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Multiblock alicyclic/aliphatic polyesters. Evaluation of the polymer's susceptibility to enzymatic degradation

Summary — Multiblock polyesters were prepared from dimethyl 1,4-cyclohaxanedicarboxylate (DMCD) (or dimethyl terephthalate, DMT) and 1,4-butanediol (1,4-BD) with dodecanedioic acid (DA). Polymers were prepared by two-stage technique namely transesterification and polycondensation from the melt. The multiblock polyesters obtained were characterized by viscosity measurements, differential scanning calorimetry and tensile test. Polymers were exposed, *in vitro*, to *Rhisopus delemar* lipase solution. The enzymatic degradation was monitored by changes in molecular weight (from gel permeation chromatography) and estimated by the weight loss of the polyester films in a buffer solution with the enzyme at 37°C. Performed studies have shown that the enzyme *Rhisopus delemar* is able to alter the structure of the alicyclic/aliphatic polymer (PCD-DA) to a greater extent than the structure of aromatic/aliphatic copolyester (PBT-DA).
Key words: multiblock copolymers, alicyclic polesters, biodegradation.

Considerable attention has been focused over the recent years on biodegradable polymers. These are of great importance for environmental and biomedical applications. Among such copolymers, synthetic aliphatic polyesters have been widely investigated [1—3], however they exhibit some disadvantages, especially regarding their mechanical properties. Therefore, blending or copolymerization technique can be successfully applied to produce biodegradable polymers with improved physical properties [4, 5].

The incorporation of aromatic structures to aliphatic polymers will lead to formation of multiblock copolymers. Multiblock copolymers are extensively studied and well documented in the literature [6—8]. Numerous papers and patents have been devoted to the synthesis, structure and properties of copoly(ester-*b*-ester)s [9, 10], copoly(ester-*b*-ester)s [11, 12] or copoly(ester-*b*-amide)s [13].

Multiblock polymers consist of different types of segments that alternate along the macromolecule. Each segment of the copolymers has its own characteristic properties, *i.e.* glass transition and melting temperature. In copolymers consisting of two different blocks, one of them possesses a low crystallizability and low bond cohesion energy. These segments, which impart elastomeric properties to the polymer material, are often called the "soft segments". Other segments usually possess high elastic modulus [14—16] and, therefore are often referred to as "hard segments". The lack of miscibility (microphase separation) between polymer segments, which depends primarily on molecular weight and segment structure, is an important characteristic of multiblock copolymers, especially in view of potential practical aspects.

Multiblock copolymers are characterized by good mechanical properties in a wide range of temperatures, especially in the low-temperature region. Major end-use markets for multiblock copolymers include the mechanical and automotive industries. They are also finding their way into medical, sport and electronic devices [17].

The systematic studies of various multiblock polyesters [18—20] have been undertaken in our laboratory. All of studied materials were composed of aromatic and aliphatic sequences and exhibited high extensibility, good reversibility after strain and good processability.

Recent efforts in our laboratory have focused on methods of broadening the structural variation in multiblock polyesters replacing aromatic segments by alicyclic sequences. There is apparently little literature data concerning material characteristics and especially biodegradability of alicyclic/aliphatic copolyesters although a number of biodegradable polyesters have been developed up to now and some of them, like poly(hydroxy-

butyrate) (PHB) or poly(caprolactone) (PCL) are commercially available [21].

Recently, Jung *et al.* [22] reported that alicyclic/aliphatic polyesters composed of butylene succinate and cyclohexanedimethylene succinate combine satisfactory physical properties and biodegradability.

In this work we report the results regarding the synthesis process and enzymatic degradation of alicyclic/aliphatic copolyester. The alicyclic segment content was kept constant at 60 wt. % to ensure relatively good mechanical properties. Polyester of identical weight content of aromatic segments was also prepared for comparative purposes.

EXPERIMENTAL

Materials

The high purity dimethyl 1,4-cyclohexanedicarboxylate (DMCD) was purchased from Sigma Aldrich, Germany as a 97% *trans/cis* mixture. The sample, as received, consisted of crystalline solid and colorless clear liquid. The solid was decanted from the liquid and the structural purity was verified by NMR. The solid monomer, which contained higher percentage content of *trans* isomer, was used for polymerization.

Dimethyl terephthalate (DMT) and 1,4-butanediol (1,4-BD) were purchased from Merck, Germany, and used as received.

Dodecanedioic acid (DA) was purchased from Sigma Aldrich, Germany, and was used without purification.

