Synthesis and Characterization of Novel Copoly(ester−urethane) Containing Blocks of Poly-[(*R*)-3-hydroxyoctanoate] and Poly-[(*R*)-3-hydroxybutyrate]

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ABSTRACT: Novel poly(ester−urethane) block copolymer has been synthesized by use of telechelic hydroxylated poly-[(*R*)-3-hydroxyoctanoate] (PHO-diol) with number-average molecular weight (M_n) of 2400 as the amorphous soft segment, telechelic hydroxylated poly-[(*R*)-3-hydroxybutyrate] (PHB-diol) with M_n of 2600 as the crystalline hard segment, and L-lysine methyl ester diisocyanate (LDI) as the junction unit. Reaction of the PHO-diol, PHB-diol, and LDI at a ratio of 1:1:2 in the presence of dibuthyltin dilaurate as catalyst afforded the block copolymer in 70% yield with a molecular weight (M_w) of 33 500 (GPC) and a M_n of 10 600 (VPO). The chemical structure of the new polymer was confirmed by ^{1}H NMR, ^{13}C NMR, and IR spectra. On average, 2 PHO-diol, 2 PHB-diol, and 5 LDI were incorporated in each molecule of the block-copoly(ester−urethane). The thermoplastic properties were very good with a melting temperature (T_m) of 146 °C and the glass transition temperature (T_g) of −6 °C (DSC). The mechanical properties, obtained from the stress/strain test, are comparable with other biocopolyester and block-copoly(ester−urethane).

Introduction

Poly-[(*R*)-3-hydroxyalkanoates] (PHAs) are high molecular weight biodegradable and biocompatible polymers synthesized by a wide variety of microorganisms.[1−4] While poly-[(*R*)-3-hydroxybutyrate] (PHB) is highly crystalline and hard-brittle,[2,5−7] mcl-PHAs (which contain medium chain length alkanoate monomers) are weakly crystalline and soft-sticky.[8−13] These properties limit the application of PHAs as thermoplastic materials.[14,15] However, both thermal and mechanical properties can be improved by the biosynthesis of copolymers such as poly-[(*R*)-3-hydroxybutyrate-*co*-(*R*)-3-hydroxyvalerate] (PHB-*co*-HV)[5,16−18] and other poly-[(*R*)-3-hydroxybutyrate-*co*-(*R*)-3-hydroxyalkanoate] (PHB-*co*-HA).[19−22] Among them, P(94%3HB-*co*-6%3HA) consisting of monomers of (*R*)-3-hydroxybutyrate (3HB) and (*R*)-3-hydroxyalkanoates (3HA) containing 6−12 carbon atoms showed a T_g of −8 °C, T_m of 133 and 146 °C, and interesting mechanical properties.[21]

Recently, we have transformed mcl-PHAs into low-molecular-weight, enantiomerically pure telechelic hydroxylated mcl-PHAs (mcl-PHA-diols).[23] With low melting temperature (T_m) and glass transition temperature (T_g), these diols could be excellent soft segments in block copolymers. The use of mcl-PHA-diols, together with hard segments such as telechelic hydroxylated poly-[(*R*)-3-hydroxybutyrate] (PHB-diol),[24,25] could form new block copolymers with improved properties. Moreover, the polymer properties can be easily modified by changing the ratio of mcl-PHA-diol and the hard segment. Here we report the synthesis of a novel block-copoly(ester−urethane) from telechelic hydroxylated poly-[(*R*)-3-hydroxyoctanoate] (PHO-diol), PHB-diol, and L-lysine methyl ester diisocyanate (LDI), the identification of the polymer structures, and the characterization of the physical and mechanical properties.

