Physical Characterization of Sorbitol or Glycerol Containing Aliphatic Copolyesters Synthesized by Lipase-Catalyzed Polymerization

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ABSTRACT: Copolymers of octanediol adipate and sorbitol adipate, P(OA-SA), copolymers of octanediol adipate and glycerol adipate, P(OA-GA), poly(octamethylene adipate), POA, and poly(sorbitol adipate), PSorA, were synthesized using immobilized Lipase B from *Candida antarctica* (Novozyme-435) as catalyst. The molecular weights and polydispersity indices of these polyesters were determined. The physical properties of these polyesters were investigated by thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), wide-angle X-ray scattering (WAXS), and dynamic mechanical analysis (DMA). These polymers are semicrystalline except for PSorA, which is amorphous. With increasing sorbitol or glycerol content in the polyesters, both melting and crystallization temperatures decreased. The melting temperature depression is well described by Baur's equation for random copolymers where the second comonomer is completely excluded from the crystal phase of the crystallizable first monomer. Also, the melting enthalpy decreases, and WAXS measurements confirm that the degree of crystallinity decreases upon copolymerization with either sorbitol or glycerol. Furthermore, the crystal phase is that of POA.

Introduction

Bioresorbable polymers are widely used in medical applications for controlled drug release, biodegradable sutures, implants, tissue engineering, and artificial skin.[1−8] Aliphatic polyesters and copolymers that contain lactic acid, glycolic acid, trimethylene carbonate, ϵ-caprolactone, and dioxanone are used commercially.[8−13] However, related products in demand are those with functional groups to attach bioactive moieties or that allow facile cross-linking. A difficulty encountered in producing such polymers is the need for protection−deprotection steps. This results in nonpractical processes and products. Furthermore, functional polymers often lack sufficient thermal stability to be processed thermally.

Our laboratory has developed a simple but versatile synthetic strategy to synthesize polyol-containing polyesters. Examples are copolymers of octanediol adipate with sorbitol adipate and copolymers of octanediol adipate with glycerol adipate.[14a−c] The key to the synthesis of these polymers is the use of a highly selective enzyme catalyst.[14a−c] One such catalyst that was found useful for this purpose is Novozyme-435, which consists of lipase B from *Candida antartica* immobilized on a Lewatit macroporous resin. Currently, our laboratories are studying relationships between the functional copolymer composition and its biocompatibility, physical properties, and bioresorption rates. This paper focuses on understanding how copolymer composition influences its thermal and crystalline properties.

Experimental Section

Materials. Adipic acid, 1,8-octanediol, D-sorbitol, and glycerol were purchased from Aldrich and were used without further purification. Novozyme-435 (specified activity 7000 PLU/g) was a gift from Novozymes (Denmark).

Novozyme-435-Catalyzed Polymerizations. The details of copolyester syntheses were described elsewhere.[14a,b] The following summarizes the synthetic methodology by using poly(octanediol adipate-*co*-30 mol % sorbitol adipate), P(OA-30% SA), and poly(sorbitol adipate), PSorA, as representative examples.

P(OA-30% SA). The reaction was performed in bulk (solventless) by the direct condensation polymerization of D-sorbitol (1.09 g, 6.00×10^{-3} mol), 1,8-octanediol (2.04 g, 1.40×10^{-2} mol), and adipic acid (2.92 g, 2.00×10^{-2} mol). Novozyme-435 (600 mg, 10% w/w of the monomers) dried in a vacuum desiccator (10 mmHg, 25 °C, 24 h) was transferred into a 100 mL round-bottom flask containing a melt of the D-sorbitol/1,8-octanediol/adipic acid mixture. The flask was stoppered with a rubber septum and then was placed into a constant-temperature (90 °C) oil bath with stirring. After 1−2 h, the reaction mixture was subjected to reduced pressure (40 mmHg). After 48 h, the reaction was terminated by the addition of chloroform and the removal of the enzyme by filtration. The chloroform filtrates were combined, and much of the chloroform was removed by rotary evaporation; the remaining concentrated polymer solution was precipitated in methanol. The precipitate was isolated by filtration and then dried in a vacuum oven to give the product in 80% yield.

