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# Properties and structure of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) filaments for fused deposition modelling

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## ABSTRACT

Fused deposition modelling (FDM) is a process of additive manufacturing allowing creating of highly precise complex three-dimensional objects for a large range of applications. The principle of FDM is an extrusion of the molten filament and gradual deposition of layers and their solidification. Potential applications in pharmaceutical and medical fields require the development of biodegradable and biocompatible thermoplastics for the processing of filaments. In this work, the potential of production of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P(3HB-co-4HB)) filaments for FDM was investigated in respect to its thermal stability. Copolymer P(3HB-co-4HB) was biosynthesised by *Cupriavidus malaysiensis*. Rheological and mechanical properties of the copolymer were modified by the addition of plasticizers or blending with poly(lactic acid). Thermal stability of mixtures was studied employing thermogravimetric analysis and rheological analyses by monitoring the time-dependent changes in the complex viscosity of melt samples. The plasticization of P(3HB-co-4HB) slightly hindered its thermal degradation but the best stabilization effect was found in case of the copolymer blended with poly(lactic acid). Overall, rheological, thermal and mechanical properties demonstrated that the plasticized P(3HB-co-4HB) is a potential candidate of biodegradable polymer for FDM processes.

**Keywords:** *Cupriavidus malaysiensis*, Fused deposition modelling, Rheological properties, Poly(lactic acid), Poly(3-hydroxybutyrate-co-4-hydroxybutyrate), 3D printing

## 1. Introduction

Poly(3-hydroxybutyrate-co-4-hydroxybutyrate), P(3HB-co-4HB) is a random copolymer of 3-hydroxybutyrate (3HB) and 4-hydroxybutyrate (4HB) and belongs to the group of bacterial polyhydroxyalkanoates (PHA). PHAs are polyesters composed of 3-hydroxy alkanic acids and are synthesized by many archaea and eubacteria under certain fermentation conditions causing metabolic stress. PHAs are accumulated in bacteria as intracellular carbon and energy storage materials in the

form of white water-insoluble inclusions. These biopolymers have attracted much attention due to their biocompatibility and biodegradability. PHAs have thermoplastic behaviour and are relevant for a wide range of packaging, agricultural, cosmetic, pharmaceutical and medical applications [1-4]. The existence of more than 100 types of monomers that can be involved in the polymerization of PHAs makes this group of polymers highly attractive due to the possibility to tune their properties according to the requirements of different applications [5].

Physico-chemical properties of PHA depend on the type of monomer unit, chain length, functional groups, and molecular weight. The best-investigated and commercially available polymer among PHAs is poly (3-hydroxybutyrate) (P3HB). P3HB is a short-chain length PHA with a high degree of crystallization, high stiffness, high brittleness and low elasticity. Moreover, the molten P3HB is extremely sensitive to moisture, shear stress and temperature that exceeds its melting temperature [6]. Therefore, its processing window is very narrow and thermal degradation often occurs already during melt-processing and causes irreversible changes in the final properties of the polymer. These characteristics limit the range of application of P3HB and methods suitable for its processing. Biosynthesis of copolymers such as poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (P(3HB-co-3HV)), poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (P(3HB-co-3HHx)), and poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P(3HB-co-4HB)) have been reported as a possible way how to modify physico-mechanical and biological properties according to the needs of the application [6]. Recent literature presented also interest in the biosynthesis of terpolymers or quarterpolymers to tune properties of biodegradable PHAs taking into account their application [7]. The biological production of PHA with a broader range of monomers in the structure is much complex and expensive than the production of pure P3HB. The highest potential of PHAs with flexible mechanical properties is in tissue engineering and drug delivery [8]. One of the commercially available flexible PHA is P(3HB-co-4HB) produced, for example, by Green Bioscience (Tianjin, China) is a typical representative of resorbable biocompatible material with flexible properties [9]. P(3HB-co-4HB) as thermoplastic is processable by melt processing methods, e.g. extrusion, injection moulding and compression moulding. However, the final properties of P(3HB-co-4HB) products are highly influenced by the thermal degradation proceeded by at least an initial random degradation and subsequent 0th-order kinetics of a weight loss [10]. The range of the thermal degradation of P(3HB-co-4HB) varies mainly with the processing time and temperature but, of course, mechanical stress and several further processing operations also play a significant role [10,11].

Recently, additive manufacturing (3D printing) technologies came to the forefront of thermoplastic melt processing methods. Several 3D printing methods could be more or less applicable for PHAs, including stereolithography, selective laser sintering, fused deposition modelling (FDM) and bioprinting [12]. FDM is a relatively novel method of additive manufacturing that is used for the creation of 3D objects according to a pre-drawn 3D CAD (computer-aided design) model. Its basic principle is a fusion of filaments with a defined diameter (usually 1.75 mm), extrusion of the melted material from a nozzle and formation of 3D objects using layer-by-layer technique. The most important advantage is the flexibility in design and material properties (multifunctional). This methodology has found its place in many industries (medicine, engineering, aerospace, art, education) where it is often used to create prototypes before the production itself. It was also established in current medicine for the production of medical or dental implants or supporting tools for the patients. There is already a relatively broad variety of materials for 3D printing modelling on the market. Thermoplastics are preferred because the processing of metals and ceramics is more challenging. The main disadvantage of industrial prototypes after their end life usage is a huge negative impact on the environment due to the plastic waste accumulation in terrestrial and aquatic ecosystems. There were several attempts to eliminate it by using recyclable polymers or using biodegradable polymers [13,14]. Especially in the

case of medical application, the 3D printed materials should be biodegradable and biocompatible [15]. 3D micro-fibrous biodegradable materials have found their application in tissue integration because pores may support better integration of cells into the 3D microfibrinous system [16]. Therefore, there is a high demand to develop biodegradable polymers that would be processable by additive manufacturing, such as fused deposition modelling. Neat poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (P3HBV) degrade during extrusion very fast and therefore their final molecular weight becomes so low after melt processing that the formed filaments are too brittle and do not reach the required dimensions [17]. It has been shown that a mixture of P3HB or P3HBV with PLA containing the addition of a plasticizer may reach suitable rheological and mechanical properties for the preparation of filaments and FDM [18,19]. Since 2018 PLA/PHA filaments have been offered by filaments producers, such as ColorFabb (The Netherlands), Prusa Research and Filamentum (Czech Republic). As the composition of PLA/PHA filaments offered on the trademark is unclear [12], their applicability in the medical area is not fully accepted yet. Recently, we have shown that P3HB-co-3HHx purchased from Kanaeka Corporation, KITA-KU Osaka, Japan under the name PHBH X131A without any additives fulfils criteria for filaments production and fused deposition modelling [17].

The interest in the 3D modelling of medical implants and personalized drug delivery materials is increasing rapidly, while the fused deposition modelling of these materials could contribute to a faster and cheaper production [20-22]. Therefore, the further development of medical polymers in addition to common thermoplastics that would be processable by FDM is needed. PHAs are of particular interest. One of the PHAs relevant for melt-processing is poly(3-hydroxybutyrate-co-4-hydroxybutyrate). This copolymer exhibits mechanical properties typical for thermoplastic elastomers [23,24]. The purpose of this paper is to carry out the characterization of rheological behaviour and thermal stability of P3HB-co-4HB and to assess its processability by fused deposition modelling under real conditions of 3D printing.