Experimental Section

Materials. Telechelic hydroxylated poly-[(*R*)-3-hydroxyoctanoate] (PHO-diol, $M_n = 2400$)[23] and telechelic hydroxylated poly-[(*R*)-3-hydroxybutyrate] (PHB-diol, $M_n = 2600$)[24] were prepared according to the published procedures and predried by azeotropic distillation with 1,2-dichloroethane. L-Lysine methyl ester diisocyanate (LDI) was purchased from Kyowa Hakko Kogyo Co. Ltd. (Japan) and freshly distilled at 0.025 mbar and at 103 °C before use. All other reagents and solvents were purchased from Fluka, all puriss: dibutyltin dilaurate, low-boiling petroleum ether, methanol, tetrahydrofuran (THF), and chloroform were used without further purification; dichloromethane was distilled; and 1,2-dichloroethane was dried by reflux in a Soxhlet apparatus filled with molecular sieve A4 (pore size 4 Å) for 6 h and distilled.

Synthesis of Block-Copoly(ester−urethane) (PUHO-HB). Telechelic hydroxylated poly-[(*R*)-3-hydroxyoctanoate] (PHO-diol) (4.55 g, 1.90 mmol, $M_n = 2400$ by VPO) and telechelic hydroxylated poly-[(*R*)-3-hydroxybutyrate] (PHB-diol) (5.00 g, 1.92 mmol, $M_n = 2600$ by VPO) were dissolved in dry 1,2-dichloroethane (150 mL) in a 250 mL three-neck flask and refluxed under an argon atmosphere in a Soxhlet apparatus filled with molecular sieve A4 (pore size 4 Å) overnight. 110 mL of 1,2-dichloroethane was then distilled off, leaving a viscous solution containing 8.1 ppm water that was determined by Karl Fischer titration. L-Lysine methyl ester diisocyanate (LDI, 807 mg, 3.81 mmol) and dibutyltin dilaurate (50 mg, 0.08 mmol) were added, and the mixture was stirred at reflux. Samples were taken at different time points for GPC and viscosity measurement to follow the polymerization. The

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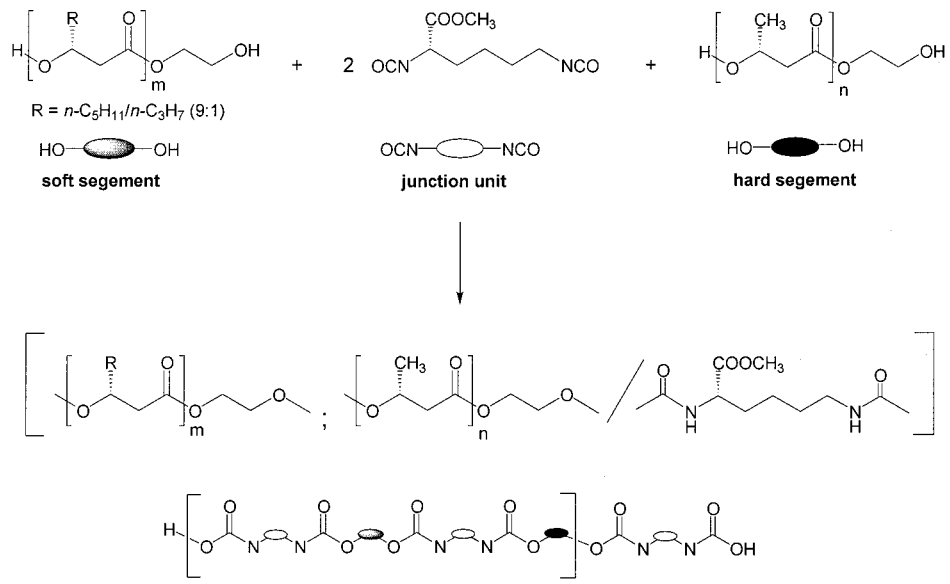
Scheme 1



reaction was stopped at 143 h by cooling to room temperature. The mixture was added dropwise into MeOH (600 mL) and stirred vigorously for 2 h, and the white precipitate was collected by filtration. The crude product was dissolved in dichloromethane (50 mL), and the solution was added dropwise into a solution of low boiling petroleum ether (800 mL) and MeOH (100 mL). After vigorously stirring for 2 h, the fine precipitate was collected by filtration and washed with MeOH (3 × 10 mL), low boiling petroleum ether (3 × 50 mL), and water (5 × 200 mL). Drying under high vacuum at 80 °C for 4 days afforded 7.20 g (70%) of PUHOHB.

