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Optimized Synthesis of Salicylate-based Poly(anhydride-esters)

Robert C. Schmeltzer, Theodore J. Anastasiou and Kathryn E. Uhrich (✉)

Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ 08854-8087

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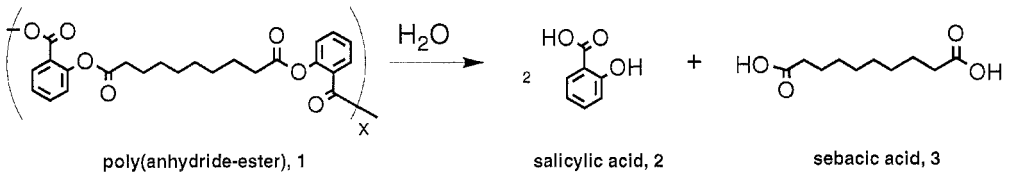
Summary

The synthesis of a salicylate-based poly(anhydride-ester) was optimized to improve the overall efficiency and quality of the polymer. First, a new approach for the preparation of the polymer precursor minimizes the overall number of synthetic steps and increases the overall yield. Second, the melt-polymerization apparatus was modified to include dynamic mixing, which yields polymer with increased molecular weights on both the milligram and gram scale.

Introduction

Previously, our laboratory reported the synthesis of poly(anhydride-esters) (**1**) comprising salicylic acid (**2**) as novel degradable biomaterials.[1-3] An important feature of homopolymer **1** is that it degrades into naturally occurring compounds, salicylic acid (**2**) and sebacic acid (**3**) as outlined in Scheme 1. These polymers are unique in that the drug is chemically incorporated into the polymer backbone, not attached as a side group[4-7]. This feature has two significant advantages. First, high amounts of drug can be incorporated into the polymer, for comparison, polymer **1** is approximately 60 % by weight. Second, the drug is released in a controlled fashion as a function of the biocompatible linker molecule, which is sebacic acid (**3**) in polymer **1**. Many examples of salicylate-based polymers exist, yet these materials are limited because they have low, therapeutically unfeasible concentrations of salicylate in the backbone[8] or are simply homopolymers of salicylic acid[9-10] (i.e., no linker molecules) such that release of salicylate cannot be readily modified.

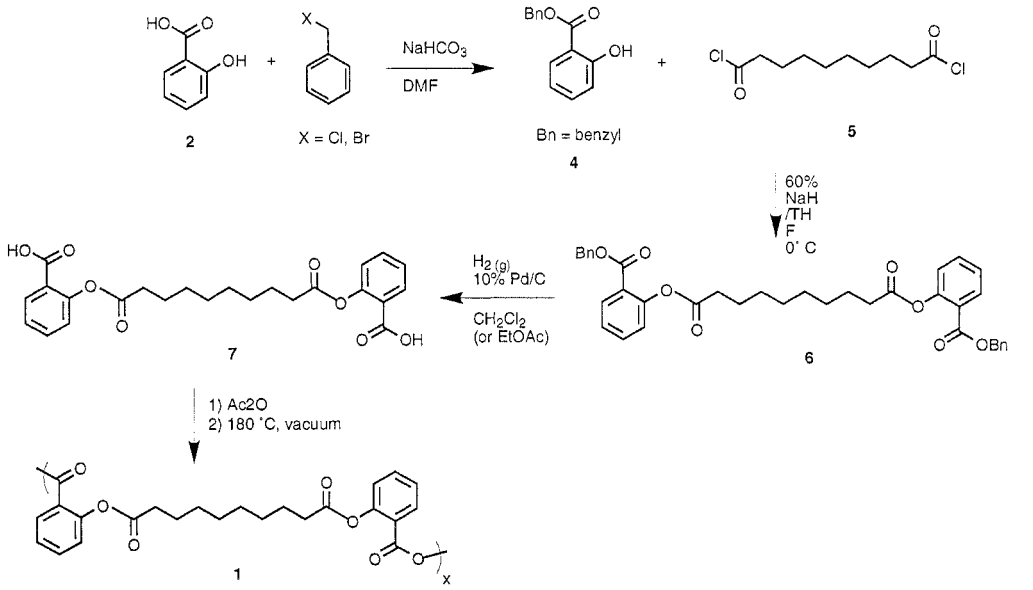
Although polymer **1** was synthesized with success on the milligram and gram laboratory scales, the number of synthetic steps must be reduced to produce the polymer on the kilogram scale in high yield. Our initial synthetic route for poly(anhydride-esters) was based upon synthetic schemes developed for polyanhydrides, which were first explored for use as textiles[11] and later exploited as biodegradable polymer for medical applications[12-23].