## **2. Materials and methods**

### *2.1. Microorganism, growth conditions and copolymer extraction*

The production strain, *Cupriavidus malaysiensis*, DSM 19379 was purchased from DSMZ-German Collection of Microorganisms and Cell Cultures GmbH (Braunschweig, Germany) and maintained as a cryo-conserved culture at -80 °C in the presence of glycerol. The 24-h shaken inoculum was prepared by the cultivation of the bacterium on nutrient broth medium (10 g l<sup>-1</sup> peptone, 2 g l<sup>-1</sup> yeast extract, and 10 g l<sup>-1</sup> beef extract) at 30 °C and 160 rpm. For PHA production, a mineral salt medium (MSM) reported elsewhere [25] was applied, which was supplemented by 10 g l<sup>-1</sup> 1,4-butanediol and 5 vol% inoculum. The biosynthesis was performed in Erlenmeyer flasks (volume 500 ml) containing 200 ml of MSM media (30 °C, 160 rpm, 72 h).

Cell biomass after 72-h cultivation was harvested by centrifugation (6000 rpm, 10 min, and -4 °C), purified in distilled water and analysed gravimetrically. The biomass concentration was expressed as cell dry weight (CDW). The PHA content of dried cells was determined by gas chromatography (Trace GC Ultra, thermos Scientific, USA) as reported previously [26]. Commercially available P(3HB-co-3HV) (Sigma Aldrich, Germany) and P(3HB-co-4HB) (Metabolix, USA) were used as external standards and benzoic acid (Lachner, Czech Republic) was used as an internal standard.

PHA was extracted from lyophilized biomass in chloroform (70 °C, 2 h) and precipitated in ethanol. The precipitated sample was lyophilized and stored in a vacuum-sealed bag.

## 2.2. Materials

For the modification of PHA processability in an extruder phenolic antioxidant, chain extender, plasticizers, and additional biopolyester were used. Dibutyl phthalate (DBP, Merck, Darmstadt, Germany) and acetyl tributyl citrate (Citrofol BII, Jungbunzlauer Austria AG, Wien, Austria) were tested as plasticizers. Irganox 1010 (phenolic antioxidant) was used for the protection against thermo-oxidative degradation (BASF, Ludwigshafen, Germany) and Joncryl ADR 4468 was used as a chain extender for modification of the PHA processability in an extruder. Poly(lactic acid) (PLA 2003D, NatureWorks, Minnetonka, MN, USA) with a D-isomer content of 4% was applied for blending with PHA to modify its viscosity.

Filaments were prepared by mixing of PHA biosynthesised within this study and other additives in an Xplore Micro Compounder twin screwdriver (Xplore Instruments BV, Sittard, The Netherlands) with a 15 ml mixing chamber at 165 °C. The composition of mixtures is shown in Table 1. In the dosing phase, the mixture was loaded at 50 rpm. In the second phase, the mixture was homogenized for 5 min at 100 rpm. In the last phase, the mixture was drained at a speed of 30 rpm in the form of the filament. The specimens to test the processability of filaments by fused deposition modelling were printed with 3D printer Original Prusa I 3MK3S (Prusa Research, Czech Republic) at a nozzle temperature of 180 °C, a printing bed temperature of 60 °C and maximum volumetric print speed of 15 mm<sup>3</sup> s<sup>-1</sup> through a 0.4 mm nozzle.

**Table 1** Composition of mixtures.

Sample	P3HB-co-4HB (g)	PLA (g)	Irganox (g)	Joncryl (g)	DBT (g)	Citrofol BII (g)
M1	10	–	0.2	0.1	–	–
M2	10	–	0.2	0.1	0.2	–
M3	10	–	0.2	0.1	–	0.2
M4	5	5	0.2	0.1	–	0.2

## 2.3. Analyses

FTIR spectrum of the isolated PHA was obtained by employing an Attenuated Total Reflectance (ATR) technique using a Nicolet iS50 spectrometer (Thermo Fisher Scientific, Waltham, USA). All measurements were taken at room temperature (in an air-conditioned room) on the built-in diamond ATR crystal. FTIR spectrum was recorded over the range 4000-400 cm<sup>-1</sup> at 4 cm<sup>-1</sup> resolutions and represented an average of 64 scans. The spectrum of the clean dry diamond ATR crystal in the ambient atmosphere (air) was used as the background for infrared measurement.

Molecular weight (M<sub>w</sub> weight-average) and polydispersity of the isolated PHA was measured by size exclusion chromatography (SEC Infinity 1260 system equipped with PL gel MIXED-C column, Agilent Technologies, USA) coupled with Dawn Heleos II multiangle light scattering (MALS) detector (Wyatt Technology, USA) and Optilab T-rEX differential refractometer (dRI, Wyatt Technology, USA). 50 µl of

the sample was injected into the system in three repeated measurement cycles under 0.6 ml min<sup>-1</sup> flow rate of a mobile phase (chloroform, HPLC purity). The obtained molecular weights of individual samples were calculated by ASTRA software (Wyatt Technology, version 7.3.2) using the value of the refractive index increment of P(3HB-co-4HB) (dn/dc = 0.0305 ml g<sup>-1</sup>). The dn/dc value was determined in this study by the dRI batch measurement as the slope of the concentration dependence of the refractive index values of P(3HB-co-4HB) solutions.

Thermal properties of PHA and processed samples were determined by differential scanning calorimetry (DSC Q2000 TA instruments, New Castle, DE, USA) in nitrogen (50 ml min<sup>-1</sup>). Approximately 5 mg of samples were sealed in aluminium pans and analysed by temperature procedure composed of a first heating cycle from 20 °C to 190 °C with one-minute isotherm, cooling to -60 °C with one-minute isotherm and second heating cycle from -60 °C to 190 °C. The heating/cooling rate was 10 °C min<sup>-1</sup>. From the first and second heating cycle melting temperature (T<sub>m</sub>) with the melting enthalpy (ΔH<sub>m</sub>) (endothermic peak) and the temperature of cold crystallization (T<sub>cc</sub>) with the melting enthalpy of cold crystallization (ΔH<sub>cc</sub>) (exothermic peak) were determined. In case of a double melting peak the melting enthalpy was presented as a sum of both melting peaks. The values of glass transition temperature (T<sub>g</sub>) were determined from the second heating scan. Specific melting enthalpy as an indicator of crystallinity degree of compounds was calculated as follows:

$$\Sigma H = \Delta H_m - \Delta H_{cc} \quad (1)$$

The thermal behaviour of the isolated copolymer and prepared samples was investigated by thermogravimetric analysis (TGA) using a TGA Q50 analyser (TA Instruments, New Castle, DE, USA). In the experiment, about 5 mg of the sample was heated from 30 °C to 600 °C at a heating rate of 10 °C min<sup>-1</sup> in a nitrogen atmosphere (60 ml min<sup>-1</sup>). From the thermogravimetric curve (TGA) the onset temperature of decomposition (T<sub>onset</sub>) was determined. Simultaneously, from the differential thermogravimetric curve (DTGA) the degradation temperature at maximum process rate (T<sub>max</sub>) was identified.