Characterization Techniques. ^{1}H NMR spectra were measured on a Bruker ASX-400 spectrometer at 330 K in DMSO-d_6. COSY (^{1}H, ^{13}C) NMR spectra were measured on the same instrument with a delay time of 5.5 μs at 300 K in DMSO-d_6. Chemical shifts are given in ppm relative to tetramethylsilane. IR spectra (film) were recorded on a Bruker Vector 22 spectrometer at room temperature. Molecular weight distribution was determined by gel permeation chromatography (GPC) in THF at room temperature with a Knauer chromatograph equipped with a differential refractive index detector with two PLGel mixed 5 μm columns (7.5 mm × 600 mm) at 85 bar and at 45 °C. M_w was estimated from the retention volume based on polystyrene standards. Number-average molecular weights (M_n) were determined in chloroform at 25 °C by vapor pressure osmometry (VPO) with a Corona Wescan C 32A machine. Melting temperature (T_m) and glass transition temperature (T_g) were obtained by differential scanning calorimetry (DSC) with a Mettler-DSC 30 instrument equipped with Me-70329 cooler and a Tc15/TA controller. The samples in a 40 μL aluminum carrier were heated in the first scan from −100 to 200 °C with a heating rate of 10 °C/min, cooled from 200 to −100 °C at a cooling rate of −10 °C/min, and then heated for the second scan. The stress/strain curves were recorded on a MECMESIN M 1000E deformation apparatus at ambient temperature and at a rate of deformation of 20 mm/min with a load cell capable of measuring forces up to 10.8 N and a sample film of 14.8 × 9.8 × 0.32 mm^3. The water content in solution was determined by Karl Fischer titration on a Metrohm 684 KF coulometer. Wide-angle X-ray diffraction patterns were recorded on a Siemens D5000 diffractometer with samples of a thickness of 1 mm and with Cu Kα radiation. A glancing incidence setup (glancing angle 1.5°) was used for the measurement, scanning the 2θ range from 5° to 40°. The step size was 0.05° and the measurement time 10 s per step. A Soller slit and a monochromator were mounted in front of the scintillator to obtain a parallel and monochromatic beam. The peak positions were fitted by the Pearson VII method using the TOPAS P software of the D5000 diffractometer.

Results and Discussion

Poly-[(*R*)-3-hydroxyoctanoate] (PHO) is the most studied mcl-PHA and can be produced in large amounts. Transesterification of PHO with ethylene glycol afforded telechelic PHO-diol with a M_n of 2400 (VPO) in 91% yield.[23] This readily available enantiomerically pure material is a sticky liquid with $T_g = -56$ °C, and it was chosen therefore as the soft segment for the preparation of block copolymers. The telechelic diol from poly-[(*R*)-3-hydroxybutyrate] (PHB-diol) with a T_m of 149 °C and an M_n of 2600 (VPO) was prepared according to the established procedure[24] and used as the hard segment. To demonstrate the concept, poly(ester−urethane) was chosen as a model target block copolymer. Moreover, polyurethanes have been used in medical practice,[25,26] and the new polymer containing PHO and PHB blocks could provide improved biocompatibility and biodegradability in addition to the desired physical and mechanical properties. Since aromatic diisocyanate derived polyurethane can generate toxic, mutagenic, and carcinogenic aromatic amine during degradation, aliphatic L-lysine methyl ester diisocyanate (LDI)[25] was chosen for the junction unit. Degradation of the corresponding polyurethane should give the nontoxic lysine derivative. Comparing to the biosynthetic polymer products, the ratio of the soft and hard segments and the junction unit can be more easily controlled in the chemical synthesis procedure, permitting modifications of polymer properties. As an example, PHA-diol, PHB-diol, and LDI were used in a molar ratio of 1:1:2 in this study.

