Enzymatic hydrolysis studies on novel eco-friendly aliphatic thiocopolyesters

Matteo Gigli [a], Andrea Negroni [a], Michelina Soccio [b], Giulio Zanaroli [a], Nadia Lotti [a,*], Fabio Fava [a], Andrea Munari [a]

[a] Dipartimento di Ingegneria Civile, Chimica, Ambientale e dei Materiali, Università di Bologna, Via Terracini 28, 40131 Bologna, Italy
[b] Departamento de Fisica Macromolecular, Instituto de Estructura de la Materia, C.S.I.C., Calle Serrano 121, 28006 Madrid, Spain

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ABSTRACT

Copolymers of poly(butylene succinate) (PBS) containing thiodiethylene succinate sequences with different molecular architectures (PBSPTDGS) were prepared via reactive blending in the presence of a Ti-based catalyst. In particular, a block copolymer with long sequences and a random one have been investigated for their biodegradability. The parent homopolymer PBS has been also synthesized via the usual two-stage melt polycondensation and used as reference polymer in the study.

The hydrolitic treatment carried out using lipase from *Candida cylindracea* ([E] = 50 U/ml, T = 30 °C and pH = 7.0), showed that copolymers biodegraded more rapidly and extensively than PBS.

ATRIR analysis and DSC measurements also performed on the resulting samples showed that the enzyme simultaneously attacks the amorphous phase and the PTDGS crystalline phase, which is characterized by lower packing density and degree of perfection with respect to the PBS one. This result suggested that the higher degradability of the copolymers under study can be correlated both to the crystallinity degree and to the nature of the crystalline phase. NMR analysis, performed to detect changes in composition of the copolymers during the degradation, demonstrated that enzyme hydrolysis preferentially targeted ester groups of TDGS sequences because of their higher hydrophilicity.

1. Introduction

In 2011, the worldwide plastic production was 280 million tons and more than 99% was due to polymeric materials obtained from fossil resources. Petroleum deriving plastics are extensively used (i.e., packaging of food, pharmaceuticals, cosmetics, detergents and chemicals, among others) not only due to their excellent mechanical properties, low cost, light weight and high energy effectiveness, but also for their stability, durability and chemical and biological inertness. The dramatic increase in production and the very low biodegradability of such plastics focused public attention on a potentially huge environmental accumulation and pollution problem that could persist for centuries. An effective way to mitigate this problem is to develop promising biodegradable polymers and to promote their industrial production and use. The major advantages of biodegradable plastics are (1) they can be composted with organic wastes and their carbon can be converted into organic matter for soil; (2) their use will not only reduce negative impacts on wild living organisms caused by dumping of conventional plastics but will also lessen the labor cost for the removal of plastic wastes from the environment since they are naturally degraded; (3) their decomposition will help to increase the longevity and stability of landfills by reducing the volume of garbage disposed; and (4) they could be converted to useful monomers and oligomers by microbial and/or enzymatic treatment.

There are three broad categories of biodegradable polymers: naturally occurring (like cellulose, starch, chitin etc.), modified natural polymers (like cellulose acetate, chitosan, esterified starch etc.), and those obtained by chemical polymerization of biobased building blocks or by micro-organisms. In addition to biopolymers, some synthetic chemical polymers, such as aliphatic polyesters, combine the features of biodegradability and biocompatibility with physical and chemical properties comparable with some of the most extensively used oil-based polymers, like LDPE, PP, etc. Poly(butylene succinate) (PBS), characterized by good mechanical properties and thermal stability, is one of the most representative, generally acknowledged and extensively used biodegradable aliphatic polyester [1,2]. Therefore, several studies have been performed to investigate its mechanical properties [3–5] and biodegradability [6–9], as well as its crystal structure [10], crystallization and melting behavior [11–13]. However, PBS exhibits a slow biodegradation rate due to its high degree of crystallinity [14]. One strategy of promoting PBS biodegradability is to copolymerize it with different diacids or diols. Till now, particular attention has

* Corresponding author. Tel.: +39 051 2090354; fax: +39 051 2090322.
E-mail address: nadia.lotti@unibo.it (N. Lotti).

been paid on random copolyesters, such as poly(butylene succinate-co-ethylene succinate), poly(butylene succinate-co-propylene succinate), and poly(butylene succinate-co-butylene adipate) [15–21]. Recently, PBS has been also copolymerized with poly(lactic acid) [22], resulting in improved degradation rate and introduction of different desirable properties. Biodegradable multiblock copolymers of PBS have been also investigated [23–27]. Generally, PBS copolymers biodegrade faster than homopolymer mainly because of their limited crystallinity.

It is worth emphasizing that the biodegradation rate of a polymer normally depends on several factors, such as the chemical structure, hydrophilic/hydrophobic ratio, molecular weight, solid-state morphology, i.e. crystallinity degree, distribution of crystal regions, crystal size and perfection [28,29].

A simple strategy to synthesize new biodegradable polymers is the reactive blending approach. Reactive blending consists of simply mixing two or more homopolymers in the molten state in the presence of a catalyst. In this economic and solvent-free synthetic route exchange reactions among functional groups belonging to the different homopolymers lead to the formation of block copolymers. Therefore, when the starting homopolymers are selected with tailored and desired physicochemical characteristics, it is possible to produce copolymers in which the final properties can be favorably modulated, depending on the type, relative amount and distribution of the comonomeric units along the polymer chain, which in turn are controlled by reaction conditions such as initial feed and reaction time.