Rheological measurements were performed on the MCR 92 rheometer (Anton Paar, Austria) using steel parallel plate-plate geometry with a roughened surface (diameter of 25 mm, profiled at 1 x 0.5 mm, a gap of 400 μm). The experiments were carried out at least in duplicates, under a controlled temperature of 180 °C (copolymer and its mixtures) or of 185 °C (PLA). Each sample was equilibrated for 2 min at a constant measured temperature before the measurement (conditioning). During the measurement, the upper-heated chamber was used for the accurate conditioning of the sample (the temperature was kept at a constant value during the whole measurement) cooling/heating by air (2 dm<sup>3</sup> min<sup>-1</sup>). Firstly, the linear viscoelastic region (LVR) for isolated and purified copolymer was determined by a strain sweep experiment. The dynamic strain sweep test was performed under the following conditions: 2 min for conditioning before measurement, deformation cycle of 0.1-100%, an oscillation frequency of 6.28 rad s<sup>-1</sup> and temperature of 180 °C. Thermo-mechanical stability of P(3HB-co-4HB) before polymer processing and of the copolymer mixtures M1 -M4 was investigated using an oscillatory time-test. During the time-sweep measurement, the amplitude of deformation (strain) and the frequency of oscillation were kept at a constant value of 0.7% (chosen from LVR) and 6.28 rad s<sup>-1</sup>, respectively. The time development of viscoelastic properties was determined over 40 min with a time interval of 10 s per measuring point.

Mechanical properties of the filaments based on P3HB-co-4HB were measured at room temperature on an Instron 3365 (Instron, Buckinghamshire, England) universal testing machine at a deformation rate of  $1 \text{ mm s}^{-1}$ .

The morphology of filaments cross-sections was observed using a Schottky field emission scanning electron microscope (FESEM) (Nova NanoSEM 450, FEI). The specimens were cryogenic fractured and coated with a thin layer of Au/Pd. The microscope was operated in a high-vacuum mode at an acceleration voltage of 5 kV.

### 3. Results and discussion

#### 3.1. Properties of isolated PHA

The gravimetric analysis revealed the production of  $3.8 \pm 0.2 \text{ g l}^{-1}$  biomass after 72-h cultivation in Erlenmeyer flasks. The results of gas chromatography turned out that the biomass contained  $65.4 \pm 3.8\%$  of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) [P(3HB-co-4HB)] with  $10.2 \pm 0.8 \text{ mol}\%$  of 4HB. ATR-FTIR spectrum of the isolated PHA, displayed in Fig. 1 shows the corresponding absorption bands of P(3HB-co-4HB) referring to their content of aliphatic and ester molecular moieties. Interpretation of the absorption bands has been carried out according to the literature data [27-29].

A sharp and intensive band located at  $1720 \text{ cm}^{-1}$  is attributed to symmetric C=O stretching in alkyl-ester groups. Further characteristic absorption bands concerning aliphatic ester can be deduced from the deeper evaluation of fingerprint regions  $1300\text{-}1160 \text{ cm}^{-1}$  and  $1100\text{-}1030 \text{ cm}^{-1}$ .

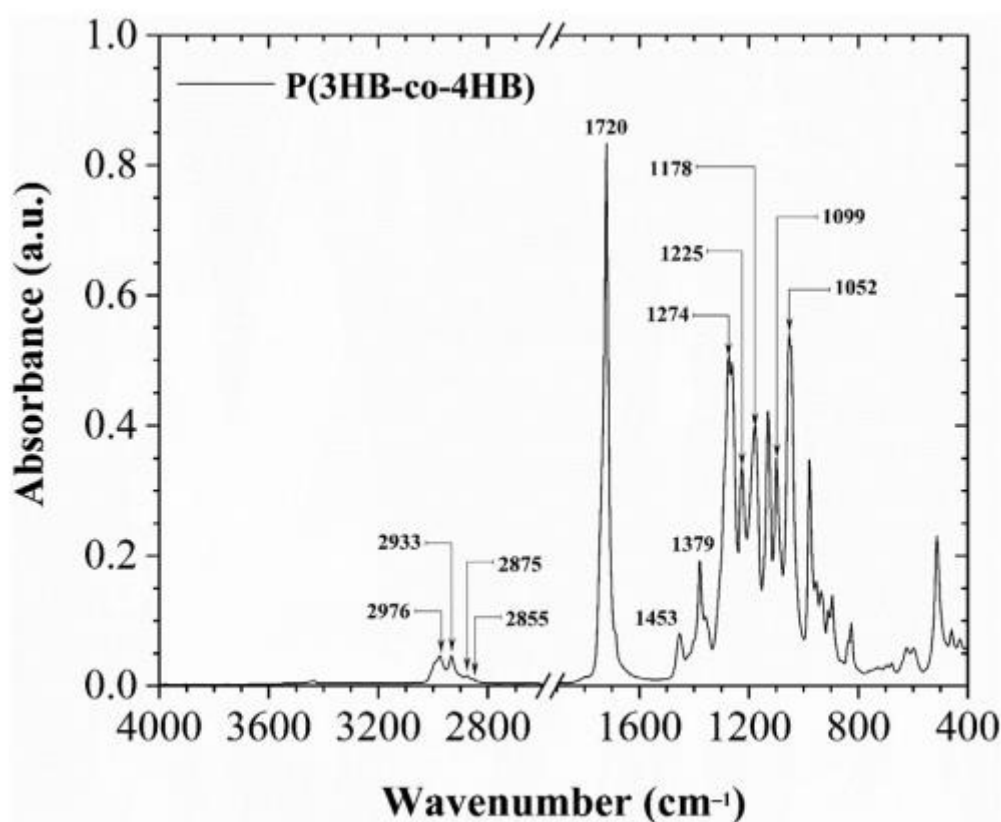


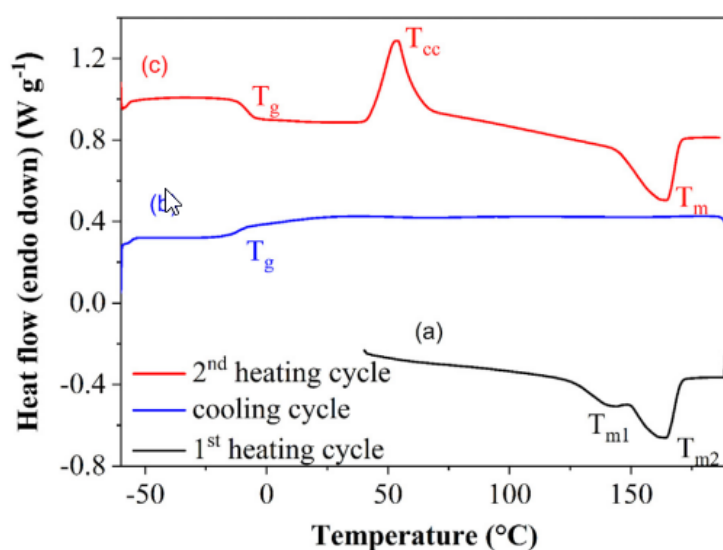
Fig. 1. FT-IR spectra of isolated PHA synthesized by *Cupriavidus malaysiensis*.

The first region is characterized by sharp and intensive C—O stretching bands at 1274  $\text{cm}^{-1}$  and 1225  $\text{cm}^{-1}$ . The relatively intensive band at 1178  $\text{cm}^{-1}$  can be ascribed to C-C-O stretching in alkyl-ester groups. The letter fingerprint zone is characterized by two bands at 1099  $\text{cm}^{-1}$  and 1052  $\text{cm}^{-1}$  corresponding to the O-C-C symmetric vibration mode in alkyl-esters [8].

The ATR-FTIR spectrum of the isolated P(3HB-co-4HB) is mainly characterized by absorption bands of aliphatic moieties. The presence of aliphatic groups is revealed by the bands at 2976  $\text{cm}^{-1}$  and 2933  $\text{cm}^{-1}$  which is attributed to asymmetric C—H stretching in methyl and methylene groups [30]. The symmetric valence vibrations of aliphatic groups (i.e. -CH<sub>3</sub> and -CH<sub>2</sub>-) occur in the spectrum as weak bands at around 2875  $\text{cm}^{-1}$  and 2855  $\text{cm}^{-1}$ . The deformation vibrations of the -CH<sub>2</sub>- and -CH<sub>3</sub> groups at 1453  $\text{cm}^{-1}$  and 1379  $\text{cm}^{-1}$  occur in the spectrum.

