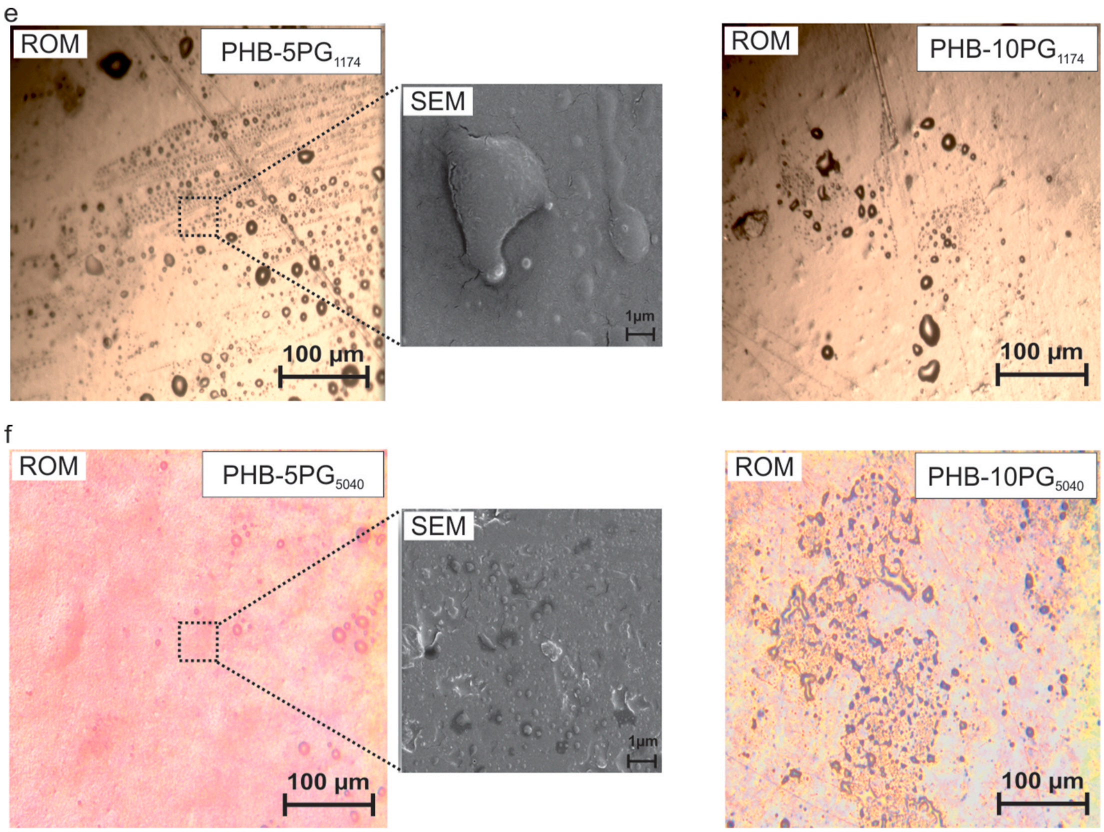

Fig. 1. (*continued*).

10, 20, and 30%, w/w). Similarly, Seoane et al. [33] reported that PHB nanocomposites with 20% GTB showed cross sections similar to neat PHB. Besides, the addition of GTs induced less rough PHB films. In accordance with those observation, Requena et al. [37] stressed that the addition of fatty acids (lauric acid or stearic acid) enhanced the roughness of PHB films surface fracture, thus indicating a different rearrangement of the polymer molecules due to the specific interactions with these less polar plasticizers. In the case of PHB films with PGs, they exhibited additive migration to the materials surface, regardless of the molar mass of the polyglycerol and its concentration (Fig. 1e and f). This phenomenon could be appreciated in ROM micrographs by the presence of additive drops, as well as in SEM images by the occurrence of holes due to evaporation. In accordance with Menčík et al. [38], additive migration is the main disadvantage of the polymer plasticizing process, leading to a loss of material elasticity and ductility. This phenomenon involves the additive diffusion from bulk towards the material surface and its evaporation [39]. It is important to highlight that additive leakage from the material can be the cause of the surrounding media contamination, which is relevant if this material will be used for food or biomedical application, among others. Many factors affect the additive migration such as its type and concentration, molar mass, branching, and polarity. External attributes of incompatibility between polymer and additives cause tackiness or exudation [10], as in the case of samples containing G and PGs. This phenomenon may cause dirt pick up and marring of the final materials.

Crystallization is a process associated with partial alignment of polymer chains, starting from nucleation and followed by subsequent growth [40]. Many properties (mechanical, optical, electrical, etc.) depend on the detailed structure of crystalline materials. Thus, it is highly important to understand how crystals grow and how different morphologies result from growth processes which can be controlled via variables such as temperature or plasticizer concentration [41]. Polymer crystallization can be studied by isothermal and non-isothermal mode. Isothermal crystallization is the most useful for theoretical analysis and comparison with experimental results. However, non-isothermal crystallization has more practical value from an industrial point of view since processing, such as extrusion and thermoforming, usually occur under non-isothermal conditions [41]. Fig. 2 shows POM photographs of PHB spherulites after isothermal melt crystallization at 25 °C for 1 h. As it can be observed, morphology of pure PHB exhibited a ring banded spherulitic structure with the characteristic maltese cross. Similar morphology is typical for traditional [42], poly(3-hydroxybutyrate) (PHB) [43–45], and poly(3-hydroxybutyrate-co-3 hydroxyvalerate) (PHB-co-HV) [46]. It is generally believed that the formation of ring banded spherulites is attributed to the periodical lamellae twisting along the radial growth direction of the spherulites [47]. When GTA and GTB were added, different band spacing of the spherulitic structure was evidenced regardless of the additive concentration (Figs. 3 and 4). Similar observation was reported by Seoane et al. [33]. Band spacing reduction could be attributed to the existence of stress build up during crystallization [48]. According to Ma et al. [49], regularity is related with the total intermolecular interaction between the two components, which causes a significant modification of crystal growth processes.