Magnesium-titanate catalyst (obtained according to recipe in [23]) was diluted in butanol to a concentration of 10 wt. %.

Polymer synthesis

All polymer material was obtained in a two-stage process, namely transesterification and polycondensation in the melt. The synthesis process was carried out in a "pressure-vacuum" reactor made of stainless steel.

The reaction mixture consisting of dimethyl 1,4-cyclohexanedicarboxylate or dimethyl terephthalate, 1,4-butanediol and magnesium-titanate catalyst was heated up to 200°C at heating rate of 1.5°C/min in the reactor for transesterification. The molar ratio of BD and DMCD (or DMT) was as 1.8:1. The reaction was finished when more than 95% of the stoichiometric amount of methanol evaporated. Oligomers of butylene cyclohexanedicarboxylate (or butylene terephthalate) were obtained under these conditions and under reduced pressure. α,ω-dicarboxy-terminated aliphatic acid, namely 1,10-dodecanedioic acid (DA) was added along with a catalyst during the second stage of the reaction. Polycondensation was carried out in a polycondensation reactor heated to 225—230°C and at pressure of 0.5—0.6 mm Hg. The process was considered complete on the basis of the observed power consumption of the stirrer motor when the product of highest melt viscosity was obtained (up to a constant value of power consumption by the stirrer). The reaction mass was extruded by means of compressed nitrogen into water, and granulated.

Characterization

The limiting viscosity number [η] of the polymers was measured at 30°C using polymer solutions in $PhOH/C_2Cl_3H$ (50/50 mol %).

Differential scanning calorimetry (DSC) was performed with Perkin-Elmer (DSC-2) apparatus. The samples, weighing between 22—25 mg, were dried in a vacuum at 70°C. The process was carried out in a triple cycle "heating-cooling-heating", in the temperature ranges -100 to 220°C and -50 to 220°C. Heating and cooling rate was 10°C/min. Temperature and enthalpy calibration were carried out using indium standard. Glass transition temperature (T_g) was determined from the temperature diagrams as the temperature corresponding to the upper inflection point. Melting point (T_m) and crystallization temperature (T_c) were determined as corresponding to the maximum of the endothermic curve and the minimum of the exothermic curve, respectively.

A Shodex (JM Science, Grand Island, NY, USA) linear GPC SE 61 column packed with 5 mm Pl-gel MIXED-C (Polymer-Laboratories) were employed for molecular weight analysis (GPC apparatus from Spectra Physics 8800).

Tensile data were collected at room temperature with an Instron TM-M tensile tester at a crosshead speed of 20 cm/min. Dumbbell-shaped samples were prepared according to ASTM D 1897-77. Stress data were calculated as the ratio of force to initial cross-section area. Stress at break, yield stress and elongation values were averaged over 4—6 measurements for each sample.

Enzymatic degradation was performed with using *Rhisopus delemar* lipase (specific activity 60 unit/g from Bio Chemika Fluka). The film specimen (20 mm × 20 mm; about 130 μm thickness) was placed in a vial containing 20 mL of phosphate buffer (pH 7.4) with or without 3 000 units of the enzyme. The vial was incubated at 37°C for various periods of time (1, 2, 3 and 6 weeks, respectively). The phosphate buffer/enzyme solution was replaced every 3 days to keep enzyme activity at a desired level throughout the experiment. After incubation, the film was washed with distilled water and dried at 50°C in vaccuo until a constant weight was reached. Weight loss averaged for three specimens was taken as the result.

RESULTS AND DISCUSSION

Synthesis

The objective of this study was the synthesis of the block polymers of poly(butylene cyclohexanedicar-

boxylate-dodecanedioic acid) (PCD-DA) with alicyclic units, which are alternated along macromolecule with amorphous aliphatic segments. Condensation method typical for the poly(butylene terephthalate) (PBT) polyester [24, 25] was used for synthesis purposes. Temperature of the process was kept at 230°C, since alicyclic units are more temperature sensitive. Alicyclic/aliphatic copolyester (PCD-DA) (I) was compared with aromatic/aliphatic copolyester [PBT-DA, poly(butylene terephthalate-dodecanedioic acid)] (II). Obtained polymers were all white and had high values of the limiting viscosity number (Table 1).

Table 1. Limiting viscosity number of copolymers

Code	W_h, %	DP_h	η, dL/g
PBT-DA	60	2.05	1.08
PCD-DA	60	1.99	1.26

PBT-DA — multiblock copolymer consisting of aromatic and aliphatic units,
PCD-DA — multiblock copolymer consisting of alicyclic and aliphatic units,
W_h — weight content of alicyclic (aromatic) segments,
DP_h — degree of polymerization of alicyclic (aromatic) segments calculated from the amount of monomers at entrance of the reaction,
η — limiting viscosity number.