The appearance and absorption bands of ATR spectrum suggest that the chemical structure and composition of the isolated PHA sample correspond to the structural moieties typical for P(3HB-co-4HB) copolymer [31]. Therefore, it can be concluded that the ATR-FTIR spectroscopy provided valuable information on the quality and purity of the isolated PHA sample.

The rheological, thermal and mechanical properties are impacted by the molecular weight of the copolymer. The molecular weight of copolymer depends on the type of bacterial strain, composition of production media, fermentation strategy, copolymer composition and isolation procedure. The published data of Mw of P(3HB-co-4HB) in the literature were found in the range of approximately 20-1060 kDa [25,28,32,33]. The molecular weight (Mw) of the isolated P(3HB-co-4HB) in this study was  $460.2 \pm 8.0$  kDa with a polydispersity of  $1.6 \pm 0.3$ . Fig. 2 shows the DSC thermograms of the first heating scan, cooling and second heating scan, respectively. The isolated copolymer displayed double melting temperature at 141.3 and 164.1 °C with a specific melting enthalpy of  $42.3 \text{ J g}^{-1}$  (the enthalpy sum for both melting peaks  $T_{m1}$  and  $T_{m2}$ ). Double melting behaviour indicates two types of crystals with different thermal stability. The relatively high value of the specific melting enthalpy corresponds with an isolation procedure, where precipitation of polymer in ethanol followed by lyophilisation supported the crystallization of copolymer. The cooling scan revealed that the melted copolymer was not able to crystallize at the cooling rate of  $10 \text{ }^\circ\text{C min}^{-1}$ . The copolymer stayed nearly entirely amorphous. The second heating scan shows a cold crystallization peak at 53.4 °C with the heat of cold crystallinity of  $28.8 \text{ J g}^{-1}$  and a melting peak at 164.5 °C with the heat of melting of  $30.0 \text{ J g}^{-1}$ .



**Fig. 2.** DSC thermograms of isolated copolymer: (a) first heating scan at  $10 \text{ }^\circ\text{C min}^{-1}$  (b) cooling scan at  $10 \text{ }^\circ\text{C min}^{-1}$  and second heating scan at  $10 \text{ }^\circ\text{C min}^{-1}$ . [Color figure can be viewed in the online issue].



From this data, the fused enthalpy ( $\sum H = \Delta H_m - \Delta H_{cc} = 0.2 \text{ J g}^{-1}$ ) proved that copolymer after quenching received almost amorphous character. The copolymer has the glass transition temperature at  $-7.4 \text{ }^\circ\text{C}$ .

Research practices and published data in the scientific literature show that poly(3-hydroxybutyrate-co-4-hydroxybutyrate) has been thermally unstable at a temperature above  $170 \text{ }^\circ\text{C}$ . The range of thermal degradation, which follows a kinetic model of random chain scission at ester groups, depends on the applied processing temperature and exposition time [11]. Therefore, in this work, we modified P(3HB-co-4HB) with additives, including a plasticizer, an antioxidant, a crosslinking agent, or by mixing with poly(lactic acid). The efficiency of two kinds of plasticizers, dibutyl phthalate and acetyl tributyl citrate have been tested in this work. Dibutyl phthalate belongs to the phthalate esters applications of which should be eliminated due to the concerns in terms of toxicological, ecotoxicological and environmental reasons [34]. On the other hand, acetyl tributyl citrate is presented as a non-toxic plasticizer with an excellent toxicological and eco-toxicological profile [35]. The main idea to blend PLA with P3HB-co-4HB is that both are biodegradable and biocompatible polyesters, although their thermal stability in the melt stage is different. Based on our previous experiments and results we have found that PLA is relatively stable in the melt stage during melt processing, e.g. extrusion. Therefore, we have suggested blending PLA with PHA copolymer.

### *3.2. Thermal and rheological properties of filaments*

Mixtures of the copolymer with additives were blended and extruded as filaments with a thickness varied from 1.07 to 1.76 mm. Filaments were prepared by melt mixing of polymer and additives and discharging the mixture at a stable rate of 30 rpm for all samples. The required final filament thickness was 1.75 mm. However, the thickness of the filaments is also depended on the mixture's viscosity. Polymer viscosity was affected by temperature, the composition of compounds, shear rate and molecular weight. Table 2 shows that the molecular weight of compounds M1-M4 after extrusion compared to the original value of the isolated copolymer decreased by 34.5-71.3% depending on the composition of the mixture. In the case of very low viscosity, the polymer mixture might be drained faster than was expected and thus the resulting filament could be much thinner compared to the initial expectations. This situation happened in the case of extruded sample M1, in which complex viscosity after extrusion reached only  $77.7 \text{ Pa s}$ , and the obtained filament achieved the thickness of approximately 1.10-1.20 mm. The modified composition of the P(3HB-co-4HB) copolymer by the addition of plasticizer remarkably enhanced the viscosity of compounds M2-M4, which was sufficient for extruding filaments with the required thickness in the thickness range between 1.65 and 1.76 mm (FESEM micrographs of filaments can be found in the Supplementary Information, see Fig. S1). Furthermore, the formulation of the compounds affected the surface morphology of the filaments (Fig. S2, Supplementary Information). The surface of the PHA filament without the addition of a plasticizer was smooth, while the morphology of the M2 and M3 filaments showed a more complex and rougher texture due to the presence of added plasticizer. The surfaces of the M4 filament compared to the M2 and M3 filaments were finer and the surface roughening due to the plasticizer was much milder only in the form of needles.

**Table 2** Molecular weight properties of P(3HB-co-4HB) filaments.

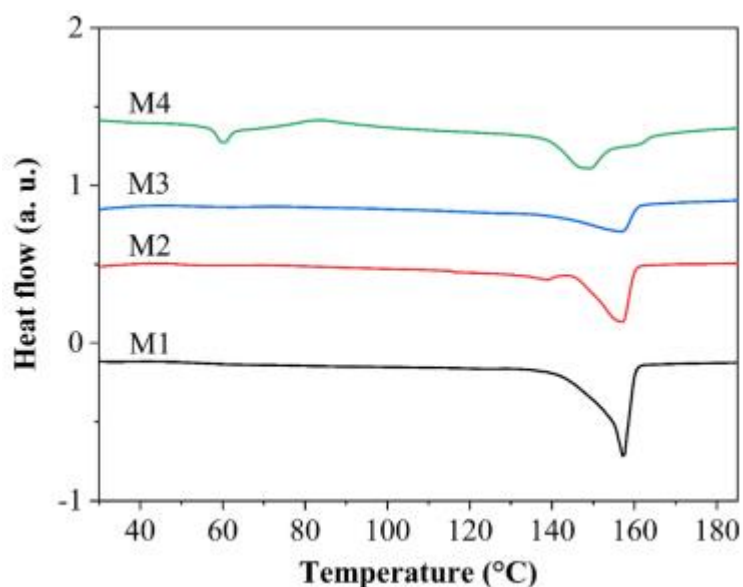
Sample	SEC MALS	
	$M_w$ (kDa)	$\bar{D}$ (a.u.)
M1	132.0 ± 3.4	1.50 ± 0.12
M2	212.4 ± 2.2	1.26 ± 0.03
M3	301.1 ± 24.8	1.20 ± 0.21
M4	142.3 ± 1.9	1.43 ± 0.03

