

## “Sweet Polyesters”: Lipase-Catalyzed Condensation–Polymerizations of Alditols

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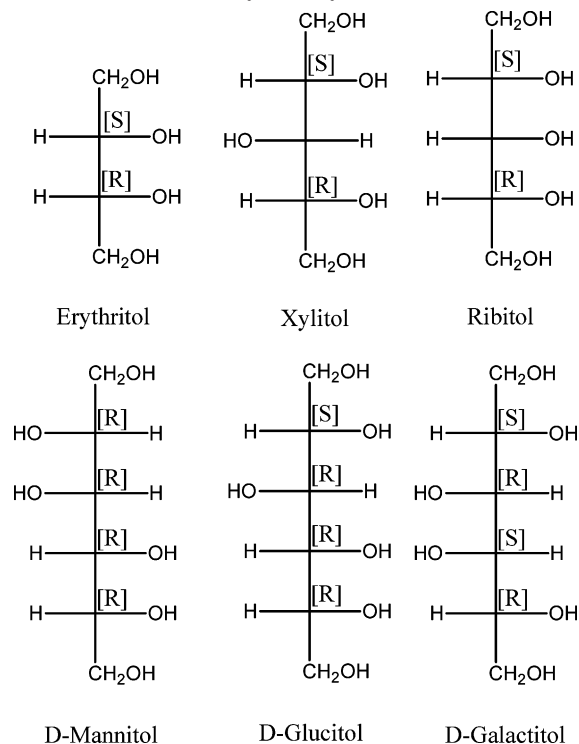
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Alditol polyols are readily renewable, inexpensive, and harmless to the environment. By incorporation of polyols into aliphatic polyesters, functional linear or hyperbranched polymers can be prepared with specific biological activities and/or that respond to environmental stimuli.<sup>1</sup> Polyesters with carbohydrate or polyol repeat units in chains have been prepared by chemical methods.<sup>2a–d,4a–c,8</sup> In some cases, the reaction conditions led to hyperbranched polymers (HBPs).<sup>4a–c,8</sup> The highly branched architecture of HBPs leads to unusual mechanical, rheological and compatibility properties.<sup>4–8</sup> These distinguishing characteristics have garnered interest for their use in numerous industrial and biomedical fields.<sup>8</sup> Chemical routes to linear polyol–polyesters require elaborate protection–deprotection steps.<sup>2a–d</sup> Furthermore, condensation routes to hyperbranched polymers generally require harsh reaction conditions such as temperatures above 150 °C and highly acidic catalysts.<sup>4a–c,8</sup>

Single-step chemical routes to HBPs from multifunctional monomers, without protection–deprotection chemistry, leads to randomly branched polymer topologies. To achieve perfectly branched polymers researchers have used stepwise synthetic methods to prepare dendrimers. A need exists for new, simple synthetic methods that do not rely on protection–deprotection methods to prepare both functional linear polymers and polymers with improved control over branching. A promising approach to address these challenges is the use of isolated enzymes as catalysts for polymerization reactions. Lipases are already well-established catalysts for regioselective esterification of low molar mass substrates at mild temperatures (30–70 °C).<sup>12</sup> Early work assumed that activation of carboxylic acids by electron withdrawing groups was needed to perform enzyme-catalyzed copolymerizations of polyols.<sup>13a–j</sup> Furthermore, since polyols (e.g., glucitol) are generally insoluble in nonpolar organic media, polar solvents were used.<sup>13f–j</sup> Unfortunately, these solvents cause large reductions in enzyme activity.<sup>13f–j</sup>

Recently, our laboratory reported copolymerizations without activation of the diacid or adding solvent.<sup>14a,b</sup> The monomers were combined so they formed monophasic mixtures, *Candida antarctica* Lipase B (CALB), physically immobilized on Lewatit beads (N435), was then added. For example, a hyperbranched copolyester with 18 mol % glycerol–adipate units was formed in 90% yield, with  $M_w = 75\,600$  (by SEC–MALLS),  $M_w/M_n = 3.1$ , and 27 mol % of glycerol units that are branch sites.<sup>14a</sup> Also, N435 catalyzed the polymerization of D-glucitol and adipic acid, in-bulk, with high regioselectivity (85 ± 5%) at the primary hydroxyl groups, to give a water-soluble product with  $M_n = 10\,880$  and  $M_w/M_n = 1.6$ . Time-course studies of glycerol copolymerizations showed that, while the reaction is under