Synthesis of Poly(sorbitol adipate) (PSorA). The reaction was performed in bulk (solventless) by the direct condensation polymerization of D-sorbitol (1.82 g, 1.00×10^{-2} mol) and adipic acid (1.46 g, 1.00×10^{-2} mol). Novozyme-435 (328 mg, 10% w/w of the monomers) dried in a vacuum desiccator (10 mmHg, 25 °C, 24 h) was transferred into a 100 mL round-bottom flask containing a melt of the D-sorbitol/adipic acid mixture. The flask was stoppered with a rubber septum and then was placed into a constant-temperature (90 °C) oil bath with stirring. After 6 h, the reaction mixture was subjected to reduced pressure (40 mmHg). After 48 h, the reaction was terminated and worked up by dissolving the reaction mixture in methanol, removing the enzyme by filtration, and then stripping the solvent by rotary evaporation. The product was dried in a vacuum oven (10 mmHg, 30 °C, 24 h) and was then analyzed as is (i.e., without a dissolution and precipitation step).

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Table 1. Thermal Properties of Octandiol Adipate (O-A) and Sorbitol Adipate (S-A) Copolymers

sample	mol compos O:S	wt % S-A	$M_n \times 10^{-3}$	PDI	T_m (first scan) (°C)	ΔH_m (first scan) (J/g)	T_c (cooling scan) (°C)	T_g (DMA) (°C)	T_m (second scan) (°C)	ΔH_m (second scan) (J/g)
POA	100:0	0	21.6[a]	1.8	74	136	51	−28	72	113
10% sorbitol	91:9	10	16.9[a]	3.3	66	101	42	−10	62	80
20% sorbitol	82:18	20	17.7[a]	2.4	62	82	34	3	58	67
30% sorbitol	71:29	32	20.3[a]	2.3	58	59	21	7	54	51
50% sorbitol	53:47	50	7.01[c]	1.8	43	33	−13	n.d.	45	22
PSorA	0:100	100	10.9[b]	1.6				n.d.		

[a] Molecular weight by GPC relative to polystyrene. [b] Absolute molecular weight by GPC-MALLS in DMF. [c] Absolute molecular weight by GPC-MALLS in THF.

All the other polymers were prepared by similar methods with the following exceptions: (i) the reaction temperature for octanediol adipate/glycerol adipate copolymerizations was 70 °C, and (ii) the synthesis of POA was performed at 70 °C for 72 h with 1 wt % Novozyme-435. The polymers were dissolved in a minimum volume of chloroform and then precipitated by slow addition to methanol. The yields of the precipitated products were ≥70%.

Instrumental Methods. Nuclear magnetic resonance (NMR): Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker Instruments Inc. DPX 300 spectrometer at 300 and 75.13 MHz, respectively. Further details of the NMR experiments and display of spectra along with peak assignments are described elsewhere.[14a,b]

The relative molecular weights of the polymers were determined by gel permeation chromatography (GPC) using a Waters HPLC system equipped with a 510 pump, a 717 autosampler, and a 410 refractive index detector and a three column set consisting of Waters Stryragel HR4, HR3, and HR1 in series. Chloroform was used as the eluent at a flow rate of 1.0 mL/min. Polystyrene standards (11 in total) with molecular weight range from 900K to 580 (from Polymer Laboratory) were used to calibrate the system. The relative molecular weights were calculated by using TriSEC GPC software version 3.0 (Viscotek, Corp.). The absolute molecular weights of PSorA and copolyester with 50% sorbitol were measured by gel permeation chromatography−multiangle light scattering (GPC-MALLS). The GPC system consisted of a Waters 510 pump, a 717plus autosampler, and a Wyatt Optilab DSP interferometeric refractometer coupled to a Wyatt DAWN DSP multiangle laser light-scattering photometer (Wyatt Technology, Santa Barbara, CA); a two column set consisting of Waters Stryragel HR4 and HR2 in series was used to chromatograph the samples. The Wyatt DSP was calibrated by toluene and normalized by polystyrene standard with molecular weight 30K in THF or DMF with 10 mmol of LiBr. The flow rate is 1.0 mL/min. The software used for data collection and processing was ASTRA (supplied by Wyatt Tech.). The specific refractive index increments (dn/dc) in corresponding solvent were determined at 632.8 nm using a Wyatt Optilab DSP interferometeric refractometer by the on-line method, in which two assumptions were used: (1) the sample is 100% pure, and (2) the sample is 100% eluted from the column.

Thermogravimetric analysis (TGA) measurements were performed with a TA Instruments TGA2950 thermogravimetric analyzer in a nitrogen atmosphere at a heating rate of 10 °C/min.