**Table 3** Thermal properties of P(3HB-co-4HB) filaments.

Sample	DSC														TGA	DTGA
	1st heating cycle						2nd heating cycle						$T_{onset}$ (°C)	$T_{max}$ (°C)		
	$T_{cc}$ (°C)	$\Delta H_{cc}$ (J g <sup>-1</sup> )	$T_{m1}$ (°C)	$T_{m2}$ (°C)	$\Delta H_m$ (J g <sup>-1</sup> )	$\Sigma H$ (J g <sup>-1</sup> )	$T_g$ (°C)	$T_{cc}$ (°C)	$\Delta H_{cc}$ (J g <sup>-1</sup> )	$T_{m1}$ (°C)	$T_{m2}$ (°C)	$\Delta H_m$ (J g <sup>-1</sup> )				
M1	-	-	-	157.2	28.5	28.5	-8.5	57.6	25.0	126.8	156.7	29.8	4.8	235.7	247.8	
M2	-	-	138.8	157.2	21.5	21.5	-8.8	63.5	21.9	128.1	157.6	22.9	1.0	240.0	253.4	
M3	-	-	-	157.1	14.0	14.0	-10.6	72.9	21.7	129.9	157.3	21.8	0.1	240.7	253.6	
M4	83.2	1.9	149.2	160.5	17.2	15.3	-8.9/57.0	75.7	5.9	131.3	160.1	7.2	1.3	258.3	268.2/328.0	

Specific melting enthalpy,  $\Sigma H = \Delta H_m - \Delta H_{cc}$ .

The thermal properties of filaments are displayed in Table 3. As expected due to the thermal instability and the modification of the mixture composition by using the additives, the thermal behaviour of compounds was different compared to that of the neat copolymer. DSC data derived from 1st heating scan inform about thermal properties of filaments (Fig. 3). The main detected differences were in lower values of melting temperature and the specific melting enthalpy. The decrease in melting temperature by about 7 °C is largely related to lower detected molecular weight values. Moreover, the data show that the addition of plasticizers (DBP and citrofol BII) modified the crystallization and melting behaviour of filaments M2-M4 compared to filament M1. The melting endotherm of filaments M1 and M3 display a single  $T_m$  of between 126.5 and 162 °C. The addition of 2 wt% DBP (M2) caused a double melting behaviour at 138.8 and 157.2 °C and loss of melting enthalpy of about 24.6% compared to filament M1. The filament with 2 wt% of citrofol BII showed only a single melting peak at 157.1 °C but with about 50.9% lower melting enthalpy compared to the filament without plasticizer (M1). The filament M4 produced by blending of the copolymer with PLA (50/50) and 2 wt% of citrofol BII showed a double melting peak due to the presence of two kinds of crystallites arisen from copolymer and PLA. Similarly to the copolymer, also compounds M1-M4 were not able to crystallize during the cooling scan. The addition of both types of plasticizer slightly shifted values of glass transition temperature to the lower values. The filament M4 displayed two  $T_g$  values at -8.9 °C and 57 °C, which are typical for polymer blends such as P3HB-co-4HB and PLA, respectively. Data from second heating scans show the cold crystallization of filaments and double melting behaviour with values of final specific melting enthalpy in the range 0.1 to 4.8 J g<sup>-1</sup>.



**Fig. 3.** DSC first heating scans of P3HB-co-4HB filaments. [Color figure can be viewed in the online issue]. [Color figure can be viewed in the online issue].

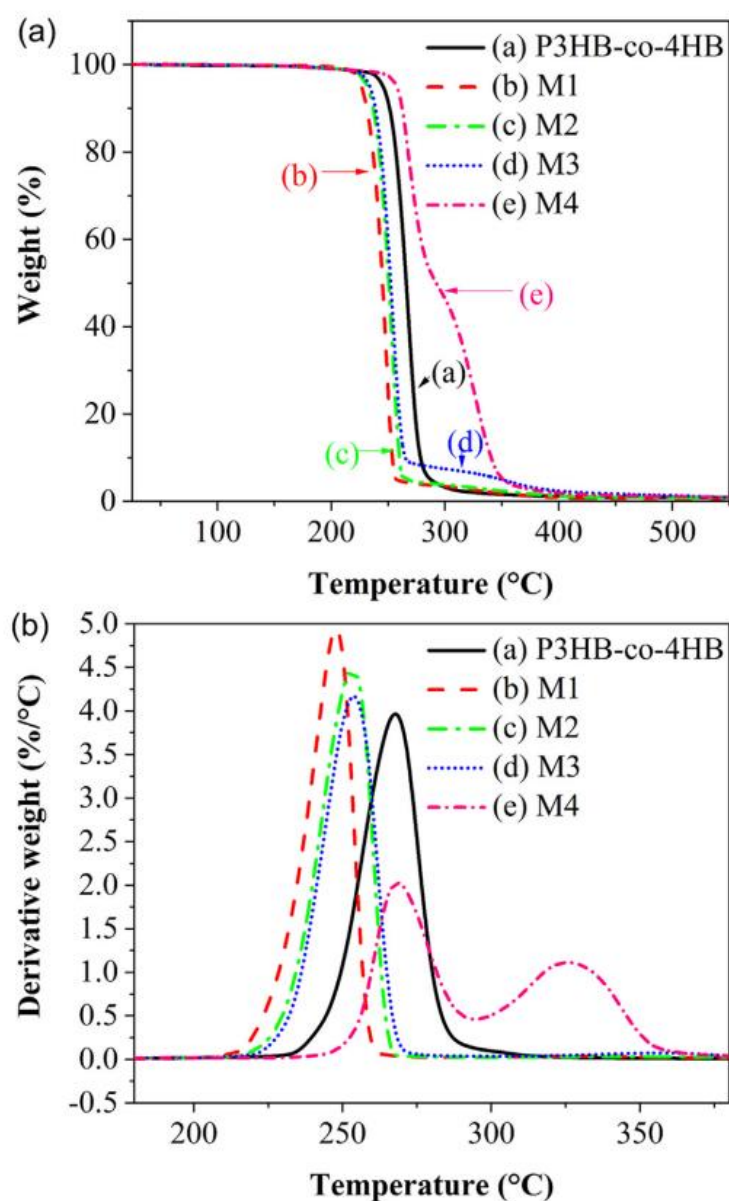
Such low values of specific melting enthalpy indicate the almost complete amorphous character of the sample after melting and consequent cooling (1st heating scan and cooling scan). The crystals were predominantly formed during the 2nd heating scan. Thermograms from second heating scans are shown in Fig. S3 (Supplementary Information). The lowest ability to crystallize displayed the compound M4. From the point of long-term stability, it can be concluded that the mixing of the copolymer with PLA may be an advantage for the balanced amorphous character of the mixture, stable even after exposure to a different temperature history.

Thermogravimetric analysis was used to assess the thermal stability of P3HB-co-4HB filaments in the air (Fig. 4) up to 550 °C. The identified values of  $T_{onset}$  and  $T_{max}$  are reported in Table 3. The TGA and DTGA curves show that the addition of both plasticizers and mainly blending of the copolymer with PLA contributed to the higher thermal stability of copolymer. The addition of plasticizers improved the thermal stability of the PHA mixture by about 4.3-5.0 °C. The PHA/PLA plasticized with citrofol BII was the most stable material with a  $T_{onset}$  value higher at about 23.1 °C compared to PHA filament M1. The PHA/PLA blend displayed two sharp weight losses at 225.7 °C and 290.0 °C due to the content of two polymers (Fig. 4b). The lower value identifies the onset temperature of PHA decomposition and the higher value is the onset temperature of PLA decomposition.