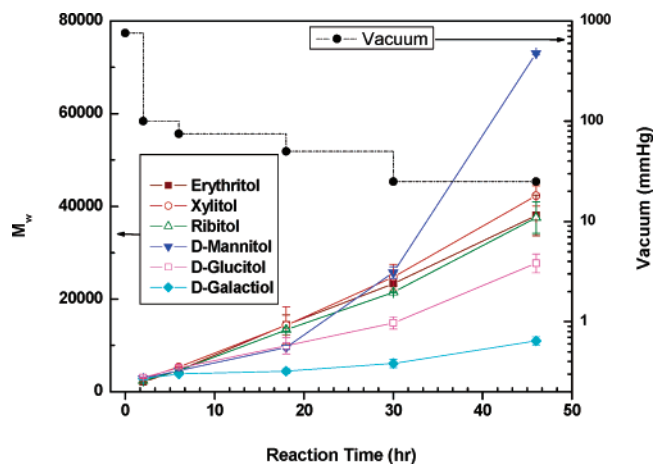
Scheme 1. Structure of Alditols Used in This Study for N435-Catalyzed Polymerizations



kinetic control, linear chains (determined by NMR) were formed. Hence, the product formed at 18 h was linear. However, by extending the reaction to 42 h, pendant hydroxyl esterification transpired giving HBPs. In contrast, when chemical methods are used, glycerol has been used to introduce branching into polyesters.<sup>15</sup>

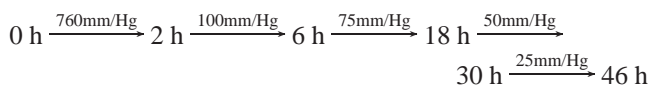
Lipase regioselectivity can be varied by many parameters such as substrate structure, lipase structure, lipase immobilization, reaction medium, time and temperature. To better understand factors of reaction time and substrate structure (e.g., chain length, stereochemistry), a series of 4-, 5- and 6-carbon natural polyols were selected as monomers for N435-catalyzed polymerizations. The structures of alditol polyols used herein along with their stereochemical configurations are displayed in Scheme 1. Bulk terpolymerizations of these substrates with 1,8-octanediol and adipic acid were performed. Adipic acid, 1,8-octanediol, erythritol, xylitol, ribitol, D-glucitol, D-mannitol, and D-galactitol were purchased from the Aldrich Chemical Co. N435 was a gift from Novozymes (Bagsvaerd, Denmark). All reagents were purchased in the highest purity available and were used without further purification. Polymerizations were performed by the identical procedure published elsewhere<sup>14a–b</sup> except for modifications in reactor hardware, vacuum regulation, and pressure used for water removal (see below). In summary, reactions were carried out in-bulk for up to 46 h at 90 °C using N435. Since N435 has 10%-by-wt CALB,<sup>16</sup> the weight ratio of CALB to monomer is 1%. Monomers were transferred to glass vessels and mixtures were heated to 130 °C for about 0.5 h. Solid reactants melted and/or dissolved forming a homogeneous liquid. The temperature was lowered to 90 °C and the reaction mixture remained as a homogeneous liquid but increased in viscosity. Polymerizations were performed in an Argonaut Advantage Series 2050

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**Figure 1.** Time-course study of molecular weight increase as a function of polyol structure. Polymerizations were performed at 90 °C, catalyzed by N435. The right Y-axis showed the schedule of applied vacuum during reactions.

personal synthesizer. The unit has five parallel reactors each with independent temperature control and magnetic stirring. Each glass reactor (2.7 cm by 19.5 cm) was directly attached to one of five ports of a vacuum manifold. A digital vacuum regulator (J-KEM scientific, model 200) was placed between the manifold and the vacuum pump. This system permitted reactions with different polyols, replicates and controls to be run side-by-side under nearly identical vacuum conditions. Polymerizations were performed with a molar ratio of adipic acid to 1,8-octanediol to polyol of 1.0:0.8:0.2 and 0.04 mol (about 6 g) of total reactants. Periodically, 50 mg aliquots were removed from reactions for analysis. Vacuum was applied in reactions according to the following schedule:



Although this series of reaction stages is nonoptimized, it takes into account the following concerns that arose based on experimental observations. No vacuum during the first 2 h allows reactants to oligomerize without evaporation of volatile monomers. Slow decrease in the pressure from 2 to 30 h avoids turbulent bubbling and foaming. Furthermore, when low pressure (e.g., 25 mmHg) was applied throughout reactions of polyols with diacids there was an increased frequency of cross-linked product formation due to chemically mediated reactions (unpublished results). To minimize these events, 25 mmHg was applied only during the last stage (30–46 h) to drive reactions toward formation of high molecular weight products.