Block copolymers were prepared by the reaction between the −NCO groups of LDI and the primary or the less active secondary −OH groups of the telechelic diols (Scheme 1). Since water can react with LDI to give carbamic acids and thus stop the polymerization, the reaction was carried out under anhydrous conditions and under an argon atmosphere. The PHO-diol and PHB-diol were dried by azeotropic distillation with 1,2-dichloroethane, and the moisture in the solvents was removed by reflux in a Soxhlet apparatus with molecular sieve granules A4 (pore size 4 Å) overnight. The water content in the reaction system should be as low as possible, preferably lower than 10 ppm. Preliminary results showed that there was no polymerization at water content of 60 ppm.

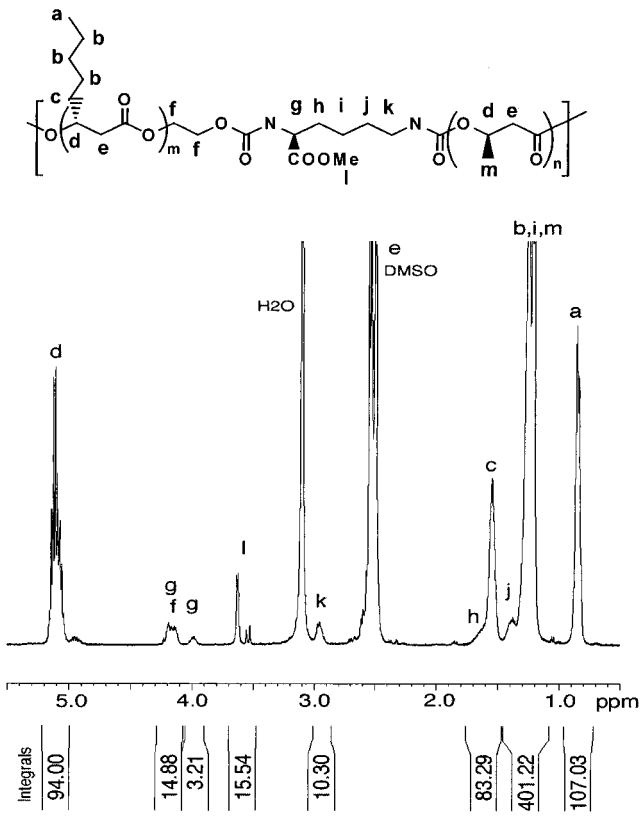


Figure 1. ^{1}H NMR spectrum of the block copoly(ester−urethane) PUHOHB at 330 K in DMSO-d_6.