Scheme 1. Hydrolytic degradation of poly(anhydride-ester).

Based on the original procedure, the polymer precursor is synthesized in three steps as outlined in Scheme 2 with an overall yield of less than 50%. The carboxylic acid of **2** was converted to a benzyl ester to give benzyl salicylate (**4**), with a free phenol group for further reaction with sebacoyl chloride (**5**) to yield **6**. The benzyl protecting groups of **6** were reductively cleaved to give the desired diacid (**7**), which is the monomer precursor. To form polymer **1**, the diacid (**7**) is first acetylated with acetic anhydride to form the monomer, a mixed anhydride, which then undergoes a melt condensation polymerization.

The low overall yield is primarily attributed to the final deprotection step, in which the benzyl ester is removed. As deprotection proceeded, compound **7** would coat the Pd/C catalyst, regardless of the polar solvents and temperatures evaluated. In addition, the procedure outlined in Scheme 2 requires repeated recrystallizations of the diacid (**7**) to get compounds of sufficient purity for the subsequently activation and polymerization steps.



Scheme 2. Previous synthesis of poly(anhydride-ester).

Lower molecular weight polymers obtained by this procedure is attributed to the impurity of the monomer precursor, **7**, and to the polymerization apparatus. During the melt condensation process, dark colored patches were observed within the polymerization apparatus. Thus, we explored alternate polymerization methods to enhance mixing, this approach has two goals: to reduce localized "hot spots" and to enhance polymer contact to ultimately increase the molecular weight. This report details our successful method to (*i*) improve polymer precursor yields and (*ii*) increase polymer molecular weights.

Experimental

Materials

Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on either a Varian 200 MHz or 300 MHz spectrometer. Samples (5-10 mg) were dissolved in the appropriate deuterated solvent ($CDCl_3$ or DMSO-d_6) using the solvent as the internal reference. Infrared (IR) spectra were measured on a Mattson Series spectrophotometer by solvent-casting samples onto a NaCl plate. Melting points (T_m) were determined on a Thomas-Hoover apparatus.

Methods

Weight-averaged molecular weights (M_w) and polydispersity indices (PDI) were determined by gel permeation chromatography (GPC) on a Perkin-Elmer (PE) LC system consisting of a Series 200 refractive index detector, a Series 200 pump, and an ISS 200 autosampler. A DEC Celebris 466 computer running PE TurboChrom 4 software was used for data collection and processing, and to automate the analysis via PE-Nelson 900 Interface and 600 Link. Samples (5 mg/ml) were dissolved in THF, filtered through 0.45 μm poly(tetrafluoroethylene) (PTFE) syringe filters (Whatman, Clifton, NJ) and immediately injected. Samples were resolved on a Jordi DVB mixed-bed GPC column (7.8 x 300 mm) (Alltech, Deerfield, IL). Molecular weights were calibrated relative to narrow molecular weight polystyrene standards (Polysciences, Dorval, Canada).

*Salicylate-based diacid (**6**)*

Salicylic acid (**2**; 1.2 g, 8.4 mmol) was dissolved in a solution of THF (3.0 ml) and pyridine (9.0 ml). Sebacoyl chloride (**5**; 1.0 g, 4.2 mmol) was added dropwise via syringe over 5 minutes to the stirring reaction solution set in an ice bath (~0 °C). The reaction mixture warmed to room temperature, stirred for 2 more hours, then poured over an ice-water slush (150 ml). After acidifying to pH~2 with concentrated HCl, **7** was isolated by vacuum filtration, washed with water (3 x 50 ml), and air-dried. Yield: 97% (white powder). ^{1}H-NMR ($CDCl_3$): δ 8.13 (d, 2H, ArH), 7.61 (t, 2H, ArH), 7.35 (t, 2H, ArH), 7.12 (d, 2H, ArH), 2.63 (t, 4H, CH_2), 1.82 (m, 4H, CH_2), 1.48 (b, 8H, CH_2). IR (NaCl, cm^{-1}): 3400-2700 (COOH), 1760 (C=O, ester), 1700 (C=O, ester). Anal. Calcd: C, 65.18; H, 5.88. Found: C, 64.50; H, 5.73. T_m = 128-131 °C.

