Novel degradable poly(anhydride-esters) for controlled drug release

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Abstract

This project centers on the synthesis of novel poly(anhydride-esters), which are biodegradable polymers consisting of two salicylic acid molecules linked by carboxylic acid derivatives. Several poly(anhydride-esters) have already been synthesized (Erdmann et al., 1999) with demonstrated biocompatibility (Macedo, 1999) and degradative properties (Erdmann and Uhrich, 1999) desirable for controlled delivery of salicylic acid. However, their glass transition temperatures (T_g's) are several degrees below body temperature (37 C), which limits the number of potential medical applications. The goal of our project is to develop poly(anhydride-esters) with T_g's higher than 37 C. To achieve this goal, we performed multi-step syntheses of a series of polymers varying the linker structure between salicylic acid moieties. Linker molecules included derivatives of succinic acid, terephthalic acid, 4,4'-oxybis(benzoic acid), and 1,4-cyclohexanedicarboxylic acid. All monomers, oligomers and polymers were characterized by proton nuclear magnetic resonance spectroscopy (to verify chemical structure), gel permeation chromatography (to measure molecular weight and polydispersity index), and differential scanning calorimetry and thermal gravimetric analysis (to determine thermal properties). All four of the new polymeric materials had T_g's above 75 C, markedly higher than the T_g's of the first generation of poly(anhydride-esters). Because the terephthalate-based polymer showed the highest degree of polymerization, it is our polymer of choice for future *in vitro* degradation studies in aqueous media.

Introduction

Background

Poly(anhydride-esters) are polymeric compounds consisting of salicylic acid moieties bridged by linker structures. An example of the polymer structure is shown in Figure 1 (A). These compounds contain two types of important bonds - anhydride and ester bonds shown in Figure 1 (A) in blue and red respectively. In the presence of water, both bonds may degrade hydrolytically, releasing salicylic acid (B) and sebacic acid (C). Salicylic acid (B) is the active form of aspirin, an anti-inflammatory agent, and sebacic acid is currently used in drug delivery systems (Brem et al., 1995). *In vivo* mice studies have indicated that this polymer assists in wound healing and that it promotes bone growth (Macedo, 1999).

The release of salicylic acid (B) via bond hydrolysis opens up a variety of possibilities for creating drug delivery systems. Potential applications include treatment of inflammatory bowel disease, dental implants, and tissue scaffolding. The studies on the degradation rate, the rate of release of salicylic acid as a function of pH, have shown that this polymer takes three months to degrade in acidic and neutral environment, but at basic pH it degrades in 19-40 hours (Erdmann and Uhrich, 1999). Since the upper gastrointestinal tract is acidic or neutral, this polymeric drug can reach the intestines undamaged, and release salicylic acid directly to the lower intestinal tract to treat the disease. Aspirin is also used in dentistry, for example, in cases of tooth breakage when there cannot be an immediate operation. In this procedure, the fast influx of salicylic acid irritates the surrounding tissue. Slower release of the medication, provided by the poly(anhydride-esters) would be a gentler solution.

A drawback to the poly(anhydride-ester) structure shown in Figure 1 is its low T_g (glass transition temperature). This is a temperature at which a polymer goes from a solid into a rubbery state. The T_g of this polymer is several degrees below body temperature (37 $^{\circ}$C), which means that it becomes a soft, sticky material when placed in the body. Because the polymer is being developed for use as a suture material, it is undesirable for the polymer to become soft during the suturing process. Therefore, poly(anhydride-esters) with T_g's higher than body temperature need to be developed.

Purpose

Our goal is to develop poly(anhydride-esters) with T_g's higher than body temperature by modifying the structure of the polymer backbone. Ultimately, we also want to investigate the effect that structural modifications of the polymer backbone have on the polymer thermal properties and the release rate of salicylic acid. To preserve the presence of salicylic acid (B) and the configuration of anhydride and ester bonds the strategy that we have used has been modification of the linker structure (R) in Figure 2.

Results and discussion

The linker structures that we incorporated into the polymer are derivatives of succinic acid (SA) (I), terephthalic acid (TA) (II), 4,4'-oxybis(benzoic acid) (OBA) (III),and 1, 4-cyclohexanedicarboxylic acid (CDA) (IV). These structures are shown in Figure 3. These four compounds cover structural extremes, from a short aliphatic chain in SA, which allows for free rotation around the carbon-carbon bonds, to a flat phenyl ring of TA. The structures of CDA and OBA are intermediate in the extent of bending and rotation, OBA being more like TA, and CDA being closer to SA.