In case of films with G, the PHB degradation induced by this additive presence perhaps is the responsible that causes distortion of crystal forms and it may also provokes chemical changes in polymer chains (Fig. 5a and b). Regarding films containing PGs, lamellae on the top surface of spherulite appears irregularly twisted and bended. There are no longer continuous ring bands, but only irregular spots of bulges versus concave regions, as already reported by Hsieh and Woo [50]. This morphology can be seen in Fig. 5c, d, and 5e for PHB-5PG$_{1174}$, PHB-5PG$_{5040}$, and PHB-10PG$_{5040}$, respectively. Lenticular-shape radial cracks are visible only on the ridge band (bulge band) of spherulites on the top surface and they are oriented in the radial direction. The ridge band is composed of parallel lamellae plates that are perpendicular to top surface of polymer films; thus, cracks between the parallel edge-on

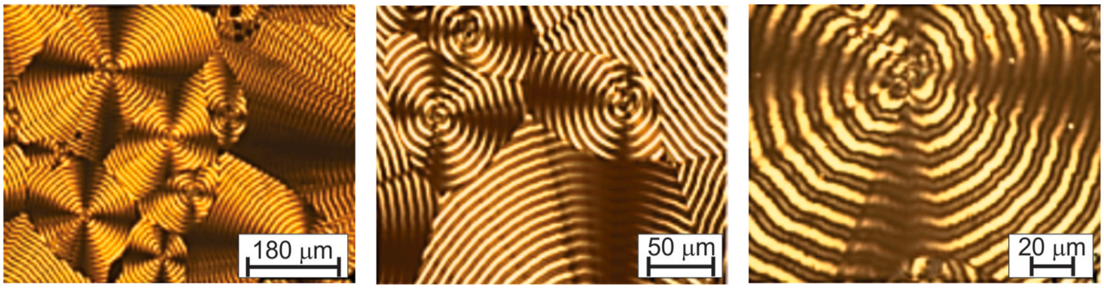

Fig. 2. Ring-banded spherulites of polyhydroxyalcanoate (PHB) films isothermally crystallized at 25 °C.

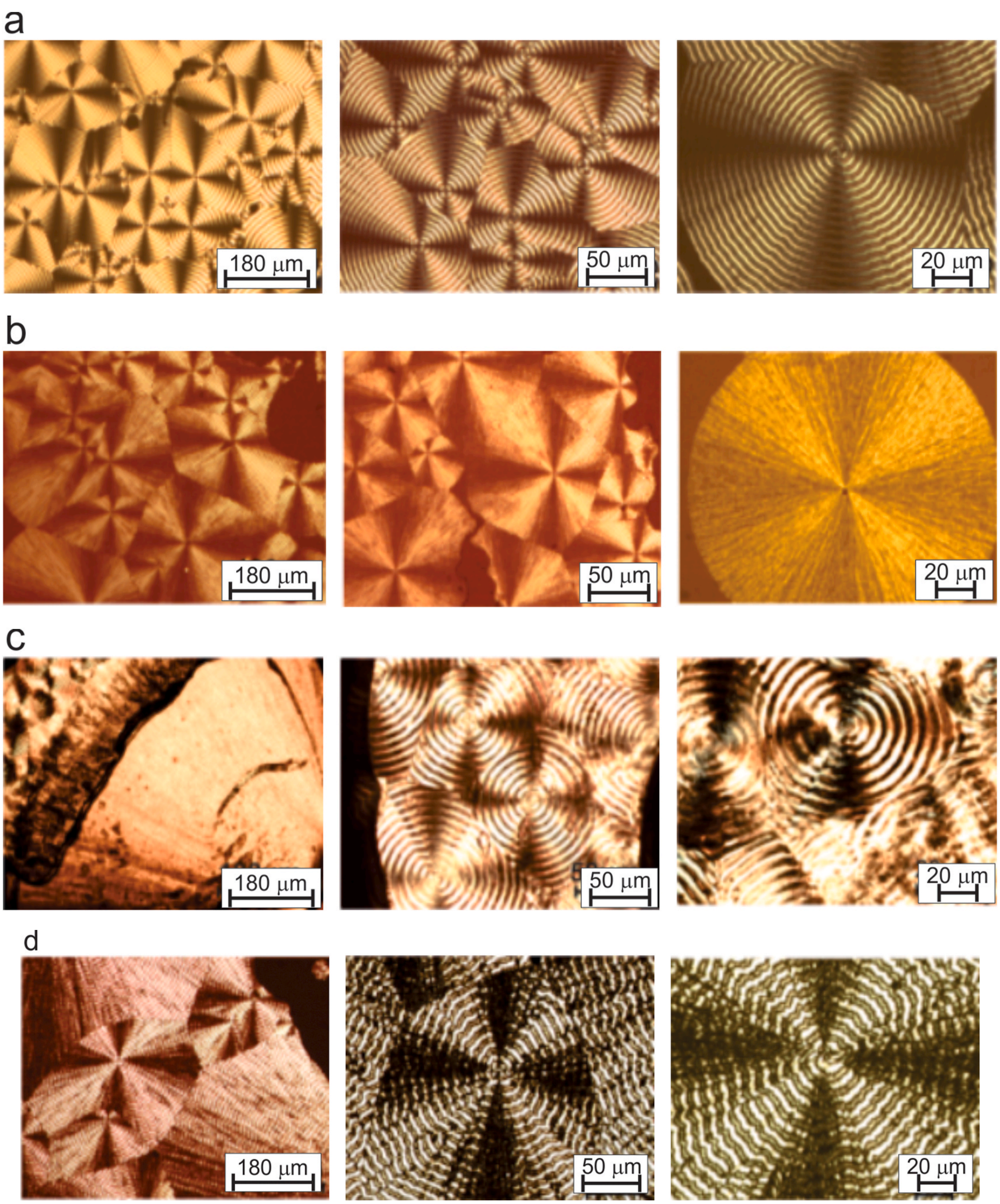

Fig. 3. Ring-banded spherulites of polyhydroxyalcanoate (PHB) films with different concentration of glycerol triacetate (GTA) isothermally crystallized at 25 °C: a) 5% GTA, b) 10% GTA, c) 20% GTA, and d) 30% GTA.