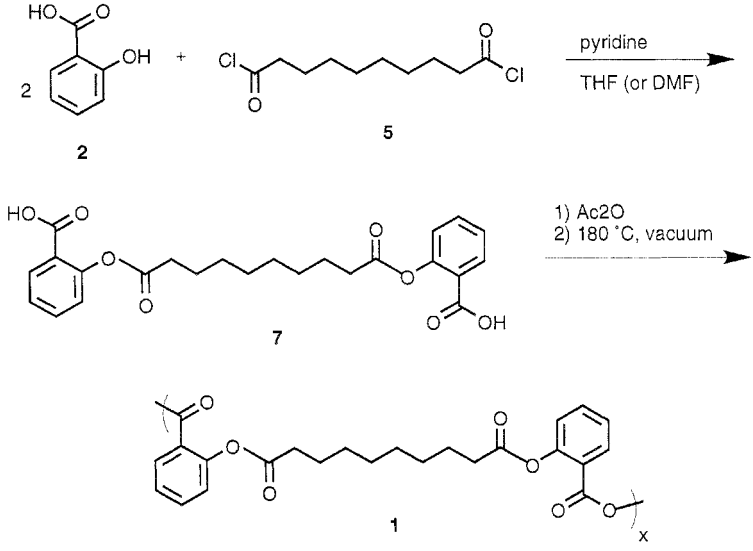
Polymerization

The diacid (**7**) was activated into monomer using previously described methods.[24-25] In brief, the diacid (**7**) was added to an excess of acetic anhydride (100 ml), then stirred at temperature until a homogenous solution is observed (approximately 120 min). The monomer is isolated by removing excess acetic anhydride under vacuum, and then washed with diethyl ether (50 ml). Monomer (500 mg) was placed in the appropriate reaction vessel, which was heated to 180 °C using a temperature controller (Cole Parmer) in a silicone oil bath under high vacuum (<2 mmHg) for 1 to 3 h. During this time, the melt was actively stirred at ~100 rpm by the overhead stirrer (T-line Laboratory Stirrer, Talboys Engineering). Polymerization was complete when the viscosity of the melt remained constant and/or solidified. The polymer was cooled to room temperature, dissolved in a minimal volume of methylene chloride (15 ml), and precipitated into a 20-fold excess of diethyl ether (300 ml). Polymer properties are given in Table 1. Yield: quant. (pale tan solid). ^{1}H-NMR (DMSO-d_6): δ 8.20 (d, 2H, ArH), 7.95 (t, 2H, ArH), 7.75 (t, 2H, ArH), 7.40 (d, 2H, ArH), 2.20 (t, 4H, CH_2), 1.55 (m, 4H, CH_2), 1.25 (b, 8H, CH_2). IR (NaCl, cm^{-1}): 1792, 1740 (C=O, anhydride), 1760 (C=O, ester).

Results and Discussion

Diacid Synthesis

The method previously developed in our laboratory to synthesize the diacid (**7**), which is the monomer precursor to polymer **1**, yielded moderate quantities.[2] Thus, synthetic methods that would provide diacid (**7**) in fewer reaction steps, with minimal purification, and higher overall yields were explored (Scheme 3).



Scheme 3. Optimized synthesis of poly(anhydride-ester).

By modifying a method reported by Pinther and Hartmann[26], the optimal reaction conditions were defined: the *free* salicylate (**2**) is directly coupled with the diacyl chloride (**5**) in an appropriate solvent containing pyridine to give diacid **7** (Scheme 3). Pinter and Hartmann[26] converted aliphatic acids into acyl chlorides by treating with thionyl chloride, the diacyl chlorides were subsequently reacted with 4-hydroxybenzoic acid in the presence of stoichiometric amounts of pyridine in dioxane. As our polymers may be used for medical purposes, we needed to replace dioxane as the solvent and to decrease the pyridine concentration, if possible. Although several solvents were evaluated, our preference is tetrahydrofuran (THF), a low-boiling, polar solvent.

A stoichiometric amount of pyridine is used to deprotonate the salicylate (**7**); the pyridine also acts as a catalyst to form an acyl pyridinium ion[27], which reacts with the free phenolate. Therefore, formation of the acyl pyridinium ion, which is known to react more rapidly with alcohols than acyl chlorides[27-28], eliminates the need to protect the carboxylic acid of the salicylate (**2**). This method also eliminates further purification, except for washing with an appropriate solvent, because of the large solubility differences between the product (**7**) and the reaction's potential by-products. Resultant conversions are quantitative and isolated yields are greater than 90%.

This one-step procedure has been applied to a variety of related diacids, where aminosalicylates[29-30] are used in place of salicylic acid (**2**) and various alkyl- and aryl-based acyl chlorides[31-32] are used in place of the sebacoyl chloride (**4**). Given the simplicity and ease of diacid isolation, this one-step method is our preferred choice for preparing a variety of diacids that will undergo melt condensation to yield poly(anhydride-esters).