On the basis of the scenario described above, copolymers of PBS containing diethylene succinate sequences (PBSPDGS) with different molecular architecture have been recently prepared in our laboratories via reactive blending in the presence of Ti-based catalyst: PBS biodegradation rate was indeed enhanced and modulated, through a targeted modification of its hydrophilicity, flexibility, and capability of crystallizing [6,30].

In the present study, block copolymers containing butylene succinate (BS) and tiodiethylene succinate (TDGS) blocks of different length, obtained by melt mixing PBS and poly(thiodiethylene succinate) (PTDGS), have been taken into consideration [31]. It has to be emphasized that PTDGS differs from PBS for the presence of a sulfur atom in the glycol sub-unit. The solid-state properties and wettability of the polymers under investigation were correlated to their enzymatic degradability. Lastly, the biodegradation rate of these copolymers has been compared with that of PBSPDGS copolymers previously studied [6] in order to evaluate the effect of the presence of sulfur atom in the polymeric chain in place of ether-oxygen one.

2. Experimental part

2.1. Materials

Dimethylsuccinate (DMS), 1,4-butanediol (BD), thiodiethylene glycol (TDEG), and titanium tetrabutoxide (TBT) (Sigma–Aldrich) were reagent grade products; DMS and BD were used as supplied, whereas TDEG and TBT were distilled before use. Dichloromethane (DCM), 2-chloroethanol (CE) and ethanol (EtOH) were purchased by Sigma–Aldrich and were used without any further purification. Lipase from *Candida cylindracea*, and pure buffer salts were purchased from Sigma–Aldrich.

2.2. Synthesis of homopolymers

Poly(butylene succinate) (PBS) and poly(thiodiethylene succinate) (PTDGS) were synthesized in bulk as described elsewhere [31]. The obtained polymers were purified and kept under vacuum at room temperature for several days to remove the residual solvent.

2.3. Synthesis of poly(butylene/thiodiethylene succinate) copolyesters

Poly(butylene/thiodiethylene succinate) copolyesters were obtained by melt mixing non-purified PBS and PTDGS (1:1 M ratio of the repeating units) as previously reported [31] and subsequently purified. To obtain the block copolymer (PBSPTDGSblock), the sample was taken from the reactor after 20 min reaction time, whereas in the case of random copolymer (PBSPDGSrandom) a mixing time of 240 min was necessary.

2.4. Film preparation

Films of PBS homopolymer and of PBSPTDGS copolymers were obtained by hot pressing the polymers between Teflon sheets in a Carver press for 2 min at a temperature $T = T_m + 40\,^\circ C$. The 0.2 mm thick films were cooled to room temperature in press by using running water. Before degradation tests, the films were stored at room temperature for at least two weeks in order to attain equilibrium crystallinity.

2.5. Physicochemical characterization

2.5.1. NMR spectroscopy
The polymer structure, composition, and sequence distribution were determined by means of ^{1}H NMR spectroscopy accordingly to the procedure described elsewhere [31]. The samples were dissolved (15 mg mL^{-1}) in chloroform-d solvent with 0.03% (v/v) tetramethylsilane added as an internal standard. The measurements were carried out at room temperature, employing a Varian INOVA 400 MHz instrument.

2.5.2. Gel-permeation chromatography
Molecular weight data were obtained by gel-permeation chromatography (GPC) at 30 °C using a 1100 Hewlett Packard system equipped with a PL gel 5 m MiniMIX-C column (250 mm/4.6 mm length/i.d.) and a refractive index detector. In all cases, chloroform was used as eluant with a 0.3 mL min^{-1} flow and sample concentrations of about 2 mg mL^{-1} were applied. Polystyrene standards in the range of molecular weight 2000–100,000 were used.

2.5.3. Differential scanning calorimetry
Calorimetric measurements were carried out by means of a Perkin Elmer DSC7 instrument equipped with a liquid sub ambient accessory and calibrated with high purity standards (indium and cyclohexane). The external block temperature control was set at −120 °C and weighed samples of c.a. 10 mg were encapsulated in aluminum pans and heated up to 40 °C above fusion temperature at a rate of 20 °C/min. The glass-transition temperature T_g was taken as the midpoint of the heat capacity increment Δc_p associated with the glass-to-rubber transition. The melting temperature (T_m) was determined as the peak value of the endothermal phenomenon in the DSC curve. The specific heat increment Δc_p, associated with the glass transition of the amorphous phase, was calculated from the vertical distance between the two extrapolated baselines at the glass transition temperature. The heat of fusion (ΔH_m) of the crystal phase was calculated from the areas of the DSC endotherm. Repeated measurements on each sample showed excellent reproducibility.

2.5.4. Wide-angle X-ray measurements
X-ray diffraction patterns were carried out by means of an X'Pert PANalytical diffractometer, in reflection mode set up, equipped with a fast X'Celerator detector and a Cu K$_\alpha$ radiation source.

The indexes of crystallinity (χ_c) were calculated from the X-ray powder diffraction profiles as the ratio between the crystalline