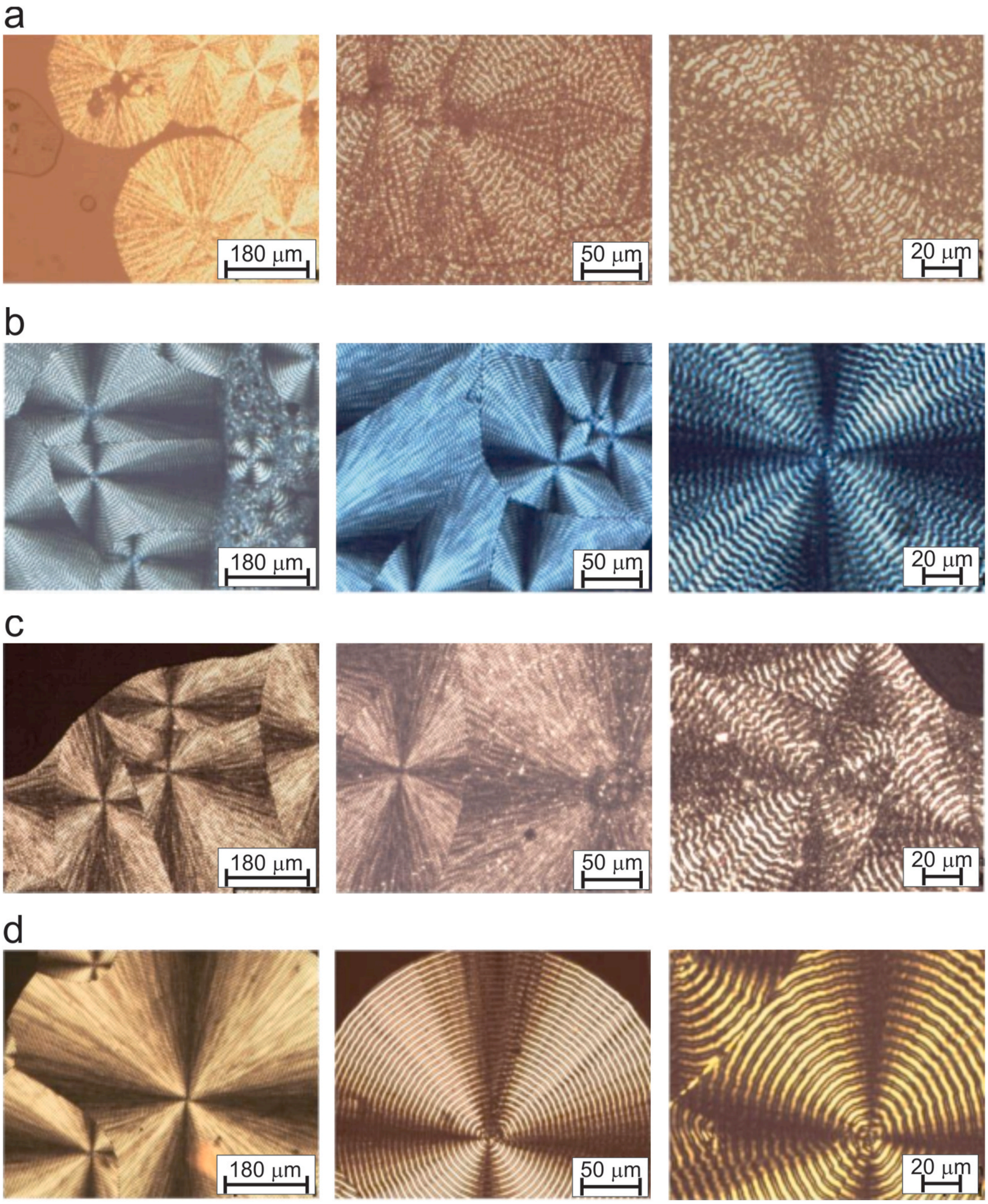

Fig. 4. Ring-banded spherulites of polyhydroxyalcanoate (PHB) films with different concentration of glycerol tributyrate (GTB) isothermally crystallized at 25 °C: a) 5% GTB, b) 10% GTB, c) 20% GTB, and d) 30% GTB.

crystals are visible, which clearly show that voids are not only on the top surface but also on the hidden interior exposed by the fracture surface [51].

4.3. Crystalline structure

XRD analysis was carried out in order to evaluate the effect of glycerol-based additives on crystalline structure of PHB. Diffractograms of pure PHB and samples with 5% of different additives are shown in Fig. 6. Pure PHB presented the typical α-type structure due to melting and crystallization processes [52]. Yokouchi et al. [53] reported that PHB crystallizes in an orthorhombic lattice structure. Accordingly, XRD pattern of PHB presented two strong intensity peaks at 13.6° and 16.9° assigned to (020) and (110) planes, respectively [54]. Besides, less intensity peaks at 21.6° and 22.6° corresponding to (101) and (111) reflection planes were also observed [54,55]. Signals detected at 25.4° and 27.2° were attributed to (130) and (040) planes, respectively [56]. In addition to the presence of orthorhombic α-form crystals with helical chain conformation, PHB also possesses a small amount of β-form crystals with zigzag conformation as revealed by the diffraction shoulder located at 20.2°. Wang et al. [57] observed a similar crystalline structure for electrospun PHB fibers. The presence of β-form crystals indicates a high level of molecular stretching in the amorphous region between the α-crystalline lamellae [53]. This crystalline β-form is characteristic of PHB samples that were molded by thermo-compression [58]. XRD diffractograms of samples processed in the presence of studied additives resulted similar to the diffractogram corresponding to pure PHB since the same reflection peaks were detected. Even though the addition of