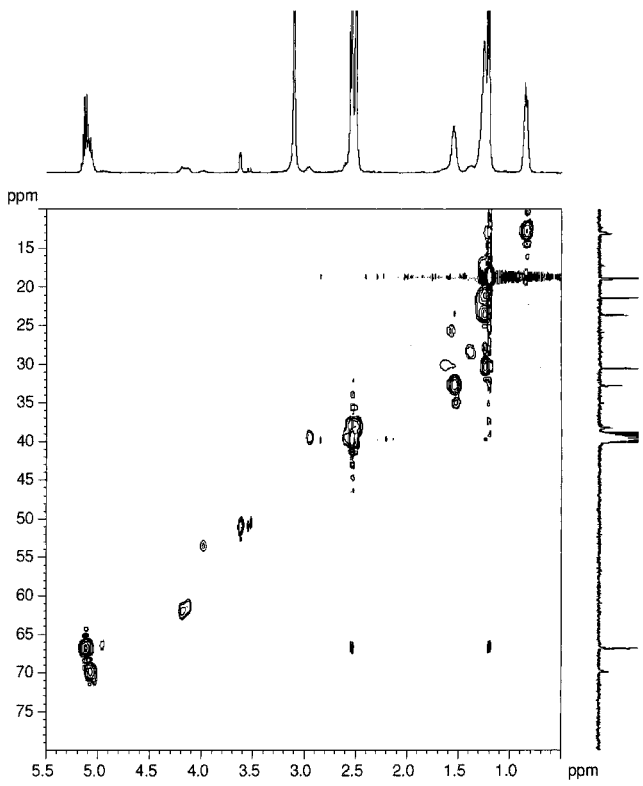


Figure 2. COSY (^{1}H, ^{13}C) NMR spectrum of the block copoly(ester−urethane) PUHOHB at 300 K in DMSO-d_6 (shown only 10−80 ppm from ^{13}C NMR).

It was shown that a high concentrated solution resulted in rapid polymerization, and 25% (w/v) of diols in 1,2-dichloroethane was therefore used. The junction unit LDI was freshly distilled before use. As a catalyst, a small amount of dibutyltin dilaurate (0.5% w/w) was used. The reaction temperature was maintained between 75 and 80 °C to avoid possible formation of side products or polymer degradation. The reaction was followed by gel permeation chromatography (GPC) of samples taken from the mixture at different times.

After 143 h, the growth of the polymer chain stopped. The crude product was precipitated in methanol to remove the unreacted LDI and small molecular side products. Subsequent precipitation in low boiling petroleum ether removed tin hydroxide generated from the catalyst. Washing with methanol, low boiling petroleum ether, and water followed by drying under high vacuum at 80 °C for 4 days afforded the poly(ester−urethane) (PUHOHB) in 70% yield. A molecular weight (M_w) of 33 500 for PUHOHB was determined by gel permeation chromatography, and M_n was established as 10 600 by vapor pressure osmometry (VPO).

In the IR spectrum, two broad absorption bands at 3530−3350 cm^{-1}, corresponding to the two terminal −OH groups of the starting materials PHO-diol and PHB-diol, were nearly absent, indicating the polymerization was successful. The ester and urethane structures were confirmed by the strong absorption at 1735 cm^{-1}. The absorption at 1520 cm^{-1} corresponded to the amide N−H in the new polymer.

The structures were further confirmed by NMR analyses. The chemical shift assignments from the ^{1}H NMR spectrum are given in Figure 1. The proton assignments for the ester domains were adopted from earlier work,[23] and protons **g**−**k** were assigned with help of a COSY (^{1}H, ^{13}C) NMR spectrum, shown in Figure 2. The assignment of the ^{13}C chemical shifts is listed in Table 1. The NMR signals of the methyl group proton **l** and the proton **g** of the incorporated LDI are multiple peaks, indicating nonequivalent chemical surroundings that exist due to the various ways of coupling between LDI and the primary or secondary OH groups of the PHO-diol or the PHB-diol.

Table 1. Assignment of Chemical Shifts of PUHOHB in the ^{13}C NMR Spectrum

position	δ (^{13}C)	position	δ (^{13}C)	position	δ (^{13}C)
a	13.1	**CO** (HO, HB)	168.4	**j**	28.6
b	21.4, 30.5, 23.6	**f**	61.9	**k**	39.5
c (HO)	32.8	**g**	53.4	**l**	50.8
d (HO)	69.9	**h**	30.2	**m**	18.3
e	38.3	**i**	28.1	**d** (HO)	66.8

For a PHB-diol with a M_n of 2600, the average number of HB monomer units (n) is 29.5. For a PHO-diol containing C8 and C6 monomer in a 90:10 ratio, with a M_n of 2400, the average number of HO monomer units (m) is 16.8. In the ^{1}H NMR spectrum, the intensity of the methyl group proton **a** of the PHO block is 107.0, so that the integral value of the proton **d** from PHO block should be 107.0/3 = 35.7. The total integration of proton **d** from the PHO and PHB blocks is 94.0; thus, the intensity of proton **d** from the PHB block can be calculated to be 94.0 − 35.7 = 58.3. Accordingly, the ratio of the PHO-diol and the PHB-diol incorporated in the new polymer can be deduced to be (35.7/16.8): (58.3/29.5) = 1:1.