A TA Instruments DSC 2920 differential scanning calorimeter was used for calorimetric analysis at a heating rate of 20 °C/min.

Dynamic mechanical measurements were carried out on compression-molded bars (35.4 mm × 12.7 mm × 1.9 mm) with a TA Instruments DMA 2980 dynamic mechanical analyzer from −150 to 25 °C (heating rate = 3 °C/min, frequency = 3 Hz).

Wide-angle X-ray scattering (WAXS) spectra were collected with a Philips powder diffractometer with nickel-filtered Cu Kα radiation (λ = 0.1542 nm, 40 kV, 30 mA). The degree of crystallinity (χ_c) was evaluated as the ratio of the crystalline peak areas to the total area under the scattering curve.[15]

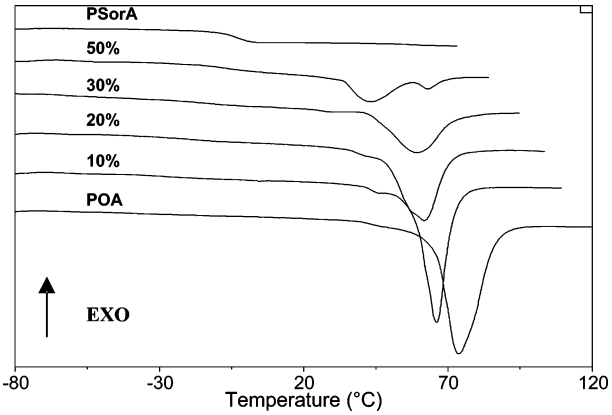

Figure 1. DSC curves (first scan) of copolymers of octanediol adipate and sorbitol adipate (content in mol % marked on curves).

Results and Discussion

New methods for lipase-catalyzed polymerizations allowed the synthesis of P(OA-SA) built from the monomers sorbitol (Sor), octanediol (O), and adipic acid (A). The polymerizations were performed assuming that, of the six sorbitol hydroxyl groups, only the two primary hydroxyls are reactive in the polymerization. Thus, polymerizations were performed using a 1:1 molar ratio of adipic acid to sorbitol plus octanediol. A copolymer with 10% sorbitol means that the molar ratio of sorbitol to octanediol to adipic acid in the feed composition is 0.1:0.9:1. NMR analyses of the copolyesters prepared from 10, 20, 30 and 50 mol % sorbitol showed that the corresponding products contained 9, 18, 29, and 47 mol % sorbitol, respectively.[14a,b] Hence, the feed and product compositions are taken as identical since the difference between these values is within the uncertainty of the NMR measurements.

Table 1 shows the thermal properties of the copolyesters with 10, 20, 30, and 50 mol % sorbitol. Also, Table 1 shows the thermal properties of poly(octamethylene adipate), POA, and poly(sorbitol adipate), PSorA. In addition, molar compositions of the products, weight fractions of sorbitol adipate (S-A), number-average molecular weights, and polydispersity indices are also shown. The weight fractions of sorbitol adipate (S-A) were calculated from the molar compositions. The products described in Table 1 have similar molecular weights. Figure 1 shows the first scan of DSC curves of these polyesters and copolyesters. Melting endotherms with peaks at 43−74 °C were the dominant thermal transition observed in the DSC curves except for PSorA, which does not show a melting endotherm but, instead, has a glass transition at −2 °C. This indicates that PSorA is an amorphous polymer. All the P(OA-SA) copolymers, as well as POA, are semicrystalline poly-

Table 2. Thermal Properties of Octandiol Adipate (O-A) and Glycerol Adipate (G-A) Copolymers

sample	mol compos O:G	wt % G-A	$M_n \times 10^{-3}$	PDI	T_m (first scan) (°C)	ΔH_m (first scan) (J/g)	T_c (cooling scan) (°C)	T_g (DMA) (°C)	T_m (second scan) (°C)	ΔH_m (second scan) (J/g)
POA	100:0	0	21.6[a]	1.8	74	136	51	−28	72	113
5% glycerol	96:4	3	18.6[a]	2.8	69	133	47	−28	67	100
10% glycerol	92:8	6	22.3[a]	2.5	68	110	43	−26	63	84
20% glycerol	82:18	15	22.6[a]	2.4	62	97	34	n.d.	55	77
30% glycerol	71:29	24	6.1[a]	4.5	58	93	27	n.d.	48	67
50% glycerol	50:50	44	14.4[a]	2.0	42	38	3	n.d.	37	35

[a] Molecular weight by GPC relative to polystyrene.