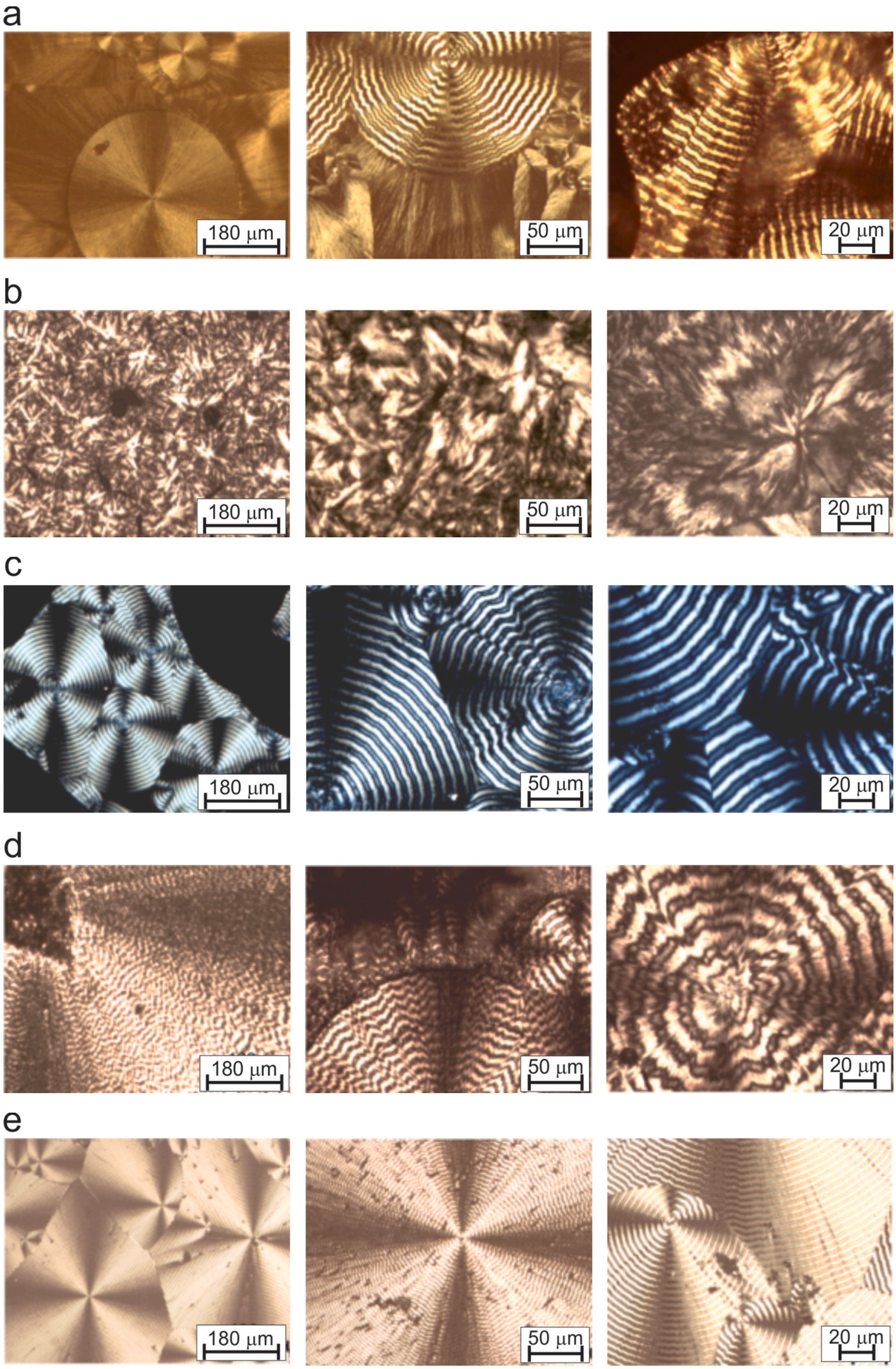

Fig. 5. Ring-banded spherulites of polyhydroxyalcanoate (PHB) films with different concentrations of glycerol (G) and polyglycerols (PGs) isothermally crystallized at 25 °C: a) 5% G, b) 10% G, c) 5% PG_{1174}, d) 5% PG_{5040}, and e) 10% PG_{5040}.

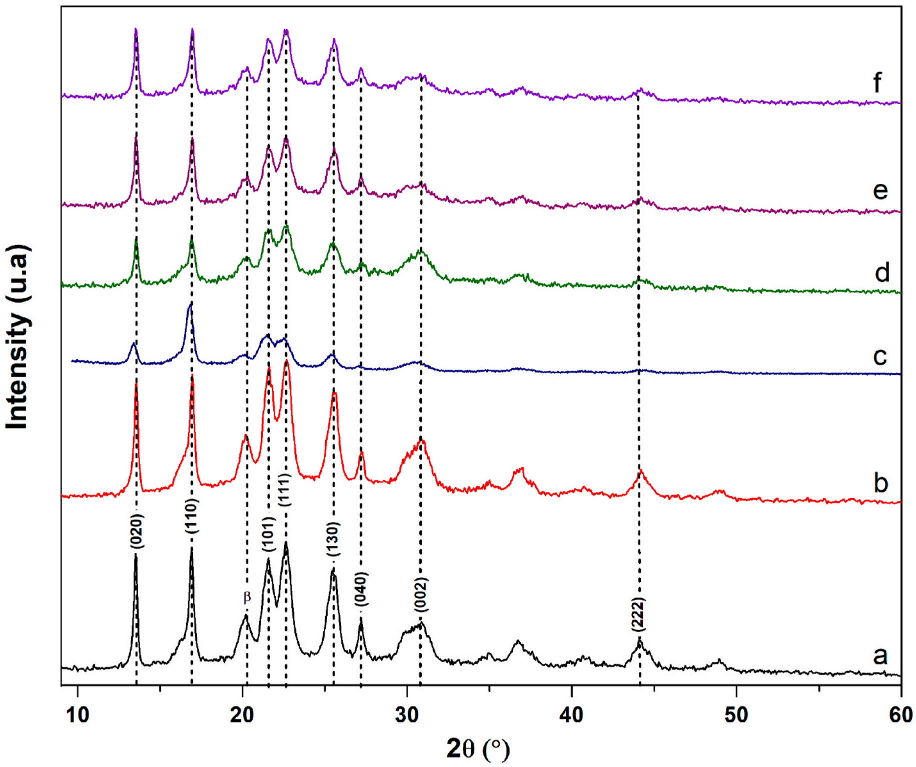

Fig. 6. XRD diffractograms of polyhydroxyalcanoate (PHB) films: a) PHB, b) PHB-5G (5% of glycerol), c) PHB-5GTA (5% of glycerol triacetate), d) PHB-5GTB (5% of glycerol tributyrate), e) PHB-5PG$_{1174}$ (5% of polyglycerol 1174), and f) PHB-5PG$_{5040}$ (5% of polyglycerol 5040).

studied additives did not affect PHB crystalline structure, diffractograms presented peaks with lower intensity, mainly in the case of PHB with GTB and PGs. Decrease in intensity of peaks in an indicative that these additives induced a decrease in the degree of crystallinity of the PHB matrix.