The intensity of the methyl proton **l** from the incorporated LDI is 15.5 and thus in good correspondence to that of the methylene proton **k** (10.3). The intensity of protons **h**, **i**, and **j** can therefore be estimated to be 10.

Table 2. Physical and Mechanical Properties of PUHOHB, PHO, PHB, P(HB-*co*-HA), and PUCL

properties	PUHOHB	PHO[a]	PHB[b]	P(HB-*co*-HA)[c]	PUCL[d]
M_w (GPC)	33 500	66 000	1170 000	1 391 500	66 000
M_n (VPO)	10 600	23 600	650 000	605 000	
M_w/M_n	2.7	2.8	1.8	2.3	
T_g (°C, DSC)	−6	−30	4	−8	−45
T_m (°C, DSC)	146	61	178	133, 146	122
E' (MP_a)[e]	213		1560	220	200
σ_{max} (MP_a)[e]	7.0		38	17	9.9
ϵ_{max} (%)[e]	6.3			27	490
σ_B (MP_a)[e]	6.9			17	7.3
ϵ_B (%)[e]	37		5	680	610

[a] Data from this study. [b] Data from ref 27. [c] Biosynthetic copolyester consisting of 94 mol % HB and 6 mol % HA; data from ref 21. [d] Poly(ester−urethane) block copolymer prepared from 17 mol % PHB-diol, 32 mol % poly(ϵ-caprolactone)diol, and 50 mol % LDI; data from ref 25. [e] Stress/strain characteristics determined on a MECMESIN M 1000E deformation apparatus at ambient temperature: Young's modulus E', tensile stress and elongation at maximum load σ_{max} and ϵ_{max} and at break σ_B and ϵ_B.

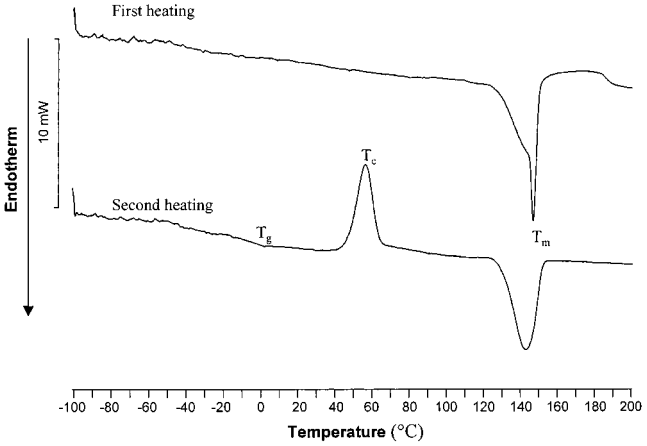


Figure 3. DSC spectrum of the block copoly(ester−urethane) PUHOHB.

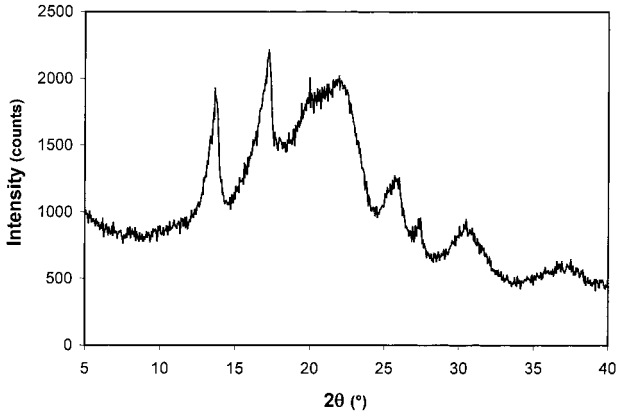


Figure 4. Diffraction intensities vs 2θ (deg) for the block-copoly(ester−urethane) PUHOHB.