The general synthetic procedure is outlined in Figure 4. Starting with benzyl protected salicylic acid (1), the activated form of linker molecule (3) is coupled with salicylic acid (1). The acid ends are then deprotected by a hydrogenation reaction, yielding the diacid structure (5). The acid ends of (5) are activated by heating to reflux temperature in acetic anhydride to give the monomer (6). Finally, the monomer (6) is polymerized in a condensation self-polymerization described by Campo et al. (1999). All stable products were characterized throughout the multi-step synthesis by ^{1}H NMR (proton nuclear magnetic resonance) spectroscopy to ensure product purity. The molecular weight (MW) and polydispersity index (PDI) of the final polymers were measured using gel permeation chromatography (GPC). Thermal properties were obtained using differential scanning chromatography (DSC), which gives T_g and T_m (melting point), and thermal gravimetric analysis (TGA), which gives the decomposition temperature, T_d , the measure of thermal stability of the material. These results are summarized in Table 1.

Conclusions

All the polymers have T_g's higher than body temperature. In addition, the values of T_d (decomposition temperature) indicate that the SA (I)-based polymer has the least thermal stability, followed by CDA- (IV), OBA- (III), and TA-(II) based materials, in ascending order. The two extreme values, those for SA (succinic acid) and TA (terephthalic acid) are not surprising. The SA linker allows for free rotation, reducing the rigidity of structure of the polymer and intermolecular bonding. The TA linker, on the other hand, is planar, so it contributes to π-interactions among the units, thereby stabilizing the polymer overall. As the TA-based polymer showed the highest degree of polymerization (highest MW and lowest PDI value), it is our polymerofchoice for future studies.

Experimental methods

Materials

All materials were purchased from Aldrich and used as received, with the exception of benzyl salicylate (1), which was distilled before use.

Methods

Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained on a Varian 200 MHz spectrometer from samples prepared by dissolving ~5-10 mg of material in either deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide (DMSO) using the solvent as the internal reference. Infrared (IR) spectra were measured on a ATI Mattson Genesis Series Fourier transform spectrophotometer. The samples were solvent-cast onto KBr pellets.

Molecular weights (MW) and the polydispersity index (PDI) were measured on a Perkin Elmer Series 200 LC system equipped with a PL-Gel column (5 m, mixed bed) operated at 60 C, Series 200 refractive index detector, Series 200 LC pump and ISS 200 autosampler. The analysis was automated using a digital Celebris 466 computer via PE Nelson 900 interface and PE Nelson 600 Link box. Data were collected and processed by using PE Turbochrom 4 software. The eluent for the analysis was tetrahydrofuran (THF) at a flow rate of 0.5 ml/min. Samples (~5 mg) were dissolved into THF (~1 ml) and filtered using 0.45 m PTFE syringe filters prior to injection into the column. Molecular weights were calibrated relative to narrow molecular weight polystyrene standards (Polysciences, Dorval, Canada).

Thermal analysis was carried out on a Perkin Elmer system consisting of Pyris 1 DSC and TGA7 analyzers with TAC 7/7 instrument controllers. Data were collected and processed on PE Pyris 1 and TGA 7 software on a digital Venturis 5100 computer. For differential scanning calorimetry (DSC), samples weighing ~5-20 mg were heated under dry nitrogen gas. Data were collected at heating and cooling rates of 30 C/min with a three cycle minimum. For thermal gravimetric analysis (TGA), samples weighing ~10 mg were heated under dry nitrogen gas at the rate of 40 C/min. Decomposition temperatures (T_d) were defined by the onset of decomposition.

Linker activation. The linker acids (**2; III and IV**) were activated by heating to reflux temperatures in excess thionyl chloride ($SOCl_2$) for 1 hr. The product (**3**) was isolated by evaporating the solvent.

Coupling reaction. Distilled benzyl salicylate (**1**) (1.0 ml) was dissolved in 20 ml of dry THF under dry nitrogen, then 6.16 mol of NaH was slowly added. After 1 hr, 2.50 mol of the linker chloride (**3**) was added, and the reaction was allowed to proceed until thin layer chromatography (TLC) using eluent of ethyl acetate:petroluem ether (30:70) indicated the disappearance of the starting material. The reactions were quenched with an aqueous solution of saturated ammonium chloride, THF was removed under vacuum, and the product (**4**) extracted with chloroform ($CHCl_3$).