4.4. Thermal properties

In order to investigate the efficiency of studied additives on PHB final properties, DSC measurements were carried out. For PHB, during the heating it two peaks were detected in the melting region: the first one can be attributed to the melting of more irregular crystals and the second one to the most ordered crystals. In accordance with Ziaee and Supaphol [59] and Liu et al. [60], the melting peak at lower temperature corresponds to primary crystals formed during cooling, and the second peak at higher temperature is due to crystals melted and recrystallized in the subsequent heating.

During cooling, a melt crystallization event, in the form of a broad peak, was observed at 82.8 °C with an enthalpy of 65.4 J/g. In the second heating scan, the glass transition at −2.2 °C and a single melting peak at 176.2 °C, with a small shoulder at lower temperature were observed. The fact that the first melting occurs as a double peak and the second melting peak is visualized as a single peak could be due to differences in the perfection of PHB crystals. During the cooling stage, formed crystals are more imperfect than that formed during the cooling after the thermo-compression process, in which crystals have more time to recrystallize and reorganize into a more perfect and stable form. Seoane et al. [33] provided a similar explanation for DSC melting behavior of PHB films obtained by casting. Considering the second heating, pure PHB presented a melting peak at 176.2 °C, with an enthalpy of 75.1 J/g and a degree of crystallinity of 51.5%. These values were close to those reported by Hong et al. [61]. The occurrence of a peak associated to cold crystallization during the second heating was not detected, unlike that reported by other authors [35,62]. According to Wellen et al. [29], PHB crystallization occurs partially during the cooling stage (from the melt) and partially during the reheating stage (as cold crystallization). The relative areas of melt and cold crystallization peaks (related to the amount crystallized) depend on the cooling rate stage. Therefore, probably during the cooling stage at 10 °C/min PHB effectively crystallized from the melt.

In the case of samples containing G and PGs, glass transition temperature values were in the range of −2.2 to −2.5 °C, regardless of the additive concentration. These results are in line with the expected effect of these additives, indicating a poor additive-PHB compatibility and the occurrence of phase separation, as it was observed by ROM and SEM. However, none of the thermograms of these samples showed the melting

Table 3
Thermal properties of PHB with additives based of glycerol: a) obtained from the second heating DSC scans and b) obtained from DTGA curves.

Sample	T_g (°C)^a	T_{m1} (°C)^a	T_{m2} (°C)^a	ΔH_m (J/g)^a	$X_{C\text{-}DSC}$ (%)^a	T_d (°C)^b
PHB	−2.3	176.2	ND	75.1	51.5	280.6
PHB-5G	−2.3	169.5	ND	89.4	64.5	267.7
PHB-10G	−2.2	164.1	ND	84.6	64.4	237.1
PHB-5GTA	−7.3	170.0	ND	61.5	44.3	277.3
PHB-10GTA	−10.4	164.1	172.5	85.4	65.0	278.3
PHB-20GTA	−11.5	163.8	172.4	77.5	66.4	277.3
PHB-30GTA	−14.4	162.9	172.3	72.62	71.1	279.4
PHB-5GTB	−4.4	166.1	172.3	92.5	66.7	264.8
PHB-10GTB	−14.4	165.9	166.1	68.1	51.8	255.9
PHB-20GTB	−23.6	158.7	166.8	72.5	62.1	273.3
PHB-30GTB	−24.6	158.4	167.0	74.3	72.7	272.5
PHB-5PG $_{1174}$	−2.4	166.6	ND	71.2	51.3	274.0
PHB-10PG $_{1174}$	−2.4	149.2	162.2	60.2	45.8	268.9
PHB-5PG $_{5040}$	−2.3	169.1	ND	82.0	59.1	269.6
PHB-10PG $_{5040}$	−2.5	160.4	164.5	80.0	60.9	270.9

ND: not detected, T_g: glass transition temperature, T_m: melting temperature, ΔH_m: enthalpy of melting, $X_{C\text{-}DSC}$: degree of crystallinity, T_d: maximum decomposition temperature.

Table 4
Melting (T_m) and boiling (T_b) temperature of additives.

Additive	T_m (°C)	T_b (°C)
Glycerol (G)	17.8 [64]	290 [64]
Glycerol triacetate (GTA)	−78 [65]	258 [65]
Glycerol tributyrate (GTB)	−75 [65]	307 [65]
Polyglycerol (PG_{1174})	190.8	NA
Polyglycerol (PG_{5040})	122–154	NA

NA: not available.

peak of the pure additives (Table 4). Requena et al. [37] reported a similar observation for PHB films with polyethylene glycol and lauric acid. In the case of samples processed with GTA and GTB, it was detected a significant reduction of glass transition temperature attributed mainly to their plasticizing effect on the PHB matrix. These results are similar to those reported by other authors [32,33] and could be attributed to a greater increment of the polymer chains mobility by the addition of low molecular weight plasticizers. DSC thermograms of samples with employed additives did not show the presence of cold crystallization