The rheological properties of neat copolymer and compounds processed in an extruder were investigated to assess their melt processability and thermo-mechanical stability. First, dynamic strain sweep tests were conducted at 180 °C (neat copolymer) and 185 °C (neat PLA) and frequency of 6.28  $\text{rad s}^{-1}$  to determine the linear viscoelastic region of the copolymer (Fig. 5). According to these data, linear melt rheological analyses were conducted at a strain of 0.7%.

Polyhydroxyalkanoates are melt-processable but prone to thermal degradation due to the fast polymer chain cleavage at a temperature not far from their melting temperatures. The range of PHAs degradation depends on various factors such as polymer chemical structure, initial molecular weight, and processing conditions (temperature, time, atmosphere, humidity and stress) [17,36]. Fig. 6 shows the results from the time sweep experiments of the neat copolymer and neat PLA in the form of complex viscosity ( $\eta^*$ ) changes at 6.28  $\text{rad s}^{-1}$  and the constant strain of 0.7% (chosen from LVR) over

a deformation period of 40 min. Both non extruded polymers, copolymer and PLA without any additives show a fast reduction of  $\eta^*$  with time at the suggested melt processing temperature and screw speeds ( $180\text{ }^\circ\text{C}$  and  $6.28\text{ rad s}^{-1}$ ). The determined 46% loss in complex viscosity even up to 5 min reflected that time is an important factor during melt processing of copolymer. PLA was also degraded but much slower. For the comparison, to a similar extent, as copolymer degrades in 5 min, PLA degrades in up to 20 min. This is one of the main reasons why PLA is preferred for fused deposition modelling and PHAs are not. The melt extrusion of neat copolymer without any additives was not possible due to the rapid thermal degradation and decrease of complex viscosity. Therefore, the composition of the copolymer was modified by the addition of phenolic antioxidant (Irganox 1010), a crosslinking agent (Joncryl) and plasticizers.



**Fig. 4.** The thermogravimetric data: (a) TGA and (b) DTGA curves for the thermal decomposition of filaments. [Color figure can be viewed in the online issue].

In all samples, the concentration of antioxidant and crosslinking agent was kept at the concentration, which was selected based on our laboratory initial experiments. The aim was to find out if the addition of plasticizer and/or PLA would fundamentally affect the resulting rheological and mechanical properties of the prepared filaments. Dibutyl phthalate and acetyl tributyl citrate have been tested as plasticizers. The reduction of viscosity of mixtures prepared by extrusion was studied for 40 min by normalizing the initial complex viscosity values at  $t = 0$  min over time (see Fig. 7). Numerical values of degradation after 5,10, 20 and 40 min are given in Table 4. The data show that the initial values of the complex viscosities of the mixtures obtained by extrusion of the copolymer with additives differ significantly from the neat P3HB-co-4HB. The initial complex viscosity of P3HB-co-4HB in the melt was similar to that of poly(lactic acid). However, 70.2% loss of complex viscosity after 5 min is unacceptable for the production of filaments by extrusion. Based on our observation, the extrusion of filaments was practically possible only with the addition of a stabilizer and chain extender. Such mixture without plasticizer (M1 filament) reached the complex viscosity of 77.7 Pa s., which was about 6 to 17.8 times lower value compared to mixtures with plasticizer or PLA (M2-M4). Theoretically, the polymer with the lower complex viscosity could contribute to the better performance of the printed samples. However, the determined values of molecular weight showed that lower complex viscosity values indicate a drop in the molecular weight of copolymer due to the large thermal degradation during extrusion. The addition of the plasticizer markedly inhibited the decrease in complex viscosity over time. The complex viscosity of the mixture M2 with DBP was about 6.1 times higher and the complex viscosity of the mixture M3 with citrofol BII was about 17.9 times higher compared to the mixture without plasticizer.

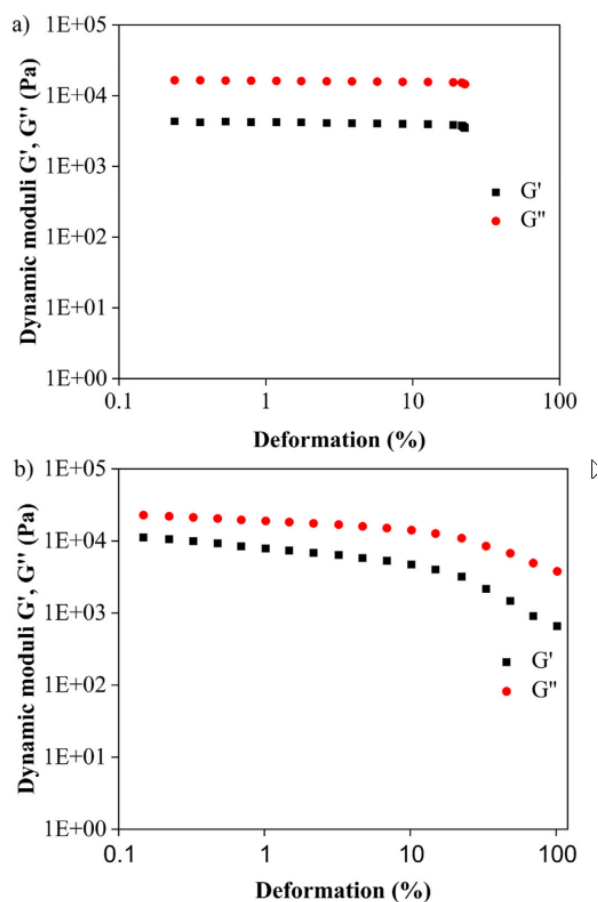


Fig. 5. Plots of storage ( $G'$ ) and loss moduli ( $G''$ ) versus deformation of (a) neat PLA at 185 °C, (b) neat copolymer at 180 °C. [Color figure can be viewed in the online issue].

The mixture M3 proved to be the most thermally stable but the extent of degradation was still critical. The degradation after 5 min was 37.2% that was still not optimal for 3D printing. The mixture M4, the 50/50 blend of the copolymer with PLA and the addition of citrofol BII was the most stable mixture showing degradation after 5 min only about 6.8%. For comparison, neat PLA pellets showed degradation after 5 min three times higher value. According to the performed rheological measurements, it can be concluded that the blending of P3HB-co-4HB with PLA seems to be important for the improvement of thermal stability during melt processing.

### 3.3. Mechanical properties of filaments

Table 5 summarizes the mechanical properties of filaments made from biosynthesised P3HB-co-P4HB. The mechanical properties correspond with the molecular weight of polymers. The cleavage of polymer chains due to thermal degradation contribute to the deterioration of mechanical properties.