The overlapping signal groups at 1.55 ppm (**c**, **h**) and 1.23 ppm (**b**, **i**, **j**, **m**) can be separated to calculate the correct intensity of protons **c** and **m**, which again resulted in the 1:1 ratio of PHB- and PHO-diol. The value M_n = 10 600 (VPO) for PUHOHB indicates that on average four diols are connected. On the basis of the intensity of proton **l** and proton **d** of the polymer, it can be concluded that two PHA-diols, two PHB-diols, and five LDI are incorporated on average in the new polymer. This suggests that the major terminating end groups are the carbamic acids.

A T_m of 146 °C was found in the DSC of the new poly(ester−urethane) block copolymer (Figure 3). In the X-ray diffraction pattern of PUHOHB (Figure 4), several peaks were observed in the measured 2θ range, indicating partial crystallinity. The high peak width reveals a small size of crystalline phase, estimated to be well below 1 μm. Comparing with the pure PHB material,[28] PUHOHB shows an identical diffractogram. This indicates that the observed T_m is due to the low-molecular-weight PHB-diol block. A T_g of −6 °C was found in the DSC of PUHOHB (Figure 3), which is most probably due to the PHB-diol block. No T_g corresponding to the PHO domains can be clearly observed in the DSC until −100 °C. Comparing with PHO (T_g of −30 °C and T_m of 61 °C) and PHB (T_g of 4 °C and T_m of 180 °C), the new poly(ester−urethane) block copolymer shows much better thermoplastic properties.

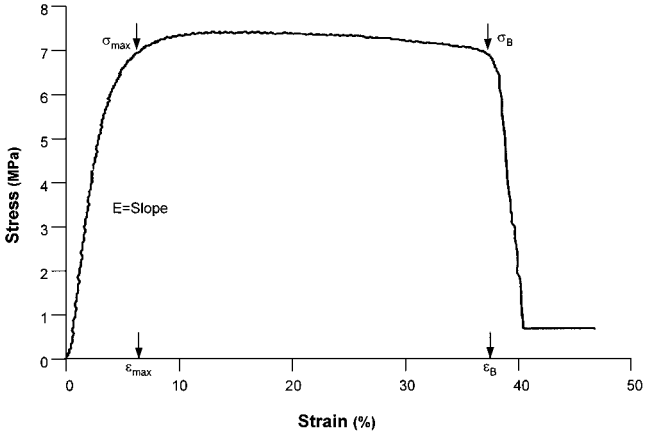


Figure 5. Stress−strain curve of the block copoly(ester−urethane) PUHOHB.

From the stress/strain curve (Figure 5), the Young's modulus (E') was deduced to be 213 MPa. The tensile stresses at maximum load (σ_{max}) and at break (σ_B) were 7.0 and 6.9 MPa, respectively. These properties are similar to those of the biocopolyester P(94%3HB-*co*-6%3HA)[21] and the block copoly(ester−urethane) PUCL[25] synthesized from 17 mol % PHB-diol, 32 mol % poly(ϵ-caprolactone)diol, and 50 mol % LDI (Table 2). The elongations at maximum load (ϵ_{max}) and at break (ϵ_B) for PUHOHB were 6.3% and 37%, respectively. These relatively low values can be probably improved by incorporating less PHB-diol hard segment and more PHO-diol soft segment in the block copolymer. For application in special medical devices, PUHOHB with a T_m of 146 °C will allow for thermal sterilization, which is an important criterion in such applications. In comparison with PUCL, PUHOHB contains only block esters obtained from biopolymers and should thus be more biocompatible.

In summary, enantiomerically pure PHO-diol has been proven to be an excellent soft segment in the

preparation of block copolymers. For the first time, a new polymer that contains both soft and hard segments derived from biopolymer PHAs has been chemically synthesized. The prepared model polymer PUHOHB shows excellent thermoplastic properties and good mechanical properties and is a potentially useful material for medical applications.

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