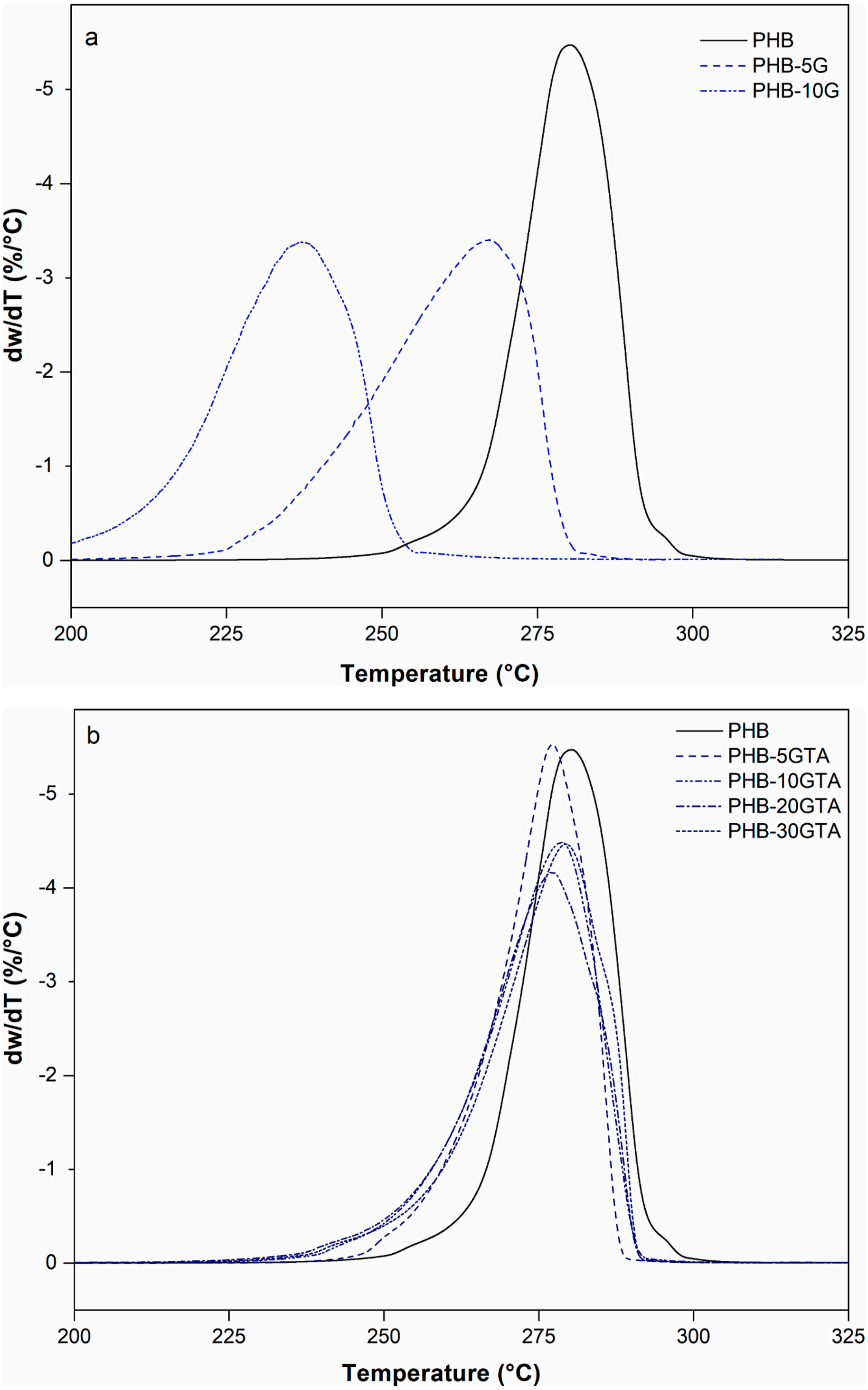

Fig. 7. First derivative thermogravimetric (DTGA) curves of polyhydroxyalcanoate (PHB) films: a) PHB with glycerol (G), b) PHB with glycerol triacetate (GTA), c) PHB with glycerol tributyrate (GTB), d) PHB with polyglycerol 1174 (PG_{1174}) and e) PHB with polyglycerol 5040 (PG_{5040}).