Differential Scanning Calorimetry (DSC)

The DSC heating and cooling scans of the PCD-DA and its aromatic homologue PBT-DA are shown in Fig. 1, whereas other thermal transitions are collected in Table 2.

Table 2. Thermal transitions from DSC

Material	T_{g1} °C	T_{c2} °C	T_{g2} °C	T_{m2}, °C I run	T_{m2}, °C II run	ΔH_{m2} J/g	$T_{m2}-T_{c2}$ °C	T_{g1}/T_{m2} °C
PBT-DA	-26	121	51	162	164	31.8	43	0.16
PCD-DA	-41	30	43	80	85	21.4	55	0.48

T_{g1} — glass transition of the soft segments;
T_{c2}, T_{g2}, T_{m2} — crystallization, glass transition and melting temperature, respectively, of the hard segments;
ΔH_{m2} — melting enthalpy of the hard segments.

A low-temperature single soft segment glass transition was observed for both polymers (Fig. 1). Comparison of the soft segment glass transitions for the alicyclic and aromatic segments-based material indicates that the alicyclic polymer exhibits lower soft segment glass transition than the comparable polymer with aromatic segments. Such a decrease in soft segment glass transition temperature is associated with a higher degree of phase separation.

The introduction of alicyclic sequences to the polymer backbone appears to inhibit polymer crystallization (Fig. 1a and Table 2). The polymer crystallizes at 30°C, whereas its aromatic analogue has the crystallization temperature of 121°C. One of the possible causes of suppression of the crystallization process in alicyclic polymer is due to the high mutual miscibility between components of the aliphatic soft segments and quasi-hard (alicyclic) segments.

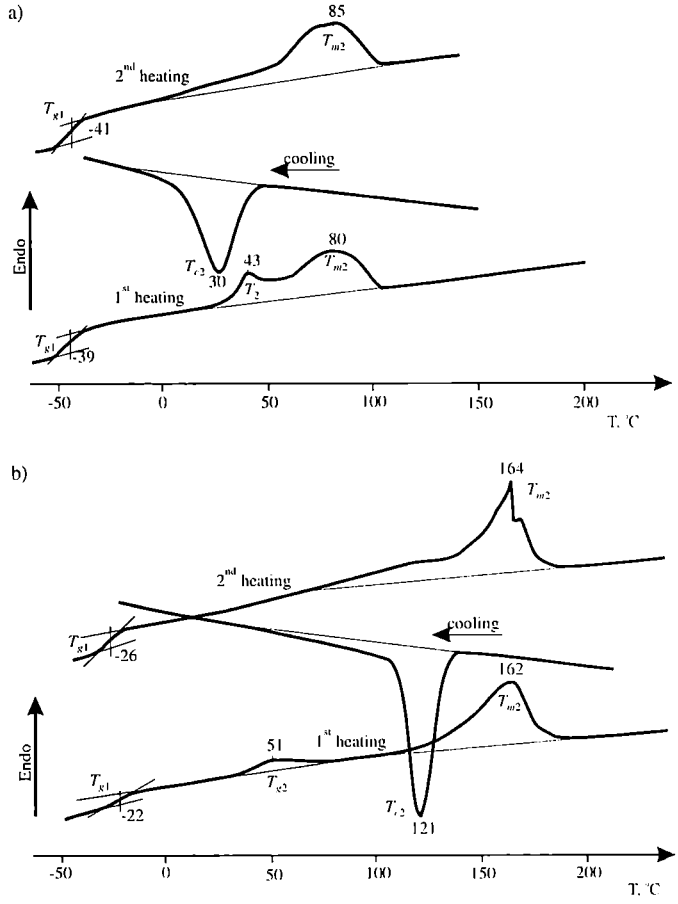

Fig. 1. DSC scans of multiblock copolymers: a — PDC-DA, b — PBT-DA

The same behaviour was also observed in suppression of melting temperature T_{m2} of polymers (Table 2). It is worth noting that for the PCD-DA polymer, disappearance of lower melting endotherm was noted in second heating run (Fig. 1a). This behaviour can indicate that the lower melting crystals are formed at much slower growth rate than the higher melting crystals, as was observed also for polymers based on succinic acid and 1,4-cyclohexanedimethanol [22].