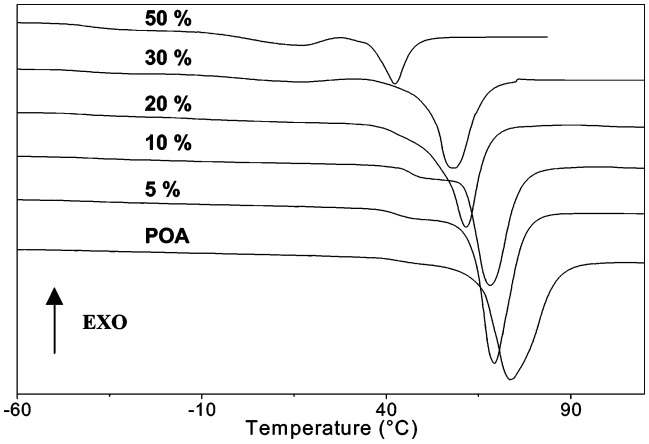

Figure 2. DSC curves (first scan) of copolymers of octanediol adipate and glycerol adipate (content in mol % marked on curves).

mers. It is seen clearly that, with increasing sorbitol content, the melting temperature decreases. Because of the high crystallizability of POA and of POA-rich copolymers, we did not succeed in quenching the samples from the melt in the DSC to a fully amorphous state. Hence, the glass transition temperature (T_g) of these semicrystalline polyesters was obtained by dynamic mechanical analysis (DMA); the α relaxation of the tan δ vs temperature spectrum was taken as the glass transition temperature. The T_g values are listed in Table 1 and increase as the amount of sorbitol in the copolymer was increased. This change with copolymer composition is reasonable based on the basis of the T_g difference of the reference homopolymers (POA and PSorA).

The results from similar analyses for the octanediol adipate (O-A)/glycerol adipate (G-A) copolymers, P(OA-GA), are presented in Table 2 and Figure 2. Also, in this case, with increasing amounts of G−A in the copolymer both the melting temperature and melting enthalpy decrease.

Thermogravimetric analysis (TGA) of these copolymers was performed. The TGA curves of P(OA-30 mol % SA) and P(OA-30 mol % GA) are shown in Figure 3. Inspection of these thermograms shows that the copolymers have excellent thermal stability.

Figures 4 and 5 show plots of the melting temperature depression for P(OA-SA) and P(OA-GA) copolymers as a function of the sorbitol adipate and glycerol adipate comonomer content, respectively. The curves drawn in Figures 4 and 5 have been calculated according to Flory's [16] (eq 1) and Baur's [17] (eq 2) equations for random copolymers:

$$1/T_m - 1/T_m^0 = -(R/\Delta H_u) \ln x \qquad (1)$$

$$1/T_m - 1/T_m^0 = -(R/\Delta H_u)(\ln x - 1/\xi) \qquad (2)$$

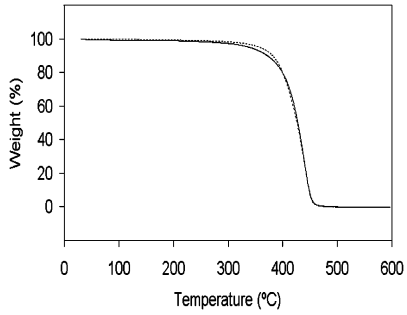

Figure 3. TGA curves of poly(octanediol adipate-30 mol % sorbitol adipate), P(OA-30 mol % SA) (continuous line), and poly(octanediol adipate-30 mol % glycerol adipate), P(OA-30 mol % GA) (broken line).

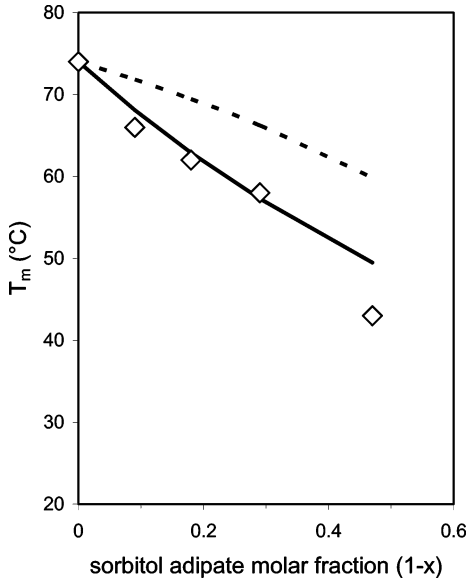

Figure 4. Melting temperature (T_m, from first scan) as a function of sorbitol adipate molar fraction in octanediol adipate−sorbitol adipate (OA-SA) copolymers: broken line, eq 1; continuous line, eq 2.