Molecular weight increase as a function of time was used to assess differences in polyol reactivity. The relative weight-average molecular weights were determined by size exclusion chromatography (SEC) in our lab<sup>15b</sup> using a PLgel HTS-D column. Figure 1 shows the increase in polyol-polyester weight-average molecular weight ( $M_w$ ) as a function of reaction time and polyol structure. Standard deviation, shown as error bars in Figure 1, was calculated from three experiments. Polyol-polyester  $M_w$  at 46 h decreased as follows: D-mannitol ( $73.0 \pm 0.4$  K) > erythritol ( $38.1 \pm 4.4$  K), xylitol ( $42.3 \pm 2.2$  K), ribitol ( $38.4 \pm 2.9$  K) > D-glucitol ( $27.7 \pm 2.0$  K) > galactitol ( $11.0 \pm 0.9$  K). This trend was identical at 18 and 30 h except for mannitol which, at these reaction times, did not give the highest polyol-polyester  $M_w$ . Instead, mannitol polyol-

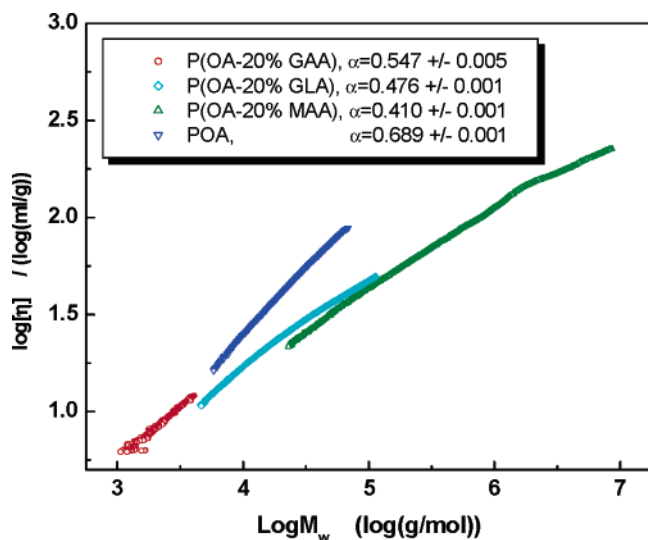
polyester  $M_w$  was similar to that for glucitol at 18 h and erythritol/xylitol/ribitol at 30 h. Thus,  $M_w$  for the mannitol copolymerization increased rapidly from 18 h relative to copolymerizations with other polyols.

Relative to higher temperature (>150 °C) chemically catalyzed polyesterification reactions, polymerizations between alcohol and acids catalyzed by CALB can be performed at 90 °C or lower. However, lowering the reaction temperature increases the reaction medium viscosity. This raised the question as to whether the applied vacuum, that largely determines water-removal efficiency, is a sensitive parameter controlling the polymerization rate. To probe this question, the standard deviation of triplicate reactions was determined by carrying out polymerizations using both a digital vacuum regulator and a conventional needle valve to control the applied vacuum. Values of percent error in Figure 1 ranged from 0.4 to 4.4 K. When these experiments were performed using a needle valve, the percent error values for the same series of reactions ranged from 13.7 to 39.6 K. Thus, without tight regulation of vacuum, experimental results were not reproducible. This result should be carefully considered by others that review related previous literature or begin new research on enzyme-catalyzed polyol-polyester condensation polymerizations.

As shown in Scheme 1, the alditols studied herein have different chain lengths and/or stereochemistry. Given the inherent selectivity of enzymes, it was anticipated that N435-catalyzed polyesterification of these substrates would occur at different rates. However, the relative order of their reactivity during polymerization reactions was unpredictable.

While this study does not include a systematic series of 4-, 5-, and 6-carbon alditol polyols with all possible permutations of stereochemical configurations, this set of polyols does allow one to begin exploring general structural characteristics that may be responsible for different reactivity. First, the influence of substrate chain length was considered. Erythritol, the only 4-carbon substrate, showed moderate reactivity for high polymer synthesis (see Figure 1). D-mannitol, D-glucitol, and D-galactitol, all 6-carbon substrates, showed high, intermediate, and low reactivity for high  $M_w$  polymer formation. From these results, no apparent correlation can be made between polyol chain length and its polymerization activity. Relative to terminal primary hydroxyl moieties, the nearest asymmetric and *pseudo*-asymmetric centers for chiral and achiral (*meso*) polyols, respectively, are at  $\beta$ -carbons. One explanation for polyol reactivity is it's determined by the stereochemical configuration of carbons closest to terminal primary hydroxyl groups ( $\beta$ -carbons). The stereochemical configuration of these carbons is [R]-[S] or [S]-[R] except D-mannitol that is [R]-[R] (Scheme 1). None of the polyols have an [S]-[S] structure. Thus, it may be that the terminal [R]-[R] configuration of D-mannitol is a key factor that led to rapid formation of D-mannitol polyol-polyesters of high  $M_w$ . This agrees with studies by Hult and co-workers on the selective for [R]-alcohols in CALB-catalyzed transesterification reactions.<sup>17,18</sup>