SA. Yield: 99%. ^{1}H NMR ($CDCl_3$): 8.07 (d, 2H, Ar-H), 7.55 (t, 2H, Ar-H), 7.40 (m, 12H, Ar-H), 7.10 (d, 2H, Ar-H), 5.29 (s, 4H, -CH_2-), 2.71 (s, 4H, -CH_2-).

TA. Yield: 100%. ^{1}H NMR ($CDCl_3$): δ 8.15 (s, 4H, Ar-H), 7.65 (t, 2H, Ar-H), 7.40 (t, 2H, Ar-H), 7.26 (s, 2H, Ar-H), 5.22 (s, 4H, -CH_2-).

OBA. Yield: 70%. ^{1}H NMR ($CDCl_3$): δ 8.15 (t, 2H, Ar-H), 7.60 (t, 2H, Ar-H), 7.40 (t, 2H, Ar-H), 7.25 (d, 2H, Ar-H), 7.10 (d, 2H, Ar-H), 5.20 (s, 4H, -CH_2-).

CDA. Yield: 100%. ^{1}H NMR ($CDCl_3$): δ 8.05 (d, 2H, Ar-H), 7.55 (t, 2H, Ar-H), 7.40 (m, 12H, Ar-H), 7.05 (d, 2H, Ar-H), 5.30 (s, 4H, -CH_2-), 2.55-0.85 (br, 4H, cyclohexyl H).

Deprotection. The product of the coupling reaction (**4**) was dissolved in ~50-70 ml of methylene chloride (CH_2Cl_2), followed by addition of 10% palladium on carbon. Hydrogen gas was allowed to bubble through the reaction mixture until complete disappearance of the starting material as monitored by TLC. The product (**5**) was isolated by filtering the reaction mixture through Celite, and rinsing with appropriate solvent system.

SA. Yield: 100%. ^{1}H NMR (DMSO): δ 7.95 (d, 2H, Ar-H), 7.70 (t, 2H, Ar-H), 7.40 (t, 2H, Ar-H), 7.20 (d, 2H, Ar-H), 2.95 (s, 4H, -CH_2-).

TA. Yield: 69%. ^{1}H NMR (DMSO): δ 8.35 (s, 4H, Ar-H), 8.05 (d, 2H, Ar-H), 7.75 (t, 2H, Ar-H), 7.50 (d of d, 4H, Ar-H).

OBA. Yield: 73%. ^{1}H NMR (DMSO): δ 8.20 (s, 4H, Ar-H), 8.00 (d, 2H, Ar-H), 7.70 (t, 2H, Ar-H), 7.50-7.30 (m, 8H, Ar-H).

CDA. Yield: 83%. ^{1}H NMR (DMSO): δ 7.95 (d, 2H, Ar-H), 7.65 (t, 2H, Ar-H), 7.40 (t, 2H, Ar-H), 7.20 (d, 2H, Ar-H), 2.80-0.90 (br, 4H, cyclohexyl H).

Activation and polymerization. The deprotected product (**5**) was heated to reflux temperature in excess acetic anhydride for 1.5 hours, followed by evaporation of excess solvent. The monomer (**6**) was self-polymerized under vacuum at 180°C until solidified. The product (**7**) was dissolved in minimum amount of CH_2Cl_2 then precipitated in diethyl ether.

SA. Yield: 28%, light brown powder. ^{1}H NMR ($CDCl_3$): δ 8.20 (br, Ar-H), 7.40 (br, Ar-H), 7.10 (br, Ar-H), 2.25 (s, -CH_2-). MW: 2,200; PDI: 1.5. T_d (°C): 297; T_g (°C): 103.

TA. Yield: 43%, brown-gray powder. MW: 7,200; PDI: 1.1. T_d (°C): 363; T_g (°C): 89. IR (cm^{-1}): 1790 (anhydride C=O), 1746 (ester C=O), 1262, 1206 (C-O).

OBA. Yield: 57%. MW: 5,300; PDI: 1.2. T_d (°C): 348; T_g (°C): 114.

CDA. Yield: 15%, brown fluffy solid. ^{1}H NMR ($CDCl_3$): 8.28 (br, Ar-H), 8.02 (br, Ar-H), 7.27 (br, Ar-H), 7.35-7.12 (br, Ar-H), 2.80-1.50 (br, cyclohexyl Hs). MW: 1,500; PDI: 1.9. T_d (°C): 336; T_g (°C): 79.