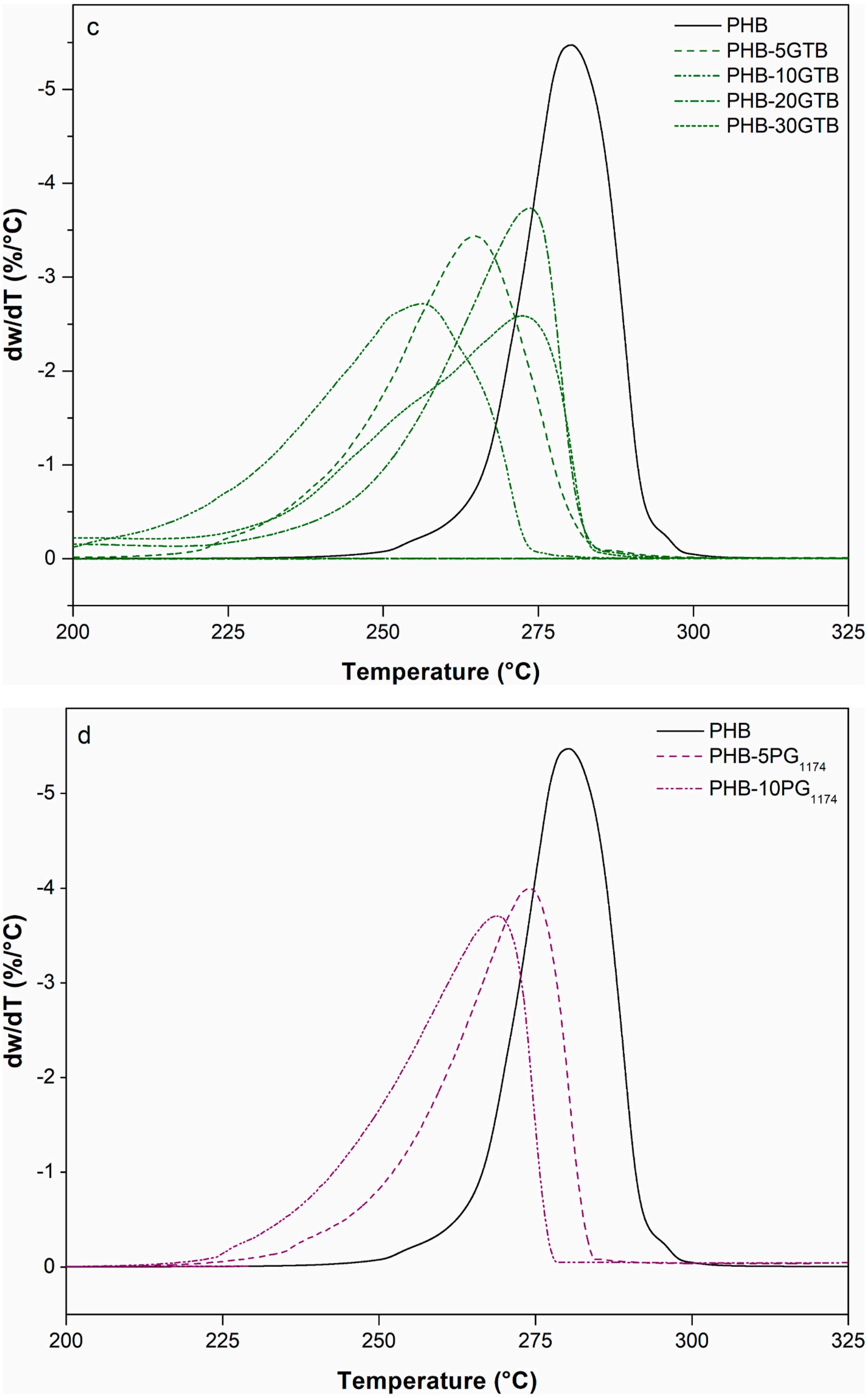

Fig. 7. (*continued*).

during the second heating. Regarding melting temperatures, PHB-5G, PHB-10G, PHB-5PG$_{1174}$, and PHB-5PG$_{5040}$ showed only one peak, meanwhile thermograms of the other studied samples evidenced the presence of two melting peaks. In agreement with Wellen et al. [29], multiple melting peaks may be attributed to the melting of crystals with different size and thermal stability. As it can be observed, additives incorporation decreased the temperature associated to the melting peak of pure PHB. These results indicate that studied additives affected the crystallinity of PHB, reducing crystallite sizes or forming more imperfect crystals that consequently exhibited a lower melting point. Similar results were reported by Seone et al. [33] for PHB nanocomposites plasticized with GTB. According to Choi et al. [62], melting temperature reflects the lamella thickness; therefore all plasticized samples presented thinner lamellae, in comparison to pure PHB. As it was reported by Râpă et al. [63] for PHB plasticized with triethyl citrate (TEC), acetyl o-tributyl citrate (ATBC) and tributyl citrate (TBC), the decrease in melting temperature implies that processing temperature window is increased. Besides, these authors stressed that this is in good agreement with a lower viscosity recorded for PHB loaded with plasticizers, making them easier for processing. In general, PHB crystallinity was affected by

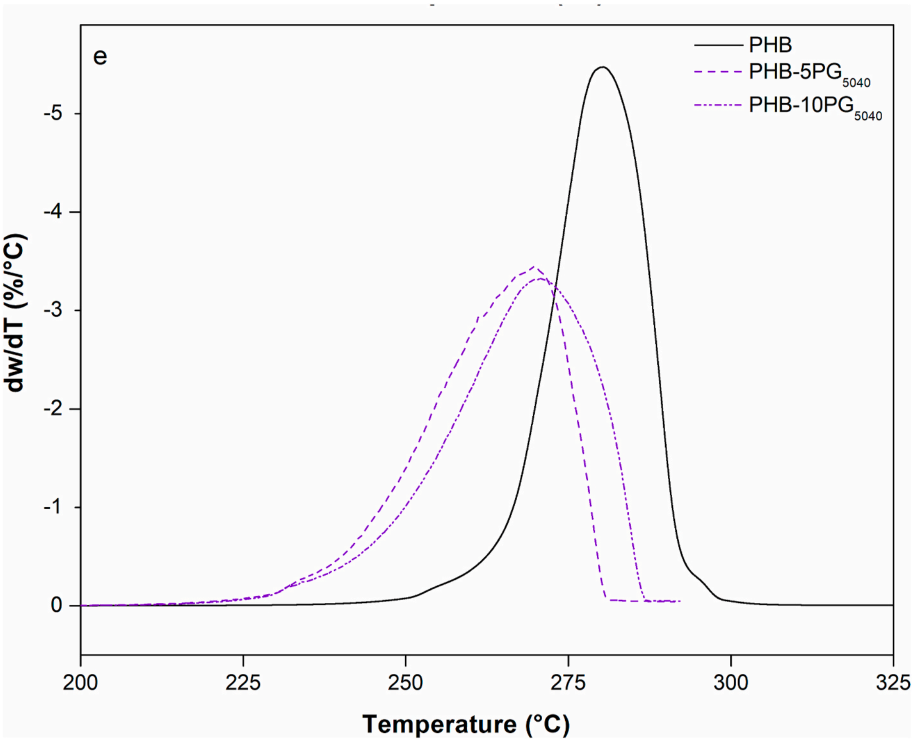

Fig. 7. (*continued*).

the addition of additives, increasing the heat of fusion and degree of crystallinity. In accordance with these results, Seaone et al. [33] observed that GTB addition slightly increased the degree of crystallinity of PHB and they concluded that this plasticizer could act as a nucleating agent.

Thermal stability is another property that affects polymers processability. TGA was performed in order to analyze the effect of studied additives on PHB thermal stability. The main reaction of PHB thermal degradation involves chain scission, which results in a rapid decrease in the molar mass [66]. First derivative thermogravimetric curves (DTGA) for studied samples are shown in Fig. 7. Thermal degradation of pure and additive PHB occurred through one step, which is characterized by a single peak on DTGA curves associated to the maximal rate of thermal degradation [67]. Maximum decomposition temperatures (T_d) of all studied samples are included in Table 3. When glycerol was added to PHB, matrix thermal degradation occurred at lower temperatures. Similar behavior was observed by the addition of studied PGs. In agreement with Lehrle et al. [68] and Hirt et al. [69], this prodegradative effect of glycerol is caused by its ability to enter a trans-esterification reaction and T_d decreases due to the shortening of chains. PHB samples containing GTA showed that mass loss started at lower temperatures, in comparison with pure PHB. This observation could be attributed to the additive vaporization. However, the temperature corresponding to the maximum rate of weight loss (T_d) of PHB did not change significantly with GTA addition within the studied concentration range (5–30%). In the case of samples with GTB, mass loss started at lower temperatures than pure PHB due to additive vaporization, similarly to samples with GTA. Analogous results were reported by Arrieta et al. [70] and Seoane et al. [33]. Cyras [71] stressed that this shift of degradation peak to lower temperatures could be attributed to the incorporation of esters groups of GTB molecules, which promote PHB catalytic degradation reactions and reduce T_d. Films PHB-20GTB and PHB-30GTB presented higher T_d values than those with 5 and 10%. In accordance with Seoane et al. [33], these results could be related to the increment in the degree of crystallization induced by the addition of high percentages of GTB, which could recover PHB thermal stability, increasing T_d.