Certainly, the reactivity of secondary hydroxyl groups of alditols will also affect chain growth. Indeed, it is the combined reactivity of primary and secondary hydroxyl moieties that will ultimately determine chain growth and branching that dictate polymer properties (e.g., viscosity). To evaluate differences in branching among polyol-polyesters, their dilute solution properties were compared by using exponent  $a$  of the  $[\eta]$ - $M$  relationship. The  $[\eta]$ - $M$  relationship, also known as the Kuhn-Mark-Houwink-Sakurada relationship, was determined by conducting size exclusion chromatography with online concen-



**Figure 2.** Intrinsic viscosity  $[\eta]$  as a function of the molar mass  $M$  for polyol containing polyesters and linear poly(octamethylene adipate) in THF solution at  $T = 25\text{ }^{\circ}\text{C}$ .

tration (Wyatt Optilab DSP interferometer, Santa Barbara, CA), multiangle light scattering (MALS) (Wyatt HELEOS) and viscosity (Wyatt Viscostar) detectors. Absolute molar mass and intrinsic viscosity of each elution moment was determined from RI and MALS and the viscosity response by the corresponding detector. Even though the chemical composition may be the same, branched polymers have higher densities in solution than their linear structural isomers and, therefore, lower intrinsic viscosities.<sup>19</sup> Figure 2 displays plots of  $\log[\eta]$  vs  $\log M_w$  ( $[\eta]$ – $M$  relationship) determined by SEC-MALS–vis analyses of poly(octamethylene adipate) (POA, linear) and terpolymers with 80 mol % OA and 20 mol % D-galactitol–adipate (GAA), D-glucitol–adipate (GLA), and D-mannitol–adipate (MAA) (P[OA-20%GAA], P[OA-20%GLA] and P[OA-20%MAA], respectively). D-Galactitol, D-glucitol, and D-mannitol are each 6-carbon sugars that give copolymers of relatively low, intermediate, and high  $M_w$ , respectively (see above). Slopes of lines in Figure 2 gave exponent  $a$  values of 0.688, 0.547, 0.476, and 0.410 for POA, P(OA-20%GAA), P(OA-20%GLA) and P(OA-20%MAA), respectively. Lower  $a$  values indicate increased branching along chains. Therefore, plots in Figure 2 suggest combined reactivity at primary and secondary hydroxyl groups increases in the following order: galactitol < D-glucitol < D-mannitol. These 6-carbon alditols have 2, 3, and 4 [R]-secondary hydroxyl groups, respectively. Previous studies showed that CALB more rapidly acylates [R]-secondary hydroxyl groups.<sup>17,18</sup> Assuming reactivity at primary hydroxyl groups remains high, increased reactivity at [R]-secondary hydroxyl groups will lead to branching. Therefore, larger molecular weight chains formed by D-mannitol may be due to high reactivity at [R]-primary hydroxyl groups in addition to reactivity at [R]-secondary hydroxyl groups leading to higher molecular weight chains through branching. It is also noteworthy that differences in  $a$  could in-part be due to configurational effects since polymer conformation may be affected by polyol stereochemistry.

In summary, an expanded set of naturally derived polyols was assessed for their potential to form high molecular weight polyol–polyesters by N435 catalysis. All substrates were polymerized forming polyol–polyesters with  $M_w$  that ranged from 11 K (galactitol) to 73 K (D-mannitol). No correlation was found between sugar reactivity and its chain length. By using a parallel reactor system and by performing replicate experiments

with both a digital vacuum controller and a needle valve, the importance of precise vacuum control on reproducibility of experiments was established. Plots of  $\log[\eta]$  vs  $\log M_w$  were prepared from SEC-MALS–vis analyses of poly(octamethylene adipate) and adipate-1,8-octanediol-alditol (D-galactitol, D-glucitol, D-mannitol) terpolymers. Comparison of exponent  $a$  values from slopes of these plots showed copolymers from D-mannitol had the largest degree of branching and, therefore, the greatest propensity for combined reactivity at both primary and secondary hydroxyl groups. An explanation proposed is that the higher reactivity of D-mannitol during copolymerizations was due to: (i) the [R]–[R] stereochemical configuration of both carbons closest to terminal primary hydroxyl groups ( $\beta$ -carbons) (ii) there is no chain regioisomerism in D-mannitol, and (iii) that all secondary hydroxyl group carbons are in the [R]-configuration. This study provides a foundation for future work with this expanded set of polyols and reproducible reaction conditions to elucidate details of regioselectivity and branching during enzyme-catalyzed polyol–polyester polymerizations.

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