The difference between the melting and crystallization temperatures expresses undercooling prior to crystallization. The lower this value is, the higher is the crystallization rate of the system. The fast crystallizing PBT homopolymer shows a T_{m2}-T_{c2} of 32°C in a DSC scan at 10°C/min heating and cooling [15, 20]. The T_{m2}-T_{c2} for synthesised block copolymer of poly(butylene terephthalate-dodecanedioic acid) (PBT-DA) consisting of aromatic/aliphatic units is 43°C, whereas the undercooling for a alicyclic/aliphatic PCD-DA copolyester is 55°C. This indicates that alicyclic copolymers crystallize slower than PBT-based multiblock copolyesters.

T_{g1} values for the PCD-DA multiblock copolymer the are of -41°C, the T_{g1}/T_{m2} ratio is of the order of 0.48 and crystallization is very slow (a T_g/T_m ratio of 0.16 was found for its fast crystallizing aromatic analogue). The crystallization of alicyclic/aliphatic copolymer becomes more difficult because of the mutual miscibility between components of the aliphatic soft segments and alicyclic segments, as discussed above.

Tensile Properties

The tensile properties of samples at room temperature are summarized in Table 3. As expected, polymer containing alicyclic units show lower values of stress at break and yield stress as well as of elongation at break. Better mechanical properties of PBT-based analogue (PBT-DA sample) can be easily explained by resulting of crystalline domains, which are responsible for good mechanical properties in thermoplastic elastomers [16].

Table 3. Mechanical properties of polymers

Code	W_h, %	σ_r, MPa	σ_e, MPa	ε, %
PBT-DA	60	19.4	11.4	590
PCD-DA	60	8.0	7.5	307

σ_r — stress at break,
σ_e — yield stress,
ε — elongation at break.

Enzymatic degradation

The enzymatic degradation of studied polymers has been monitored by changes of molecular weight (from GPC), by weight loss as well as optical microscopy. Synthesized polymers exhibit similar and rather high values of molecular weight (Table 4). A higher decrease in M_n can be observed for the PCD-DA copolyester after exposure of polymer films to enzyme in buffer solution. Weight loss for that copolyester was also more distinct than for its aromatic/aliphatic analogue (Table 5). Blank test without the lipase was performed in a buffer solution at 37°C. No weight loss was observed for polymer films incubated for 6 weeks without lipase. Figure 2 shows the optical micrographs of the PCD-DA copolyester film before degradation, and Figures 3—5 shows

Table 4. Molecular weight loss from GPC

Code	W_h, %	M_n	M_w	M_n/M_w	M_n*)	M_w*)	M_n*)/M_w
PBT-DA	60	56 500	91 800	1.62	52 300	88 460	1.69
PCD-DA	60	57 600	98 600	1.71	48 500	90 100	1.85

*) after enzymatic hydrolysis.

Table 5. Weight loss after enzymatic hydrolysis

Code	Weight loss, %		
	1 week	3 weeks	6 weeks
PBT-DA	0	0	3
PCD-DA	2.1	4.5	9.8

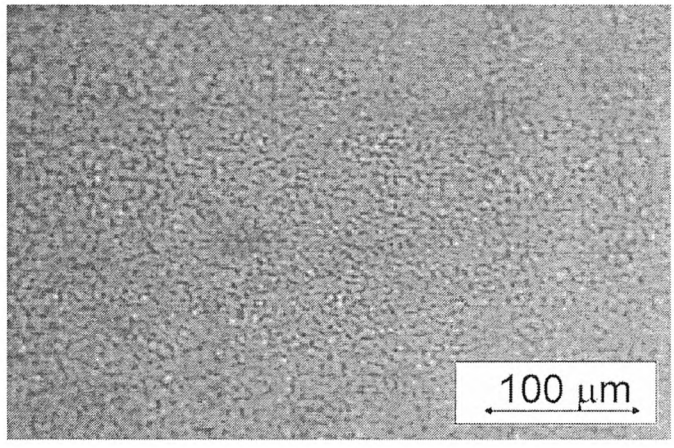

Fig. 2. Optical micrograph of PCD-DA polymer film before exposure to Rhisopus delemar