where T_m and T_m^0 are the melting temperatures of the copolymer and the pure crystallizing homopolymer (POA), respectively; x is the molar content of the crystallizable monomer, and ΔH_u is the melting enthalpy per mole of repeating unit of the crystallizable polymer (at 100% crystallinity). Since no experimental value of ΔH_u is available, $\Delta H_u = 43$ kJ/mol was used, a value derived from the group contribution additivity method.[18]

In eq 2, $\xi = 1/[2x(1 - x)]$ represents the average length of the crystallizing POA sequences. Both eq 1 and eq 2 assume that the comonomer (glycerol adipate or sorbitol adipate) is completely excluded from the POA crystals. However, unlike eq 1 by Flory, Baur's equation takes into account that the crystallizable monomer sequences shorten with increasing comonomer content. It is evi-

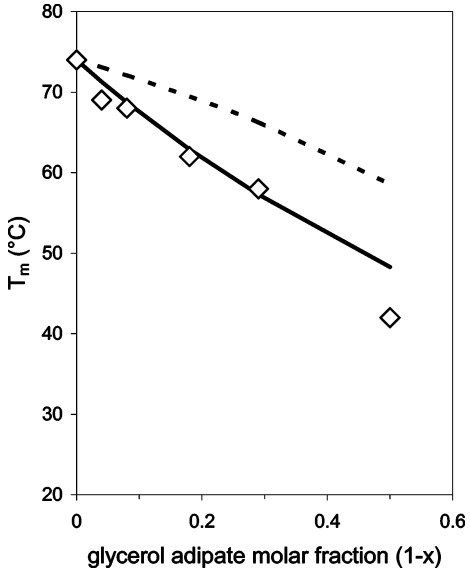

Figure 5. Melting temperature (T_m, from first scan) as a function of glycerol adipate molar fraction in octanediol adipate−glycerol adipate (OA-GA) copolymers: broken line, eq 1; continuous line, eq 2.

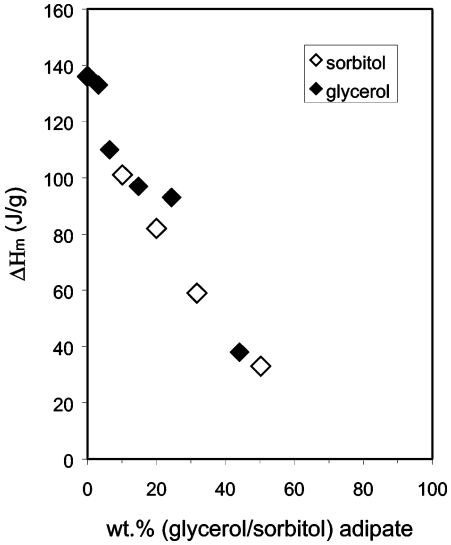

Figure 6. Melting enthalpy of copolymers of octanediol adipate and glycerol adipate and copolymers of octanediol adipate and sorbitol adipate as a function of composition.

dent from the curves of Figures 4 and 5 that eq 2 provides a much better fit to the experimental results than eq 1. This shows that the average length of the crystallizing sequences is an important factor that determines the melting temperature of the analyzed copolymers. Moreover, on the basis of the assumptions of Baur's equation, it is deduced that the comonomer units (GA or SA) do not enter the crystal lattice of POA.

Figure 6 shows the dependence of the melting enthalpy on the polyol content for P(OA-SA) and P(OA-GA) copolymers. The comonomer content is expressed as weight fraction (instead of mole fraction) since the melting enthalpy is given on a weight basis (J/g). The same behavior is shown by the melting enthalpy of both copolymers. It is clear that the introduction of the polyol units in the polyester chain hinders crystallization. Furthermore, a given weight content of comonomer, whether GA or SA, causes similar crystallinity decrease.