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Table 1. Polymer Properties Summary

Linker	SA	TA	OBA	CDA
MW	2,200	7,200	5,300	1,500
PDI	1.5	1.1	1.2	1.9
T_d (°C)	297	363	348	336
T_g (°C)	103	89	114	79

Figure 1. Hydrolytic degradation of poly(anhydride-esters)

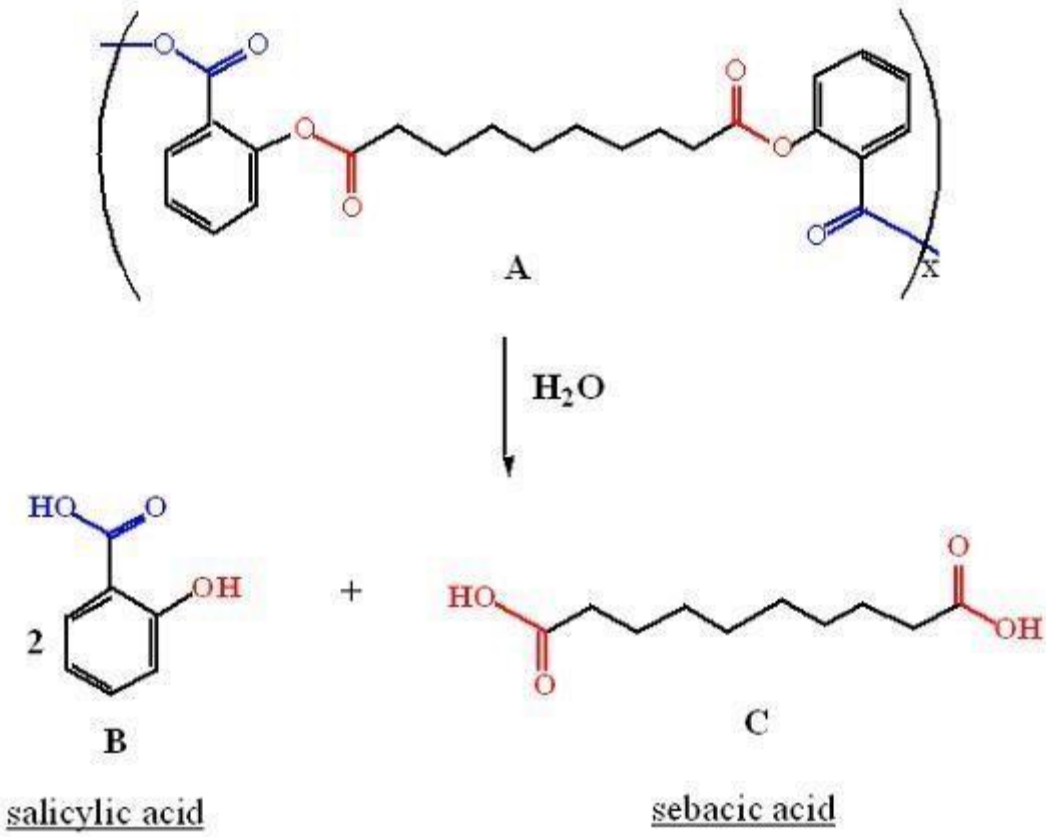


Figure 2. General poly(anhydride-ester) structure

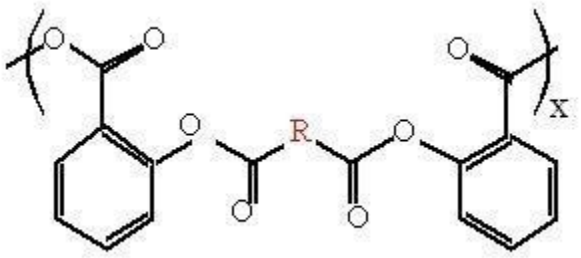
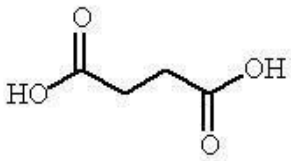
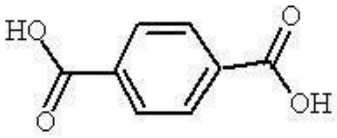


Figure 3. Linker structures



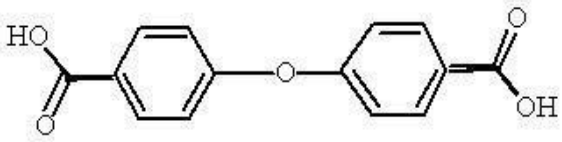
I

SUCCINIC ACID (**SA**)



II

TEREPHTHALIC ACID (**TA**)



III

4,4'-OXYBIS(BENZOIC ACID) (**OBA**)

IV

1,4-CYCLOHEXANEDICARBOXYLIC ACID (**CDA**)

Figure 4. General poly(anhydride-ester) synthesis