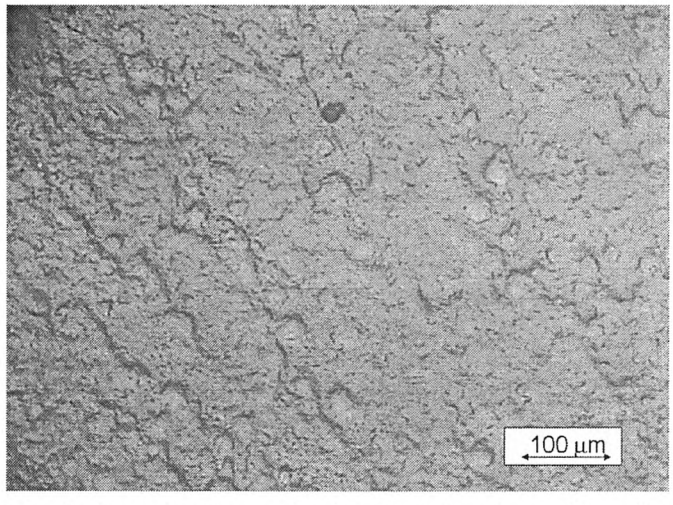

Fig. 3. Optical micrograph of PCD-DA polymer film after exposure to Rhisopus delemar, after 1 week of incubation at 37°C

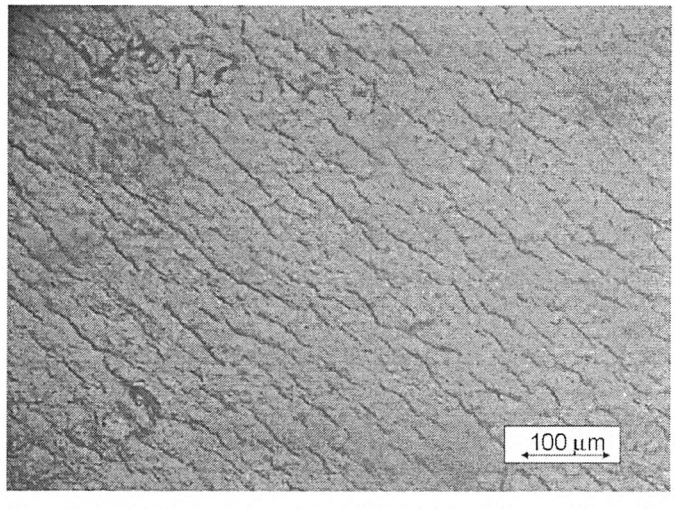

Fig. 4. Optical micrograph of PCD-DA polymer film after exposure to Rhisopus delemar, after 3 weeks of incubation at 37°C

the same polymer in the course of degradation. At the early stage of degradation the copolyester film maintained a relatively smooth surface; however, a rough surface with distinct cracks was developed at the later stages, *i.e.* after 3 and 6 weeks of incubation, respectively (Figures 4 and 5).

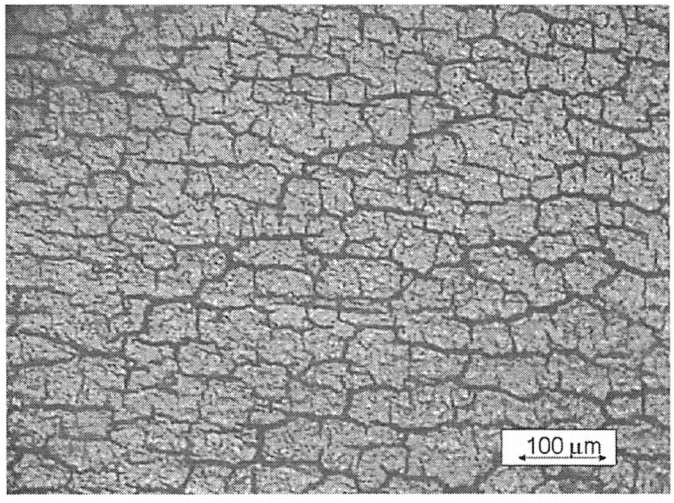

Fig. 5. Optical micrograph of PCD-DA polymer film after exposure to Rhisopus delemar, after 6 weeks of incubation at 37°C

CONCLUSIONS

The enzyme *Rhisopus delemar* used for the experiment is able to alter the structure of the alicyclic/aliphatic polymer to a greater extent than the structure of aromatic/aliphatic copolyester. But, it was demonstrated here that a far more stable polymer, such as aromatic/aliphatic copolyester, is also affected by enzyme.

When biodegradable polymers are defined as natural or synthetic materials with susceptibility toward microbial and/or enzymatic degradation [26], it can be concluded that newly synthesised alicyclic/aliphatic copolyester could be considered to be a biodegradable polymer. Moreover, future work will be focused on preparation of the series of polymers with a varying monomer composition in order to define the dependence between chemical composition of the alicyclic/aliphatic copolyesters and their susceptibility to degradation.

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