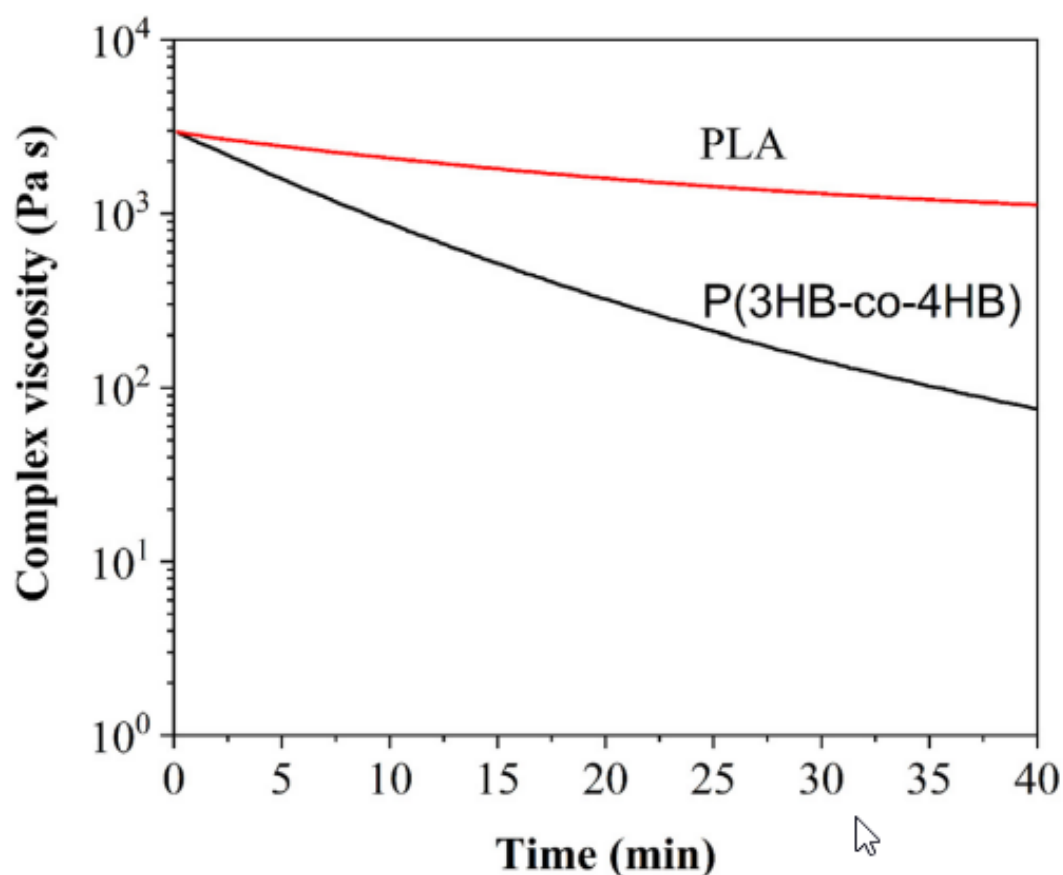
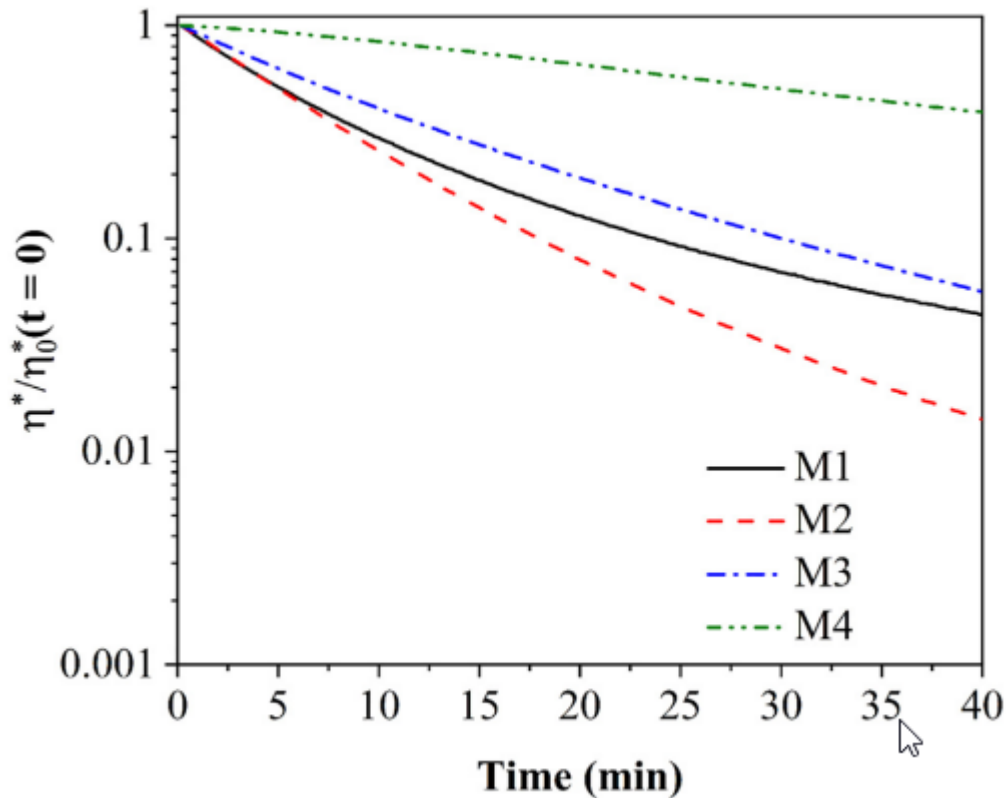


Fig. 6. Complex viscosity  $n^*$  versus time at 6.28 rad s<sup>-1</sup> of copolymer at 180 °C and PLA at 185 °C. [Color figure can be viewed in the online issue].



**Fig. 7.** Evolution of complex viscosity,  $\eta^*(t)/\eta^*(t=0)$  versus time of M1 (P3HB-co-4HB), M2 ((P3HB-co-4HB with dibutyl phthalate), M3 (P3HB-co-4HB with citrofol BII), and M4 (P3HB-co-4HB/PLA blend with citrofol BII). [Color figure can be viewed in the online issue].

The filaments for FDM should exhibit sufficient mechanical properties to withstand physical stress arisen during fused deposition modelling without failure. All tested filaments displayed high values of tensile strain at break showing a high ability to resist mechanical changes without cracking.

**Table 4** Complex viscosity of neat P3 HB-co-4HB and PLA as well as compounds formed in the form of filaments at 180 °C and 185 °C (PLA pellets) and 6.28 rads<sup>-1</sup> after 5,10,20 and 40 min of evolution time.

Sample	E modulus (MPa)	Tensile strength at maximum (MPa)	Tensile strain at break (%)
M1	51.9 ± 11.7	16.1 ± 1.5	1346 ± 264
M2	75.9 ± 15.3	21.6 ± 2.3	1695 ± 154
M3	68.5 ± 13.2	18.3 ± 3.2	1720 ± 230
M4	118.2 ± 0.4	20.1 ± 2.3	397 ± 76



**Table 5** Mechanical properties of P3HB-co-4HB filaments.

Sample	$\eta^*$ (Pa s) at 0 min	Degradation (%) after time of			
		5 min	10 min	20 min	40 min
PLA pellets	2947.5	29.3	45.9	55.8	62.04
P3HB-co-4HB powder	2943.2	70.2	89.1	95.1	97.4
M1 filament	77.7	48.5	70.3	87.2	95.6
M2 filament	475.1	49.3	74.1	98.4	99.7
M3 filament	1387.1	37.2	59.2	80.8	94.3
M4 filament	1324.5	6.8	15.9	34.3	60.7