Table 5
Mechanical properties and water vapor permeability of PHB films with different additives.

Sample	E (MPa)	σ_m (MPa)	ϵ_B (%)	WVP × 10^{14} (g m^{-1} s^{-1} Pa^{-1})
PHB	61.9 ± 9.6	31.8 ± 3.5	1.00 ± 0.15	77.0
PHB-5G	27.3 ± 4.4	29.4 ± 2.0	0.92 ± 0.13	56.0
PHB-10G	31.2 ± 6.0	18.8 ± 2.8	0.39 ± 0.08	49.5
PHB-5GTB	52.9 ± 6.9	33.5 ± 7.4	1.24 ± 0.27	75.0
PHB-10GTB	37.6 ± 6.3	31.6 ± 1.7	2.50 ± 0.14	325.0
PHB-20GTB	21.2 ± 2.0	22.6 ± 2.2	3.53 ± 0.15	787.0
PHB-30GTB	16.2 ± 1.8	19.5 ± 1.8	3.98 ± 0.50	794.0
PHB-5GTA	43.1 ± 2.3	23.1 ± 4.1	1.59 ± 0.40	0.13
PHB-10GTA	45.7 ± 4.9	25.8 ± 3.5	1.10 ± 0.20	0.03
PHB-20GTA	35.1 ± 3.6	25.5 ± 4.2	1.70 ± 0.30	0.10
PHB-30GTA	39.1 ± 3.8	28.4 ± 1.9	2.40 ± 0.10	0.19
PHB-5PG_{1174}	56.3 ± 11.2	15.5 ± 2.5	0.32 ± 0.08	13300
PHB-5PG_{5040}	50.9 ± 6.9	28.8 ± 7.4	0.77 ± 0.19	886
PHB-10PG_{5040}	41.5 ± 9.1	11.0 ± 2.7	1.31 ± 0.11	3010

E: Young's modulus, σ_m: maximum tensile strength, ϵ_B: elongation at break, WVP: water vapor permeability.

4.5. Mechanical properties

The influence of glycerol-based additives on mechanical properties of PHB was evaluated through tensile tests. Values of Young's modulus (E), maximum tensile strength (σ_m), and elongation at break (ϵ_B) are presented in Table 5. Mechanical properties of pure PHB evidenced the brittleness and stiffness of this polymer with a high Young's modulus and a low elongation at break, which limit its range of applications [72]. If additives act as plasticizers, it is expected an improvement in PHB mechanical properties by reducing E and σ_m and increasing ϵ_B [34]. In the case of G and PGs, PHB thermal degradation induced by these additives led to a shortening of polymer chains and, consequently, a negative effect on mechanical properties of samples since, despite E and σ_m diminished, ϵ_B values suffered a significantly reduction. When GTA and GTB were incorporated to PHB matrix, mechanical properties were modified considerably since a significantly decrease in E and σ_m, as well as an increment in ϵ_B were achieved. These results suggest a plasticizer effect resulting from the presence of those additives in the PHB matrix in accordance with Rahman et al. [73]. Among the studied additives, GTB was the most effective plasticizer for PHB. Particularly, adding 30% GTB allowed a reduction of 74% and 39% in E and σ_m, respectively; meanwhile an increment of around 300% in ϵ_B values was obtained, in accordance with Seoane et al. [33]. Plasticization reduces the relative number of polymer-polymer interactions thereby decreasing the rigidity of the three-dimensional structure, allowing deformation without rupture [64]. Accordingly, GTA and GTB reduced any restraint imposed on the separation of the lamellae during the mechanical test and probably inhibited the relative chain motion or reduced the force of secondary intermolecular bonds between PHB chains [74]. In addition, El-Hadi et al. [32] reported that the addition of GTA and GTB to PHB leads to a lower glass temperature and lower crystallinity, forming numerous, small and imperfect crystallites. Therefore, an increase in the elongation at break and a decrease in the yield stress is observed [75].

4.6. Water vapor permeability (WVP)

WVP values of studied materials are presented in Table 5. The effect of additives incorporation on WVP of PHB films depends on the compound type and concentration. In the case of GTB and PGs, the addition of these additives increased WVP, probably due to the increment of free volume that favors water vapor diffusion. WVP in PHB-GTB films value was higher than that of pristine PHB, corroborating that GTB is miscible in the PHB matrix [33]. Besides, Requena et al. [37] stressed that water solubility is promoted in the more polar plasticized regions, which also contributes to the increase in permeability values. The major increase in WVP values was observed for PGs addition, probably attributed to the high molar mass of these additives and their high hydrophilic character. Also, the incorporation of additives with higher number of chains and molecular structures highly branched such as PGs, surely generated an increment to free volume and increased WVP [18,33]. In the case of PHB samples with G and GTA, a decrease in WVP was observed. In accordance with Follain et al. [76], the improvement of barrier properties is usually correlated to the tortuosity effects induced by the impermeable domains (crystalline phase) within the material. Besides, Requena et al. [37] stressed that WVP decrease could be attributed to the rearrangement of matrix components such as a secondary crystallization or a possible phase separation as in the case of PHB samples with glycerol.

5. Conclusions

The effect of different glycerol-based additives on PHB films was studied. Glycerol triesters (GTB and GTA) led to homogeneous PHB films, within the assayed concentrations range (5–30%). Besides, GTB and GTA addition significantly decreased the stiffness and the maximum tensile strength, yielding to more extensible PHB films. GTB and PGs increased the water affinity of PHB, promoting an increase in films WVP. All plasticizers slightly decreased PHB thermal stability; GTB gave rise to the most heat-resistant plasticized films, while G, PGs, and GTA highly promoted the heat-sensitivity of PHB. Among the studied additives, GTB was the most effective plasticizer for PHB films, although a high amount would be required to fulfill PHB mechanical properties to certain requirements. Finally, it is not recommended to use G and PGs due to the significant prodegradative effect, giving worse thermal stability and poor mechanical properties than pure PHB.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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