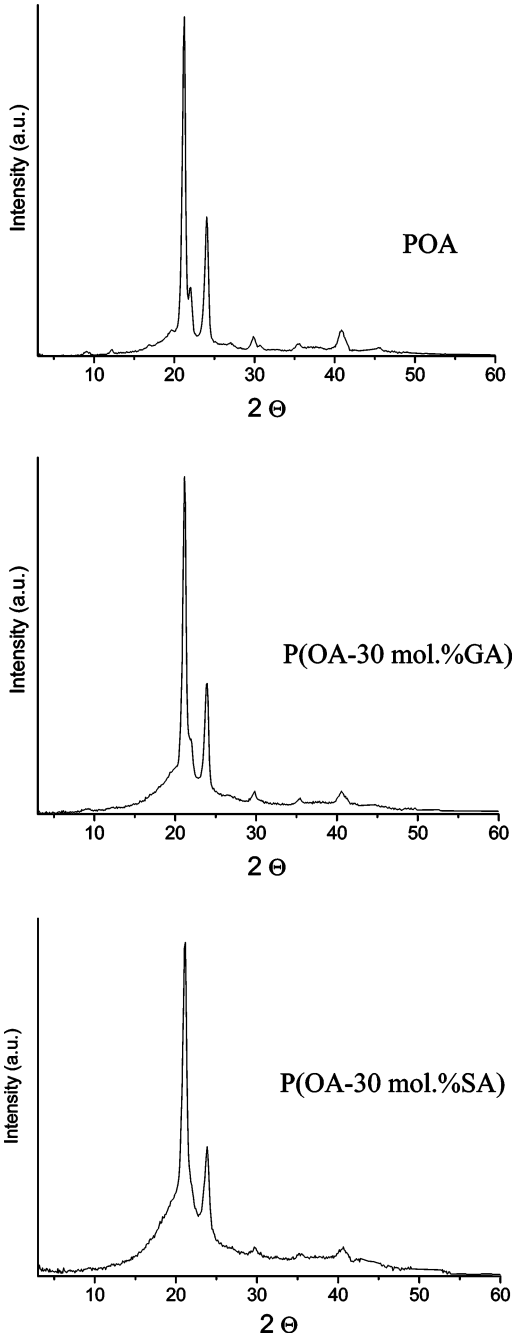

Figure 7. WAXS diagrams of POA and of the P(OA-SA) and P(OA-GA) copolymers containing 30 mol % of glycerol or sorbitol.

WAXS analysis of P(OA-SA) and P(OA-GA) copolymers showed that, in all cases, the only crystal phase was that of POA. As an example, Figure 7 compares the diffractograms of P(OA-30 mol % SA) and P(OA-30 mol % GA) copolymers with that of POA. The degrees of crystallinity calculated from the WAXS curves are 65, 45, and 32% for POA, P(OA-30 mol % GA), and P(OA-30 mol % SA), respectively. Hence, the WAXS measurements corroborate the DSC experimental results shown above, i.e., that the degree of crystallinity of the polyester decreases upon copolymerization with either glycerol or sorbitol. This behavior is common to crystallizable polymers upon copolymerization.[19a,b] The degree of crystallinity derived by WAXS shows that when the same molar content (30%) of either sorbitol or glycerol is incorporated into the copolymers, sorbitol

causes a stronger crystallinity decrease than glycerol. However, if the comparison is carried out on a weight content basis (the X-ray diffraction result depends on the mass of the analyzed sample), very similar results are obtained for the two copolymers P(OA-SA) and P(OA-GA). Furthermore, the decreases in crystallinity with added polyol content in the copolymer found by WAXS are in very good agreement with the DSC enthalpy results of Figure 6.

Conclusions

With increasing sorbitol or glycerol content, both melting and crystallization temperatures decrease for the copolymers of octanediol adipate and sorbitol adipate and copolymers of octanediol adipate and glycerol adipate. The same behavior is shown by the melting enthalpy of both copolymers. This is attributed to polyol units along the polyester chain which disrupt crystallinity. WAXS measurements confirm that the degree of crystallinity of these polyesters decreases upon copolymerization with either sorbitol or glycerol. The concurrent decrease in the degree of crystallinity with increasing contents of hydrophilic polyol units in the polyester chain indicates that these copolyesters will have "tunable" degradation rates. In other words, by increasing the polyol content, we expect that the susceptibility of the copolymer to hydrolytic degradability can be increased. Adjustable bioresorption rates, safe building blocks, and the ability to link bioactive moieties to functional groups along the chain make these copolyesters attractive candidates for use as bioresorbable materials.

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