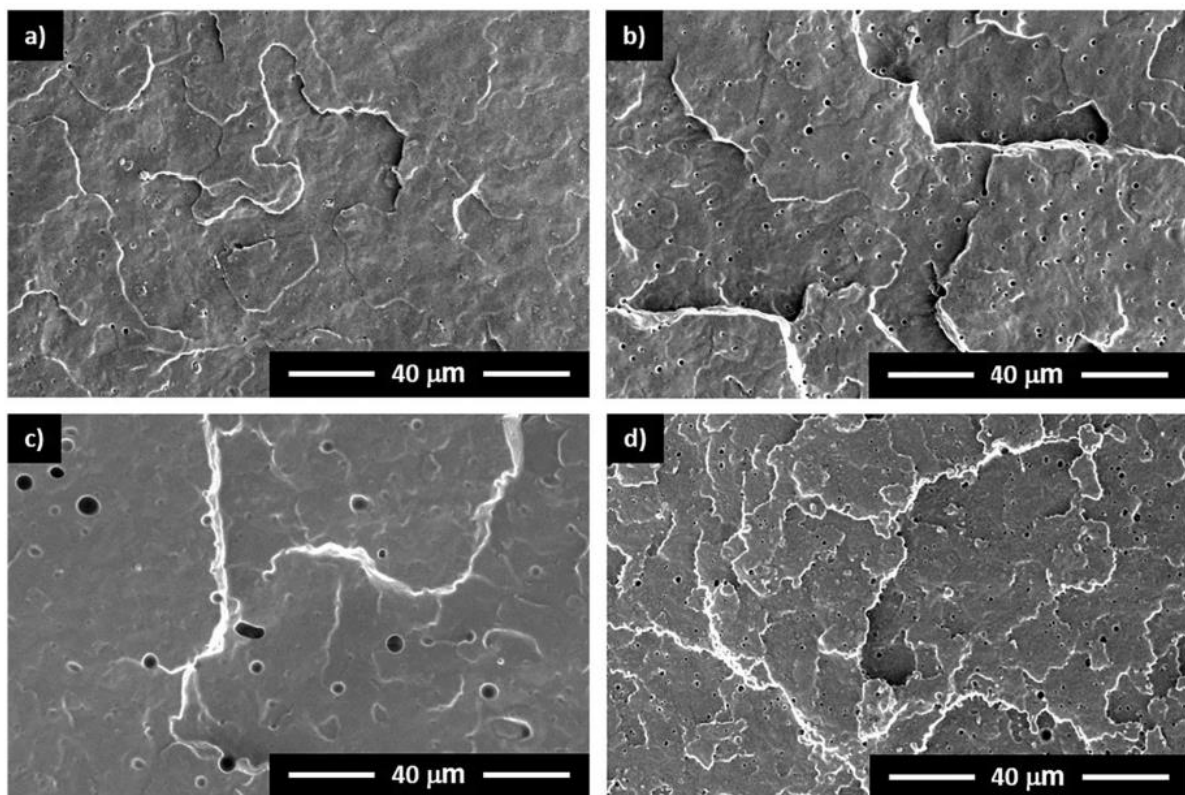
The M2 and M3 filaments with added plasticizers DBP and citrofol BII displayed higher values of Young moduli, tensile strength at maximum and tensile strain at break compared to non-plasticized filament M1. The increase of flexibility of polymers is a typical effect of plasticizers. However, the higher values of tensile strength and E-modulus determined for plasticized filaments confirmed that plasticizers being incorporated into PHA hindered the thermal degradation. The reason for heat stabilization may be that both plasticizers are hydrophobic low molecular organic compounds, and therefore may effectively hinder the hydrolytic degradation of polyesters [37]. The tensile strength value of the PHA/PLA blend is comparable with that of plasticized PHA but the value of E-modulus is approximately about one time higher than PHA filaments. The value of tensile strain at break of PHA/PLA plasticized blend reached one fourth value of tensile strain at break of non-plasticized PHA. This value is more than 16 times higher compared to pure PLA filament [17]. Considering the fused deposition modelling process, all filaments except M2 were suitable for the 3D printing process (see Fig. 8).

**Fig. 8.** 3D printed specimens formed from filaments based on (a) P3HB-co-4HB (M1), (b) P3HB-co-4HB with 2 wt% of citrofol BII (M3) and (c) blend of P3HB-co-4HB/PLA 50/50 with 2 wt% of citrofol BII (M4).



However, the print speed for work with filaments M1 and M3 had to be decreased to 60% of the value used for filament M4. Therefore, these materials can be used in commercial 3D printers with printing protocol similar to a 3D printing from PLA, the most widespread biodegradable polymer on the market of filaments, but at a slightly lower temperature compared to PLA (180 °C for P(3HB-co-4HB) versus 240 °C for PLA). The filament M2 clogged the 3D printing extruder, the material was sticky and formed lumps, so it was not possible to print specimens from it.

Scanning electron microscopy (FESEM) was employed to discern the surface morphology of the fractured filaments. At the first sight, all FESEM micrographs show pores (Fig. 9). These pores did not cause the deterioration of mechanical properties of the filaments as was determined by tensile testing (Table 5). The presence of pores in filaments processed by extrusion has also been described in other works [38,39]. Their origin is explained as a consequence of gas evolution during the production of filaments by extrusion [38]. On the other hand, polymers are well known to be sensitive to electron beam irradiation. The primary effects of the interaction of electrons with organic matter are inelastic scattering processes, which yield ionisation and break chemical bonds. Secondary effects are mainly chain scission or crosslinking, mass loss, fading of crystallinity, heat generation, and charging-up [40]. Therefore, it is reasonable to assume that these pores are formed during the morphology investigation. This feature occurred on the morphology of all tested samples despite the coating of the freeze-fractured surface by a thin layer of Au/Pd prior investigation and the "low-dose" technique employed for image acquisition. The surface of all tested filaments was relatively smooth which is attributed to the low degree of crystallinity. The smoothest fracture surface exhibited the filament M3 with the addition of citrofol BII. This plasticizer caused a 50.9% decrease in P3HB-co4HB crystallinity. The slight surface roughening was observed in the M4 filament due to the blending of PHA with PLA.



**Fig. 9.** FESEM micrographs of filaments cross-sections: (a)M1 (P3HB-co-4HB), (b) M2 (P3HB-co-4HB with dibutyl phthalate), (c) M3 (P3HB-co-4HB with citrofol BII), and (d) M4 (P3HB-co-4HB/PLA blend with citrofol BII).

#### 4. Conclusions

Filaments for further fused deposition modelling with defined diameters based on poly(3-hydroxybutyrate-co-4-hydroxybutyrate) with 10 mol% 4HB were successfully prepared by the extrusion process. Experimental investigation on the role of plasticizer addition into P(3HB-co-4HB) or its mixing with poly(lactic acid) on the improvement of the rheological behaviour of mixture and mechanical properties of filaments have been conducted. As the most thermally stable mixture was proved a blend of P(3HB-co-4HB) with PLA plasticized with citrofol BII was proved when the complex viscosity of this blend decreased in the melt at 180 °C after 10 min by only 15.9% compared to the 70.3% decrease in the complex viscosity observed in the case of the non-plasticized copolymer.

Compared with the non-plasticized specimen, adding dibutyl phthalate or citrofol BII into copolymer could increase E modulus, tensile strength at maximum and tensile strain at break of filaments based on P3HB-co-4HB. Specimens of copolymer plasticized with 2 wt% of citrofol BII reached tensile strain at a break of 1720%. Also blending of P3HB-co-4HB with PLA increased values of E modulus and tensile strength at maximum but decreased the tensile strain at the break into the value of 397% (16 times higher compared to the value for a pure PLA filament [17]). Acetyl tributyl citrate as a biobased alternative to phthalate plasticizers was well dispersed in P(3HB-co-4HB) as well as in PHA/PLA blend producing single-phase morphologies. The filaments with citrofol BII were ductile and sufficiently thermo-mechanically stable for fused deposition modelling. The most thermo-mechanically stable mixture was P(3HB-co-4HB)/PLA blend plasticized with 2 wt% citrofol BII. Moreover, this sample was identified as the most suitable formulation for fused deposition modelling because the 3D process in a commercial printer was smooth without any difficulties.

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