Polymer Synthesis

The polymerization apparatus was redesigned to give higher molecular weight materials on both the milligram and gram scale. In our initial work, we utilized the polymerization methods described by Langer[33] and Domb[34]. In brief, the poly(anhydride-esters) (**1**) were synthesized by melt condensation polymerization using a side-arm test tube containing a magnetic stir bar (Figure 1), attached to a gas-vacuum manifold. The monomers were melt-polymerized at 180 °C under vacuum (<2 mmHg) until the melt solidified, and the reaction vessel was flushed every 15 min with dry nitrogen with stirring. Incomplete mixing, due to increased viscosity of the polymer melt after the reaction proceeded, resulted in prolonged polymerization times and low molecular weights, even at the milligram scale. In addition, portions of the polymer melt would locally decompose due to incomplete mixing, resulting in polymers that were dark brown and sometimes charred.

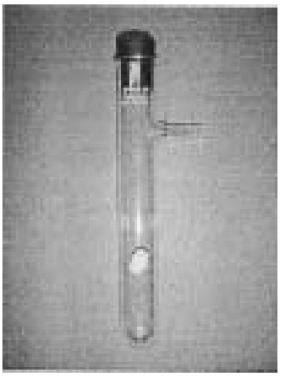


Figure 1. Side-arm test tube containing magnetic stir bar

To overcome these issues, we investigated methods to provide more homogenous mixing of the melt. By using simple, inexpensive, and readily available components (Figure 2), a polymerization apparatus was constructed that actively stirred the molten monomer, while maintaining a high vacuum (<2 mmHg). Both small (<1 g) and medium (1 g – 100 g) scale methods use a typical laboratory stirring motor.

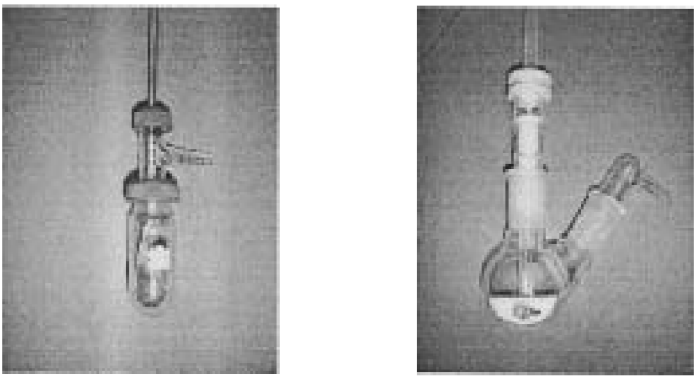


Figure 2. (a) (*left*) Small (<1 g) and (b) (*right*) medium scale (1 g–100 g) polymerization vessel.

On the small scale (<1 g), the polymerization vessel is constructed from microscale glassware components with 14/10 joints (see Figure 2a). A cylindrical bottom vial (10 ml) is equipped with a vacuum adapter; the included o-rings and screw-top joints ensure a vacuum seal, and create a modular system. The stirring shaft is constructed by shaving the edges of the spoon end of a stainless steel lab spoon-spatula (9") to fit through the 14/10 joint of the vial. The spatula end is left flat, which allows the shaft to interlock with the stirring motor. The joint and o-ring at the top of the vacuum adapter form a vacuum-tight fit around the shaft. Sealing the vessel with the supplied septa facilitates storage of the final polymer.

On the medium scale (1 g – 100 g), the polymerization apparatus is similar but 125 –250 ml two-necked round-bottom flasks with 24/40 joints are used as the reaction vessel (see Figure 2b). A vacuum joint is installed in one neck, while the other neck holds a Teflon vacuum-stirring adapter. The stirrer assembly consists of a glass stirring shaft and Teflon paddle (19 mm x 48 mm). After the polymerization, a standard-taper stopper seals the flask.

Several changes were observed with the change in polymerization techniques. First, using the modular apparatus in conjunction with the overhead mechanical stirrer, poly(anhydride-esters) were prepared with weight-averaged molecular weights around 30,000. In contrast, using our initial system (i.e., magnetic stirrer), polymer molecular weights were typically below 10,000[2]. Second, slightly elevated glass transition temperatures were observed with the increased molecular weights. For the poly(anhydride-ester) described herein comprised of salicylate and sebacate, the glass transition temperature was raised from 23.5 °C (magnetic stirrer) to 27.0 °C (mechanical stirrer). This aspect was extremely important for the continued development of homopolymer **1**, glass transition temperatures above room temperature significantly enhance the processing capabilities of these polymeric materials. Third, the poly(anhydride-esters) are consistently white in color, as opposed to brown, which is an important indicator of purity and especially significant for using homopolymer **1** in medical applications.

Conclusions

By redesigning a generally utilized polycondensation method to prepare poly(anhydrides), higher molecular weight poly(anhydride-esters) were obtained in near-quantitative yields at higher purity.

Acknowledgements

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